Universidade Federal de Goiás

Instituto de Ciências Biológicas

Programa de Pós-Graduação em Ecologia e Evolução

Padrões de ocorrência e composição de girinos do Cerrado: importância da interação fenótipo-ambiente e do espaço

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Março/2016

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País:	Brasil	UF:	DF	CNPJ:	00889834/0001-08	
Título:	Padrões de ocorrência e composição de girinos do Cerrado: importância da relação fenótipo-ambiente e do espaço					
Palavras-chave:	Anfíbios; Comunidades; Ecomorfologia; Morfologia; Morfometria geométrica;					
Título em outra língua:	Ocurrence patterns and composition of Cerrado tadpoles': importance of phenotype-environment interaction and space					
Palavras-chave em outra língua:	Amphibians; Community; Ecomorphology; Morphology; Geometric morphometrics					
Área de concentração:	Ecologia e Evolução					
Data defesa: (dd/mm/aaaa)	18/03/2016					
Programa de Pós-Graduação:	Ecologia e Evolução					
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Padrões de ocorrência e composição de girinos do Cerrado: importância da interação fenótipo-ambiente e do espaço

Tese apresentada a Universidade Federal de Goiás como parte das exigências do Programa de Pós-graduação em Ecologia e Evolução para obtenção do título de Doutor em Ecologia e Evolução.

Núbia Carla Santos Marques

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Santos Marques, Nubia Carla
Padrões de ocorrência e composição de girinos de anuros do Cerrado: importância da relação fenótipo-ambiente e do espaço [manuscrito] / Nubia Carla Santos Marques. - 2016.
viii, 83 f.: il.

Orientador: Prof. Dr. Fausto Nomura.
Tese (Doutorado) - Universidade Federal de Goiás, Instituto de Ciências Biológicas (ICB) , Programa de Pós-Graduação em Ecologia e Evolução, Goiânia, 2016.
Bibliografia. Apêndice.
Inclui gráfico, tabelas.

1. Anfíbios. 2. Comunidades. 3. Ecomorfologia. 4. Morfologia. 5. Morfometria geométrica. I. Nomura, Fausto, orient. II. Título.

Padrões de ocorrência e composição de girinos do Cerrado: importância da interação fenótipo-ambiente e do espaço

Dissertação apresentada a Universidade Federal de Goiás como parte das exigências do Programa de Pós-graduação em Ecologia e Evolução para obtenção do título de Doutor em Ecologia e Evolução. Composta pela banca examinadora: Dr. Fausto Nomura (UFG) Orientador; Dr. Rogério Pereira Bastos (UFG) membro interno; Dra. Priscilla de Carvalho (UFG) membro interno; Dr. Vitor Hugo M. do Prado (UEG) membro externo; Dra. Paula Cabral Eterovick (PUCMG) membro externo.

AGRADECIMENTOS

Durante a realização desse trabalho algumas pessoas colaboraram para o meu desenvolvimento pessoal e profissional. Gostaria de agradecer ao meu orientador Dr. Fausto Nomura que durante seis anos me ajudou a entender um pouco mais a Ecologia e a Herpetologia. A Dra. María Florência Vera Candioti que me recebeu no Museo Miguel Lillo na Argentina durante meu período de Doutorado sanduíche, a acolhida foi essencial durante esse período.

Ao Laboratório de Herpetologia e Comportamento Animal e Laboratório de Funcionamento de Comunidades que foi minha casa durante todo esse tempo, principalmente os amigos Renan, Fernanda Fava, Arthur, Muryllo, Nathane, Maurivan, Priscilla, Raísa, Vinícius, Leandro e Fernando.

As minhas amigas de apartamento que viraram amigas para a vida: Vanessa e Ludmila. Obrigada por todos os momentos alegres e discussões ecológicas.

Aos meus amigos, Bruna, Natalia, Aline, Laila, Rafael.

A minha família, mãe, pai, irmãos, sobrinhos e cunhadas.

Agradeço a CAPES pela bolsa concedida e ao projeto “Girinos do Brasil” que permitiu as coletas de campo e a viabilidade do trabalho.

RESUMO

Girinos de anuros que ocorrem no Cerrado podem ser encontrados em diversos tipos de habitats, sendo essa plasticidade de hábitats possível devido as adaptações morfológicas que asseguram uma boa performance em diferentes contextos ecológicos. Nesse presente estudo, nós investigamos como fatores ambientais e espaciais podem explicar os padrões de ocorrência e distribuição de girinos do Cerrado, além da interação com a variação morfológica dos indivíduos. A ocorrência dos girinos foi explicada por variáveis ambientais e sete interações morfologia-ambiente, sendo as mudanças morfológicas mais comuns: variação no tamanho do corpo e da cauda e mudanças nas posições dos olhos, narina e boca. A composição e a variação morfológica da assembleia de girinos são significativamente afetadas por fatores espaciais, mas fatores ambientais locais também desempenham um importante papel definindo a variação morfológica dos girinos, embora em um menor grau. Abordamos as guildas ecomorfológicas dos girinos e sugerimos que em guildas maiores pode haver variação morfológica atreladas as variáveis ambientais, enquanto nas menores mudanças no comportamento de forrageio e na seleção de hábitat são mais comuns. O presente estudo fornece informações adicionais sobre fatores ambientais e espaciais que contribuem para os padrões de ocorrência e distribuições das espécies de anuros. Fatores abióticos, por exemplo presença de vegetação e uso da terra ao redor da poça, também podem estar agindo mudando o fenótipo dos indivíduos e determinando a ocorrência e a composição das assembleias. Fatores espaciais também são determinantes em assembleias de girinos, sendo um grupo que possui baixa capacidade de dispersão. Girinos estão, em geral, limitados a poça que foi previamente escolhida pelo adulto, e processos neutros como nascimentos e mortes aleatórias na população podem operar nas assembleias.

