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Environmental and spatial factors affect the composition and morphology of tadpole assemblages

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Abstract

Understanding how communities compositions are affected by environmental and spatial factors are essential to provide knowledge about the distributions of species. Furthermore, these factors can play a role in species morphological variation. Tadpoles can be found in different types of aquatic microhabitats, showing a considerable amount of morphological diversity. We hypothesized that tadpole morphological diversity is controlled by ecological and spatial factors other than assemblage attributes, and that tadpole assemblage composition is affected by spatial factors. To test these hypotheses, we recorded the abundance of tadpoles from different ponds, identified eight environmental variables that represented local and landscape descriptors of the ponds, recorded the spatial coordinates of the ponds, and measured the morphological variation of assemblages. Spatial factors significantly affected the composition of tadpole assemblages, while both spatial and environmental factors affected morphological variation. The ability of tadpoles to alter their morphology in response to environmental factors might be a result of poor oviposition site choice, and this probably interacts with spatial factors to control the assemblage composition of tadpoles. Morphological variation is advantageous for tadpoles as it allows them to adjust their morphology to environmental conditions. This study has demonstrated how factors that control the assemblage composition of tadpoles also drive their morphological diversity.

Keywords

Amphibian; Environmental filter; Geometric morphometrics; Neutral process; Variation partitioning
Introduction

Understanding the process that drives the distribution and abundance of species is a central theme in community ecology (Gleason 1917; Brown et al. 1995; Matthews and Whittaker 2014). Environmental factors such as moisture, temperature, and physical habitat structure can significantly affect species diversity on a local and regional scale (Tamme et al. 2010; Stein et al. 2014). Thus, sites with similar environmental factors will tend to support assemblages with similar species composition (Legendre and Fortin 1989). Other important factors that affect the distribution and abundance of species are the geographic distance between different habitats and biotic processes such as predation, competition, and dispersion (McCarthy and Lindenmayer 2000; Ehrlén and Morris 2015). A greater geographic distance between available habitats presents various challenges to the movement of individuals, affecting the probability of successful colonization of distant habitats (Legendre and Fortin 1989) and leading to spatial structuring in the composition of assemblages. Therefore, it is expected that closely located habitats will have a similar species composition. Thus, the presence of a species within a given habitat is the result of an environmental and spatial filtering process which selects for species which are better adapted to such filters (Webb et al. 2010).

Tadpoles are the larval stages of anurans and can be found in different habitats, including terrestrial, semiterrestrial and aquatic. Aquatic tadpoles can be found in microhabitats as diverse as streams, temporary and permanent ponds, or phytotelmata (i.e. water held in plants parts or cavities; Inger 1985; Altig and McDiarmid 1999a, 1999b). Experimental studies conducted in mesocosms show that tadpoles displays different morphological adaptations according to the level of intra and interspecific competition (e.g. relative small tails and larger bodies; Relyea 2002, 2004; Relyea and Hoverman 2003) predation (e.g. relative larger tails and small bodies; Relyea 2001,
and heterogeneity of environmental factors (Lind and Johanson 2007; Michel 2011). These adaptations are important to permit tadpoles to occupy different microhabitats.

In general, tadpoles are restricted to the developmental site selected by the adults during oviposition (Wells 2007). Therefore, the selection of the microhabitat made by adults is important for the survival of tadpoles (Resetalits and Wilbur, 1989; Werner et al. 2007). Occasionally, adults anurans may select unfavorable habitats for oviposition, and the survival of the tadpoles depends on their ability to respond to habitat threats (Petranka et al. 1994; Rudolf and Rodel 2005). This can be achieved by selecting the most appropriate patch of microhabitat that provides the best feeding opportunities or, alternatively, by changing their morphology to escape predators (induced defenses) (Miner et al. 2005; Gianoli and Valladares 2012). Many studies, in different spatial scales, highlight the importance of spatial factor (e.g. two spatial scales, among and within ponds; Michel 2011) and environmental factors (e.g. broad-scale spatial patterns of pond canopy cover and pond morphology, Provete et al. 2014; local scale, Both et al. 2009) in the distribution and abundance of tadpoles' assemblages. Regarding adults' amphibians, seems that spatial and environmental factors play a role on the distribution of assemblages (e.g. regional scale; Parris 2004; Keller et al. 2009) as well as local and regional factors (Werner et al. 2007). However, there is a lack of studies that show how both spatial and environmental factors contribute to tadpole morphological variation, since the high phenotypic plasticity of individuals favors their adaptation to different habitats and environments.

