Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

ORIGINAL RESEARCH

# Investigating the behavioral responses of nektonic and benthic tadpoles elicited by the presence of a predatory nektonic fish

Y. C. M. de Souza[1], F. S. Annibale[2], F. Nomura[3] & D. C. Rossa-Feres[4]

[1]Graduate Program in Biodiversity, São Paulo State University – UNESP, São José do Rio Preto, SP, Brazil
[2]Universidade Virtual do Estado de São Paulo – UNIVESP, Bady Bassitt, SP, Brazil
[3]Department of Ecology, Goiás Federal University – UFG, Goiânia, GO, Brazil
[4]Department of Biological Sciences, São Paulo State University – UNESP, São José do Rio Preto, SP, Brazil

**Keywords**
feeding behavior; induced defenses; *Oreochromis niloticus*; *Physalaemus nattereri*; predator–prey interactions; *Scinax fuscovarius*; swimming activity; vertebrate predator.

**Correspondence**
Yasmim Caroline Mossioli de Souza, Graduate Program in Biodiversity, São Paulo State University – UNESP, São José do Rio Preto 15054-000, SP, Brazil.
Email: mossioli.souza@unesp.br

Editor: Anthony Herrel
Associate Editor: Débora Moreno Azócar

Received 20 May 2024; revised 3 February 2025; accepted 17 March 2025

doi:10.1111/jzo.70020

## Abstract

Different defensive strategies are used by tadpoles to avoid or escape from predators, and it is possible that differences in the microhabitat of prey and predators influence the defensive strategies used by tadpoles. Therefore, we experimentally tested whether the presence of a nektonic fish predator (*Oreochromis niloticus*) reduces the time of displacement, increases the latency to start foraging, and reduces the amount of food consumed by nektonic (*Scinax fuscovarius*) and benthic (*Physalaemus nattereri*) tadpoles. Contrary to our expectations, the presence of the predator did not affect the behavior of the nektonic tadpoles. Conversely, however, benthic tadpoles reduced their displacement time in the presence of the predator. It is possible that, due to the ability of nektonic predators to occupy the entire water column, they may trigger defensive behaviors in benthic prey, while the silvery coloring of nektonic tadpoles would reduce their detectability by aquatic predators. Thus, nektonic tadpoles were less reactive toward the presence of predators, despite sharing the same microspatial niche, whereas benthic tadpoles seem to be more reactive toward predators, despite the fact that they do not share their microspatial niche.

## Introduction

Tadpoles are exposed to a wide variety of predators (e.g. Mamede & Nomura, 2021; Martins et al., 2021; Melo et al., 2021; Mettler et al., 2021), and present different defense mechanisms against predation (e.g. Balestrieri et al., 2019; Brown et al., 2019; Rae & Murray, 2019; Van Buskirk et al., 2003). In principle, defense mechanisms can be classified into two types (*sensu* Brodie Jr. et al., 1991): strategies to avoid predators (e.g. camouflage, use of refuges and decreased levels of swimming and foraging activities), and strategies to escape from predators (e.g. burst speed, unpalatability and deflection of the attacks of predators toward non-essential body parts, such as the tail).

Benthic and nektonic tadpoles exhibit different strategies to avoid predators. For example, benthic tadpoles, meaning those that occupy the bottom of aquatic habitats (McDiarmid & Altig, 1999), generally decrease swimming activity as a response to predation risk (e.g. Eterovick et al., 2020; Nomura et al., 2013). This strategy is efficient to avoid ambush predators, such as Odonata naiads, as it reduces encounter rates (Heyer et al., 1975; Pritchard, 1965). However, this strategy is not efficient against active predators, such as fishes, which are visually oriented and capable of detecting even small movements of the prey (Nomura et al., 2011; Wellborn et al., 1996). Nektonic tadpoles, which mainly occupy the middle of the water column (McDiarmid & Altig, 1999), generally perform quick movements and evasive maneuvers to escape from predators (e.g. Azevedo-Ramos et al., 1992; Watkins, 1996).

It could be assumed that tadpoles which share the same microhabitat with a predator would display stronger defensive behaviors compared to their responses toward predators that occupy different microhabitats. For example, de Souza et al. (2024) observed that benthic tadpoles of *Physalaemus* *nattereri* (Steindachner, 1863) reduce their swimming activity in the presence of benthic predators (Odonata naiads), while nektonic tadpoles of *Scinax fuscovarius* (Lutz, 1925) do not. However, there are few empirical tests on how the predator's microhabitat preferences could predict the display of defensive strategies by tadpoles.

In this study, we hypothesized that predators elicit behavioral changes in prey species when they share the same microspatial niche, but not when predator and prey have different microspatial preferences. In such conditions, the presence of a nektonic fish predator, as *Oreochromis niloticus* (Linnaeus, 1758), would (1) reduce the displacement time, (2) increase the latency to start foraging, and hence, (3) decrease food consumption of nektonic tadpoles, as the tadpoles of *S. fuscovarius*. We perceived *O. niloticus* and *S. fuscovarius* as appropriate model species to test these predictions, as both the fish and the tadpole forage in the same microhabitat, which is the middle of the water column (Rossa-Feres & Nomura, 2006; Wudneh, 1988), and both occupy farm dams and other reservoirs (Vasconcelos & Rossa-Feres, 2005). Conversely, we predicted that the presence of the nektonic fish predator (1) would not reduce the displacement time, (2) the latency to start foraging, and (3) food consumption of the benthic tadpoles of *P. nattereri*, as they forage in different depth ranges (Rossa-Feres & Nomura, 2006; Wudneh, 1988).

## Materials and methods

### Species, collection and acclimatization

Tadpoles of the treefrog *S. fuscovarius* have a nektonic habit, compressed body, big and lateral eyes, high fins, a flagellum at the tip of the tail, and silver-grayish coloration, with small dark spots scattered all over the body (Rossa-Feres & Nomura, 2006). Tadpoles of the frog *P. nattereri* have a benthic habit, globular body, small and dorsal eyes, low fins, and brown coloration, with pigmented tail muscle (Rossa-Feres & Nomura, 2006). Both species are widely distributed throughout central Brazil, are locally abundant (Rossa-Feres & Nomura, 2006), and are considered of least concern regarding extinction risk (IUCN SSC Amphibian Specialist Group, 2023a, 2023b). These tadpoles can be found in a variety of aquatic habitats, such as temporary and permanent ponds and large artificial tanks (Vasconcelos et al., 2011; Vasconcelos & Rossa-Feres, 2005).