ABSTRACT

Tadpoles of anuran species that occur in Cerrado can be found in several types of habitats. This habitat plasticity is possible because tadpoles have a high diversification in morphological adaptations that ensure an optimal performance in different ecological contexts. In this study, we were interested to investigate how environmental and spatial factors can explain the patterns of occurrence and distribution of tadpoles in Brazilian Cerrado and the interaction with tadpoles' morphological variation. We found that occurrence of tadpoles was explained by environmental variables and seven morphology-environment interactions. The most common tadpoles morphological change is related to body and tail size, and in eyes, nostril and mouth positions. Tadpoles' assemblage composition and morphological variation have significantly affected by spatial factors, but local environmental factors also play a major role defining morphological variation, although to a small degree. We discuss ecomorphological guilds of tadpoles and suggest that in larger guild (benthic tadpoles) can have morphology-environment interaction, while in the smaller ones (nektonic tadpoles) changes in feeding behavior and habitat selection is more common. There is a lack of knowledge about amphibian diversity, abundance and occurrence in Brazilian Cerrado and our study provide additional information about environmental and spatial factors that contribute to the patterns of occurrence and distribution of species. Abiotic factors (*e.g.* vegetation in ponds and land use) and spatial factors can contribute to individuals' phenotype changes and play a role in patterns of occurrence and tadpoles' assemblage composition.

SUMÁRIO

Introdução Geral

A identificação dos processos bióticos e abióticos que agem determinando padrões de ocorrência e abundância das espécies é há muito tempo discutida por ecólogos (McArthur & Levins 1967; Norton 1991; Chase & Leibold 2003). Alguns fatores são apontados como determinantes nos padrões de ocorrência e composição das assembleias, como competição e predação (Morin 1983; Gascon 1991), traços morfológicos e comportamentais (Toft 1985), fatores ecológicos e históricos (Eterovick & Fernandes 2001), fatores espaciais (Parris 2004) e interações entre fatores biológicos e ambientais (Wilbur 1967). Em assembleias de girinos de anuros podemos ter diferentes fatores agindo e determinando os padrões de ocorrência, composição e abundância nos diferentes habitats.

Girinos de anuros podem ocorrer em diversos tipos de hábitats como poças permanentes e temporárias, ambientes lóticos, além de plantas epífitas (Inger 1985; Altig & McDiarmid 1999), sendo notável a necessidade de adaptações. As adaptações morfológicas conferem aos indivíduos maiores chances de sobrevivência e prosperidade no ambiente, sendo a plasticidade fenotípica (*i.e.* capacidade de um genótipo gerar diferentes fenótipos em resposta a mudanças no ambiente) um importante processo agindo nas populações. Um método para detectar a variação morfológica nos indivíduos é a morfometria geométrica, o qual retira o efeito do tamanho, posição e rotação com o uso de sobreposição das formas (método de Procrustes), sobrando somente a diferença na forma geral entre os indivíduos (Zelditch *et al.* 2012). Esse método tem se mostrado eficiente e nós o utilizamos como forma de extrair as variáveis morfológicas dos girinos.

O bioma Cerrado é composto por distintas paisagens que vão desde campos abertos, campos fechados e matas de galeria (Klink & Machado 2005) o que resulta em alta heterogeneidade ambiental suportando uma grande riqueza de espécies. Porém, é hoje um bioma ameaçado, principalmente por expansão de monoculturas (soja, milho) e pastagens, ocorrendo mudanças na estrutura ambiental e refletindo em como a fauna e a flora interagem com os fatores ambientais (Klink & Machado 2005). Entender as interações biológicas e ambientais nos padrões de ocorrência e composição das espécies é de grande importância para conseguirmos conservar as espécies e desacelerar o processo de extinção causada por fatores humanos.

Nesse sentido, esse trabalho foi dividido em três capítulos onde focamos em entender como fatores ambientais e espaciais podem explicar a ocorrência e composição de assembleia de girinos do Cerrado e sua interação com a variação morfológica dos indivíduos. No primeiro capítulo "*The role of environment and morphology in the pattern of occurrence of Brazilian Cerrado tadpoles*" fizemos uma seleção de modelos com variáveis ambientais e morfológicas para identificar aquelas que melhor explicam a ocorrência dos girinos, e procuramos entender se existem interações positivas e negativas entre as variáveis ambientais e os traços morfológicos dos indivíduos. No segundo capítulo "*Environmental and spatial factors affects composition and morphology of tadpoles assemblages at Cerrado*" quantificamos o quanto a abundância e a morfologia das espécies seria explicada por fatores ambientais e espaciais. No terceiro capítulo "*Phenotype-environment interaction in tadpoles: phenotypic plasticity in ecomorphological guilds***"** focamos em girinos de diferentes guildas ecomorfológicas, que são agrupamentos de espécies baseados na morfologia geral, ecologia e comportamento alimentar, e a sua relação com as variáveis ambientais.

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The role of environment and morphology in the pattern of occurrence of Brazilian Cerrado tadpoles

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Abstract

Tadpoles of anuran species that occur in Cerrado can be found in several types of habitats. This habitat plasticity is possible because tadpoles have a high diversification in morphological adaptations that ensure an optimal performance in different ecological contexts. Even in a same habitat, abiotic factors that can be representative of habitat heterogeneity can generate morphological divergence in tadpoles, through phenotypic plasticity, to reduce predation risk or competition intensity. In this study, we were interested to investigate (1) which set of morphological traits and environmental variables best explains the occurrence of tadpoles and (2) to what extent are morphological traits of tadpoles associated with environmental variables. To accomplish these goals, we collected tadpoles in 86 ponds from the Brazilian Cerrado, measured a set of environmental descriptors of these ponds,

extracted morphological traits of tadpoles, using morphometric geometrics technics, and used a GLMM approach to model the occurrence of tadpoles and to evaluate the significance of trait-environment relationship. Seven morphology-environment interactions were important to explain occurrence of tadpoles and this provide additional information about patterns of occurrence of amphibian species. The most common tadpoles morphological change is related to body and tail size, and in eyes, nostril and mouth positions. Our results showed that local structure affect species occurrence and morphology of tadpoles, and there are more than one morphological source variation in tadpoles, as phenotypic plasticity and anthropogenic stressors.