The aim of this study was to investigate the relative influence of environmental and spatial factors on the composition and morphological variation of tadpoles’ assemblages. We hypothesized that spatial factors control the composition of tadpole
assemblages, as it depends on the likelihood of the adults finding suitable sites for reproduction (e.g. adult breeding site choice according to predation and competition, Resetarits and Wilbur 1989; presence of parasites, Kiesecker and Skelly 2000; presence of pesticides, Takahashi 2007; ponds chemistry, Banks and Beebee 1987). Amphibians have low dispersion abilities (Wells 2007) thus closer sites tend to have similar assemblage compositions. Conversely, we expected that the total morphological variation of a given assemblage is controlled by spatial (e.g. predators changes over a spatial scale, Van Buskirk 2017) and environmental factors (e.g. body shape and oral morphology, Vences et al. 2002). Spatial factors can be a result of adult breeding site choice, since tadpoles are restricted to sites previously selected by adults. Thus, changes in morphology would be associated with their ability to adapt their phenotypes to their imposed environment, to compensate errors made by the adults when selecting a microhabitat for tadpole development. Environmental conditions can induce phenotypic changes in organisms (i.e. phenotype-environment association) and select phenotypes that confer the highest fitness; we therefore expected a high correlation between morphology and environment.

Methods

The Cerrado is the second largest biome in Brazil and is composed of different patches of dry and gallery forests surrounded by savannah and grasslands (Ribeiro and Walter 2008). There are two seasons, the wet season lasts from October to March and the dry season from April to September. Mean temperatures range from 22°C to 27°C (Ribeiro and Walter 2008). The particular organization of vegetation types results in the high environmental heterogeneity typical of the Cerrado biome, leading to an equally diverse biota (Nogueira et al. 2009).
To quantify the relative contribution of different environmental and spatial factors on the composition and morphology of tadpole assemblages, we sampled 88 ponds over a period of four years (2010–2013) in Cerrado areas from the state of Goiás and Minas Gerais (Brazil) (Fig. 1). All ponds were randomly selected and sampled once, during the wet season. Pond length ranged from 1.5 to 400 m (average 89.19 m) and pond width from 1 to 212 m (average 45.31 m), with a depth between 0.03 and 5 m (average 0.92 m). All tadpoles sampling were restrict to the margin of the ponds. Most ponds were permanent and lentic, and all ponds are located in open areas.

**Composition matrix**

Tadpole sampling was performed using a wired dip net 30 cm in diameter with a mesh size of 3 mm. The net was swiped through the ponds in all available habitat, always first in benthic surface and then in water column, for a period of one hour; the ponds contained clumps of aquatic vegetation and were approximately 0.9 m deep. The tadpoles collected were anesthetized with benzocaine (5%), fixed with formalin (10%), identified in the laboratory using taxonomic keys (e.g., Rossa-Feres and Nomura 2006) or specialized articles, and deposited in the Zoological Collection of the Federal University of Goiás (ZUFG 534-628; ZUFG 927-978, ZUFG 1056-1084, ZUFG 1192-1215, ZUFG 1281-1312, ZUFG 1335-1376, ZUFG 1779-1803, ZUFG 1928-1940, ZUFG 1962, ZUFG 2583). Species abundance per pond sampled were organized in a composition matrix (Table S1) which was then submitted to a Hellinger transformation (Dray and Dufour 2007) before data analysis. The Hellinger transformations express each tadpole abundance value as a proportion in relation to total sum of tadpoles collected at ponds, removing the effect of multiple zeros in our composition matrix.
**Morphological matrix**

