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Article

Differential behavioral responses of benthic and nektonic tadpoles to predation at varying water depths

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Abstract

Predators influence microhabitat selection and activity level of tadpoles, but it is still unclear how such responses to predators differ among species and how water column's depth influences this predator–prey interaction. Here, we experimentally tested whether the presence of Odonata water nymphs influenced the spatial use and activity of benthic and nektonic tadpoles in different food availability contexts. Benthic tadpoles occupied and consumed more food at the bottom level, irrespective of predator's presence. However, when predators were at the bottom, benthic tadpoles remained close to the cages, suggesting a typical "stay-still" defensive behavior known for *Physalaemus nattereri* (Steindachner, 1863). Nektonic tadpoles occupied shallower depths on predator presence, and they also consumed less food and avoided the predator by selecting food sources far from it. When the predator was at the bottom level and food was available, the distance of tadpoles to the cage tended to be smaller. *Scinax fuscovarius* (Lutz, 1925) tadpoles were more active when food was absent regardless of predator's presence. When food was available, these tadpoles generally occupied and consumed more food at the bottom level. Tadpole responses depended not only on predator presence but also on a complex net of factors, which included tadpole habit, antipredatory behavior, and availability and location of food.

Key words: feeding behavior, induced defenses, *Micrathyria* sp., niche occupancy, *Physalaemus nattereri*, *Scinax fuscovarius*, swimming activity

Résumé

Si les prédateurs influencent la sélection de microhabitats et le niveau d'activité des têtards, les variations entre espèces de ces réactions aux prédateurs et l'influence de la profondeur dans la colonne d'eau sur cette interaction prédateur–proie ne sont pas bien établies. Nous vérifions de manière expérimentale l'existence d'une éventuelle influence de la présence de naïades d'odonates sur l'utilisation de l'espace et l'activité de têtards benthiques et nectoniques dans différents contextes de disponibilité de nourriture. Les têtards benthiques occupent le fond de la colonne d'eau et y consomment plus de nourriture, qu'un prédateur soit présent ou non. Toutefois, quand des prédateurs sont présents au fond, les têtards benthiques demeurent près des cages, ce qui indiquerait un comportement défensif d'immobilisation typique de *Physalaemus nattereri* (Steindachner, 1863). Les têtards nectoniques occupent des profondeurs moins grandes en présence d'un prédateur et consomment aussi moins de nourriture et évitent le prédateur en sélectionnant des sources de nourriture éloignées de ce dernier. Quand le prédateur se trouve au fond et que de la nourriture est disponible, la distance entre les têtards et la cage a tendance à être plus faible. Les têtards de *Scinax fuscovarius* (Lutz, 1925) sont plus actifs en l'absence de nourriture, qu'un prédateur soit présent ou non. Quand de la nourriture est disponible, ces têtards occupent généralement le fond de la colonne d'eau, où ils consomment plus de nourriture. Les réactions des têtards dépendent non seulement de la présence d'un prédateur, mais aussi d'un éventail complexe de facteurs qui comprend l'habitude et le comportement anti-prédation du têtard et la disponibilité et l'emplacement de nourriture. [Traduit par la Rédaction]

Mots-clés : comportement d'alimentation, défenses induites, *Micrathyria* sp., occupation de niches, *Physalaemus nattereri*, *Scinax fuscovarius*, activité de nage

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Introduction

Predation is an important evolutionary pressure for species and one of the main mechanisms that regulate community structure (Futuyma and Slatkin 1983; Begon et al. 1986). Apart from direct effects (e.g., mortality), predators indirectly affect the morphology (e.g., Relyea et al. 2021; Sergio et al. 2021), life history (e.g., Lent and Babbitt 2020; Najafi et al. 2021), and physiology (e.g., Joshi et al. 2017; Florencio et al. 2020) of prey. Predators may also modify the behavior of preys by reducing foraging (Rae and Murray 2019; Mamede and Nomura 2021), increasing the use of refuge sites (Semlitsch and Reyer 1992; Hartman and Lawler 2014), stimulating specific escape behaviors (Sousa et al. 2011), and changing the habitat selection (Chuang et al. 2019; Pacheco et al. 2019).

Indeed, the ability to identify the presence of a predator is crucial for prey survival (Ferrari et al. 2010). In aquatic systems, preys, such as tadpoles, evaluate predation risk by visual, mechanical, and chemical cues produced by predators (Hettyey et al. 2012; Eterovick et al. 2018; Chuirazzi et al. 2021). When tadpoles recognize predators, they often use aquatic vegetation as a refuge (Babbitt and Tanner 1998), and this strategy decreases the predation rate (Kopp et al. 2006). Tadpoles also tend to reduce their activity in predator's presence and avoid sites close to the predator (Brown et al. 2019; Mogali et al. 2020), thus reducing the chance of being found. However, either immobility or sheltering in refuges leads tadpoles to decrease foraging time, which in turn negatively affects the growth and developmental rates during their metamorphosis (Skelly and Werner 1990; Van Uitregt et al. 2016). Therefore, the responses of tadpoles to predators constitute a trade-off between behaviors that reduce predation risk and behaviors that favor their growth and development. Yet, it is still unclear whether predators influence how tadpoles make use of different depth levels along the water column and how different morphological attributes of tadpoles may influence such responses.

Differences in body morphology affect the probability of occurrence of tadpoles in different habitats (e.g., lentic or lotic environments: McDiarmid and Altig 1999) and microhabitats (e.g., surface or deep areas: Altig and Johnston 1989). Nektonic tadpoles, with higher fins and compressed body, occupy mostly midwater depths, whereas benthic tadpoles, with low fins and depressed body, occupy the bottom of water bodies (Altig and Johnston 1986; McDiarmid and Altig 1999). Differences in tail musculature are also related to the depths occupied by tadpoles of different species along the water column (Jordani et al. 2019). Taking into account the potential effects of predator occurrence probability and food availability, here we used controlled experiments to investigate whether benthic and nektonic tadpoles can also change their behavior (water column occupancy, swimming activity, and foraging activity) in response to changes in the context of predator occurrence and food availability. As benthic tadpoles are morphologically and physiologically restricted to the bottom of water bodies (Altig and Johnston 1989; Gee and Waldick 1995; McDiarmid and Altig 1999; Tu et al. 1999), we hypothesized that the presence of predators would not influence the depth where these tadpoles usually remain and forage (i.e., close to the bottom). However, as benthic tadpoles usually remain far from the predator (Van Buskirk and Arioli 2005; Nunes et al. 2013), we predicted that these tadpoles would select areas as far as possible from the predator by changing their foraging area horizontally. Nektonic tadpoles are more flexible in terms of occupying different water column depths (McDiarmid and Altig 1999; Schulze et al. 2015). For this reason, we hypothesized that these tadpoles could adjust their vertical and horizontal use of space depending on the presence of the predator and its position along the water column (at midwater or on the bottom). Finally, as detected by previous studies (Orizaola et al. 2013; Berec et al. 2016; Schalk 2016), we predicted tadpoles to be less active in the presence of the predator.