Key Words

Amphibians; ecomorphology; GLMM approach; Geometric morphometrics; phenotypic plasticity

Introduction

The Brazilian Cerrado is the second largest Brazilian biome and is composed by different phytophisiognomies, as savannas, grasslands, dry forests and gallery forests (Klink & Machado 2005). This elevated environmental heterogeneity of the Cerrado biome result in a highly diverse niche space and an equally diverse biodiversity (Nogueira *et al.* 2009), which makes the biome to be considered as one of the world biodiversity hotspots and considered a priority area for directing conservation efforts (Mittermeier *et al.* 2004). Currently, 209 species of amphibians were registered to the Cerrado biome, with 108 species (51%) endemic (Valdujo *et al.* 2012), but this number

is expected to be higher because the Cerrado biome is still poorly sampled (Nomura, unpublished data).

The majority of anurans species that occur in Cerrado have a larval phase. These tadpoles can be found occupying several types of habitats and microhabitats, as temporary or permanent ponds, streams and phytotelmatas (Inger 1986, Altig & McDiarmid 1999a,b). Differences in the physical (*e.g.* size, width, depth; Eterovick 2003), chemical (*e.g.* pH, temperature; Noland & Ultsch 1981), landscape (*e.g.* matrix type, conservation level of native vegetation; Souza-Queiroz *et al.* 2015) and biotic (*e.g.* presence of competitors and predators; Azevedo-Ramos *et al.* 1999) traits of habitats can affect the rate of development and predation risk, for example, and regulates the tadpole diversity. The optimum habitat in which tadpoles perform its development is controlled not only by historical factors, but also by contemporaneous effects, as predation and competition, being a result of a mixed influence of adaptive convergence and common evolutionary history (Marques & Nomura 2015).

The influence of contemporaneous effects are resultant of the high phenotypic plasticity of tadpoles (*i.e.* the capacity of a genotype to display different phenotypes as a response to a change in the environment; Miner *et al.* 2005, Gianoli & Valladares 2012). Tadpoles are restricted to the site selected by the adults during the oviposition (Wells 2007). These site restrictions make tadpoles sensitive to environmental changes, and microhabitat selection is important to tadpoles' survival, once they can select the most appropriate microhabitat that provides site refuge and feeding opportunities. Tadpoles' microhabitat selection can result in a considerable amount of morphological diversity, as a consequence of the adaptations necessary to live in the diversity of habitats in which they can be found (Orton 1953, 1957; Altig & Johnston 1989). Phenotypic plasticity allows the tadpoles to make fine tune morphological adjustments

to the microhabitat selected, and permit the tadpoles to express significant morphological variation among ponds in response not only to habitat traits, but to the presence of predators and competitors (Langerhans *et al.* 2007, Michel 2011, Relyea 2002). However, the abiotic factors, mainly those related to habitat heterogeneity, can also generate morphological divergence in tadpoles (Michel 2011). As a result, we can expect a phenotype-environment association for tadpoles.

In this study, we aim to answer the questions: i) Which set of morphological traits and environmental variables best explains the occurrence of tadpoles? ii) to what extent are morphological traits of tadpoles associated with environmental variables? To answer these questions, we represented the morphological variation among sites in the Cerrado biome using the geometric morphometric approach (Zeldith *et al.* 2012), and associate this variation to environmental variables and the distribution of tadpoles of anuran species.

Material and Methods

Data acquisition

We sampled 86 ponds, throughout three years (2010-2013) in State of Goiás, Brazil (Tab.S1). The tadpoles' were captured using a wired dip net with a mesh of 3 mm and 30 cm of diameter that was swiped during one hour in the ponds margins, clumps of aquatic vegetation and water with 1.5m depth. A total of 486 individuals of 41 species were collected. The tadpoles collected were anesthetized, killed, and identified in laboratory using taxonomic keys (*e.g.* Rossa-Feres & Nomura, 2006) or specialized articles, and deposited in the Zoological Collection of the Federal University of Goiás (ZUFG). After tadpole identification, we organized the information of

presence-absence of each species found in each pond sampled in an occurrence matrix (Tab. S2).

Tadpole Habitat descriptors

We described the ponds where the tadpoles were sampled based on local and landscape descriptors: pond dimensions (greatest length, greatest width and maximum sampled depth); pond margin type (cliffed, plane, sloped, excavated); pond bottom substrate (rock, stones, coarse gravel, gravel, sand, clay, mud and leaf litter); percentage of each vegetation type inside the pond margins (none, submerged, floating, upright herbaceous, shrub, tree and taboa); percentage of each vegetation type at the pond margins (none, herbaceous undergrowth, erect herbaceous, shrub, arboreous and taboa) and percentage of land use within 500m of the pond perimeter (nature vegetation, pasture and short crops). All traits were estimate as an percent estimative, that were posteriorly transformed in a semi-qualitative variable, to reduce the variation in the estimative of the observer, as follow: 0%=0; 1-20%=0.1; 21-40%=0.3; 41-60=0.5%; 61-80%=0.7; 81-100%=0.9**.** These environmental variables can reflect the pond heterogeneity, the availability of microhabitats and the land use change.

Using the environment descriptors, we created six independent indexes (Tab. S1) based on the Nessiman *et al.* (2008) approach and adapted for tadpoles as a measure of habitat heterogeneity (Costa and Nomura, 2016). To calculate the indexes, for each pond trait (pond dimensions, pond marginal type, pond bottom substrate, percentage of each vegetation type inside the pond margins, percentage of each vegetation type at the pond margins and percentage of land use within 500m of the pond perimeter) we

assigned a score, that was posteriorly weighted for maximum scores (*i.e.* all features has the same weight in the analysis). Then, we obtained, for each pond trait, an average of all analyzed parameters resulting in a value that range between 0 to 1. In this way, we have six indexes for each pond that reflects the individual contribution for each pond trait for total heterogeneity.