We found these tadpoles in a temporary aquatic habitat located in a pasture area (20°50′48.7″ S, 49°28′27.4″ W) in the municipality of Mirassol, in the northwest part of the state of São Paulo, Brazil. We collected the tadpoles (150 for each species) with a wire mesh net (32 cm in diameter and with mesh size of 3 mm$^2$), transported them in plastic bags with water from the site where they had been collected, and accommodated them in isothermal boxes of expanded polystyrene with frozen thermal bags to avoid overheating. Tadpole collections were performed in January 2022 (*P. nattereri*) and February 2023 (*S. fuscovarius*).

Before the experiments, we acclimatized the tadpoles to laboratory conditions for 2 days in five glass aquaria (30 × 20 × 30 cm) with 30 tadpoles in each, filled with 16.2 L of dechlorinated and constantly aerated water, covered with blue plastic adhesive to reduce the stress caused by external movements, and containing artificial vegetation to simulate the natural conditions of the collection site. We housed the tadpoles under a controlled photoperiod regime (12 h of light and 12 h of darkness), with water temperature at 26.64 ± 0.80°C, simulating the natural conditions of the collection site. The tadpoles were fed algae and krill as powdered commercial food (Sera Micron®, Heinsberg, Germany) *ad libitum*. The aquaria were siphoned daily to remove leftover food and feces. For the experiments, we selected tadpoles of similar size (*S. fuscovarius*: 27.46 ± 3.37 mm; *P. naterreri*: 24.88 ± 1.62 mm) and between the stages 25 and 36 of development (Gosner, 1960), as these stages are mainly involve body growth and not developmental changes (McDiarmid & Altig, 1999).

The tilapia fish *O. niloticus* is a nektonic generalist predator (Wudneh, 1988) introduced in Brazil in the 1970s for food production but, due to management errors and accidents, it is now found reproducing in natural habitats, like rivers and lakes (Gomiero & Braga, 2006; Langeani et al., 2007), including areas that are used by anurans for reproduction such as farm dams and other reservoirs (Vasconcelos & Rossa-Feres, 2005). Although the fish used in our experiment were not obtained from an aquatic habitat shared with the tadpoles of *S. fuscovarius* and *P. nattereri*, this fish species is a suitable predator model because it is widely distributed in Brazil (Rocha et al., 2023) and is a known tadpole predator (Nomura et al., 2011). One hundred fish specimens were obtained from a fish farming tank (10 × 12 × 1.60 m) located in an experimental area at the Campus of the São Paulo State University, São José do Rio Preto, Brazil (20°47′06.8″ S, 49°21′44.1″ W). We collected the fish with a mesh cast net (30 mm, 210/18 wire, height 2.30 and 16 m) and acclimatized them for 15 days before the experiments, in a polypropylene tank (95 cm in diameter by 72 cm in depth) filled with 400 l of dechlorinated and constantly aerated water. We cleaned the tank weekly to remove excess food and waste. During this process, we only replaced a percentage (<25%) of the water volume, to maintain a stable environment (e.g. Gauy et al., 2018; Gonçalves-de-Freitas et al., 2008). The fish tank was kept under a controlled photoperiod regime (12 h/12 h) and constant water temperature (28°C, maintained by two heater Atman 200 w), simulating the conditions of the collection site. The fish were fed daily with commercial fish food (AGROMIX®, Jaboticabal, Brazil – 32% crude protein) *ad libitum*. Three days before the experiment, the fish were taken to the experimental room and individually conditioned in aquariums. For the first 48 h, we fed the fish with extra tadpoles of *S. fuscovarius* and *P. nattereri*. Fish were then fasted for 24 h before the beginning of the experiment to stimulate tadpole predation. Predation by fish during the experiment was important in order to obtain all kinds of chemical predation cues (kairomone, disturbance cue and alarm cue; Ferrari et al., 2010). Fish used in the experiment measured 7.60 ± 0.50 cm, which was an adequate size to prey on tadpoles, as evidenced by preliminary tests (YCMS, personal observation).

## Experimental design

We carried out the experiments between 10:00 and 11:00 AM in aquaria of the same dimensions as those used in tadpole acclimatization, but without the blue adhesive plastic on one of its sides (20 × 30 cm) to allow behavioral observations. A galvanized steel net (30 × 20 cm; 2.5 × 2.5 mm mesh) was placed into the aquarium, vertically dividing it into two unequal compartments (24 cm for the larger compartment and 6 cm for the small compartment). The net allowed the water to flow between the two aquarium parts and, consequently, the transmission of chemical, mechanical, and visual cues in the water. Each treatment consisted of exposing tadpoles of *S. fuscovarius* (nektonic, *N* = 25) or of *P. nattereri* (benthic, *N* = 25) to the *O. niloticus* predator in the experimental aquaria described above. We added one fish predator and one conspecific tadpole (nektonic or benthic) in the larger compartment, and one tadpole (nektonic or benthic), our focal animal, in the small compartment (Fig. 1). Our control group consisted of a single focal tadpole per aquarium (*N* = 25 for *S. fuscovarius*; *N* = 25 for *P. nattereri*) released in the small compartment of the experimental aquarium (Fig. 1). Focal tadpoles were kept fasted and alone in an individual aquarium 24 h before the experiment. The conspecific tadpole was not subjected to any additional acclimatization, as it was used only to feed the fish.