To extract the morphological information, we selected up to five tadpoles from each species per pond that were within the Gosner stages of 35 to 42. A total of 511 individuals were measured. Each tadpole was placed in a petri dish and positioned in the lateral view using a water-based gel. We then captured images (Alpha 100 camera; Sony) using a tripod to standardize the distance between the camera and each tadpole (20 cm). Based on these images, we defined and extracted 23 landmarks for shape comparisons (modified from Van Buskirk 2009). Landmarks are anatomical references that represent the shape of an organism (Zelditch et al. 2012). We ensured that these landmarks could be recognized in all individuals sampled to guarantee biological correspondences (Sneath and Sokal 1973). To remove any effects of size, position, or rotation in shape variation within each landmark data set (Zelditch et al. 2012) we performed a Procrustes transformation (Rohlf 1990). The Procrustes transformation reduces the differences among landmark configurations from the centroid of the average shape (i.e., the average distance for all landmarks from the center of gravity; Zelditch et al. 2012). After applying this transformation, we calculated the partial-warp scores (Zelditch et al. 2012) to represent deformities in the external morphology of tadpoles. To represent assemblage morphological variation, we calculated the mean of partial-warp scores of tadpoles for each of the 88 ponds. To summarize the deformities in the external tadpole morphology, we performed a principal component analysis (PCA) and extracted the eigenvectors that cumulatively represented at least 90% of the total variation (Table S2). We then used these eigenvectors in the posterior statistical analysis. The landmarks were defined and extracted with the tpsUtil (Rohlf 2013a) and
Habitat descriptors of tadpoles

We used a standardized table to visually describe the heterogeneity of the sampled ponds, using the following descriptors (for more detailed information see Table S3): pond position (forest, open area, or inner or outer edge of the forest border), pond type (lentic or lotic), pond hydroperiod (permanent or temporary), pond dimensions (greatest length, greatest width, and maximum depth), pond margin type (cliff, plane, sloped, or excavated), pond bottom substrate (rock, stones, coarse gravel, gravel, sand, clay, mud, or leaf litter), percentage of each vegetation type inside the pond (none, submerged, floating, upright herbaceous, shrub, tree, or *Typha domingensis*), and percentage of each vegetation type surrounding the pond (none, herbaceous undergrowth, erect herbaceous, shrub, arboreous, or *Typha domingensis*). A semi-qualitative variable approximated all percentage estimates: 0% = 0; 1–20% = 0.1; 21–40% = 0.3; 41–60 = 0.5%; 61–80% = 0.7; and 81–100% = 0.9. These percentage estimates were used as continuous variables in subsequent analysis.

The environmental matrix was then transformed with a principal coordinate analysis (PCoA) to generate the independent environmental variables (Table S4). We compared the ecological information among all tadpoles using the Gower index of distance, which is generally used for a matrix of mixed data (Pavoine et al. 2009). The Gower index was proposed to measure general similarity among entities, which includes continuous data and fuzzy variables, which is the case for our ecological variables. We included all principal components that were necessary to explain 90% of
the environmental variation in the posterior statistical analysis. The analyses were performed using the R software (version 3.1).

Spatial matrix

To construct the spatial matrix, we recorded the geographical coordinates of all sites sampled using a global positioning system (GPS; Table S5). The geographical information was transformed into spatial variables by the principal coordinates of neighbor matrices method (Table S6; PCNM; Borcard and Legendre 2002), which is based on a matrix of Euclidean distances between the samples. We used the spatial matrix only for eigenvectors that were positive with significant spatial structure, as measured by Moran’s I coefficient (values higher than 0.1). If Moran’s I coefficient values is 0, it’s means that spatial autocorrelation does not exist in the dataset; while if Moran’s I coefficient values are higher than 0, it’s mean that there is positive spatial autocorrelation in the dataset. Moran’s coefficient is the most commonly used measure of spatial autocorrelation to identify spatial structures (Dray 2011). Thus, our spatial matrix represented the positive spatial relationships between the sampled sites (Dray et al. 2006).

Data analysis

To quantify how much of the composition and morphological variation could be explained by environmental and spatial factors, we used the variance partition method (pRDA; Diniz-Filho et al. 1998; Desdevises et al. 2003; Peres-Neto et al. 2006). This method allowed us to partition the total variance of a given response matrix into four fractions: i) the variation fractions that was explained exclusively by environmental variables; ii) the variation fraction that was explained exclusively by spatial variables;
iii) the variation fraction that was explained by the spatially structured environmental variation; and iv) the variation fraction that was not explained by our predictive matrices, referred to as the residuals of the analysis. To test our hypothesis, we constructed two models; one used the composition matrix as the response matrix and another model to predict the morphological variation between sites. In both models, we used the environmental matrix and the spatial matrix as predictor matrices. After evaluating the total contribution of the spatial factors and environmental variables, we compared both terms in the model using an ANOVA-like permutation test to calculate the significance in terms of the pRDA model (Legendre and Legendre 1998). All analyses were performed using the R software (version 3.1).