Materials and methods

Species and acclimatization

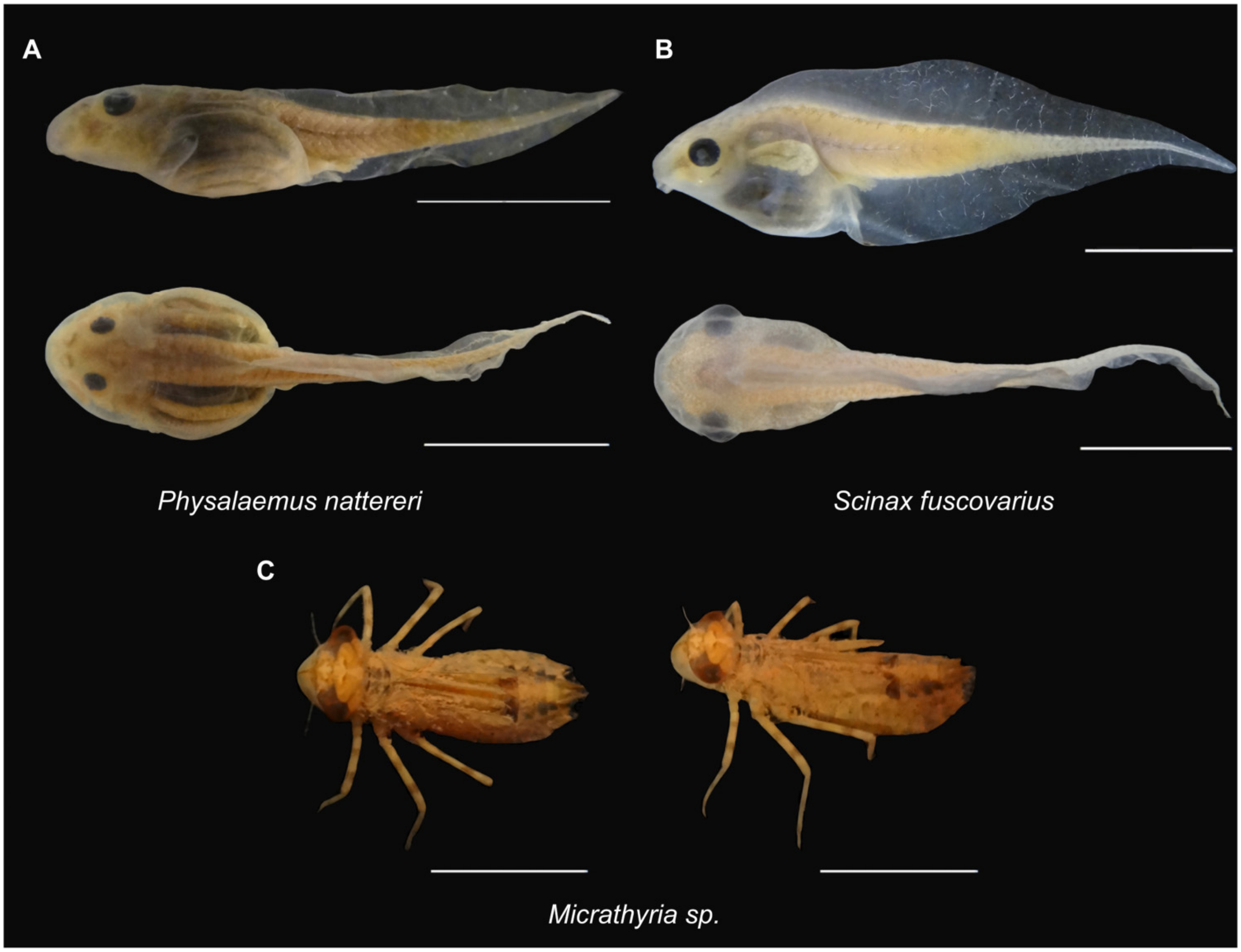
The experiments were performed with tadpoles of *Physalaemus nattereri* (Steindachner, 1863) and *Scinax fuscovarius* (Lutz, 1925). *Physalaemus nattereri* tadpoles (Fig. 1A) have a benthic habit, globular body, low fins, and small and dorsal eyes (Rossa-Feres and Nomura 2006). *Scinax fuscovarius* tadpoles (Fig. 1B) have a nektonic habit, compressed body, high fins, a flagellum at the tip of the tail, and big and lateral eyes (Rossa-Feres and Nomura 2006). They have a similar diet, mostly composed of Bacillariophyceae, *Trachelomonas* Ehrenberg, 1833, and *Oedogonium* Hirn, 1900 (Rossa-Feres et al. 2004; do Prado et al. 2009).

Odonata water nymphs, species of *Micrathyria* Kirby, 1889 (Fig. 1C), are important and efficient predators of tadpoles (Brockelman 1969; Gascon 1989; Arribas et al. 2018). The water nymphs present a "sit and wait" behavior, in which the predator chooses a site to capture prey (Heyer et al. 1975). They have an extensible lip that works as a hinge, located in the ventral part of the head, that quickly stretches to capture prey (Pritchard 1965).

Tadpoles and water nymphs were collected with a wire mesh net with 32 cm diameter and 3 mm^2 mesh, between January and February 2018, in two temporary ponds located in a pasture area (pond 1: 20°50′48.7″S, 49°28′27.4″W; pond 2: 20°50′50.3″S, 49°28′29.5″W) in the municipality of Mirassol, São Paulo State, Brazil. Tadpoles of both species and water nymphs are common, abundant, and coexist in temporary ponds across the region (Sousa et al. 2011; Nomura et al. 2013). After capturing the specimens, tadpoles and nymphs were transported separately in plastic bags containing water from the ponds where they were collected and accommodated in isothermal expanded polystyrene boxes to prevent overheating.

Tadpoles and water nymphs were separately acclimatized in laboratory for 2 days before the beginning of the experiments. During this period, the animals were kept in polyethylene aquaria filled with constantly aerated dechlorinated water. The experiments were conducted under a controlled photoperiod regimen (12 h of light and 12 h of darkness) with air temperature between 28 and 30 °C, which kept water temperature around 26 °C, simulating the natural conditions of

Fig. 1. Lateral and dorsal view of (A) *Physalaemus nattereri* (Leptodactylidae, Anura) and (B) *Scinax fuscovarius* (Hylidae, Anura) tadpoles, and (C) water nymphs of *Micrathyria* sp. (Odonata). Images provided by K.O.R. Picheli. [Color online.]



the ponds. Tadpoles were fed ad libitum with commercial fish food based on algae and krill (Sera Micron®), and water nymphs were fed with tadpoles that were not used in the experiments.

