Lurking in the depth: Pond depth predicts microhabitat selection by *Rhinella icterica* (Anura: Bufonidae) tadpoles at two different sampling scales

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Abstract

Habitat selection has long been a central theme in ecology and has historically considered both physiological responses and ecological factors affecting species establishment. Investigating habitat selection patterns at different scales can provide important information on the relative roles of the environmental factors influencing the organisms’ abilities to use their surrounding habitat. This work aimed at investigating which environmental factors determine habitat selection by *Rhinella icterica* tadpoles, and also took the opportunity to investigate how the scale in which tadpoles and environmental data are sampled might influence the habitat selection results. A total of 2,240 tadpoles were counted in the whole sampling area, and while substrate cover and depth were the variables that better explained the abundance of tadpoles at the larger scale (plot level), depth and water turbidity better explained tadpoles’ abundance at the smaller scale (subplot level). The results suggest that avoiding predation by matching the background color is a likely process explaining tadpoles’ occupancy at both scales. Depth is known to influence tadpole habitat use in the tropics, and although its combination with turbidity and substrate cover varied between scales, our study suggests that sampling at different scales might not affect the inferred ecological processes driving habitat selection. This information might also be useful to predict tadpoles’ responses to micro-environmental perturbations and help in guiding the choice of parameters that should be taken into account when analyzing the effects of habitat degradation in Atlantic Forest amphibian populations.

Key Words

Atlantic Forest, conservation, Cururu Toad, depth, distribution, habitat selection, substrate cover, water turbidity

Introduction

There is currently little doubt that habitat loss and degradation are among the major threats to biodiversity worldwide (Venter et al. 2006; Betts et al. 2017). Habitat selection has long been a central theme in ecology and has historically considered physiological requirements and other limiting factors, such as competition and environmental conditions, as important factors affecting species establishment (Huey 1991). Therefore, investigating habitat selection patterns can help biologists to more efficiently determine how these abiotic and biotic characteristics will influence the individual’s abilities to use their surrounding habitat (MacArthur and Levins 1964; Whitham 1978), and evaluate the effects of habitat degradation on these patterns (Serrano and Astrain 2005; Campbell et al. 2018).

In ectotherms, the biotic and environmental factors affecting habitat selection, such as predation pressure...
and temperature, will certainly influence population dynamics over time (Huey 1991; Bradford et al. 1992). For amphibians, and specifically their aquatic tadpoles, habitat occupancy depends on many environmental characteristics of the water bodies they inhabit, such as temperature, oxygen concentration, depth, substrate coverage and surrounding aquatic vegetation (Laurila 1998; Nie et al. 1999; Torres-Orozco et al. 2002; Provete et al. 2014; Clevenot et al. 2018). These factors will directly affect the capacity of tadpoles to thermoregulate, which is linked to their abilities to select the microhabitat that provides better foraging opportunities (Noland and Ultsch 1981), and also influence their abilities to colonize other habitats (Clevenot et al. 2018). Furthermore, tadpoles’ ecomorphological traits variation within a population can be largely explained by the availability of microhabitats (Jordan et al. 2019) and resource partitioning (Eterovick and Fernandes 2001). Hence, intra-specific phenotypic plasticity in response to micro-environmental variations will also influence tadpole habitat use (Fatorelli and Rocha 2008; Jordan et al. 2019). Taken together, these patterns indicate that understanding the mechanisms behind tadpole habitat selection may help explain the factors that determine amphibian population structure and fluctuations, especially in the megadiverse Neotropical region.

The Atlantic Forest is one of the most diverse and understudied ecosystems in the world (Morellato and Haddad 2000; Ribeiro et al. 2009). This diversity is mirrored in amphibian reproductive patterns, and the Atlantic Forest harbours the largest number of amphibian reproductive modes known to date (Haddad and Prado 2005). Our understanding of amphibian reproductive modes and its implication for conservation, population ecology, and community structure has advanced in the last decades (Crump 2015), and this development was accompanied by a continuous growth of research on tadpole ecology (Altig 2018). Only approximately 30% of the anuran species in the world even had their tadpoles described (McDiarmid and Altig 1999) and, although ~60% of Brazilian tadpoles have already been described (Provete et al. 2012), most of what we know on habitat selection involves choices made by reproducing adults (Buxton and Sperry 2017).

Researchers working on tadpole ecology and habitat selection must always take practical decisions on how to effectively sample tadpoles in the field. In most cases, individuals are sampled near the stream or pond edges using dip-nets (Laurila 1998; Both et al. 2009; Provete et al. 2014), or using localized traps (Torres-Orozco et al. 2002), but efforts to sample the entire extension of the water bodies are rarely employed (Jordan et al. 2019). Many factors might influence these practical decisions, such as the particular characteristics of field sites and the local water bodies, as well as the time and resources available for sampling. However, sampling at the edges of the water body might bias habitat selection inferences, since space occupancy by tadpoles might be reflecting the particular characteristics of these places, and not taking into account how tadpoles effectively use the habitat considering the whole range of environmental conditions and biotic interactions. For example, in ponds or slow flowing streams, edges might be more shaded and the substrate might be more covered with leaves because of the surrounding vegetation. Since the results found in habitat selection studies could be influenced by the scale on which the data was collected (Morris 2003; McGarigal et al. 2016), sampling tadpoles and environmental data at different scales might uncover different ecological patterns. At larger scales, we can expect that environmental variables that are more related to tadpole space use, such as depth and water flow, will be more important to explain the distribution of individuals in the habitat. On the other hand, at smaller scales, especially close to the edge of the ponds, predation might be a more important factor affecting the distribution of individuals (Heyer et al. 1975), and habitat structure such as the presence of surrounding vegetation and leaves in the substrate will determine the presence of such individuals (Kopp et al. 2006). Hence, habitat selection patterns at larger scales will reflect the processes to which these individuals are responding in order to forage (Pearman 1993; Both et al. 2009; Domingos et al. 2015) whereas, at smaller scales, will reflect individuals behavior in order to avoid predation (Bridges 2002).