Morphological matrix

To extract the morphological information of tadpoles, we photographed all individuals between Gosner's stages 35 and 42 in lateral view. Then, we positioned the tadpoles in a petri dish, with ultrasound gel, in lateral view and take photographs using a camera positioned with a tripod, that standardized the distance from the camera and the tadpole in 20 cm. The pictures allow us to extract the landmarks of all individuals. The landmarks provide anatomical references that represent the shape of organisms and, ideally, should be recognizable in all individuals, being homologous and having biological correspondences (Sneath & Sokal 1973). We define 23 landmarks that describe tadpole morphology in lateral view (modified from VanBuskirk 2009) and used them to perform a geometric morphometric analysis. In the geometric morphometric, each landmark is represented by an x and y coordinate in a Cartesian plane and we use a set of landmarks to represent the organism shape, after excluding the effect of size, position, and rotation (Zelditch *et al.* 2012). We transformed our landmarks coordinates by the Procrustes method (Rohlf 1990) that reduced the differences between landmarks configurations from the centroid of the average shape (*i.e.* the average distance among all landmarks from the gravity center; Zelditch *et al.*

2012), and obtained the partial warps scores that represent the deformation in external morphology of tadpoles. To summarize the deformation information we perform a Principal Component Analysis (PCA) and extract the two first eigenvectors (PW1 and PW2) and used them in posteriorly statistical analysis. The landmarks were obtained using the tpsUtil (Rohlf 2013) and tpsDig2 (Rohlf 2013b) softwares.

Data analysis

We evaluated which set of morphological traits and environmental variables best explained tadpoles' occurrence and the significance of trait-environment relationship using a generalized linear mixed models - GLMM - approach (Jamil *et al* 2013). The GLMM is a class of statistical model in ecology that is general and powerful (Zuur *et al*. 2009; Jamil *et al.* 2013). The GLMM allowed us to identify the set of morphological traits and environmental variables that account for species occurrence data, and provides the significance of trait-environment relationship (Jamil *et al.* 2013). We used the procedure described by Jamil *et al.* (2013) that use the tiered forward selection approach to multiple traits and multiple environmental variables (Jamil *et al.* 2012). The tiered forward selection approach have three tiers, the first one the random factors are selected; in the second, the fixed effects; in the third tier, nonsignificant terms are removed using a modified Akaike information criterion (SigAIC; Jamil *et al.* 2012). In this approach, environmental variables enter the model as random terms and fixed terms, and traits entered as fixed terms (Jamil *et al.* 2012). Thus, we selected models with lower SigAIC values to describe species occurrence and evaluated the significance of trait-environment relationship. All analysis was performance in software R (version 3.2) with the package lme4 (version 1.1; Bates *et al.* 2015).

Results

We built a parsimonious regression model from all environmental variables and morphological traits using tiered forward selection. In the first tier, the best model (*i.e.* the one with lower SigAIC value; Tab.1) include all environmental variables. In the second tier, the main effects of all environmental variables were added to models as fixed terms. Then, the interactions between morphological traits and each environmental variables were successively added in models and stopped when SigAIC values added are higher than the last model. A total of eight interactions was added to the model, and one interaction (pond bottom substrate:PW2) was deleted in third tier because they increased the SigAIC values. The two most parsimonious models that explain the occurrence of tadpoles in Brazilian Cerrado contains all environmental variable (tier one) and seven morphology-environment interactions (tier two; Tab. 1). In terms of morphological plasticity of tadpoles the first principal component (PW1) explained 45.5% of general shape variation and reflects mostly the tadpoles' body shape, tail shape, eyes deviations, and nostril and mouth positions (Fig. 1). The second principal component (PW2) explained 17.6% of general shape variation and reflects mostly tadpoles' tail and body shapes (Fig. 1).

Vegetation type at the pond margins, pond dimensions and land use are positively associated with PW1; vegetation type inside the ponds margin and pond marginal type are negatively associated with PW1; pond dimensions and land use are negatively associated with PW2 (Tab.2). Figure 1 show general tadpoles shape change in morphological space and we can related this with selected interactions. Thus, in ponds with high values of vegetation type at ponds margins tadpoles developed relative small bodies, relative larger tails, deviations in eyes, nostril and mouth positions. Ponds with low values of vegetation type inside the ponds margins and pond marginal type

tadpoles developed larger bodies, small tails, deviations in eyes, nostril and mouth positions. Pond dimensions and land use both are positively correlated with PW1 and negatively with PW2. Thus, tadpoles in ponds with higher and low values of pond dimensions and land use developed relative larger bodies, larger tails, but just in pond with high values we can observed the deviations in eyes, nostril and mouth positions.

Table 1. Tiered forward selection of environmental variables and morphological traits in models explaining tadpoles' occurrence probability. The best model and interaction (represented by :) giving the lowest SigAIC is added in each row (represented by +). The best model is indicated in bold. Veg. PM= percentage of each vegetation type at pond margin; Veg. IM= percentage of each vegetation type inside pond margin; Pond BS= pond bottom substrate; Pond MT= pond marginal type.

Tier	Effects	SigAIC
Random effects		
1	(1|species) + (1|sites)	27248.2
1	(..+ Pond MT | species)	26436.7
1	(..+ Veg. IM| species)	25839.3
1	(..+ Land use | species	25761.7
1	(..+ Pond dimension | species)	25682.4
1	(..+ Veg. PM | species)	25613.9
1	(..+ Pond BS | species)	**25612.3**
Fixed effects		
2	+All environmental variables	25621.7
2	+PW1+Veg. IM:PW1	25527.2
2	+Pond MT:PW1	25503.9

2	+Veg.PM:PW1	25472.4
2	+Pond dimension:PW1	25452.4
2	+Land use:PW1	25437.1
2	+PW2+Land use:PW2	25426.2
2	+Pond dimension:PW2	**25423.2**
2	+Pond BS: PW2	25425.9
3	Pond BS:PW2 Deleted	25425.9

Table 2. Interactions between selected environmental variables and morphological traits. Sign of estimate values show that interactions is positive or negative. Veg. PM= percentage of each vegetation type at pond margin; Veg. IM= percentage of each vegetation type inside pond margin; Pond BS= pond bottom substrate; Pond MT= pond marginal type.