We used a camera (HDR-CX440, Sony), positioned 80 cm away from the front of the aquarium, to register all tadpole behaviors, always operated by the same observer (YCMS). At this distance, neither the predators nor the tadpoles seemed to react to the presence of the observer. The predator was kept in the experimental aquarium 24 h before the beginning of the experiment. Five minutes after we released the focal tadpole into the experimental aquarium, we released the conspecific tadpole in the larger part of the aquarium and, after the predation of the conspecific tadpole by the fish, we recorded the focal tadpole's displacement time for 3 min. In the control group, we started filming 5 min after placing the focal tadpole into the small part of the aquarium, also for 3 min. After this period for activity measurement, we started another filming session of 26 min to evaluate the foraging activity of tadpoles. To this end, we added a glass plate (29 × 19 cm), with a layer of food on the surface, vertically on the aquarium's uncovered side. We prepared the glass plate using a mixture of water and the commercial food (Sera Micron®), in a concentration of 80 mg.mL$^{-1}$ (Venesky et al., 2013). We brushed 2.0 mL of this solution on one surface of the glass plate, forming a uniform layer of food in a delimited area (27.5 × 3 cm). Then, we let the glass plate naturally dry over 24 h, so that the food was firmly adhered to the plate's surface to be used for the sessions to evaluate tadpole foraging activity. We observed and recorded two aquaria per day and randomized

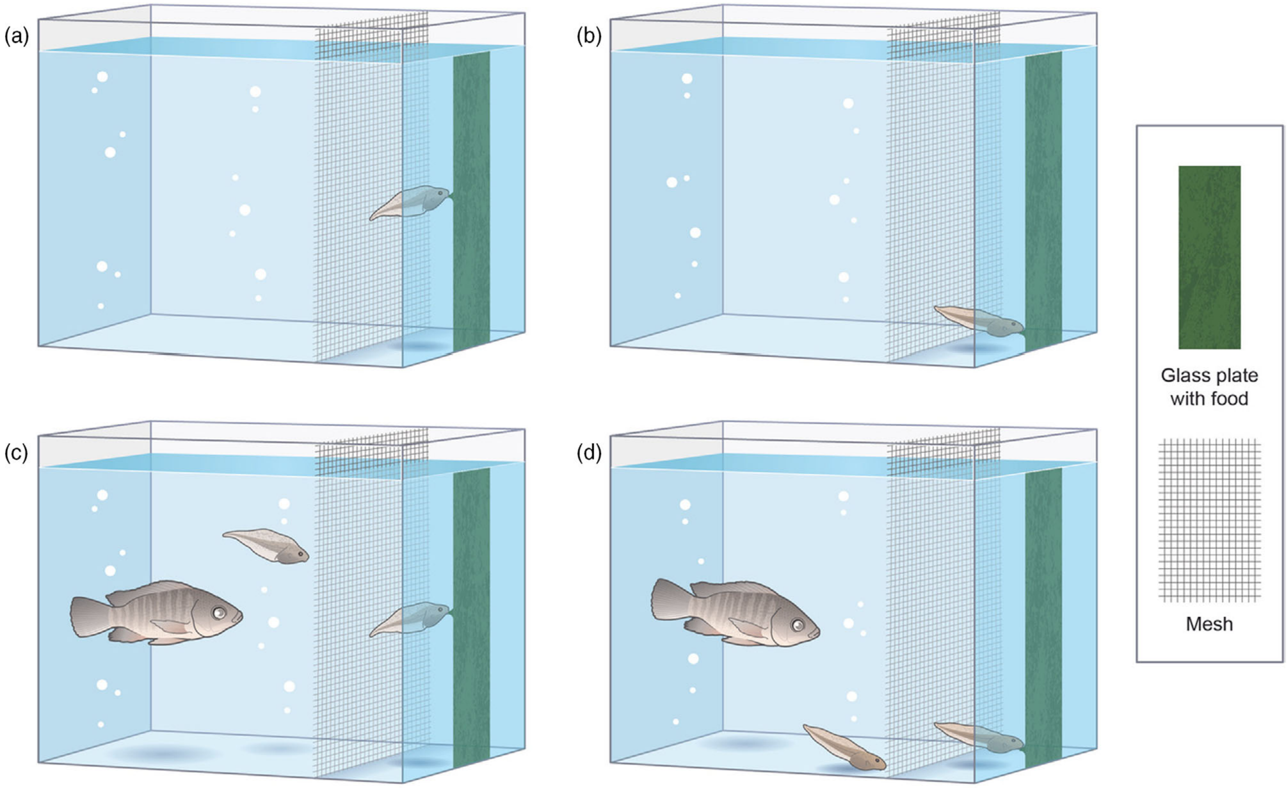

**Figure 1** Experimental design scheme: controls (a, b) and treatments (c, d), conducted separately for nektonic (a, c) and benthic (b, d) tadpoles.

the order of control and treatment groups. As such, the experiment lasted for 25 days for each studied species. Each tadpole and fish was used only once in each observational unit and the experiment was carried out separately for each species. After the experiment, we anesthetized all tadpoles in a solution of 10% lidocaine, preserved them in a 1:1 solution of alcohol (70%) and formaldehyde (15%), and deposited them into the Amphibian Scientific Collection (DZSJRP Amphibia-Tadpoles) at the Department of Biological Sciences of UNESP, São José do Rio Preto, Brazil (DZSJRP 3578.01 and 3579.01). We returned the fish to the tanks from which they had been obtained.

## Data collection

For each focal tadpole, we recorded its time of displacement, latency to forage, and amount of food consumed from the videos recorded during the experiments. We quantified the total displacement time in seconds, as the sum of all swimming intervals during the experimental session. After the positioning of the glass plate with food, as described above, we started to count the time until the focal tadpole started to feed. We called this time latency to forage. We established a maximum latency time of 26 min to prevent habituation to the stressful condition of the aquarium or attenuation of the chemical signals within the water (e.g. Ferrari et al., 2007; Peacor, 2006). Both situations could reduce the assessment accuracy of predation risk by the tadpoles and influence their behavioral response to the predator. We considered the amount of food consumed as the proportion of the food scraped from the glass plate. To measure this food consumption, the glass plates were digitized (HP© LaserJet M1132) and analyzed using the Particle Analysis tool from ImageJ® software (Schneider et al., 2012).