For the experiments, we selected tadpoles and water nymphs of similar sizes within each species (*P. nattereri*: 28.68 ± 2.13 mm; *S. fuscovarius*: 28.93 ± 3.76 mm; *Micrathyria* sp.: 17.98 ± 2.23 mm). While feeding the water nymphs during acclimatization period, we confirmed that they preyed on tadpoles efficiently (YCMS, personal observation), regardless of differences in predator and prey sizes. We selected tadpoles between developmental stages 29 and 35 (sensu Gosner 1960), in which the major changes in tadpole morphology are related to body growth and not to developmental changes (McDiarmid and Altig 1999).

Experimental design

Experiments were developed in glass aquaria (30 cm × 20 cm × 30 cm) filled with 16 L of dechlorinated water, resulting in a water column of 27 cm depth. We covered three of each aquarium's faces with a blue plastic adhesive to avoid visual contact among tadpoles from different aquaria and to reduce the stress that other colors could cause (based on fishes; Maia and Volpato 2013). The uncovered face (30 cm × 30 cm) was marked with five columns (A–E) and nine lines (1–9), producing 45 sectors of 6 cm × 3 cm each (Fig. 2), that allowed us to determine the position of tadpoles inside the aquarium.

For each experiment, with both benthic and nektonic tadpoles, we used only one tadpole per aquarium. We also placed a wire-net cage (6 cm × 3 cm × 3 cm) with 1 mm × 1 mm mesh into each aquarium. The cages were placed at the bottom or in the middle of the water column, corresponding to the depths where the predator could be present. Half of the cages at each depth were kept empty (control groups) and the other half received one *Micrathyria* sp. water nymph (Fig. 2). This kind of cage prevented the predator from injuring the tadpole but allowed the tadpole to be in contact with the chemical cues released by the predator in the water, as well as to visualize the predator. Water nymphs were inserted into the cages 20 h before the beginning of the experiments, remaining without food during this period. Tadpoles were acclimatized into the experimental aquarium for 30 min before the experiment and also remained without food for about

Fig. 2. Experimental design exemplified with benthic tadpoles. Treatments consisted of an aquarium with food unavailable (A–D) or available (E–H), a cage positioned at the bottom (A, B, E, F) or at midwater (C, D, G, H), either empty (A, C, E, G) or with a predator (B, D, F, H), and a tadpole. Letters and lines on the aquaria's front wall represent sectors drawn to facilitate tadpole location during the experiment. The experimental design was similar for nektonic tadpoles. [Color online.]

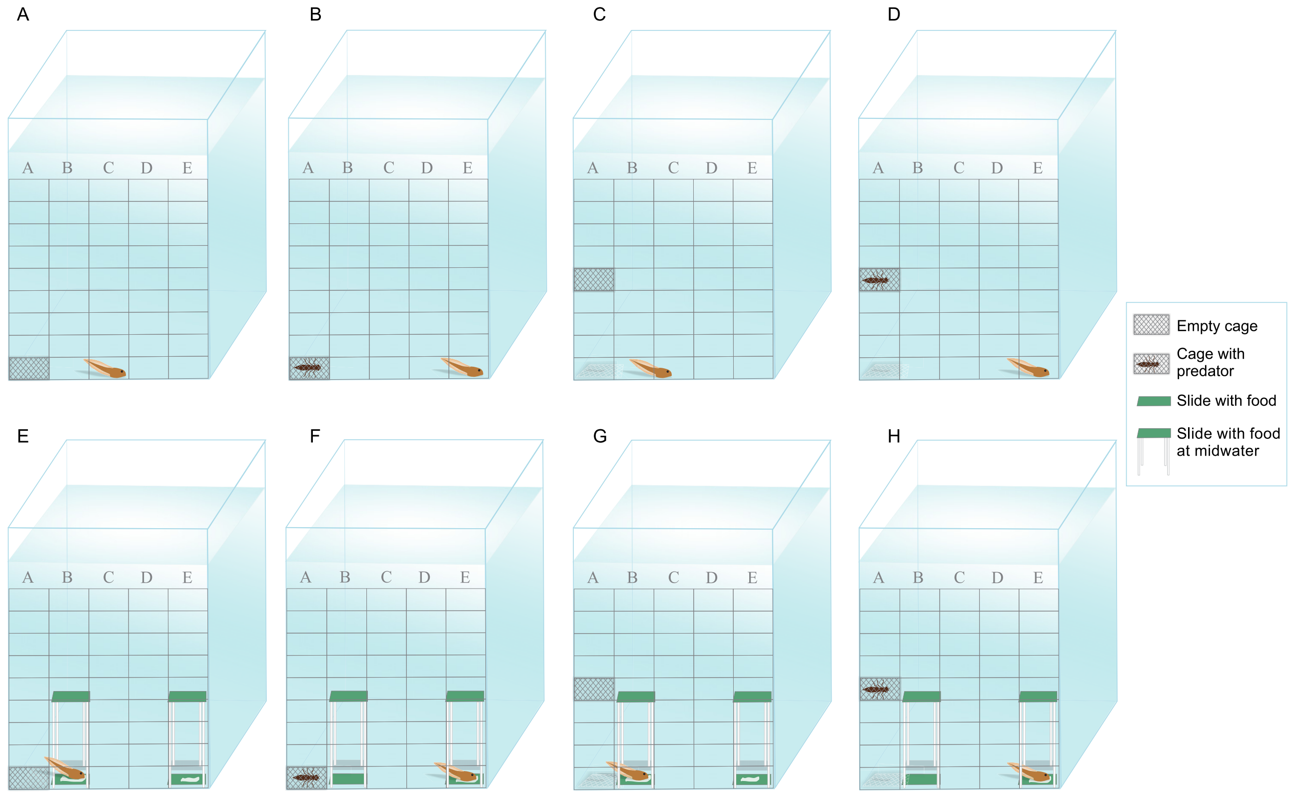


Table 1. Results from model selection evaluating the influence of predator (P), cage depth (C), and food presence (F) on depth occupied and distance to the cage and number of sectors occupied (activity) by benthic tadpoles (*Physalaemus nattereri*).

Response variable	F	C	P	F:C	F:P	C:P	AIC_c	ΔAIC_c
Depth		+					366.4	0.00
							367.7	1.35
		+	+				368.2	1.88
Distance	+	+	+				1088.4	0.00
	+	+	+		+		1089.8	1.41
	+	+	+	+			1090.1	1.75
	+	+	+			+	1090.1	1.77
		+	+				1090.2	1.87
Activity		+					471.4	0.00
							472.0	0.66
	+	+					472.1	0.72
	+						472.7	1.36
		+	+				472.8	1.44