Interactions	**Estimate**	**P value**
Veg. IM:PW1	-0.31661	<0.0001
Pond MT:PW1	-0.25166	<0.0001
Veg.PM:PW1	0.22373	<0.0001
Pond dimension:PW1	0.09555	<0.0001
Land use:PW1	0.12296	<0.0001
Land use:PW2	-0.05883	0.001
Pond dimension:PW2	-0.05241	0.001

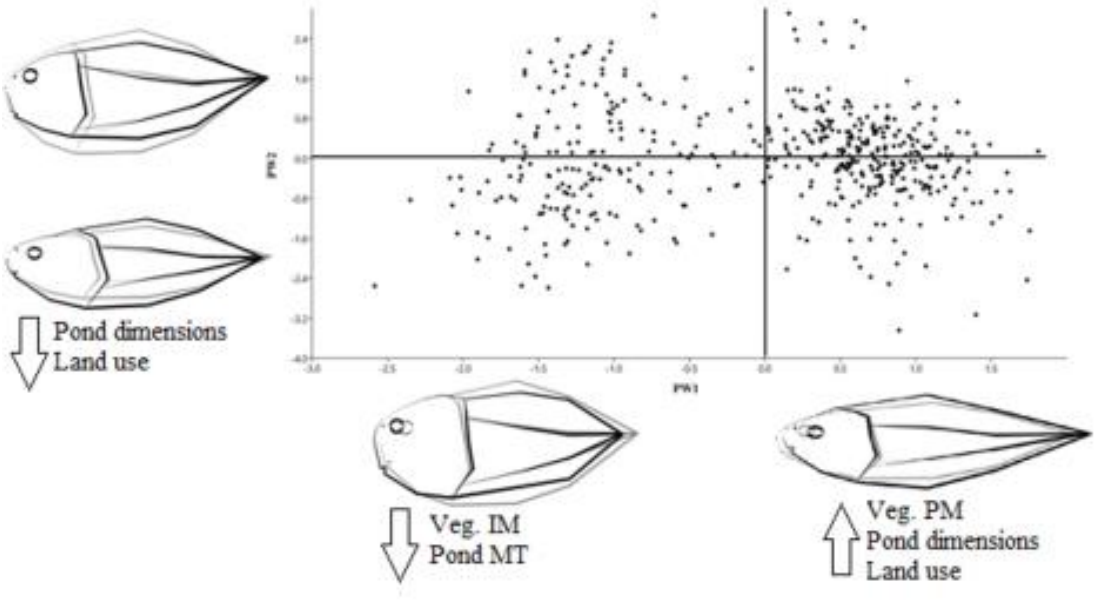

Figure 1. Procrustes plot in morphological space showing the first two relative warps (PW1 and PW2) of shape data of tadpoles of Brazilian Cerrado. Tadpole outline in grey line represent the average shape while black lines are high scores shape changes. Veg. PM= percentage of each vegetation type at pond margin; Veg. IM= percentage of each vegetation type inside pond margin; Pond MT= pond marginal type.

Discussion

Explain the pattern of species occurrence is a challenge for ecologists. Species differences in environmental requirements and in their functional traits play an important role in this search for species distribution explanation. Tadpoles is an interesting organism to study the role of environment, morphology and phenotype-environment association in patterns of occurrence because they can live in a variety of habitats and is known to have a higher phenotypic plasticity, that is a capacity to change their morphology in response to environmental changes (Miner *et al.* 2005). Our results

demonstrate that all environmental variables measured explain the pattern of occurrence of tadpoles in Brazilian Cerrado. Moreover, there is a strong phenotype-environment association in tadpoles, with seven morphological traits and environmental variables interactions, indicating that tadpoles are capable of fine tuning its morphology according to the pond traits.

Environmental variables are known to influence occurrence of tadpoles (Van Burkirk 2005). Vegetation types at and inside pond margin, pond dimensions and pond bottom substrate reflects the availability of microhabitats in ponds. Different types of vegetation and bottom substrate can offer site refuges and food for tadpoles, reducing the predation and competition rates (*e.g.* Rozas & Odum 1988; Kopp *et al.* 2006). Moreover, vegetation inside margins and vegetation at margin can provide moisture, shelter and calling sites for adults. Tadpoles have little control in the habitat type they will live (*i.e.* the adults who chooses the oviposition site) and their dispersion is limited (Altwegg & Reyer 2003; Grözinger et al. 2012; Stein & Blaustein 2015). Thus, factors which influences tadpoles and adults distribution is different. Adults' distribution has a spatial and terrestrial landscape influence (Resetaratis *et al.* 2005; Wells 2007), while tadpoles distribution are affected by ponds variables (*e.g.* size, inside vegetation, presence of predators; Ultsch *et al.* 1999). However, tadpoles' metacommunity structure can be influenced in large scale by physical characteristics of ponds (Provete *et al.* 2014). This suggests that adult reproductive behavior is driving the tadpoles' occurrence dynamic, but in a local scale, microhabitat availability influences tadpoles' survival.

Physical environment can exert an inductive and selective pressure on tadpoles' morphology. Our results showed a correlation between morphological phenotype of tadpoles and environmental variables. Tadpoles usually fit their phenotype in response to environmental conditions through phenotypic plasticity (Losos 1990, Van Buskirk

2009, Michel 2011). Phenotypic plasticity is known to act as an important strategy for organisms to cope with diverse environmental conditions (Stearns 1989; Scheiner 1993). Thus, tadpoles of Cerrado can fit their phenotypes according to environmental changes. The most common tadpoles morphological change is in body and tail size and, in some cases, there is deviations in eyes, nostril and mouth positions. Pond dimensions and land use are the environmental variables measured that have correlations with both morphological traits (PW1 and PW2). Tadpoles in ponds with higher and lower values of pond dimension and land use developed relative larger tails and larger bodies. However, in higher values of pond dimensions and land use they have deviations in eyes, nostril and mouth positions. Presence of vegetation at pond margins and inside pond margins can provide different microhabitat to tadpoles and may be a structural factor that promotes niche partition among tadpoles from different anuran species. Microhabitat selection by tadpoles differs not only according to the position of the water column that they occupy (Eterovick & Barata 2006), but also according to the association to different substrates. Thus, higher levels of vegetation at ponds margins favors tadpoles with low fins as benthic species. Low levels of vegetation inside ponds margins favors tadpoles with high tail fins as nektonic species. Nektonic species move through vegetation and habitat complexity can be a challenge to their locomotion which can favors invertebrate ambush predators (*e.g.* larval dragonflies and water bugs; Nomura *et al.* 2011).