The toad *Rhinella icterica* (Spix, 1824) is distributed throughout southern South America, from eastern Paraguay to northern Argentina, in southern and southeastern Brazil, and northwards to the state of Bahia (Frost, 2019). This species differs from other *Rhinella marina* group species mainly by the presence of a subtriangular parotid and tibial glands, as well as less developed foot webs, and tadpoles with isometric growth (Kwet et al. 2006; Lima and Pederassi 2012). Besides being a large-bodied anuran, surprisingly little is known about this species in terms of reproduction (Pereyra et al. 2015) and habitat selection (Guix et al. 1998). In the Atlantic Forest, this species can be found from 30 to 1870 meters above sea level, and adults were found occupying a wide range of natural and anthropized habitats from streams and ponds to cleared and native forests (Guix et al. 1998). Its reproductive period varies according to the local climate and, although calling males and amplexant couples can be found throughout the year, reproduction usually peaks from July to February (Bertoluci 1998; Rodrigues and Bertoluci 2002; Lima et al. 2010). Oviposition and tadpole development occur in ponds or slow-flowing streams (Dixo and Verrado 2006; Hartmann et al. 2010), and there is anecdotal evidence that, in each particular year, *R. icterica* tries to avoid interspecific competition by reproducing earlier than other *Rhinella* species (Lima et al. 2010).

In this paper, we investigated which environmental factors determine microhabitat selection of *R. icterica* tadpoles. In addition, we examined how spatial scale might influence habitat selection results. We hypothesized that the environmental variables explaining tadpoles’ abundance will differ according to the sampling scale: variables related to the physical characteristics of the pond will be more important at the larger scale,
whereas variables that provide better camouflage opportunities will be more important at the smaller scale. Thus, at the larger scale, we predict tadpole abundance will be greater in shallow and larger habitats, with higher substrate cover and slower water flow. These expectations are based on the assumption that in these conditions there are more opportunities for shelter and less risk of dragging. At the smaller scale, near to the pond edge, higher levels of vegetation cover, water turbidity, as well as substrate leaf content, will possibly provide better camouflage opportunities and a stronger role in the abundance of tadpoles.

Methods

Study site

The study area is located in the protected area ‘Reserva Natural Salto Morato’ (25°10’54″S; 48°17’52″W), municipality of Guaraqueçaba, Paraná State, Brazil (Fig. 1). According to Köppen classification, the climate of the area is CFA with an average annual temperature of 21 °C. Mean annual precipitation reaches 2000 mm, and average relative air humidity is 85% (FGBPN, 2011; Alvares et al. 2013).

The sampling site was a 30 m long temporary pond with width varying from 60 cm to approximately 6 m and maximum depth of approximately 30 cm. This pond was surrounded by vegetation, predominantly secondary forest, and emergent macrophytes in the pond margins. Since we sampled during the rainy season (August), the pond had a constant influx of water from a nearby creek, and the excess of water would escape from the opposite part of the pond through a small water channel excavated by the rain. Ultimately, this means that the pond had constant running water throughout its whole extension.

Data collection

With the help of labelled stakes we delimited 20 plots along the pond extension where it was possible to visually count the tadpoles from the margin and without entering the pond. This condition was necessary to avoid biases due to the possibility of disturbing the tadpoles if an observer entered the pond. Each plot was 1 m long, consisting of the whole rectangular area across the pond, and this delimitation comprised our larger scale data collection. For the smaller scale data collection, each plot was subdivided in two equal parts considering a central imaginary line parallel to the pond edge (Fig. 2). In other words, the larger scale measurements considered the whole area of each of these one-meter long plots within the pond, while the smaller scale considered only one side of each plot, from the center to the pond edge. Hereafter, we refer to the larger scale sampling units as ‘plots’, and to the smaller scale as ‘subplots’ (Fig. 2).

Within each plot and subplot we counted the total number of tadpole individuals (abundance), measured pond width, depth, water flow velocity, and visually estimated water turbidity, surrounding vegetation cover percentage (including emergent macrophytes present in pond margins), and percentage of the sand-soil covered by leaves (hereafter substrate cover). Tadpoles were counted by a single observer, and always considering one subplot and then the other (i.e., the plot count was the sum of both subplots). Tadpole counting was performed from the outer edge of the pond before all other measurements to avoid disturbing the tadpoles. Hence, after counting the tadpoles, water flow velocity was measured in meters per second (m/s) by the formula D/T, where D is the distance (meters) and T is time (seconds). Distance was measured using a polystyrene foam ball and a 30 cm scale, where the ball was gently placed on the water surface, parallel

Figure 1. Study site (black circle) located in the protected area of Reserva Natural Salto Morato, municipality of Guaraqueçaba, Paraná State, Brazil. The red square highlights the municipality of Guaraqueçaba.
to the streamflow direction, and next to the scale for visualization. Pond width and depth were measured (in cm) with a measuring tape. Since the pond was very shallow, it was not possible to use the standard Secchi disk protocol to estimate water turbidity. Thus, we used a Secchi disk as a proxy to estimate turbidity by visually estimating how well it could be seen (similar to a ‘percentage’ of how well the disc could be visualized). Turbidity, surrounding vegetation cover, and substrate cover were estimated by the same observer.

The width was measured only once (for the plot), and the subplot width was half of the plot width. To replicate the usual data collection in habitat selection studies, all other measurements were taken twice (depth, velocity, turbidity, vegetation cover, and substrate cover), one in the plot center and another one in the subplot center. Since *Rhinella* tadpoles, if undisturbed, tend to remain still for long periods (Jara and Perotti 2009, 2010), we believe our tadpole abundance counts were accurate, and we did not observe any tadpole disturbance before entering the pond to measure physical variables. Data were collected in a single day on 22 August 2019. After all data collection procedures, a few tadpoles were collected from each plot to confirm identification. We based our identification in the comparative data provided in Tolledo and Toledo (2010).