Results

We sampled a total of 7,553 individuals belonging to 45 species and 6 families (Table S1). The most abundant species were *Scinax similis* (Cochran, 1952) (1,476 individuals), *Physalaemus cuvieri* (Fitzinger, 1826) (1,227 individuals), and *Proceratophrys cururu* (Eterovick and Sazima, 1998) (1,052 individuals).

We used 23 pairs of partial-warps to describe the total morphological variation of 511 individuals. The first eigenvector of the PCA accounted for 45% of the total morphological variation, while the second accounted for 17% of the total morphological variation (Fig. 2). Three species had greater morphological variation: *Physalaemus cuvieri*, *Scinax fuscomarginatus* (Lutz, 1925), and *Pseudis platensis* (Gallardo, 1961). Species that were assigned to the same genera showed similar morphology (Fig. 2). We found a larger overlap of benthic species in the morphological space and a broader variation in nektonic species. The most morphologically distinguished species was the
semi-terrestrial tadpole of *Thoropa megatimpanum* (Caramaschi and Sazima, 1984), which was found just in two ponds.

The environmental variables which described the microhabitat heterogeneity of tadpoles were described in nine eigenvectors, determined by the PCoA analysis, that accounted for 92% of the total environmental variation (Table S4). The environmental variables that more contributed to the first two PCoA eigenvectors were mud and leaf litter substrate, no vegetation, submerged and upright herbaceous vegetation, no vegetation, and shrub vegetation at pond margin (Table S7). The positive spatial relationship between the sampled sites could be resumed by only three PCNM eigenvectors.

**Environmental and spatial effects**

Only the spatial factors were found to significantly affect the amount of variation in the community composition between the sampled sites (Fig. 3). Conversely, both the spatial and environmental factors significantly affected the amount of morphological variation of the tadpoles; however, the spatial effect was two-fold higher than the environmental effect (Fig. 3)

**Discussion**

As expected, we found that the tadpole assemblage composition was controlled by spatial factors and morphology by both spatial and environmental factors. The Cerrado biogeographic domain contains a mosaic of environmental types that range from humid ciliary forests to open and dry savannahs (Ribeiro and Walter 2008). In such conditions, sites that are closer each other were more likely to have a similar assemblage composition and less morphological variation.
Our results highlight the importance of adult movements that are affected by spatial factors, in the determination of the assemblage composition and morphological variation of tadpoles’ assemblages. The selection of an oviposition site by adult anurans has evolved to maximize the survival of the tadpoles (Altwegg and Reyer 2003; Grözinger et al. 2012; Stein and Blaustein 2015). Anurans consider both abiotic (e.g., pond depth) and biotic (e.g., predation) factors during their decision-making process (Van Buskirk 2005; Grözinger et al. 2012; Stein and Blaustein 2015). Thus, the choice made by the adults affect directly the survival of the tadpoles and is the main process that promotes changes in assemblage composition and tadpole morphological variation (Grözinger et al. 2012; Stein and Blaustein 2015).

Tadpole assemblage composition in Cerrado seems to be more influenced by spatial factors, which probably is a result of their low dispersal capability and the adults’ movements. In general, the spatial effects were associated with probabilistic events (e.g., random birth and death) and were found to be the main drivers of neutral assemblages (Hubbell 2001). The role of environmental and spatial factors in structure amphibian assemblage composition of adults is well documented in the literature (e.g., regional scale: Parris 2004; Afonso and Eterovick 2007; local scale: Kopp and Eterovick 2006; Ernst and Rodel 2008; Keller et al. 2009; Vasconcelos et al. 2009; Menin et al. 2011; Rohas-Ahumada et al. 2012; Iop et al. 2012; Dias-Terceiro et al. 2015), but for tadpoles, these factors are not well understood and appears to differentially affect adult and tadpole compositions (e.g., local scales: Both et al. 2009; Provete et al. 2014; Almeida et al. 2015).