## Statistical analyses

To test whether nektonic predators elicit behavioral changes on tadpoles, and whether these changes could be predicted by the tadpole's microspatial niche, we used mixed generalized linear models (Zuur et al., 2009). We tested whether the presence of the nektonic fish (fixed effect with two levels: fish present and fish absent) reduced the displacement time or increased the latency to forage (response variables, measured in seconds; Tables S1–S3) of the *S. fuscovarius* or *P. nattereri* tadpoles (models constructed separately for each species). We considered the day on which the tadpoles were tested as a random factor, with a Tweedie distribution for the link function (Zuur et al., 2009). We also constructed a similar GLMM model for food consumption (response variable, measured as a percentage; Tables S1–S3) but with a beta distribution for the link function (Zuur et al., 2009). We evaluated the fitting of our models by visual inspection of random quantile residuals (Dunn & Smyth, 1996) and then we tested the effect of the predator presence with likelihood ratio tests (Zuur et al., 2009). All models were fitted using the *glmmTMB* function of the *'glmmTMB'* package (Brooks et al., 2017) in the R software (R Core Team, 2023).

# Results

The presence of the predatory nektonic fish did not affect the displacement time (Table 1, Fig. 2), latency to forage (Table 1, Fig. 3), or food consumption (Table 1, Fig. 4) of nektonic *S. fuscovarius* tadpoles. However, the predator presence reduced the mean displacement time of benthic *P. nattereri* tadpoles almost by a factor of three (Table 1, Fig. 5). However, the presence of the fish predator did not affect the latency to forage (Table 1, Fig. 6) or the food consumption (Table 1, Fig. 7) of benthic tadpoles.

# Discussion

Contrary to our hypothesis, the presence of the predatory nektonic fish was not sufficient to elicit behavioral changes in nektonic tadpoles, but was capable of reducing the swimming activity of benthic tadpoles. It is possible that benthic tadpoles of *P. nattereri* were more reactive toward the presence of a predator in comparison with nektonic tadpoles of *S. fuscovarius*, independent of the predator's microspatial niche.

To avoid predators, nektonic tadpoles use vegetation as a refuge and for camouflaging (e.g. Babbitt & Tanner, 1998; Tarr & Babbitt, 2002). Tadpoles of *S. fuscovarius* are commonly found among the vegetation, during either day or night, holding on to the tip of leaves by their snouts (Schulze et al., 2015; YCMS, personal observation; Fig. S1). We know that the presence of dense vegetation in the environment increases the survival rates of some nektonic tadpoles, by offering them a shelter from predators (see Kopp et al., 2006). The complexity of vegetation offers physical obstacles that can hinder direct access to tadpoles for predators (e.g. Babbitt & Jordan, 1996; Baber & Babbitt, 2004). In our study, we did not include vegetation in the experimental aquaria, but we did observe tadpoles of *S. fuscovarius* holding on to the aquarium walls, glass plates, and galvanized steel net by their snouts, as they usually do with natural plants (Videos S1–S3). We interpreted such behavior as an antipredatory strategy, in which the tadpole disguises itself as a leaf (mimicry) and thus is less vulnerable to predator attacks. It is also possible that the silvery coloring of these tadpoles decreased their

**Table 1** Likelihood ratio tests for the effect of the predator presence on behavioral responses of *Scinax fuscovarius* and *Physalaemus nattereri* tadpoles, tested separately

| | *S. fuscovarius* | | | *P. nattereri* | | |
|---|---|---|---|---|---|---|
| | d.f. | $X^2$ | *P* | d.f. | $X^2$ | *P* |
| Displacement time (s) | | | | | | |
| Predator | 1 | 1.592 | 0.207 | **1** | **7.537** | **0.006** |
| Latency (s) | | | | | | |
| Predator | 1 | 0.194 | 0.660 | 1 | 0.167 | 0.683 |
| Food consumption (%) | | | | | | |
| Predator | 1 | 0.159 | 0.690 | 1 | 0.767 | 0.381 |

Significant effects are highlighted in bold.
d.f., degrees of freedom.

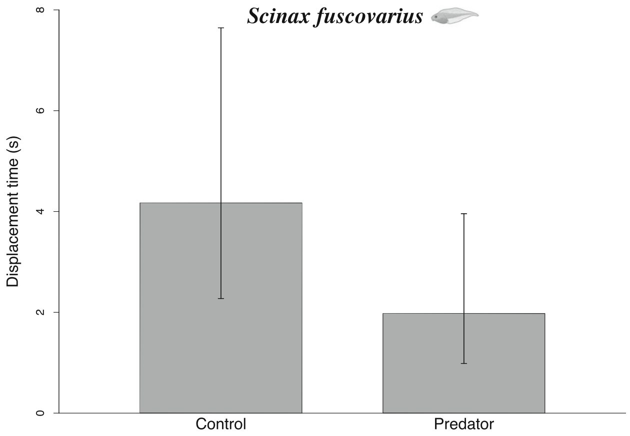

**Figure 2** Mean displacement time of *Scinax fuscovarius* tadpoles in the presence and absence of a predator. Error bars represent standard errors. Mean values and standard errors were estimated using mixed generalized linear models.

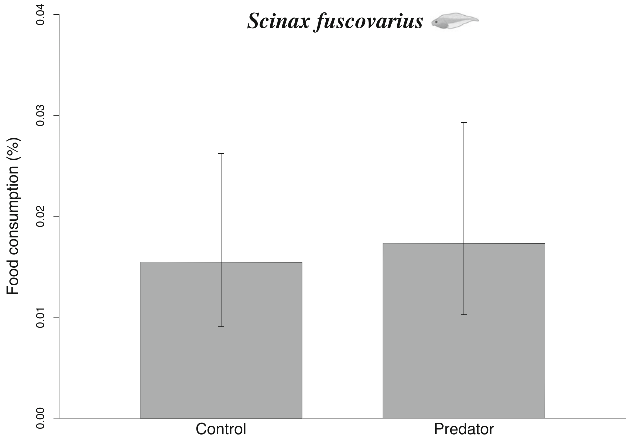

**Figure 4** Average food consumption by tadpoles of *Scinax fuscovarius* in the presence and absence of a predator. Error bars represent standard errors. Mean values and standard errors were estimated using mixed generalized linear models.