**Figure 2.** Schematic representation of the study site and pond where data was collected. The 1 m long twenty plots and subplots are represented.

**Data Analysis**

We separately analyzed the data of the plots (n = 20) and the subplots (n = 20) to compare the habitat selection results inferred in these two different ecological scales represented by the different sampling strategies. Although each plot consists of two subplots, we only analyzed the data of one randomly chosen subplot per plot, to try to reflect the most commonly adopted tadpole sampling strategy used in ecological studies.

We checked for multicollinearity of our data by calculating Variance Inflation Factors (VIF) and using a threshold of VIF > 5 to exclude variables (Sheather 2009). Since no variable exceeded this threshold at the plot or subplot scale we ran all analyses using our full dataset. Considering the geographic structure of our sampling strategy, before proceeding with the analyses we tested for spatial autocorrelation in the data using Moran’s *I* statistic by implementing a permutation test with 199 simulations in the package *spdep* (Bivand et al. 2008). Because abundance data at both scales showed spatial autocorrelation (pseudo-*p* values < 0.05), we used statistical procedures to remove such effects from the residuals of our multiple regressions analyses. Thus, for each scale, we performed Wavelet-Revised Models (WRM) with tadpole abundance as the dependent variable and environmental data as independent variables (width, depth, water velocity, turbidity, surrounding vegetation, and substrate cover). The WRM is an extension of Generalized Linear Models (GLM) developed to remove the effect of spatial autocorrelation in multiple regressions (Carl and Kühn 2010). This method is particularly suited for the analyses of our data for a multitude of reasons: first, GLM methods such as WRM do not require data to be normally distributed and are also robust to deviations from other parametric assumptions; second, the calculations can be fitted to data in different statistical distributions and; third, the WRM was developed to accommodate grid-based datasets, which is in line with the structure of our data. All WRM analyses were performed using package *spind* (Carl et al. 2018). In addition, to build the best possible models, we investigated the best fit of our data to a statistical distribution so we could use it in WRM model inference. Us-
ing package fitdistplus (Delignette-Muller and Dutang 2015) we visually inspected our data and compared the fit to the three different distributions available to run the WRM in package spinif (normal, binomial or poisson).

After building the full WRM model, we performed an automated backward stepwise model selection using the Akaike Information Criterion (AIC) to select the environmental variables that better explain tadpole abundance. In short, the analysis starts with a previously created full WRM model where abundance is explained by all environmental variables, and variables are removed in a stepwise fashion while the AIC value of the models are compared until the best model fit with the lowest AIC value is reached. Thus, for each scale, the best model explaining the abundance of tadpoles was selected by AIC, and then the significance of the variables of the best model was calculated. All statistical procedures were performed in R v3.6.0 (R Core Team, 2019).

**Results**

In total, we counted 2,240 tadpoles in the whole sampling area. Tadpole abundance at the plot scale was almost twice that of the subplot scale (Table 1). In general, environmental variables had higher values at the plot scale, except for substrate cover that was slightly higher at the subplot scale. Turbidity did not vary between scales (Table 1). Visual inspections suggested that the best distribution that should fit our plot abundance data was a normal distribution. We compared the normal, binomial and poisson distributions using the Kolmogorov-Smirnov statistic (respectively 0.213, 0.232 and 0.450) and also Cramér-Von Mises statistic (respectively 0.120, 0.179, and 1.33). For our subplot data, visual inspections also suggested that the best distribution was a normal distribution. Again, we compared the normal, binomial and poisson distributions using the Kolmogorov-Smirnov statistic (respectively 0.249, 0.254 and 0.494) and the Cramér-Von Mises statistic (respectively 0.191, 0.267 and 1.426).

Table 1. Predictor variables of tadpoles’ abundance at the plot and subplot spatial sampling scales.

| Analyzed variables | Mean ± standard deviation | Minimum – Maximum (range) |
|--------------------|--------------------------|---------------------------|
| **PLOT (n = 20)**  |                          |                           |
| Tadpoles abundance | 112.1 ± 109.4            | 0–392 (392)               |
| Depth (cm)         | 13.4 ± 7.1               | 4–23 (19)                 |
| Velocity (m/s)     | 11.1 ± 16.9              | 0–59 (59)                 |
| Width (cm)         | 172.7 ± 86.6             | 39–305 (266)              |
| Surrounding vegetation cover (%) | 46.5 ± 21.8 | 20–90 (70) |
| Substrate cover (%) | 38.7 ± 22.6             | 10–80 (70)                |
| Turbidity (%)      | 16.2 ± 8.7               | 5–35 (30)                 |
| **SUBPLOT (n = 20)** |                        |                           |
| Tadpoles abundance | 59.1 ± 52.7              | 0–141 (142)               |
| Depth (cm)         | 10.3 ± 5.7               | 2–20 (18)                 |
| Velocity (m/s)     | 3.7 ± 7.4                | 0–24 (24)                 |
| Width (cm)         | 86.38 ± 43.32            | 15.95–152.5 (133)         |
| Surrounding vegetation cover (%) | 46.0 ± 24.9  | 5–95 (90) |
| Substrate cover (%) | 39.7 ± 23.6             | 10–80 (70)                |
| Turbidity (%)      | 16.2 ± 8.7               | 5–35 (30)                 |

Hence, we fitted both our WRM models using a normal distribution. All R codes and statistical results, and the visual inspection graphs, can be found at Suppl. Material 1: R Markdown file.

At the plot scale, the stepwise model selection procedure kept substrate cover and depth as the variables that better explained abundance, while depth and turbidity were the ones selected at the subplot level (Table 2). Contrary to our expectations, the relationship of tadpole abundance and depth was positive, since there was an increase in the number of tadpoles in deeper plots (Fig. 3A). Nonetheless, the increase in tadpole abundance in plots with higher substrate cover percentage is in agreement with our hypothesis for the plot scale (Fig. 3A). In the subplot scale, tadpoles’ abundance increased with turbidity and depth (Fig. 3B). While the relationship with water turbidity is in agreement with our initial hypothesis, we did not predict that depth would influence abundance at this scale.