Percentage of land use within 500m of the pond perimeter is an estimative of the human disturbance in conversion of native vegetation in pasture or agriculture (Klink & Machado 2005). Moreover, there is an intense use of pesticides that can contaminate water pools through overspray in aerial application, lixiviation and overland flow (Goldsborough & Beck 1989; Queiroz *et al.* 2011), and in higher values of land use and

pond dimensions probably there is high levels of agrochemical dissolved in water. These anthropogenic stressor can have adverse effects on organisms through effects on various individuals traits (*e.g.* behavior and morphology; Teplitsky *et al.* 2005). Tadpoles are highly susceptible to contaminations and morphological traits could be affected by contaminants such as Glyphosate (Relyea 2005; Costa & Nomura 2015). Costa and Nomura (2015) evaluated the impacts of an agrochemical in tadpoles of *Physalaemus cuvieri* and found deviations in nostril-snout distance and eye-width, which are morphological traits associated with sensory capabilities of tadpoles. Our results show deviations in eyes, nostril and mouth positions of tadpoles, which may be due anthropogenic stressors, and can affect individuals survival reducing their competitive potential and increasing predation risks (Costa & Nomura 2015).

There is a lack of knowledge about amphibian diversity, abundance and occurrence in Brazilian Cerrado (Diniz-Filho *et al*. 2004, Diniz-Filho *et al.* 2007), and our study provide additional information about environmental factors that contribute to the patterns of occurrence of species. Furthermore, linking morphological traits and environment help us to understand some ecological and evolutionary processes (*e.g.* phenotypic plasticity, niche partitioning, predation, competition) which may play important roles in tadpoles' assemblage structure. Brazilian Cerrado is one of the global biodiversity hotspots and recent expansion of soybean cultures and cattle ranch it is threatening this biodiversity (Klink & Machado 2005). Our results indicates that there are more than one source of morphological variation in tadpoles, brings concerns about the extensive use of agrochemicals linked to agriculture expansion, which can threaten tadpoles changing their morphologies and probably changing their sensory capacity.

Acknowledgments

We thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for fellowships. We are grateful to Girinos do Brasil (SISBIOTA: grants CNPq 563075/2010-4 and FAPESP 2010/52321-7) for the financial support.

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Supporting Information

Table S1: Environmental index collected in 10 cities of Brazilian Cerrado. Veg. PM= percentage of each vegetation type at pond margin; Veg. IM= percentage of each vegetation type inside pond margin; Pond BS= pond bottom substrate; Pond MT= pond marginal type

City	Ponds	Latitude	Longitude	Land use	Veg. PM	Veg.IM	Pond BS	Pond MT	Pond dimensions
Aruanã	1	-14.87205556	-51.06838889	0.4	0.32	0.30	0	0.3	15000
Aruanã	2	-14.88897222	-51.07580556	0.4	0.48	0.20	0	0.3	3750
Aruanã	3	-14.80461111	-51.01588889	0	0.2	0.17	0	0.25	45
Aruanã	4	-14.73919444	-51.02155556	0	0.36	0.30	0.25	0.25	180
Aruanã	5	-14.77994444	-51.02394444	0	0.2	0.17	0	0.25	0.45
Aruanã	6	-14.8195	-51.02255556	0	0.4	0.17	0.1	0.25	112.5
Aruanã	7	-14.86747222	-51.04155556	0.5	0.32	0.13	0.125	0.25	30000
Aruanã	8	-14.92091667	-51.00069444	0.5	0.4	0.33	0.125	0.25	2048
Aruanã	9	-14.8755	-50.98183333	0.4	0.4	0.07	0.125	0.25	66.4
Aruanã	10	-14.93686111	-51.01291667	0.5	0.2	0.47	0.25	0.25	48000
Aruanã	11	-14.89705556	-50.91275	0.5	0.44	0.27	0.125	0.25	584
Aruanã	12	-14.67113889	-50.91275	0.5	0.6	0.37	0.125	0.25	65.7
Aruanã	13	-14.68308333	-50.91077778	0.5	0.56	0.23	0.125	0.45	293.85
Aruanã	14	-14.69419444	-50.91191667	0.5	0.24	0.17	0.125	0.25	447.25
Aruanã	15	-14.76358333	-50.96386111	0.5	0.56	0.13	0.125	0.25	975
Aruanã	16	-14.81922222	-50.97677778	0.5	0.48	0.30	0.125	0.25	1240
Aruanã	17	-14.94911111	-51.04225	0.5	0.36	0.30	0.125	0.55	32960