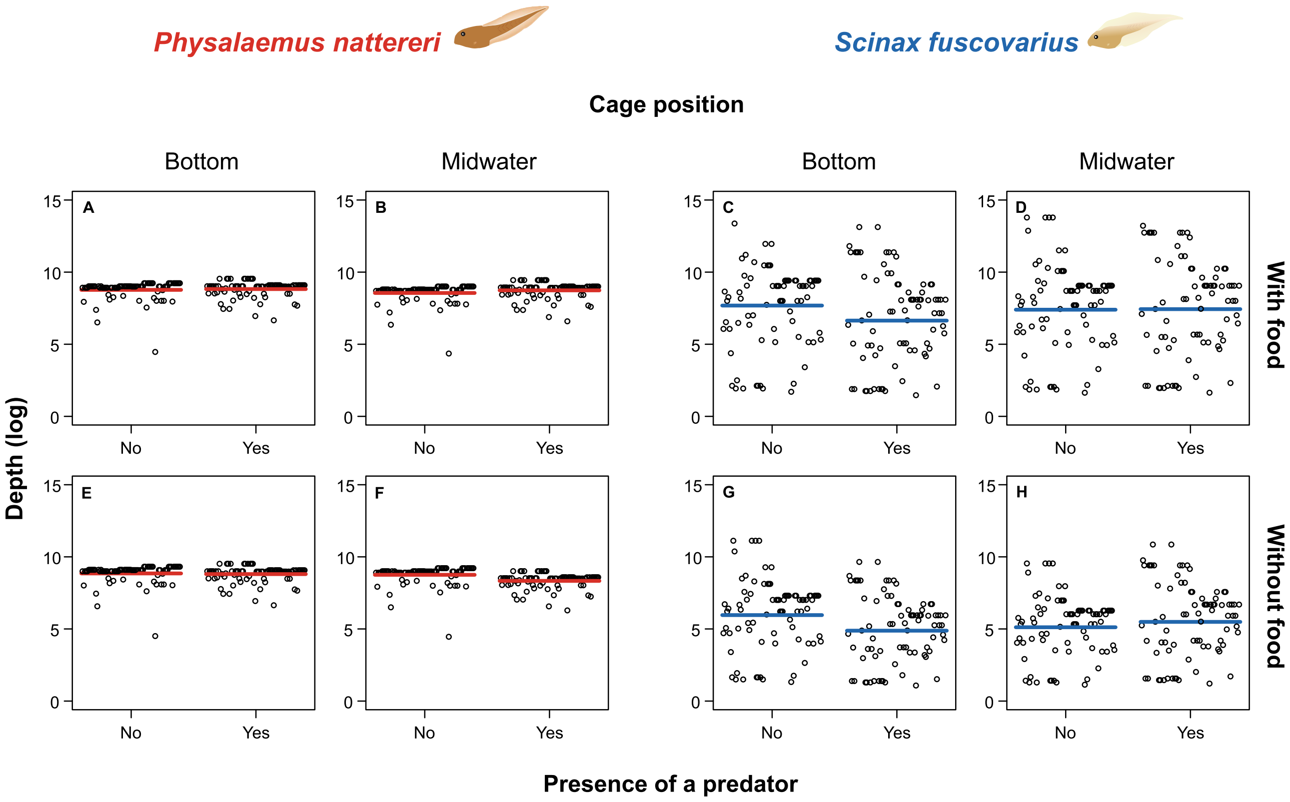
Note: The symbols ":" and "+" indicate the interaction between variables and the variable presence in the model, respectively. AIC_c, Akaike information corrected criterion for small databases; ΔAIC_c, variation between the AIC_c values in relation to the best model (i.e., the model with lesser AIC_c and single $\Delta < 2$). Only the models with $\Delta AIC_c < 2$ are presented.

20 h. This ensured that both predators and preys would need to feed during the experiments. Tadpoles and water nymphs were used only once for each trial.

To test the effect of predator on how tadpoles use the space, we used a combination of three variables: predator (absent or present), predator depth (on the bottom or at midwater), and food (absent or present). In total, benthic and nektonic tadpoles were submitted to eight combinations of these conditions (Fig. 2). Each condition had 20 replicates, which accounted for 160 replicates for each species (Supplementary Table S1).

To test the influence of the predator on tadpole food consumption rate and on selection of foraging depth, we prepared a mixture of water and the same commercial food that was used during tadpole acclimatization (Sera Micron®) at a concentration of 100 $mg \cdot mL^{-1}$ (Venesky et al. 2013). We brushed 0.3 mL of this solution on the upper part of the microscope slides (26 mm × 76 mm), producing a uniform layer of food on its extension. After this procedure, the slides were maintained in the laboratory so that the mixture could dry naturally over 24 h. After this period, the food was firmly adhered to the surface, and then we placed four slides into each aquarium, simultaneously on the bottom and at midwater and near and far from the cage (Fig. 2). Regardless of the cage depth, the slides on the left were considered near the predator, and those on the right were considered far from

Fig. 3. Depth occupied by tadpoles in the presence or absence of a predator, with a cage positioned at the bottom (A, C, E, G) or at midwater (B, D, F, H), with food available (A–D) or unavailable (E–H). Circles correspond to partial residuals and colored lines to fitted values of *Physalaemus nattereri* (red) and *Scinax fuscovarius* (blue) tadpoles. Depth data were transformed using a log link function. Graphs are based on the model that includes all interactions between variables. For statistical details see Tables 1 and 3. [Color online.]



the predator. Slides at midwater (15 cm depth) were fixed and supported by rigid plastic sticks (length, 280 mm; diameter, 4.3 mm; thickness, 0.5 mm).

After all elements were included in the aquarium (i.e., predator, tadpoles, and food, in this order), we waited for 30 min to start observing tadpole behavior to minimize potential stressful behaviors that could result from handling the animals. Observations were conducted between 11 a.m. and 3 p.m., the period of the day when tadpoles were more active (YCMS, personal observation). Each aquarium was observed for 300 s, always by the same person (YCMS), positioned approximately 80 cm away from the aquaria.