**Discussion**

Habitat selection theory predicts that individual choices will influence foraging efficiency and survivorship, which will be directly related to individual fitness (Rosenzweig 1981; Chesson 2000; Morris 2003). Understanding tadpole habitat use might help to evaluate how habitat change will influence the underlying ecological processes related to individuals’ choices (Holbrook and Schmitt 1988; Carey et al. 1992) and, thus, provide important information on how environmental disturbance might directly affect survivorship. Even though this work focuses on only one species, our results reveal useful patterns that help to fill the knowledge gaps we still have on Atlantic Forest tadpole ecology and habitat selection in particular.

Many environmental variables have been found to influence tadpole habitat selection such as water flow and substrate composition (Hoff et al. 1999; Haramura 2006). Nonetheless, water depth is usually a very strong component influencing habitat occupancy by tadpoles both at population (Pearman 1993) and community scales (Both et al. 2009). For our population of *R. icterica* tadpoles, depth was positively related to abundance both at the plot and subplot scales. The other two variables that better explained tadpole abundance were substrate cover (plot scale, Fig. 3A) and water turbidity (subplot scale, Fig. 3B). Taken together, all these variables seem to be essentially related to predation avoidance by the tadpoles which, in our particular system, can be at least partially explained by the tadpoles’ dark body color. The sand-soil at the bottom of our sampled pond was clear, in stark contrast to the *Rhinella* tadpole color. However, by staying on top of dark decomposing leaves at the bottom (the main substrate cover), the tadpoles would be less conspicuous to visually oriented predators (Nomura et al. 2013; Gontijo et al. 2018). In fact, while measuring our environmental variables we noticed that, when disturbed,
tadpoles would quickly swim through the clear sand-soil until other leaves in the nearby substrate were reached, or they would also swim to deeper parts of the pond. In line with these strategies, more turbid waters also provide better camouflaging opportunities from visually oriented predators (Gardner, 1981; Minello et al., 1987). Since predation pressure has an important role in tadpole habitat use (Eterovick and Barata, 2006), being in the deep, in more turbid water, and on top of leaves, could be directly influencing tadpole predation avoidance.

There is no information on the predation of *R. icterica* tadpoles in the wild, but it can be inferred from results of other species of the genus *Rhinella* that they should be mainly consumed by invertebrate predators such as dragonfly larvae and waterbugs (Jara and Perotti, 2009), fishes (Nomura et al. 2011), and birds (Beckmann et al. 2011). In laboratory experimental conditions, *Rhinella* tadpoles only slightly change their activity patterns in response to the presence of predators (Jara and Perotti 2010), which suggests that they may rely on other ecological strategies to escape predation in the wild, such as camouflage. Another experiment showed that although matching the background coloration did not affect tadpole mortality rate, tadpoles also decreased their activity in response to the presence of predators, and were less active in white background (Nomura et al. 2013). These laboratory habitat selection results depict a generalization of the individual’s behavioral changes, and seem to partially corroborate our conclusions that adjusting their coloration to the background is the process driving habitat selection in *R. icterica* tadpoles.

Although the selection of both substrate cover and turbidity by our analyses can be attributed to protection against predators (Semlitsch, 1993), it is worth noting that these characteristics might also be correlated with food availability (Skelly et al. 2002; Schiesari, 2006; Provete et al. 2014). *Rhinella* tadpoles primarily eat algae scraped from the bottom of the water body (Hinckley, 1963; Rossa-Feres et al. 2004) and the presence of leaves in the bottom is also linked to higher primary productivity and decomposition rates (Skelly, 1996; Skelly et al. 2004) and the presence of leaves in the bottom is also linked to higher primary productivity and decomposition rates (Skelly, 1996; Skelly et al. 2002), which provide food resources to tadpoles. Pond depth may also be determinant in tadpoles’ survival and growth, since deeper ponds tend to retain water for longer periods, allowing tadpoles to grow bigger until metamorphosis (Peltzer and Lajmanovich, 2004). However, the different combination of these variables in the plot and subplot level, and the fact that depth was selected in both scales, suggests that the more proximal explanation, i.e., decreasing predation risk, might be a better explanation for the process affecting *R. icterica* tadpoles’ habitat selection. Nonetheless, given the lack of information on tadpole habitat use in the Atlantic Forest, further studies aiming at evaluating these and other factors influencing tadpoles’ habitat use at different spatial scales are highly warranted.

According to our results, water flow was not important for tadpoles’ abundance. However, this result needs to be interpreted with caution because of the low variation of this data observed among the replicates (Table 1), and the fact that flow was not particularly high throughout the whole pond. Not surprisingly, this observation supports the idea that *R. icterica* adults prefer slow-flowing water for reproduction, reducing the risk of egg dragging.
Domingo et al. (2010). During fieldwork, we also sought for tadpoles in all the nearby creeks and other water bodies in the reserve, but with no success. We noted that these other water bodies were clearer, had fast-flowing water, and were mostly free of substrate leaves, reinforcing the mating site preferences of *R. icterica* adults. In tropical ponds, characteristics such as water flow and pond size have distinct effects on tadpole distribution (Borges Júnior and Rocha 2013), but tadpoles that inhabit ponds with slow water flow generally use sites with vegetation cover (Eterovick and Barata 2006). Predation rates are supposedly lower when the habitat has more hiding opportunities (Kopp et al. 2006), but vegetation cover did not influence the abundance of *R. icterica* tadpoles in our study site, also supporting the idea that they might predominantly rely on camouflage to avoid predation.