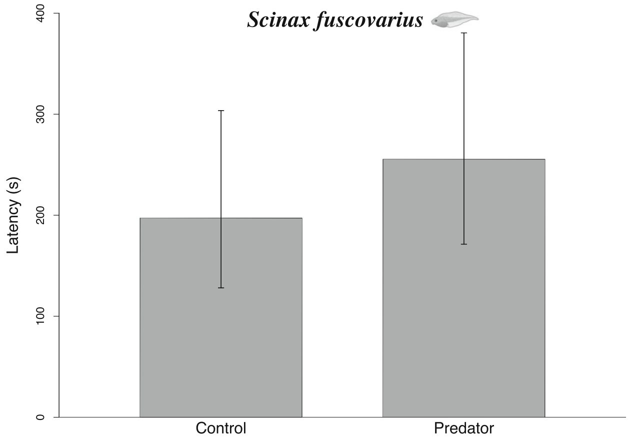

**Figure 3** Average latency time of *Scinax fuscovarius* tadpoles to start foraging in the presence and absence of a predator. Error bars represent standard errors. Mean values and standard errors were estimated using mixed generalized linear models.

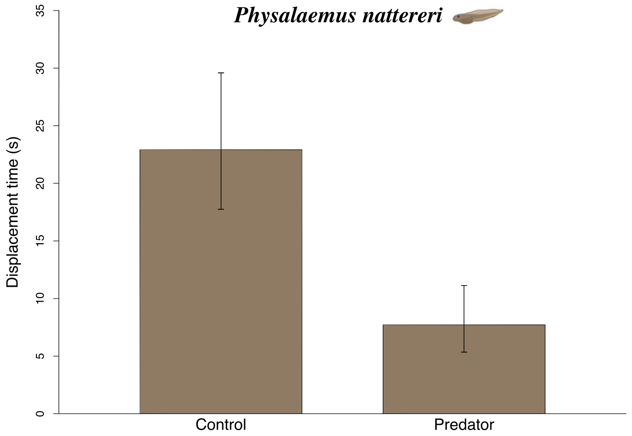

**Figure 5** Mean displacement time of *Physalaemus nattereri* tadpoles in the presence and absence of a predator. Error bars represent standard errors. Mean values and standard errors were estimated using mixed generalized linear models.

detectability by the predators and allowed them to maintain their activities in the presence of the predatory fish. For fish, the silvery coloring contributes to confusing predators attacking from below, imitating the sunlight that shines on the surface of the water (Hamilton III & Peterman, 1971; Ruxton et al., 2004). This obscures the organism when viewed from below, hindering detection by predators and functioning as an effective form of camouflage in aquatic environments (e.g. Hamilton III & Peterman, 1971; Ruxton et al., 2004). Thus, the silvery coloring provides a cryptic advantage, explaining its prevalence among nektonic tadpoles and fish (Ruxton et al., 2004). Moreover, the tadpoles of *S. fuscovarius* were less active during the experiment, regardless of the presence or absence of the predator, compared to the tadpoles of *P. nattereri*. In general, the tadpoles of *S. fuscovarius* were two times less active in the presence of the predator and more than three times less active in the absence of the predator when compared to the activity time of the benthic tadpoles. Therefore, the foraging activity of *S. fuscovarius* tadpoles could be controlled by an agoraphobic response (i.e. fear of open spaces). This tendency could be an adaptation to encourage foraging within protected microhabitats by rendering open spaces less appealing to the animal. However, this hypothesis still needs testing.

Reducing swimming or foraging activities in the presence of predators is a common antipredatory response in benthic species (e.g. Hettyey et al., 2012; Scribano et al., 2020). However, we did not expect that *P. nattereri* tadpoles would react by changing their behavior in the presence of the nektonic predator, as these species do not share microspatial niche. It is possible that due to the ability of nektonic predators to occupy the

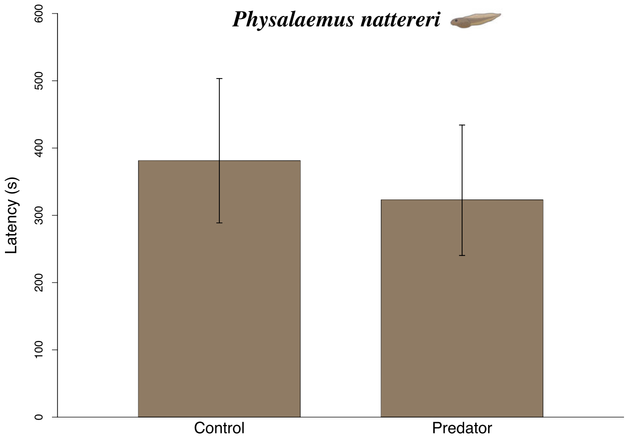

**Figure 6** Average latency time of *Physalaemus nattereri* tadpoles to start foraging in the presence and absence of a predator. Error bars represent standard errors. Mean values and standard errors were estimated using mixed generalized linear models.