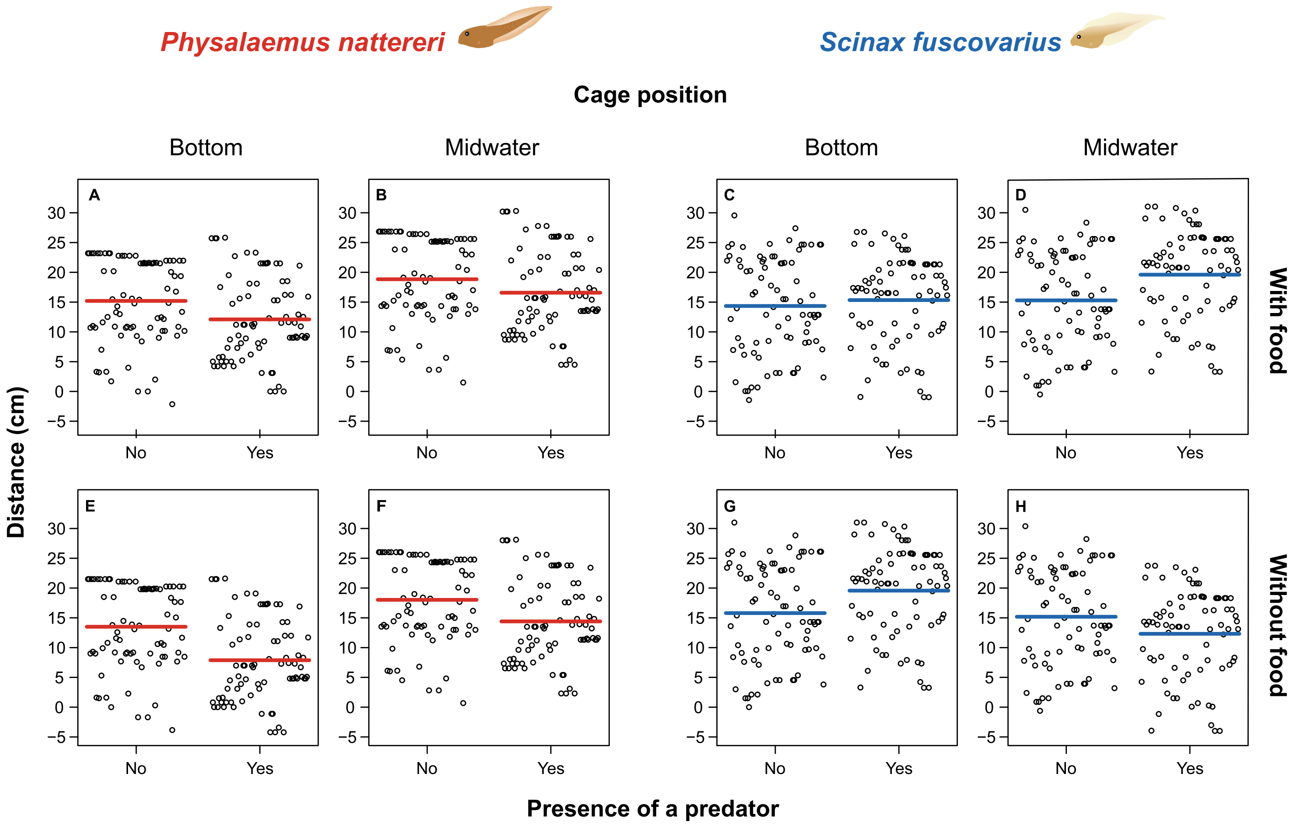
The slides with food remained in the aquarium for 4 h, the period in which the experiment was conducted. At the end of the observations, the slides with food were removed from the aquarium and allowed to dry naturally for 24 h in the laboratory. After that, each slide was scanned (HP LaserJet M1132) to produce digitized images, from which we quantified the proportion of food consumed by the tadpoles following Annibale et al. (2020). The tadpoles were immersed in a 10% lidocaine anesthetic solution and conserved in a preservative solution (1:1 alcohol (70%) and formalin (15%)). The water nymphs were preserved in alcohol 70%. The preserved specimens were deposited at the Scientific Collection of Amphibians (DZSJRP Amphibia–Tadpoles) of the Department of Zoology and Botany, UNESP, campus São José do Rio Preto, Brazil (Lots DZSJRP 3572.1, 3573.1, 3574.1, 3575.1, 3577.1, 3572.INV, 3573.INV, 3574.INV, and 3577.INV).

Data analysis

To quantify the space used by the tadpoles, we recorded which and how many sectors were occupied by the tadpoles during the observations (Supplementary Tables S2 and S3). We were then able to determine (*i*) the average depth occupied by the tadpoles and (*ii*) the average distance from the tadpole to the cage with or without the predator. Depth was measured by the total number of visits to each depth range (lines 1–9 marked in the aquarium) for each experimental treatment. As each line had a 3 cm depth, we could determine the average depth range for each individual. The distance between the tadpole and the predator was measured from the central point of the cage to the central point of each sector occupied by the tadpole. When the tadpole was positioned between two sectors, we considered the sector that contained the major part of its body. Then, we calculated the average distance from the tadpole to the cage for both with and without predator conditions.

To evaluate tadpole swimming activity during the experiment, we quantified the total number of sectors occupied by the individuals during the observations. We considered the presence of a tadpole in a certain sector when it remained for more than 5 s in it. Therefore, a more active tadpole

Fig. 4. Distance from tadpoles to the cage in the presence or absence of a predator, with a cage positioned at the bottom (A, C, E, G) or at midwater (B, D, F, H), with food available (A–D) or unavailable (E–H). Circles correspond to partial residuals and colored lines to fitted values of *Physalaemus nattereri* (red) and *Scinax fuscovarius* (blue) tadpoles. Graphs are based on the model that includes all interactions between variables. For statistical details see Tables 1 and 3. [Color online.]



occupied a higher number of sectors, whereas a less active tadpole occupied a fewer number of sectors.

We also evaluated how tadpoles used the space by analyzing their foraging rate at each location where food was available (i.e., food consumption rate and the depth where they consumed more food). We quantified the food consumed in each of the four slides as the proportion of food removed in relation to the slide area (Supplementary Tables S4 and S5). For this, we assessed the scanned images of the slides using the Particle Analysis tool of ImageJ® software (Schneider et al. 2012).

Statistical analysis

To test whether predators influenced the spatial use (i.e., depth and distance to predator), swimming activity, and foraging rate (i.e., food consumption rate and depth where tadpoles consumed more food) of tadpoles, we employed linear mixed models and generalized linear mixed models (Zuur et al. 2009). In all analyses, we included the day the tadpoles were submitted to the experiment as a random variable to control the potential variation caused by differences on the day of the experiment. We performed the analyses separately for tadpoles of each species.

In the first analysis, we tested the influence of the predator on the depth occupied by the tadpoles. For this, we considered the average depth occupied by tadpoles as the response variable and (*i*) predator (absence or presence), (*ii*) cage depth (bottom or midwater), and (*iii*) food (absence or presence) as fixed explanatory variables. All the interactions of these three variables were considered in the model. As the model residuals did not meet the normality assumption, we performed a generalized linear mixed model with gamma family and log as the link function (Zuur et al. 2009).

In the second analysis, we tested the influence of the predator on the distance of the tadpole to the cage. We used a linear mixed model (Gaussian distribution) and considered the distance from the tadpole to the cage as the response variable and (*i*) predator (absence or presence), (*ii*) cage depth (bottom or midwater), and (*iii*) food (absence or presence) as fixed explanatory variables. All interactions were considered in the model.

In the third analysis, we tested the influence of the predator on tadpole swimming activity. We used a generalized linear mixed model, assuming a negative binomial distribution. We considered the number of sectors occupied by tadpoles as the response variable and (*i*) predator (absence or presence), (*ii*) cage depth (bottom or midwater), and (*iii*) food availability (absence or presence) as fixed explanatory variables. All interactions between these three variables were considered in the model.

Finally, in the fourth analysis, we tested whether predators influenced the foraging activity of tadpoles. We considered

Fig. 5. Number of occupied sectors by tadpoles in the presence or absence of a predator, with a cage positioned at the bottom (A, C, E, G) or at midwater (B, D, F, H), with food available (A–D) or unavailable (E–H). Circles correspond to partial residuals and colored lines to fitted values of *Physalaemus nattereri* (red) and *Scinax fuscovarius* (blue) tadpoles. Graphs are based on the model that includes all interactions between variables. For statistical details see Tables 1 and 3. [Color online.]