Even though we were not able to test it, another factor that could influence tadpole microhabitat use is their toxicity. Previous studies detected toxins in the eggs and tadpoles of *Rhinella marina*, although in lower concentrations compared to adults (Flier et al. 1980; Akizawa et al. 1994; Hayes et al. 2009). For instance, *R. marina* tadpoles are toxic to some native predators and other tadpole species in Australia, where it is an invasive species (Covacevich and Archer 1975; Hamley and Georges 1985; Crossland and Alford 1998; Crossland and Azevedo-Ramos 1999; Crossland 2000). The toxins in the tadpoles’ bodies make them unpalatable, which, in turn, may favor a gregarious behavior by inclusive fitness (Waldman 1982; Griffiths and Denton 1992). Besides, the black coloration in unpalatable tadpoles might also be related to aposematism (D’Heursel and Haddad 1999). However, we did not find other tadpole species in our study site, which prevented any comparison with co-occurring species. Unfortunately, we cannot infer the impact of *R. icterica* toxicity in tadpoles’ spatial distribution and microhabitat use from our data and observations, and the currently available evidence indicates that camouflage is possibly the main factor driving habitat selection in this case. Nonetheless, *R. icterica* tadpoles do have gregarious behavior and a body coloration that is consistent with aposematism. From an ecological perspective, we suggest that further studies should focus on tadpole individuals’ spatial distribution, group sizes, and the comparison of such characteristics with sympatric species.

Although our two different sampling scales might reflect common sampling practices in tadpole ecology studies, they were probably not divergent enough to capture different ecological properties of tadpole habitat selection. Contrary to our hypotheses, all three variables selected at the plot and subplot scales are putatively related to predation avoidance, even though at least one environmental variable differed. In this regard, we suggest that sampling at a smaller scale should be enough to capture the ecological processes behind habitat selection, and future studies in tadpole habitat selection might benefit from this information during the research planning stage. Nonetheless, given that different variables were selected in each scale, our results also indicate that tadpoles are sensitive to micro-environmental changes, which suggests that small scale habitat perturbations might influence the behavior of these animals. Tadpoles might be highly sensitive to environmental disturbances (Babini et al. 2018), and our results highlight the need to consider these putative influences when planning conservation actions for Atlantic Forest amphibians.

In conclusion, although variables that predict *R. icterica* tadpoles’ habitat selection in the Atlantic Forest might be explained by the different ecological processes discussed above, avoiding predation seems to be a reasonable explanation for our results, considering that water depth was selected at both scales and correlated with substrate cover and turbidity. Our study also indicates that sampling at different scales might not affect the inference of the ecological processes behind habitat selection. In face of the concerning conservation status of the Atlantic Forest, and considering the lack of data on the ecology of many amphibian species, the conservation of anurans reproductive sites might be one of the most important conservation measures to maintain viable populations in the Atlantic Forest (Gonçalves et al. 2015). In this context, our study can also be helpful in guiding the choice of parameters that should be taken into account when analyzing the effects of habitat degradation in amphibian populations.

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**References**

Akizawa T, Mukai T, Matsukawa M, Yoshioka M, Morris JF, Butler VP (1994) Structures of novel bufadienolides in the eggs of a toad, *Bufo marinus*. Chemical and Pharmaceutical Bulletin 42: 754–756. [https://doi.org/10.1248/cpb.42.754](https://doi.org/10.1248/cpb.42.754)

Altig R (2018) Fifty-three years a tadpole. Journal of Herpetology 52: 1–5. [https://doi.org/10.1670/16-034](https://doi.org/10.1670/16-034)

Alvares, CA, Stape JL, Sentelhas PC, de Moraes Gonçalves JL, Sparovek G (2013) Köppen’s climate classification map for Brazil. Meteoroilogische Zeitschrift 22: 711–728. [https://doi.org/10.1127/0941-2948/2013/0507](https://doi.org/10.1127/0941-2948/2013/0507)

Babini MS, de Lourdes Bionda C, Salinas ZA, Salas NE, Martino AL (2018) Reproductive endpoints of *Rhinella arenarum* (Anura, Bufonidae): populations that persist in agroecosystems and their use for the environmental health assessment. Ecotoxicology and Environmental Safety 154: 294–301. [https://doi.org/10.1016/j.ecoenv.2018.02.050](https://doi.org/10.1016/j.ecoenv.2018.02.050)

https://herpetozoa.pensoft.net
Beckmann C, Crossland MR, Shine R (2011) Responses of Australian wading birds to a novel toxic prey type, the invasive cane toad *Rhinella marina*. Biological Invasions 13: 2925–2934. https://doi.org/10.1007/s10530-011-9974-1

Bertoluci J (1998) Annual patterns of breeding activity in Atlantic rainforest anurans. Journal of Herpetology 32: 607–611. https://doi.org/10.2307/15655223

Bets MG, Wolf C, Ripple WJ, Phalan B, Millers KA, Duarte A, Butchart SH, Levi T (2017) Global forest loss disproportionately erodes biodiversity in intact landscapes. Nature 441–444. https://doi.org/10.1038/nature2285

Bivand RS, Pebesma EJ, Gomez-Rubio V, Pebesma EJ (2008) Applied spatial data analysis with R. Springer, 405 pp.