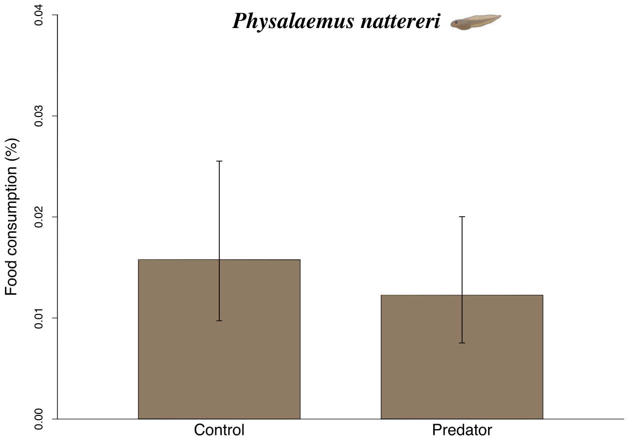

**Figure 7** Average food consumption by tadpoles of *Physalaemus nattereri* in the presence and absence of a predator. Error bars represent standard errors. Mean values and standard errors were estimated using mixed generalized linear models.

entire water column, they caused a predation pressure on benthic tadpoles as benthic predators do, which may explain their reduced swimming activity in our experiment. Since fishes could exert considerable predation pressure on tadpole populations (Heyer et al., 1975), any response that allows tadpoles to avoid fish predators would be advantageous, even if the perceived risk is greater than the actual danger. This could explain why even benthic tadpoles react to nektonic predators and why nektonic tadpoles prefer to forage among vegetation. While a reactive strategy would require context evaluation, an alternative strategy involves tadpoles not evaluating the risk but simply foraging in areas associated with predator absence. Moreover, reactive tadpoles respond to different risk sources even from predators in different spatial niches, such as spiders (e.g. Mamede & Nomura, 2021; Menin et al., 2005).

Furthermore, the efficiency of defense mechanisms of tadpoles could interact with the predators' foraging strategies (Nomura et al., 2011). Reduction in activity time would be more efficient against ambush predators, as it reduces the encounter rates between the tadpole and the predator, compared to active foragers such as the *O. niloticus* fish (Nomura et al., 2011). Conversely, due to the efficiency of fishes as predators of tadpoles (Heyer et al., 1975), avoidance mechanisms (*sensu* Brodie Jr. et al., 1991), such as changing the foraging microhabitat, would be favored by tadpoles.

Food consumption by both nektonic and benthic tadpoles was not influenced by the presence of the predator, since both species consumed little food in both the control and the treatment groups. It is possible that the time that we set to test the foraging behavior of the tadpoles was insufficient for them to find and consume the food. We had set a period of 26 min to our assay because some studies have demonstrated that the chemical cues released by predators and prey into the water degrade quickly (e.g. Ferrari et al., 2007; Peacor, 2006). We made this choice to avoid reducing the accuracy of predation risk assessment by the tadpoles, but this amount of time may not have been enough to allow the tadpoles of *S. fuscovarius* and *P. nattereri* to feed.

Another possible explanation for our results may be the prey's naivety. The 'prey naiveté' hypothesis suggests that native species may not recognize alien species as predators and thus fail to exhibit appropriate antipredator behaviors (Banks & Dickman, 2007; Cox & Lima, 2006). However, the ability of prey to detect and respond to invasive predators can evolve rapidly and appears to depend on the timing of the predator's introduction to a specific region (Hettyey et al., 2016). In the studied region, the fish *O. niloticus* has coexisted with tadpoles of the two studied species at least since 2005 (Vasconcelos & Rossa-Feres, 2005). In addition, experimental studies have shown that tadpoles in the neotropics can recognize invasive fish species as predators and modify their behavior in their presence (e.g. Nomura et al., 2011; Teplitsky et al., 2003). Furthermore, given that we combined kairomone, disturbance, and alarm cues to simulate the risk of predation, tadpoles may have exhibited a generic instead of a specific response to the predator. Therefore, more studies are needed to distinguish whether the behavioral changes in *P. nattereri* tadpoles are a consequence of the detection of the fish as a predator or of the alarm cues emitted by the consumed conspecific tadpoles.

Regardless of the cues that the benthic tadpoles of *P. nattereri* were responding to, and despite the fact that they do not share a microspatial niche, we demonstrated that they are sensitive toward nektonic predators. Nektonic tadpoles of *S. fuscovarius*, however, avoid predation by hiding in the environment (Kopp et al., 2006), and this behavior may explain the absence of behavioral changes in the presence of the predator, even from the same spatial niche. Therefore, we emphasize the need for additional experiments that would include vegetation as refuge and control tadpole coloration to understand the defensive strategies used by the nektonic tadpoles of *S. fuscovarius*. This study contributes to broadening our understanding of the influence of predators on the behavior of tadpoles, but the generalization of these results requires experimental tests performed on benthic and nektonic tadpoles of different species.

## Acknowledgements

We thank Eliane G. de Freitas and Luiz H. Florindo for supporting this study with equipment and infrastructure. We also thank Carlos E. de Sousa for his help during collections. The experiments were approved by the Institute of Environment and Renewable Natural Resources (IBAMA) and the Chico Mendes Institute for Biodiversity Conservation (ICMBio), SISBio authorization number: 18163-1 to DCR-F. The maintenance of tadpoles and tilapia in the laboratory and the experimental design were approved by the Commission for Ethics in the Use of Animals (CEUA n. 234/2021 under the responsibility of DCR-F), according to the National Council for the Control of Animal Experimentation (CONCEA). The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). The Article Processing Charge for the publication of this research was funded by the CoordenaÃ§Ã£o de AperfeiÃ§oamento de Pessoal de NÃvel Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

## Author contributions

Y.C.M.S., F.S.A., and D.C.R.-F. conceived the idea for the project and planned the experimental design. Y.C.M.S. carried out the laboratory work, performed the statistical analysis, and led the writing of the manuscript with contributions from F.S.A., F.N., and D.C.R.-F.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Funding statement

This study was funded by the Higher Education Personnel Improvement Coordination (CAPES, 88887.509980/2020–00 for Y.C.M.S.) and the National Council for Scientific and Technological Development (CNPq 304 760/2021–8 for D.C.R.-F.).

## Data availability statement

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files.

## References

Azevedo-Ramos, C., Van Sluys, M., Hero, J. M., & Magnusson, W. E. (1992). Influence of tadpole movement on predation by odonate naiads. *Journal of Herpetology*, **26**(3), 335–338.

Babbitt, K. J., & Jordan, F. (1996). Predation on *Bufo terrestris* tadpoles: Effects of cover and predator identity. *Copeia*, **1996**, 485–488.

Babbitt, K. J., & Tanner, G. W. (1998). Effects of cover and predator size on survival and development of *Rana utricularia* tadpoles. *Oecologia*, **114**, 258–262.