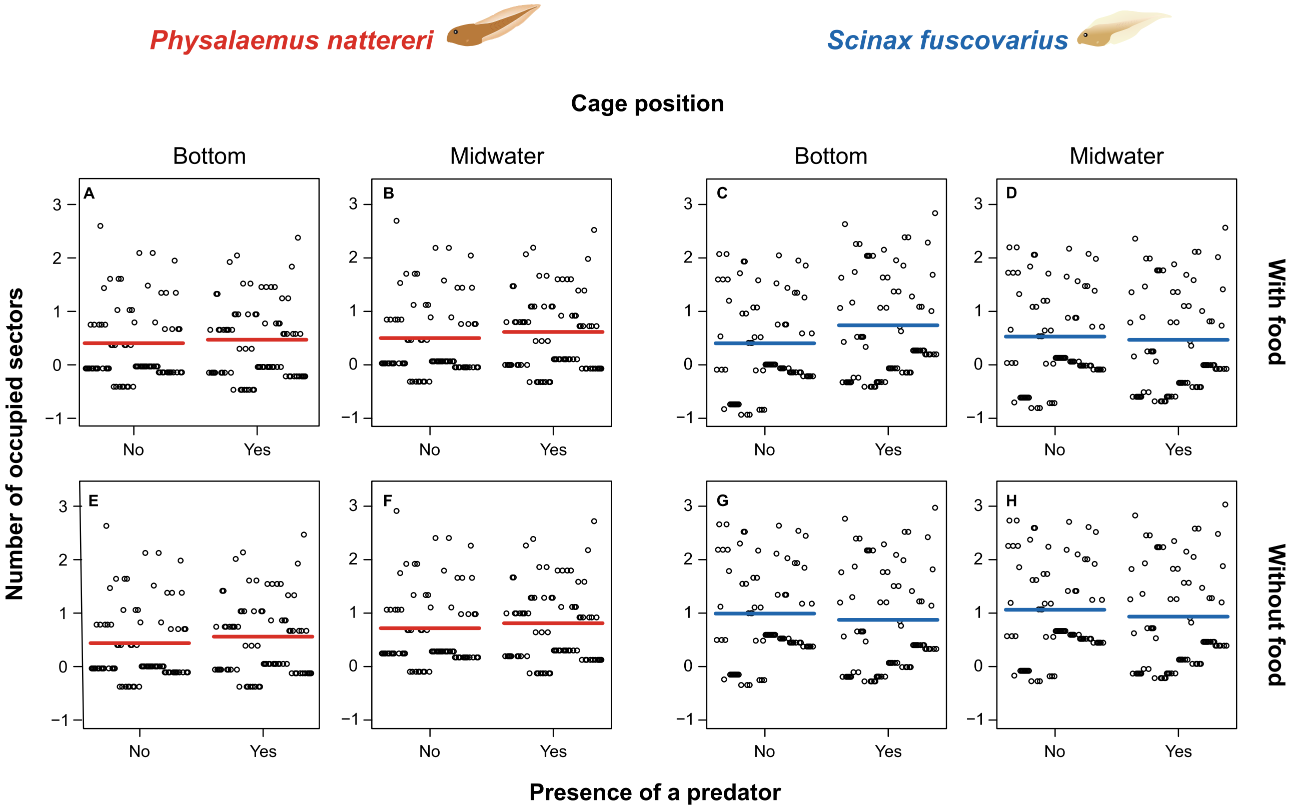


Table 2. Results from model selection evaluating the influence of predator (P), cage depth (C), depth of food (FDe), and distance of the cage to the food (FDi) on the proportion of consumed food of benthic tadpoles (*Physalaemus nattereri*).

P	FDi	C	FDe	FDi:C	FDi:FDe	AIC$_c$	ΔAIC$_c$
	+		+		+	1044.5	0.00
	+		+			1044.7	0.17
	+	+	+	+	+	1045.4	0.86
	+	+	+	+		1045.5	1.03

Note: The symbols ":" and "+" indicate the interaction between variables and the variable presence in the model, respectively. AIC$_c$, Akaike information corrected criterion for small databases; ΔAIC$_c$, variation between the AIC$_c$ values in relation to the best model (i.e., the model with lesser AIC$_c$ and single $\Delta < 2$). Only the models with ΔAIC$_c$ < 2 are presented.

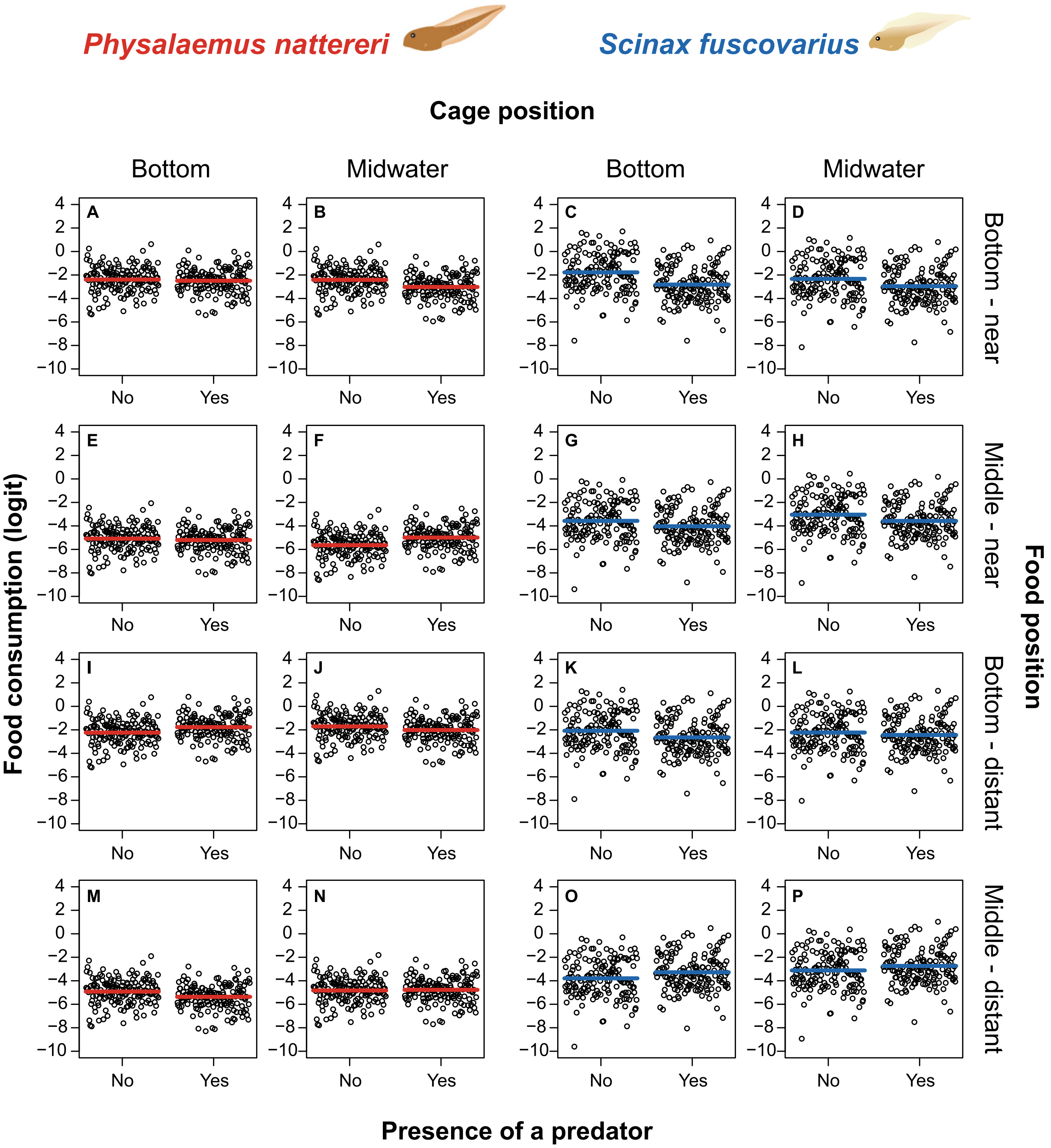
the rate of food consumed in each slide as the response variable. This allowed us to analyze not only the quantity of food consumed by tadpoles but also their foraging site preference. We also included, in this analysis, the distance from the slide with food to the cage because this variable also indicated the location of tadpoles. For this analysis, we employed a linear mixed model. We considered (*i*) predator (absence or presence), (*ii*) cage depth (bottom or midwater), (*iii*) food depth (bottom or midwater), and (*iv*) food distance (near or far from the cage) as fixed explanatory variables. All interactions of two and three ways of these variables were considered in the model. Because the response variable data (food consumption) were percentage values, we used the logit function for data normalization (Zuur et al. 2009).