Both C, Solé M, Dos Santos TG, Cechin SZ (2009) The role of spatial and temporal descriptors for neotropical tadpole communities in southern Brazil. Hydrobiologia 624: 125–138. https://doi.org/10.1007/s10750-008-9685-5

Bradford DF, Swanson C, Gordon MS (1992) Effects of low pH and aluminum on two declining species of amphibians in the Sierra Nevada, California. Journal of Herpetology 26: 369–377. https://doi.org/10.2307/1565113

Bridges CM (2002) Tadpoles balance foraging and predator avoidance: effects of predation, pond drying, and hunger. Journal of Herpetology 36: 627–634. https://doi.org/10.1670/0022-1511(2002)036[0627:TBFA02.CO;2]

Buxton VL, Sperry JH (2017) Reproductive decisions in anurans: a review of how predation and competition affects the deposition of eggs and tadpoles. Bioscience 67: 26–38. https://doi.org/10.1093/biosci/biw149

Campbell LA, Tkaczynski PJ, Mouna M, Derrou A, Ouannou L, Majolo B, van Lavieren E (2018) Behavioural thermoregulation via microhabitat selection of winter sleeping areas in an endangered primate: implications for habitat conservation. Royal Society Open Science 5: 1–12. https://doi.org/10.1098/rsos.181113

Carey AB, Horton SP, Biswell BL (1992) Northern spotted owls: influence of prey base and landscape character. Ecological Monographs 62: 223–250. https://doi.org/10.2307/2937094

Carl G, Kühn I (2010) A Wavelet-Based Extension of Generalized Linear Models to Remove the Effect of Spatial Autocorrelation. Geographical Analysis 42: 323–337. https://doi.org/10.1111/j.1538-4632.2010.00777.x

Carl G, Levin SC, Kühn I (2018) spind: an R Package to Account for Spatial Autocorrelation in the Analysis of Lattice Data. Biodiversity Data Journal 6: 1–17. https://doi.org/10.3897/BDJ.6.e20760

Chesson P (2000) Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31: 343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343

Clevenot L, Carré C, Pech P (2018) A review of the factors that determine whether stormwater ponds are ecological traps and/or high-quality breeding sites for amphibians. Frontiers in Ecology and Evolution 6: 1–12. https://doi.org/10.3389/fevo.2018.00040

Crossland MR (2000) Direct and indirect effects of the introduced toad *Bufo marinus* (Anura: Bufonidae) on populations of native anuran larvae in Australia. Ecography 23: 283–290. https://doi.org/10.1111/j.1600-0587.2000.tb00283.x

Crossland MR, Alford RA (1998) Evaluation of the toxicity of eggs, hatchlings and tadpoles of the introduced toad *Bufo marinus* (Anura: Bufonidae) to native Australian aquatic predators. Australian Journal of Ecology 23: 129–137. https://doi.org/10.1111/j.1442-9993.1998.tb00711.x

Crossland MR, Azvedo-Ramos C (1999) Effects of *Bufo* (Anura: Bufonidae) toxins on tadpoles from native and exotic *Bufo* habitats. Herpetologica 55: 192–199. https://www.jstor.org/stable/3893070

Covacevich J, Archer M (1975) The distribution of the cane toad, *Bufo marinus*, in Australia and its effects on indigenous vertebrates. Memoirs of the Queensland Museum 17: 305–310. https://www.biodydiversitylibrary.org/resource/48771202/page/183mode/lup

Crump ML (2015) Anuran reproductive modes: evolving perspectives. Journal of Herpetology 49: 1–16. https://doi.org/10.1670/14-097

Delignette-Muller ML, Dutang C (2015) fitdistrplus: An R package for fitting distributions. Journal of Statistical Software 64: 1–34. https://doi.org/10.18637/jss.v064.i04

D’Heurle A, Haddad CFB (1999) Unpalatability of *Hyla semimaculata* tadpoles (Anura) to captive and free-ranging vertebrate predators. Ethology Ecology & Evolution 11: 339–348. https://doi.org/10.1080/08927014.1999.9522818

Dixo M, Verde VK (2006) Herpetofauna de serrapielha da reserva florestal de Morro Grande, Cotia (SP), Biota Neotropical 6: 1–20. https://doi.org/10.1590/S1676-0632060000200009

Domíngos FMCB, Arantes IC, Cavalcanti DR, Jotta PACV (2015) Shelter from the sand: microhabitat selection by the bemelicolous tree frog *Scinax cuspisatids* (Anura, Hylidae) in a Brazilian restinga. North-Western Journal of Zoology 11: 27–33. http://biozoojournals.ru/nwjz/content/v11n1/nwjz_141508_Domingos.pdf

Eterovic PC, Barata IM (2006) Distribution of tadpoles within and among Brazilian streams: the influence of predators, habitat size and heterogeneity. Herpetologica 62: 365–377. https://doi.org/10.1655/01-0831(2006)62[365:DOTWAA]2.0.CO;2

Eterovic PC, Fernandes GW (2001) Tadpole distribution within montane meadow streams at the Serra do Cipó, southeastern Brazil: ecological or phylogenetic constraints? Journal of Tropical Ecology 17: 683–693. https://doi.org/10.1017/S026646740100150X

Fatorelli P, Rocha CFD (2008) O que molda a distribuição das guilas de girinos tropicais?: Quarenta anos de buscas por padrões. Oecologia Brasiliensis 12: 11. https://doi.org/10.4257/occ.2008.1204.11

Flier J, Edwards MW, Daly JW, Myers CW (1980) Widespread occurrence in frogs and toads of skin compounds interacting with the ouabain site of Na+, K+-ATPase. Science 208: 503–505. https://doi.org/10.1126/science.6245447

Gaoncalves DS, Crivellari LB, Conte CE (2015) Linking environmental drivers with amphibian species diversity in ponds from subtropical grasslands. Anais da Academia Brasileira de Ciências 87: 1751–1762. https://doi.org/10.1590/0001-3765201520140471

Gontijo ASB, Espanha J, Eterovic PC (2018) Is tadpole coloration adaptive against bird predation? Acta Ethologica 21: 69–79. https://doi.org/10.1007/s10211-017-0141-2

Griffiths RA, Denton J (1992) Interspecific associations in tadpoles. Animal Behaviour 44: 1153–1157. https://doi.org/10.1016/S0003-3472(05)80327-6