Baber, M. J., & Babbitt, K. J. (2004). Influence of habitat complexity on predator–prey interactions between the fish (*Gambusia holbrooki*) and tadpoles of *Hyla squirella* and *Gastrophryne carolinensis*. *Copeia*, **2004**, 173–177.

Balestrieri, A., Gazzola, A., Pellitteri-Rosa, D., & Vallortigara, G. (2019). Discrimination of group numerousness under predation risk in anuran tadpoles. *Animal Cognition*, **22**, 223–230.

Banks, P. B., & Dickman, C. R. (2007). Alien predation and the effects of multiple levels of prey naiveté. *Trends in Ecology & Evolution*, **22**, 229–230.

Brodie, E. D., Jr., Formanowicz, D. R., Jr., & Brodie, E. D., III. (1991). Predator avoidance and antipredator mechanisms: Distinct pathways to survival. *Ethology Ecology & Evolution*, **3**, 73–77.

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. S., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, **9**(2), 378–400.

Brown, T. A., Fraker, M. E., & Ludsin, S. A. (2019). Space use of predatory larval dragonflies and tadpole prey in response to chemical cues of predation. *The American Midland Naturalist*, **181**, 53–62.

Cox, J. G., & Lima, S. L. (2006). Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. *Trends in Ecology & Evolution*, **21**, 674–680.

de Souza, Y. C. M., Annibale, F. S., Pelinson, R. M., & Rossa-Feres, D. C. (2024). Behavioral responses of benthic and nektonic tadpoles to the presence of a benthic predator. *Hydrobiologia*, **851**, 4955–4964.

Dunn, P. K., & Smyth, G. K. (1996). Randomized quantile residuals. *Journal of Computational and Graphical Statistics*, **5**, 236–244.

Eterovick, P. C., Kloh, J. S., Figueredo, C. C., Viana, P. I. M., Goulart, M., Milan, D. T., Fonseca, M. B., Martins, Í. M., Pinheiro, L. T., Quintão, R. P., Melo, T. K. F., Magalhães, R. A., Campos, C. M., Ferreira, V. C. M., de Oliveira, A. L., & Vences, M. (2020). Background choice and immobility as context dependent tadpole responses to perceived predation risk. *Scientific Reports*, **10**(1), 13577.

Ferrari, M. C., Messier, F., & Chivers, D. P. (2007). Degradation of chemical alarm cues under natural conditions: Risk assessment by larval woodfrogs. *Chemoecology*, **17**, 263–266.

Ferrari, M. C., Wisenden, B. D., & Chivers, D. P. (2010). Chemical ecology of predator–prey interactions in aquatic ecosystems: A review and prospectus. *Canadian Journal of Zoology*, **88**, 698–724.

Gauy, A. C. d. S., Boscolo, C. N. P., & Gonçalves-de-Freitas, E. (2018). Less water renewal reduces effects on social aggression of the cichlid *Pterophyllum scalare*. *Applied Animal Behaviour Science*, **198**, 121–126.

Gomiero, L. M., & Braga, F. M. (2006). Ichthyofauna diversity in a protected area in the state of São Paulo, Southeastern Brazil. *Brazilian Journal of Biology*, **66**, 75–83.

Gonçalves-de-Freitas, E., Teresa, F. B., Gomes, F. S., & Giaquinto, P. C. (2008). Effect of water renewal on dominance hierarchy of juvenile Nile Tilapia. *Applied Animal Behaviour Science*, **112**, 187–195.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**, 183–190.

Hamilton, W. J., III, & Peterman, R. M. (1971). Countershading in the colourful reef fish *Chaetodon lunula*: Concealment, communication or both? *Animal Behaviour*, **19**, 357–364.

Hettyey, A., Roelli, F., Thuerlimann, N. I. N. A., Zuercher, A. C., & Van Buskirk, J. (2012). Visual cues contribute to predator detection in anuran larvae. *Biological Journal of the Linnean Society*, **106**, 820–827.

Hettyey, A., Thonhauser, K. E., Bókony, V., Penn, D. J., Hoi, H., & Griggio, M. (2016). Naive tadpoles do not recognize recent invasive predatory fishes as dangerous. *Ecology*, **97**, 2975–2985.

Heyer, W. R., McDiarmid, R. W., & Weigmann, D. L. (1975). Tadpoles, predation and pond habitats in the tropics. *Biotropica*, **7**, 100–111.

IUCN SSC Amphibian Specialist Group. (2023a). *Physalaemus nattereri. The IUCN Red List of Threatened Species 2023*. https://www.iucnredlist.org/species/57267/148971050

IUCN SSC Amphibian Specialist Group. (2023b). *Scinax fuscovarius. The IUCN Red List of Threatened Species 2023*. https://www.iucnredlist.org/species/55958/61407576

Kopp, K., Wachlevski, M., & Eterovick, P. C. (2006). Environmental complexity reduces tadpole predation by water bugs. *Canadian Journal of Zoology*, **84**, 136–140.

Langeani, F., Castro, R. M. C., Oyakawa, O. T., Shibatta, O. A., Pavanelli, C. S., & Casatti, L. (2007). Diversidade da ictiofauna do Alto Rio Paraná: Composição atual e perspectivas futuras. *Biota Neotropica*, **7**, 181–197.

Mamede, J., & Nomura, F. (2021). *Dendropsophus minutus* (Hylidae) tadpole evaluation of predation risk by fishing spiders (*Thaumasia* sp.: Pisauridae) is modulated by size and social environment. *Journal of Ethology*, **39**, 217–223.

Martins, Í. M., Vasconcellos, A. D. S., Mota, T., & Eterovick, P. C. (2021). Detectability is in the eye of the beholder—The role of UV reflectance on tadpole detection and predation by a passerine bird. *Behavioral Ecology and Sociobiology*, **75**, 1–14.

McDiarmid, R. W., & Altig, R. (1999). Research: Materials and techniques. In R. W. McDiarmid & R. Altig (Eds.), *Tadpoles: The biology of anuran larvae* (pp. 7–23). The University of Chicago Press.