Aruanã	18	-14.85725	-50.9795	0.5	0.28	0.20	0.2	0.25	2050
Aruanã	19	-14.83166667	-51.04433333	0.5	0.4	0.23	0.125	0.45	20000
Aruanã	20	-14.71902778	-50.98869444	0.5	0.32	0.13	0.25	0.25	100
Aruanã	21	-14.75277778	-51.02238889	0.5	0.44	0.27	0.25	0.25	40000
Aruanã	22	-14.82605556	-51.04197222	0.5	0.24	0.33	0.125	0.5	8000
Aruanã	23	-14.80155556	-51.03113889	0.3	0.36	0.50	0.2	0.25	2250
Aruanã	24	-14.83141667	-51.04341667	0.4	0.28	0.07	0.25	0.25	140
Aruanã	25	-14.82658333	-51.02747222	0.4	0.4	0.23	0.35	0.25	750
Aruanã	26	-14.81505556	-51.02105556	0.4	0.48	0.33	0.2	0.25	500
Britânia	28	-15.45538889	-51.07013889	0.5	0.4	0.13	0.15	0.3	802.9
Britânia	30	-15.36622222	-51.12383333	0.5	0.2	0.13	0.15	0.25	130000
São João da Aliança	31	-14.732806	-47.563139	0.2	0.2	0.30	0.125	0.25	900
São João da Aliança	32	-14.727667	-47.553639	0	0.68	0.47	0.225	0.25	1824
São João da Aliança	33	-14.612806	-47.541056	0.5	0.4	0.07	0.25	0.45	492.8
São João da Aliança	34	-14.659417	-47.526417	1	0.56	0.33	0.2	0.7	4560
São João da Aliança	35	-14.589833	-47.642472	0	0.72	0.50	0.275	0.35	3780
São João da Aliança	36	-14.555667	-47.628639	0	0.64	0.27	0.3	0.5	150
São João da Aliança	37	-14.662	-47.616944	0	0.68	0.13	0.5	0.6	3000
Alto Paraíso	38	-14.283112	-47.532672	0.8	0.48	0.07	0.225	0.55	18750
Alto Paraíso	39	-14.292278	-47.629333	0.5	0.6	0.43	0.275	0.35	1365
Alto Paraíso	40	-14.415833	-47.521917	0.5	0.56	0.33	0.225	0.45	216
Alto Paraíso	41	-14.500389	-47.517444	0.6	0.6	0.50	0.375	0.5	7500
Alto Paraíso	42	-14.483278	-47.591861	0	0.6	0.17	0.225	0.5	90
Alto Paraíso	43	-14.478722	-47.578139	0.3	0.76	0.17	0.225	0.5	750
Pires do Rio	44	-17.40161111	-48.35697222	0.5	0.32	0.00	0.25	0.3	864
Pires do Rio	45	-17.44069444	-48.40858333	0.5	0.4	0.47	0.25	0.55	490.578375
Pires do Rio	46	-17.40372222	-48.38327778	0.4	0.36	0.37	0.125	0.3	349.37

Pires do Rio	47	-17.32286111	-48.43755556	0.5	0.48	0.33	0.125	0.5	2940
PARNA Emas	48	-17921583	-52967694	0	0.44	0.27	0.175	0.25	4200
PARNA Emas	49	-17919389	-52967056	0	0.4	0.27	0.125	0.3	8000
PARNA Emas	50	-	-	0	0.2	0.23	0.125	0.3	11000
PARNA Emas	51	-17937611	-53081861	0	0.4	0.20	0.15	0.2	50000
PARNA Emas	52	-17887444	-53152694	0	0.2	0.17	0.125	0.25	1260
Pontalina	53	-17.42066667	-49.34783333	0.9	0.48	0.50	0.275	0.35	1337.448
Pontalina	54	-17.47480556	-49.36352778	0.5	0.52	0.23	0.175	0.25	688.8
Pontalina	55	-17.49319444	-49.27661111	0.5	0.52	0.33	0.175	0.25	39071.6
Pontalina	56	-17.36844444	-49.32308333	0.5	0.6	0.07	0.125	0.3	380
Pontalina	57	-17.43722222	-49.47577778	0.5	0.24	0.33	0.125	0.25	294.5
Pontalina	58	-17.425703	-49.370136	0.6	0.48	0.40	0.15	0.1	1000
Pontalina	59	17° 25' 57,5"	49° 20' 37.9"	0.3	0.32	0.23	0.15	0.25	8400
PARNA Sempre-Vivas	60	-17.89525	-43.76806389	0	0	0.13	0.125	0.55	0.075
PARNA Sempre-Vivas	61	-17.91672222	-43.78363056	0	0	0.10	0.275	0.5	8123.543196
PARNA Sempre-Vivas	62	-17.92216667	-43.76475	0	0	0.00	0.125	0.3	30
PARNA Sempre-Vivas	63	-17.89111111	-43.80705556	0	0.28	0.33	0.125	0.25	22.5
PARNA Sempre-Vivas	66	-17.95730556	-43.78344444	0	0.2	0.07	0.125	0.25	1.8
PARNA Sempre-Vivas	67	-17.91352778	-43.78638889	0	0	0.23	0.2	0.5	20
PARNA Sempre-Vivas	68	-17.86291667	-43.76	0	0	0.27	0.075	0.25	56.25
PARNA Sempre-Vivas	69	-17.86291667	-43.76	0	0.2	0.17	0.225	0.25	18
Serranópolis	70	-18.45033017	-52.08222597	0.5	0.4	0.33	0.225	0.45	131.0925
Serranópolis	71	-18.45033017	-52.08222597	0.4	0.64	0.63	0.275	0.7	3527.16
Serranópolis	72	-18.455928	-52.07349283	0.5	0.72	0.47	0.45	0.5	12881.1264
Serranópolis	73	-18.46905556	-52.23452778	0.5	0.4	0.33	0.125	1	860.41704
Serranópolis	74	-18.32908359	-52.16260255	0.5	0.44	0.20	0.15	0.5	1857.91
Serranópolis	75	-18.31311111	-52.15761111	0	0.76	0.67	0.25	0.55	7200