Guix JC, Montori A, Llorente G, Carretero MA, Santos X (1998) Natural history and conservation of bufonids in four Atlantic rainforest areas of southeastern Brazil. Herpetological Natural History 6: 1–12. https://www.researchgate.net/publication/236239577_Natural_history_and_conservation_of_bufonids_in_four_Atlantic_rainforest_areas_of_SE_Brazil
Haddad CF, Prado CP (2005) Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. Bioscience 55: 207–217. https://doi.org/10.1641/0006-3568(2005)055[0207:RMF-
FATJ2.0.CO;2

Hamley T, Georges A (1985) The Australian snapping tortoise Elseya latisternum: a successful predator on the introduced cane toad? Australian Zoologist 21: 607–610. https://www.scopus.com/re-
cord/display.uri?eid=2-s2.0-0022170656&origin=inward&tx-
Gid=43854a390d2b3c3ae729d9996ee36b22

Haramura T (2006) Microhabitat selection by tadpoles of Buoergeria japonica inhabiting the coastal area. Journal of Ethology 25: 3–7. https://doi.org/10.1007/s10164-006-0197-3

Hartmann MT, Hartmann PA, Haddad CF (2010) Reproductive modes and fecundity of an assemblage of anuran amphibians in the Atlantic rainforest, Brazil. Iheringia Série Zoologia 100: 207–215. https://doi.org/10.1590/S0073-47212010000300004

Hayes RA, Crossland MR, Hagman M, Capon RJ, Shine R (2009) Ontogenetic variation in the chemical defenses of cane toads (Bufo marinus): toxin profiles and effects on predators. Journal of Chemical Ecology 35: 391–399. https://doi.org/10.1007/s10886-009-9608-6

Heyer WR, McDiamrd RW, Weigmann DL (1975) Tadpoles, predation and pond habitats in the tropics. Biotropica 7: 11. https://doi.org/10.2307/2989753

Hinckley AD (1963) Diet of the giant toad, Bufo marinus (L.), in Fiji. Herpetologica 18: 253–259. http://hbs.bishopmuseum.org/fiji/pdf/ hinckley1962.pdf

Hoff K, Blaustein A, McDiamrwd R, Altig R (1999) Behavior: interactions and their consequences. In: McDiamrd RW, Altig, R. (Eds.) Tadpoles: The biology of anuran larvae, 215–239. https://pubs.
er.usgs.gov/publication/520027

Holbrook SJ, Schmitt RJ (1988) The combined effects of predation risk and food reward on patch selection. Ecology 69: 125–134. https://doi.org/10.2307/2989753

Huey RB (1991) Physiological consequences of habitat selection. The American Naturalist 137: 91–115. https://doi.org/10.1086/285141

Jara FG, Perotti MG (2009) Toad tadpole responses to predator risk: ontogenetic change between constitutive and inducible defenses. Journal of Herpetology 43: 82–88. https://doi.org/10.1670/07-229R2.1

Jara FG, Perotti MG (2010) Risk of predation and behavioural response in three anuran species: influence of tadpole size and predator type. Hydrobiology 644: 313–324. https://doi.org/10.1007/s10928-010-1196-9

Borges Junior VNT, Rocha CFD (2013) Tropical tadpole assemblages: which factors affect their structure and distribution? Oecologia Australis 17: 217–228. https://doi.org/10.4257/ocoo.2013.1702.04

Kopp K, Wachlevski M, Eterovic P (2006) Environmental complexity reduces tadpole predation by water bugs. Canadian Journal of Zoology 84: 136–140. https://doi.org/10.1139/z05-186

Kvet A, Di-Bernardo M, Maneyro R (2006) First record of Chaunus achatvali (Anura, Bufonidae) from Rio Grande do Sul, Brazil, with a key for the identification of the species in the Chaunus marinus group. Iheringia Série Zoologia 96: 479–485. https://doi.
.org/10.1590/S0073-47212006000400013

Laurila A (1998) Breeding habitat selection and larval performance of two anurans in freshwater rock-pools. Ecography 21: 484–494. https://doi.org/10.1111/j.1600-0587.1998.tb00440.x

Lima M, Pederassi J (2012) Morphometrics and ratio of body proportionality of tadpoles of Rhinella ictericia (Anura, Bufonidae) at different developmental stages. Brazilian Journal of Biology 72: 623–629. https://doi.org/10.1591/s1519-698242012000300028

Lima MS, Pederassi J, dos Santos Souza CA, Silva CdPA, Peixoto OL (2010) Distribuição e fidelidade de desenvolvimento de Rhinella ictericia (Anura, Bufonidae) no rio Cachimbau, Sudeste do Brasil. Revista Brasileira de Zoociências 12: 151–156. https://periodicos.
ufjfr.br/index.php/zooociencias/article/view/24457

MacArthur R, Levins R (1964) Competition, habitat selection, and character displacement in a patchy environment. Proceedings of the National Academy of Sciences of the United States of America 51: 3. https://doi.org/10.1073/pnas.51.6.1207

McDiarmrd RW, Altig R (1999) Tadpoles: the biology of anuran larvae. University of Chicago Press, 444 pp.

McGarigal K, Wan HY, Zeller KA, Timm BC, Cushman SA (2016) Multi-scale habitat selection modeling: a review and outlook. Landscape Ecology 31: 1161–1175. https://doi.org/10.1007/s10980-016-0374-x

Morello LPC, Haddad CF (2000) Introduction: The Brazilian Atlantic Forest I. Biotropica 32: 786–792. doi.org/10.1111/j.1744-7429.2000.tb00618.x

Morris DW (2003) Toward an ecological synthesis: a case for habitat selection. Oecologia 136: 1–13. https://doi.org/10.1007/s00442-003-1241-4

Nie M, Crim JD, Ultsch GR (1999) Dissolved oxygen, temperature, and habitat selection by bullfrog (Rana catesbeiana) tadpoles. Copeia 1999: 153–162. https://doi.org/10.2307/1447396

Noland R, Ultsch GR (1981) The roles of temperature and dissolved oxygen in microhabitat selection by the tadpoles of a frog (Rana pippins) and a toad (Bufo terrestris). Copeia 1981: 645–652. https://doi.org/10.2307/1444570