Melo, G. R., Solé, M., & Eterovick, P. C. (2021). Invisible or fearless: Tadpole response to predator cues depends on color. *Ethology Ecology & Evolution*, **33**, 99–107.

Menin, M., Rodrigues, D. D. J., & Azevedo, C. S. D. (2005). Predation on amphibians by spiders (Arachnida, Araneae) in the neotropical region. *Phyllomedusa*, **4**, 39–47.

Mettler, C. A., Aguirre-Morales, M., Harmeson, J., Robinson, W. L., & Carlson, B. E. (2021). Effects of the herbicide metolachlor and fish presence on pond mesocosm communities. *The American Midland Naturalist*, **186**, 199–214.

Nomura, F., De Marco, P., Carvalho, A. F. A., & Rossa-Feres, D. C. (2013). Does background colouration affect the behaviour of tadpoles? An experimental approach with an odonate predator. *Ethology Ecology & Evolution*, **25**, 185–198.

Nomura, F., do Prado, V. H. M., da Silva, F. R., Borges, R. E., Dias, N. Y. N., & Rossa-Feres, D. D. C. (2011). Are you experienced? Predator type and predator experience trade-offs in relation to tadpole mortality rates. *Journal of Zoology*, **284**, 144–150.

Peacor, S. D. (2006). Behavioural response of bullfrog tadpoles to chemical cues of predation risk are affected by cue age and water source. *Hydrobiologia*, **573**, 39–44.

Pritchard, G. (1965). Prey capture by dragonfly larvae (Odonata; Anisoptera). *Canadian Journal of Zoology*, **43**, 271–289.

R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Rae, J., & Murray, D. (2019). Pathogen vs. predator: Ranavirus exposure dampens tadpole responses to perceived predation risk. *Oecologia*, **191**, 325–334.

Rocha, B. S., García-Berthou, E., & Cianciaruso, M. V. (2023). Non-native fishes in Brazilian freshwaters: Identifying biases and gaps in ecological research. *Biological Invasions*, **25**, 1643–1658.

Rossa-Feres, D. C., & Nomura, F. (2006). Characterization and taxonomic key for tadpoles (amphibia: Anura) from the northwestern region of São Paulo state, Brazil. *Biota Neotropica*, **6**, 1–26.

Ruxton, G. D., Speed, M. P., & Kelly, D. J. (2004). What, if anything, is the adaptive function of countershading? *Animal Behaviour*, **68**, 445–451.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.

Schulze, A., Jansen, M., & Köhler, G. (2015). Tadpole diversity of Bolivia's lowland anuran communities: Molecular identification, morphological characterisation, and ecological assignment. *Zootaxa*, **4016**, 1–111.

Scribano, G., Balestrieri, A., Gazzola, A., & Pellitteri-Rosa, D. (2020). Strong behavioural defensive responses of endemic *Rana latastei* tadpoles induced by a native predator's odour. *Ethology*, **126**, 922–930.

Tarr, T., & Babbitt, K. (2002). Effects of habitat complexity and predator identity on predation of *Rana clamitans* larvae. *Amphibia-Reptilia*, **23**, 13–20.

Teplitsky, C., Plénet, S., & Joly, P. (2003). Tadpoles' responses to risk of fish introduction. *Oecologia*, **134**, 270–277.

Van Buskirk, J., Anderwald, P., Lüpold, S., Reinhardt, L., & Schuler, H. (2003). The lure effect, tadpole tail shape, and the target of dragonfly strikes. *Journal of Herpetology*, **37**, 420–424.

Vasconcelos, T. D. S., & Rossa-Feres, D. D. C. (2005). Diversidade, distribuição espacial e temporal de anfíbios anuros (Amphibia, Anura) na região noroeste do estado de São Paulo, Brasil. *Biota Neotropica*, **5**, 137–150.

Vasconcelos, T. D. S., Santos, T. G., Rossa-Feres, D. D. C., & Haddad, C. F. (2011). Spatial and temporal distribution of tadpole assemblages (amphibia, Anura) in a seasonal dry tropical forest of southeastern Brazil. *Hydrobiologia*, **673**, 93–104.

Venesky, M. D., Rossa-Feres, D. C., Nomura, F., de Andrade, G. V., Pezzuti, T. L., de Sousa, V. T. T., Anderson, C. V., & Wassersug, R. J. (2013). Comparative feeding kinematics of tropical hylid tadpoles. *Journal of Experimental Biology*, **216**, 1928–1937.

Watkins, T. B. (1996). Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla*. *Physiological Zoology*, **69**, 154–167.

Wellborn, G. A., Skelly, D. K., & Werner, E. E. (1996). Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, **27**, 337–363.

Wudneh, T. (1988). Temporal and spatial dynamics in the sizerelated distribution patterns of *Clarias gariepinus*, *Oreochromis niloticus*, *Barbus tsanensis* and *Barbus platydorsus*. In T. Wudneh (Ed.), *Biology and management of fish stocks in Bahir Dar gulf, Lake tana, Ethiopia* (pp. 43–52). Wageningen University.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Video S1.** *Scinax fuscovarius* tadpole supported by the snout on the wall of the aquarium. The red circle shows tadpole's location.

**Video S2.** *Scinax fuscovarius* tadpoles supported by the snout in the glass plate containing food. The red circle shows tadpole's location.

**Video S3.** *Scinax fuscovarius* tadpoles supported by the snout in galvanized steel net which divides the aquarium in two parts. The red circle shows tadpole's location.

**Figure S1.** *Scinax fuscovarius* tadpoles supported on the tip of the leaves by their snout.

**Table S1**. Mean and standard deviation of displacement time, latency to start foraging and food consumption of *Scinax fuscovarius* and *Physalaemus nattereri* tadpoles for treatment and control conditions.

**Table S2**. Data collected during the experiment in the presence (treatment) and absence (control) of predators on behavioral aspects (displacement time, latency to start foraging and food consumption) of *Scinax fuscovarius* tadpoles.

**Table S3**. Data collected during the experiment in the presence (treatment) and absence (control) of predators on behavioral aspects (displacement time, latency to start foraging and food consumption) of *Physalaemus nattereri* tadpoles.