In all analyses, we applied a model selection to identify the most parsimonious model that explained the major variability percentage with a minor number of terms, based on Akaike's information criterion corrected for small sample size (AIC$_c$; Burnham and Anderson 2002). Whenever it was not possible to identify a single most parsimonious model (i.e., with the lowest AIC$_c$ value and single $\Delta < 2.00$) or when a null model was not selected in any test, we considered only the models with the lowest AIC$_c$ value and $\Delta < 2.00$ among those selected. All analyses were performed in RStudio (version 1.1.332; RStudio 2018) using the packages lme4 (Bates et al. 2015), lmtest (Hothorn et al. 2019), car (Fox et al. 2019), MuMIn (Bartoń 2019), and visreg (Breheny and Burchett 2019).

Ethics statement

The fieldwork was performed with the permission of the Institute of Environment and Natural Renewable Resources (IBAMA) and the Chico Mendes Institute for Biodiversity Conservation (ICMBio) — Authorization and Information System

Fig. 6. Food consumed by tadpoles in the presence or absence of a predator, with a cage positioned at the bottom (A, C, E, G, I, K, M, O) or at midwater (B, D, F, H, J, L, N, P), either near the food (A–H) or far from the food (I–P). Circles correspond to partial residuals and colored lines to fitted values of *Physalaemus nattereri* (red) and *Scinax fuscovarius* (blue) tadpoles. Food consumption data were transformed using a logit function. Graphs are based on the model that includes all interactions between variables. For statistical details see Tables 2 and 4. [Color online.]



on Biodiversity (SISBio) permit number 18163-1 to DCR-F. The maintenance of tadpoles in the laboratory and the experimental design were approved by the Ethics Committee in the Use of Animals (CEUA) (number 187/2018), according to the National Council of Animal Experimentation Control (CONCEA).

Results

Overall, predator presence and food availability had a significant effect on how tadpoles used the available space. However, the effects varied between tadpoles of the two species tested in this study. Benthic tadpoles of *P. nattereri* were much

Table 3. Results from model selection evaluating the influence of predator (P), cage depth (C), and food presence (F) on depth occupied and distance to the cage and number of sectors occupied (activity) by nektonic tadpoles (*Scinax fuscovarius*).

Response variable	F	C	P	F:C	F:P	AIC_c	ΔAIC_c
Depth	+					831.3	0.00
	+		+			832.8	1.56
Distance	+	+		+		1129.2	0.00
						1129.8	0.64
	+	+	+	+		1129.9	0.65
			+			1130.5	1.27
Activity	**+**					**548.3**	**0.00**
	+		+			550.4	2.11
	+	+				550.4	2.13
	+		+		+	551.0	2.76
						551.6	3.32

Note: The symbols ":" and "+" indicate the interaction between variables and the variable presence in the model, respectively. AIC_c, Akaike information corrected criterion for small databases; ΔAIC_c, variation between the AIC_c values in relation to the best model (i.e., the model with lesser AIC_c and single $\Delta < 2$). Only the models with $\Delta AIC_c < 2$ are presented.

more conservative in terms of depth than *S. fuscovarius* nektonic tadpoles. Consequently, significant effects of food availability and presence of predators at depth were detected only in the second species. Below, we expose in detail the effects detected for tadpoles of each species. The provided numbers in this section are written in the form *x* (mean) ± *y* (standard deviation).

Benthic tadpoles

Irrespective of predator depth, benthic tadpoles of *P. nattereri* remained mostly at the bottom of the water column (Table 1; Fig. 3). The predator affected the movement of this species, with tadpoles remaining close to the cage when the water nymph was present (Fig. 4). This effect tended to be more accentuated when there was no food available and when the cage was at the bottom (Fig. 4), although interaction effects were only included in the second-best model (Table 1). Although the number of sectors occupied by benthic tadpoles was slightly higher when the cage was positioned at midwater, leaving more space available at the bottom (Table 1; Fig. 5), the number of sectors was not affected by the predator or food availability.

Food consumption was affected by the distance between the food and the cage and by the depth of the food (interaction between these variables in the first selected model; Table 2). Tadpoles consumed more food at the bottom (around 15.5% of the slide area) than at midwater (around 1.1% of the slide area), and mainly far from the cage (on average, 19% ± 17% far and 12% ± 11% near the cage; Fig. 6). Predator presence had no effect on food consumption patterns (Table 2).

Nektonic tadpoles

Predators and food availability had a significant effect on the depth, distance from the cage, and activity of *S. fuscovarius* tadpoles. Tadpoles of this species occupied greater depths when the predator was absent (average depth: 19.62 ± 8.1 cm) than when it was present (average depth: 18.33 ± 8.61 cm). This effect seemed to be more accentuated when the predator cage was positioned at the bottom (Fig. 3), although this interactive effect was not detected in the best model (Table 3). Moreover, in the absence of food, tadpoles occupied lower depths (average depth: 16.08 ± 8.85 cm), whereas in the presence of food, tadpoles remained near the bottom (average depth: 21.87 ± 6.75 cm). When the cage was at the bottom and food was available, the distance from the tadpoles to the cages tended to be smaller when predators were present (Fig. 4), although this effect was only observed in the third-best model (Table 3). Overall, tadpoles of this species were more active when food was not available, as indicated by the most parsimonious model (Table 3; $AIC_c = 548.3$ and $\Delta = 0$), which means that tadpoles visited 53% more sectors when food was unavailable (Fig. 5).