Environmental variables only slightly contributed to changes in tadpole morphology. Variables associated to the pond substrate, the vegetation available to tadpoles (i.e., those inside the pond), and the vegetation available only to adults (i.e.,
those outside the water and surrounding the pond) were more significantly correlated with morphological tadpole variation (Table S6). Vegetation types available to tadpoles and the pond substrate are estimators of microhabitat availability. Different types of vegetation and bottom substrate can offer site refuges and food for tadpoles, reducing predation and competition rates (e.g. Rozas and Odum 1988; Kopp et al. 2006). Thus, these environmental variables can induce morphological variation in tadpoles. Moreover, vegetation types, including those surrounding the pond’s margin, can provide moisture, shelter, and calling sites for adults, showing an indirect effect of the environment on tadpole morphological diversity. As tadpoles have little control of the habitat type they will be exposed during metamorphosis, the environmental variables that were associated with adults' breeding site choice could play an important role in tadpole morphological variation. However, we found evidence that tadpoles' morphological variation was a response to environmental changes, we cannot distinguish the direction of such transformation. The environmental changes perceived by tadpoles can be due evaluation errors made by adults when selecting an oviposition site. When adult anurans make poor decisions regarding site selection for oviposition, tadpoles are being forced to develop in unfavorable sites. In such conditions, morphological variations are advantageous for tadpoles as it allows them to adjust their morphology to the environmental conditions. Morphological variation might result in broader niches for tadpoles, which would suggest that adults were less selective when choosing oviposition sites, at least in Cerrado areas. Our data do not include other factors, as developmental stage, which can play a role in tadpoles’ morphological variations. These factors may have added noise to our analysis (i.e. to increase the residuals). Thus, we suggest that in future studies developmental stage be incorporated into the analysis.
The three species with broader morphological variation are from two different ecomorphological guilds: benthic (*Physalaemus cuvieri*) and nektonic (*Scinax fuscomarginatus* and *Pseudis platensis*). However, benthic tadpoles had a larger overlapping in morphological space than nektonic species. Since benthic tadpoles are restricted to the pond’s bottom, their morphology could be experiencing a constraint by the actual environmental conditions, filtering morphological traits. Conversely, nektonic species have a broader morphological variation. Since they can occupy the entire water column and explore the water surface and the pond’s bottom, these tadpoles can deal with different environmental pressures and show different morphological responses.

This study has demonstrated how factors that control the assemblage composition of tadpoles also drive tadpole morphological diversity. The effects of the neutral and niche-based process on communities depend on the spatial scale (Vellend 2010; Heino et al. 2015). Despite of many studies showed the effects of the neutral and niche-based process on the community organization, our study is the first one to demonstrate the structuring effects in greater spatial scale and address the tadpoles morphological variation. Anurans are generally considered to have low dispersion ability, and their tadpoles are usually restricted to the ponds selected by adults, with rare exceptions (e.g., Sousa et al. 2011). In general, tadpole morphological variation has mostly been studied in response to predator and competition pressures (e.g., Azevedo-Ramos and Magnusson 1999; Skelly 1997; Relyea 2002; Relyea and Hoverman 2003; Relyea 2004; Eterovick and Barata 2006; Both et al. 2011). Thus, there is a lack of studies that focus on how spatial and environmental pressures affect tadpole morphology (but see Lind et al. 2007; Michel 2011). The ability of tadpoles to adapt their morphology, even slightly, in response to environmental (both biotic and abiotic) factors can be a response to evaluation errors in the choice of the oviposition site made.
by adults; it probably interacts with spatial factors to control the morphological assemblage characteristics of tadpoles.

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References


Figure 1. Locations of 88 ponds sampled in Brazil. The ponds are located in Cerrado Biome and 78 ponds are in the state of Goiás (GO) and 10 ponds are in the state of Minas Gerais (MG).

Figure 2. Relative warps analysis of 511 tadpoles in lateral view. Thin-plate splines depict shapes with minimum and maximum values along relative warps and represented shape averages of all species. P. cu: Physalaemus cuvieri; S. fm: Scinax fuscomarginatus; P. pl: Pseudis platensis.

Figure 3. Results of partial redundancy analysis. Abundance is explained by only spatial component. Morphology is explained by both spatial and environmental components. Environment+spatial is a interaction component (according to Diniz-Filho et al. 1998).