Nomura F, De Marco P, Carvalho A, Rossa-Feres DC (2013) Does back-ground colouration affect the behaviour of tadpoles? An experimental approach with an odonate predator. Ethology Ecology & Evolution 25: 185–198. https://doi.org/10.1080/03949370.2012.742465

Nomura F, do Prado V, da Silva F, Borges R, Dias N, Rossa-Feres DC (2011) Are you experienced? Predator type and predator experience trade-offs in relation to tadpole mortality rates. Journal of Zoology 284: 144–150. https://doi.org/10.1111/j.1469-7998.2011.00791.x

Pearman PB (1993) Effects of habitat size on tadpole populations. Ecology 74: 1982–1991. https://doi.org/10.2307/1940841

Peltzer PM, Lajmanovich RC (2004) Anuran tadpole assemblages in riparian areas of the Middle Paraná River, Argentina. Biodiversi ty & Conservation 13: 1833–1842. https://doi.org/10.1023/B:BLI-
OC.0000035870.36495.fc

Pereyra MO, Vera Candidoti MF, Faivovich J, Baldo JD (2015) Egg clutch structure of Rhinella tumboldi (Anura: Bufonidae), a toad from the Yungas of Argentina, with a review of the reproductive diversity in Rhinella. Salamandra 51: 161–170. http://www.sala-
mandra-journal.com/index.php/home/contents/2015-05-405-pe-
reyra-m-o-m-f-v-candidoti-m-faivovich-d-baldo/file

Provete DB, Garey MV, Da Silva FR, Jordani MX (2012) Knowledge gaps and bibliographical revision about descriptions of free-swimming anuran larvae from Brazil. North-Western Journal of Zoology 8: 283–286. http://biozoojournals.ro/nwjz/content/view/8n2/
wjz.121124.Provete.pdf

Provete DB, Gonçalves-Souza T, Garey MV, Martins IA, Rossa-Feres DC (2014) Broad-scale spatial patterns of canopy cover and pond morphology affect the structure of a Neotropical amphibian meta-

herpetozoa.pensoft.net
community. Hydrobiologia 734: 69–79. https://doi.org/10.1007/s10750-014-1870-0

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM (2009) The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biological Conservation 142: 1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Rodrigues MT, Bertoluci J (2002) Seasonal patterns of breeding activity of Atlantic Rainforest anurans at Boracéia, Southeastern Brazil. Amphibia-Reptilia 23: 161–167. https://doi.org/10.1163/156853802760061804

Rosenzweig ML (1981) A theory of habitat selection. Ecology 62: 327–335. https://doi.org/10.2307/1936707

Rossa-Feres DC, Jim J, Fonseca MG (2004) Diets of tadpoles from a temporary pond in southeastern Brazil (Amphibia, Anura). Revista Brasileira de Zoologia 21: 745–754. https://doi.org/10.1590/S0101-81752004000400003

Schiesari L (2006) Pond canopy cover: a resource gradient for anuran larvae. Freshwater Biology 51: 412–423. https://doi.org/10.1111/j.1365-2427.2005.01497.x

Semlitsch RD (1993) Effects of different predators on the survival and development of tadpoles from the hybridogenetic Rana esculenta complex. Oikos 67: 40–46. https://doi.org/10.2307/3545093

Serrano D, Astrain C (2005) Microhabitat use and segregation of two sibling species of Calandrella larks during the breeding season: conservation and management strategies. Biological Conservation 125: 391–397. https://doi.org/10.1016/j.biocon.2005.04.010

Sheather S (2009) A modern approach to regression with R. Springer Science & Business Media, 387 pp. https://doi.org/10.1007/978-0-387-09608-7

Skelly D, Freidenburg L, Kiesecker J (2002) Forest canopy and the performance of larval amphibians. Ecology 83: 983–992. https://doi.org/10.1890/0012-9658(2002)083[0983:FCATPO]2.0.CO;2

Skelly DK (1996) Pond drying, predators, and the distribution of Pseudacris tadpoles. Copeia 1996: 599–605. https://doi.org/10.2307/1447523

Tolledo J, Toledo LF (2010) Tadpole of Rhinella jimi (Anura: Bufonidae) with comments on the tadpoles of species of the Rhinella marina group. Journal of Herpetology 44: 480–483. https://doi.org/10.1670/09-157.1

Torres-Orozco RE, Jimenez-Sierra CL, Vogt RC, Benitez-JLV (2002) Neotropical tadpoles: spatial and temporal distribution and habitat use in a seasonal lake in Veracruz, Mexico. Phyllomedusa: Journal of Herpetology 1: 81–91. https://doi.org/10.11606/issn.2316-9079.v1i2p81-91

Venter O, Brodeur NN, Nemiroff L, Belland B, Dolinsek JJ, Grant JW (2006) Threats to endangered species in Canada. Bioscience 56: 903–910. https://doi.org/10.1641/0006-3568(2006)56[903:TTE]2.0.CO;2

Waldman B (1982) Sibling association among schooling tadpoles: field evidence and implications. Animal Behaviour 30: 700–713. https://doi.org/10.1016/S0003-3472(82)80141-3

Whitham TG (1978) Habitat selection by Pemphigus aphids in response to response limitation and competition. Ecology 59: 1164–1176. https://doi.org/10.2307/1938230

Xavier Jordani M, Mouquet N, Casatti L, Menin M, Rossa-Feres CD, Albert CH (2019) Intraspecific and interspecific trait variability in tadpole meta-communities from the Brazilian Atlantic rainforest. Ecology and Evolution 9: 4025–4037. https://doi.org/10.1002/ece3.5031

Supplementary material 1

R Markdown file

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Data type: Adobe PDF file

Explanation note: This R Markdown file can be opened using any PDF reader. It contains all R codes (script) used for data exploration and analyses, as well as all results obtained at each analytical step.

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