We found that food positioning (distance to the cage and depth of the slides), predator presence, and cage depth influenced the rate of food consumption and food foraging location by tadpoles of *S. fuscovarius* (i.e., models with the interactions between these variables were selected; Table 4). Unless the food was close to the predator (consumption rate: 8% ± 11%), most treatments evidenced similar food consumption by tadpoles (approximately 13%). Additionally, tadpoles consumed more food at the bottom (approximately 16.5%) than at midwater (8%), but the consumption was even higher in the absence of a predator (20% ± 21%) than in its presence (13% ± 15%). The same model also indicated that tadpoles consumed more food at the same depth of the cages (Table 4; Fig. 6). For example, tadpoles consumed three times more food at the bottom when the cage was also at the bottom (18% ± 21%) than when the cage was at midwater (6% ± 10%), irrespective of the predator's presence. In summary, all interactions between pairs of variables were present among the selected models, hence indicating that all of them were important to explain where tadpoles foraged (Table 4).

Discussion

Here, we demonstrated that the spatial use and activity of benthic and nektonic tadpoles depended on a complex net of factors, which include tadpole habit (benthic or nektonic), antipredatory behavior, and food availability and location.

The morphology of benthic tadpoles (e.g., depressed body and low fins: Altig and Johnston 1986, 1989; McDiarmid and Altig 1999) and their physiology (negative buoyancy: Gee and Waldick 1995; Tu et al. 1999) seem to be more important in determining their occurrence at the bottom of the water column than the presence of a potential predator. Benthic tadpoles are not able to easily move to different depths as an attempt to escape or to hide from a predator; they instead use other antipredatory strategies. Immobility, for

Table 4. Results from model selection evaluating the influence of predator (P), cage depth (C), depth of food (FDe), and distance of the cage to the food (FDi) on the proportion of consumed food of nektonic tadpoles (*Scinax fuscovarius*).

FDi	C	FDe	P	FDi:C	FDi:FDe	FDi:P	C:FDe	C:P	FDe:P	AIC_c	ΔAIC_c
+	+	+	+			+	+		+	1218.3	0.00
+	+	+	+			+	+			1219.0	0.68
+		+	+			+			+	1219.4	1.09
	+	+	+				+		+	1219.7	1.32
+	+	+	+	+		+	+		+	1220.0	1.65
+		+	+			+				1220.1	1.74
+	+	+	+				+		+	1220.1	1.81
+	+	+	+		+	+	+		+	1220.2	1.82
+	+	+	+			+			+	1220.3	1.97
	+	+	+				+			1220.3	1.98
+	+	+	+			+	+	+	+	1220.3	1.99

Note: The symbols ":" and "+" indicate the interaction between variables and the variable presence in the model, respectively. AIC_c, Akaike information corrected criterion for small databases; ΔAIC_c, variation between the AIC_c values in relation to the best model (i.e., the model with lesser AIC_c and single $\Delta < 2$). Only the models with $\Delta AIC_c < 2$ are presented.

instance, is a defense behavior against "sit and wait" predators, such as Odonata water nymphs that detect visual and mechanical cues generated by prey (Pritchard 1965). Usually, benthic tadpoles respond to predator presence by reducing their activities (Ramamonjisoa et al. 2019; Scribano et al. 2020). For some species, proximity to a predator leads tadpoles to immediately cease moving and remain motionless (Azevedo-Ramos et al. 1992; Nomura et al. 2013). In our study, tadpoles of *P. nattereri* remained closer to predators than to empty cages, which, we suppose, is an indication that these tadpoles detect the water nymphs' chemical or visual cues only when they are remarkably close to them. Nomura et al. (2011) demonstrated that the motionless behavior of *P. nattereri* tadpoles (i.e., cryptic behavior), compared with other more active tadpoles (*Rhinella diptycha* (Cope, 1862)), resulted in decreased predation rates by Odonata water nymphs. Therefore, we hypothesized that *P. nattereri* tadpoles remained close to the predators in an attempt to avoid predation.

We observed that tadpoles of *S. fuscovarius* used the surface and the midwater levels in some situations (e.g., in the absence of food) and the bottom level in others (e.g., presence of food). This finding corroborates what we predicted, that these tadpoles would use the entire water column because nektonic tadpoles present morphological attributes (Rossa-Feres and Nomura 2006) that likely give them a more dynamic swimming ability. They are also able to regulate buoyancy (Gee and Rondeau 2012), which contributes to better occupancy of the water column (Altig and Johnston 1986; McDiarmid and Altig 1999). Indeed, in natural environments, it is common to observe nektonic tadpoles floating in open water or near the vegetation at midwater, either foraging, refuging, or resting (Schulze et al. 2015). However, we found that nektonic tadpoles consumed a higher quantity of food at the bottom level than at midwater. This result suggests that the spatial niche breadth of nektonic tadpoles may be wider than reported, even if we consider the presence of predator and food availability at different water depths. Furthermore, tadpole movements along the water column were accentuated in the absence of food, regardless of the predator presence, thereby suggesting that, for these tadpoles, the need for feeding may outbalance the risk of predation. Such behavior tends to put tadpoles at a greater predation risk (Anholt and Werner 1995), configuring a trade-off between foraging and predator avoidance (Werner and Anholt 1993). Interestingly, these nektonic tadpoles also remained closer to predators than to empty cages, similar to *P. nattereri* tadpoles. It is possible that both species use the motionless strategy when they detect a predator nearby, but *S. fuscovarius* tadpoles can displace along the entire water column under different conditions.

Although nektonic tadpoles tended to remain close to the cage when the water nymph was present, they consumed more food away from the predator. They also consumed more food when the predator was absent. Thus, our results showed that when these tadpoles perceive the presence of water nymphs, they feed as far away from them as possible, reduce foraging activity and stay motionless, possibly to avoid being detected. Changes in microhabitat use, as well as changes in foraging activity, are crucial strategies for tadpole survivorship, but on the other hand, these behaviors can result in the reduction of food intake and growth, affecting the developmental process of tadpoles (Van Buskirk 2002). This trade-off between surviving and growing reflects the great pressure that predation exerts on several aspects of tadpole life (Eklöv and Halvarsson 2000).

Importantly, this study contributed to a better understanding of the ecology of neotropical tadpoles, which, despite their high taxonomic diversity in Brazil, are still poorly known (Rossa-Feres et al. 2015). Further studies on predator–prey interactions can be key to helping us understand some behaviors of tadpoles that we observed here, such as staying close to predators. We encourage expanding the experiments to more species with different habits (e.g., neustonic), and under different conditions, as this may also be relevant to better comprehend community structure.

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Competing interests

The authors declare that there are no competing interests.

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Supplementary material

Supplementary tables are available with the article at https://doi.org/10.1139/cjz-2021-0236.

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