Serranópolis	76	-18.35858333	-52.00863889	0.2	0.56	0.37	0.25	0.25	899
Serranópolis	77	-18.42683333	-52.18658333	0.5	0.56	0.47	0.275	0.7	21953.1858
Caiapônia	78	-17.21066667	-51.46838889	0.9	0.2	0.17	0.225	0.3	6111
Caiapônia	79	-17.28936111	-51.96222222	0.4	0.36	0.17	0.4	0.45	7928.8
Caiapônia	80	-17.24072222	-51.46755556	0.5	0.2	0.30	0.25	0.5	1250.5472
Caiapônia	81	-17.21555556	-51.44255556	0.5	0.32	0.33	0.15	0.25	28456.17996
Chapadão do Céu	82	-18.2362222	-52.6133333	0.4	0.2	0.17	0.15	0.25	722.274
Chapadão do Céu	83	-18.2349444	-52.6062222	0.5	0.2	0.03	0.125	0.3	1025.4927
Chapadão do Céu	84	-18.0543333	-52.6368889	0.1	0.2	0.10	0.175	0.25	100047.52
Chapadão do Céu	85	-18.11688138	-52.59325175	0	0.24	0.23	0.15	0.25	320
Chapadão do Céu	86	-18.0045907	-52.72455803	0.2	0.28	0.23	0.175	0.3	1093.5
Chapadão do Céu	87	-18.23320753	-52.5796822	0.6	0.2	0.10	0.125	0.3	238.986
Chapadão do Céu	88	-18.04381139	-52.66135421	0.3	0.2	0.00	0.15	0.3	304.2208
Pires do Rio	89	-17.32713889	-48.45597222	0.4	0.24	0.40	0.125	0.3	1236.76938
Pires do Rio	90	-17.30272222	-48.30889444	0.6	0.56	0.23	0.125	0.25	9539.0208

Table S2. Occurrence data of 42 species of tadpoles in 90 ponds.

Ponds	Af	Ba	Bn	Dj	Dm	Dn	Dr	Ds.	Dm	Eb	Ec	Eo	Em	H.a	Hl	Hp	Hr	Lc	Lf	Lfu	Lgl	Llab	Llat	Lm	Lp	Ls	Lt	O.a	Pc	Pcu	Pm	Pp	Psp.	Sf	Sfv	Sp	Sr	Ss	Ssp.	Ssq	Tm	Tv
1																									x																		
2								x		x																				x													
3																														x								x					
4																														x													
5																																						x					
6								x																																			
7																																x		x									
8				x													x																	x									
9																																		x									
10																																	x	x									
11																																											
12																													x	x					x								
13						x																												x									
14																													x			x											
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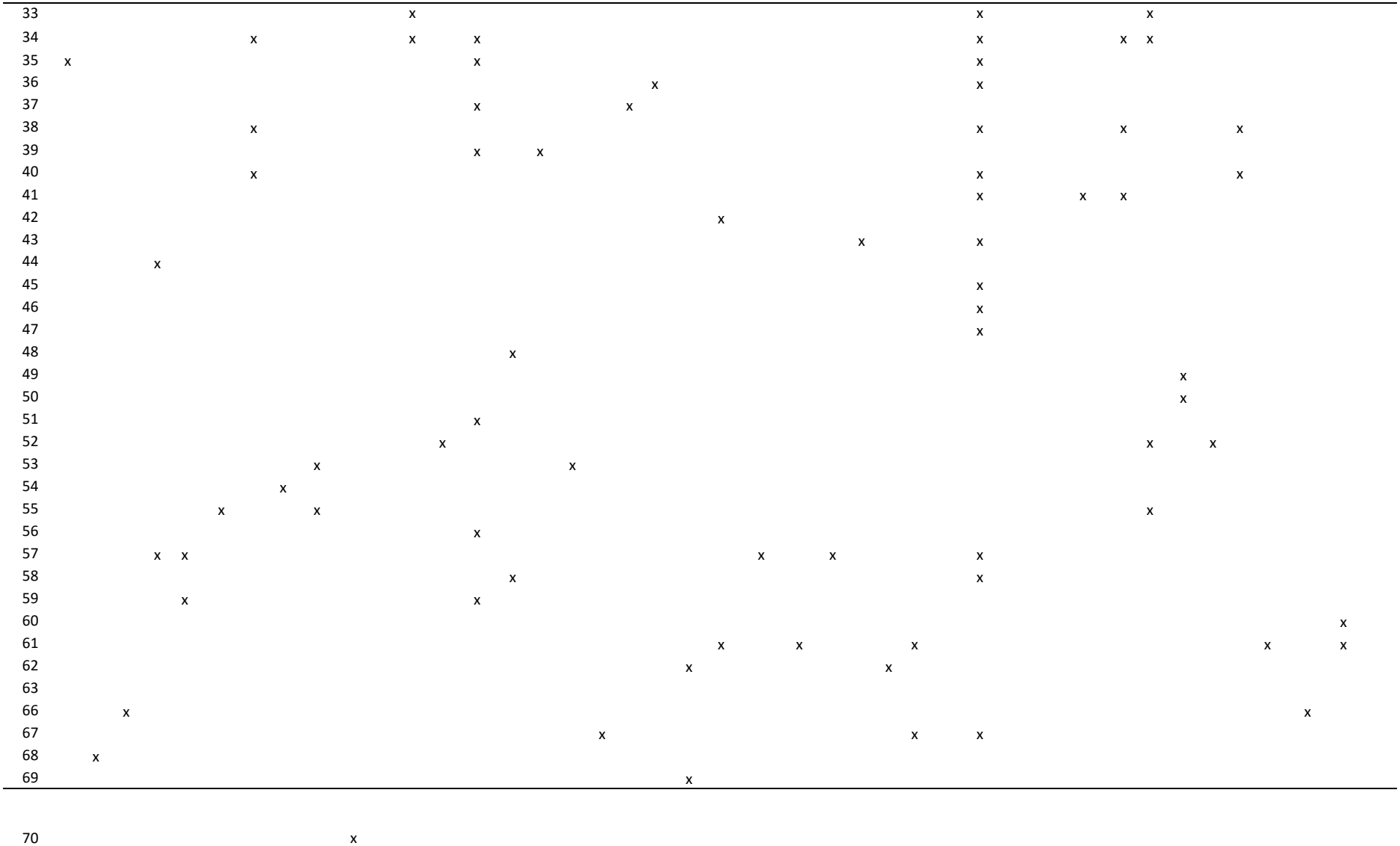
26																														x													
28								x	x																				x													
30							x				x																			x							x						
31																														x													
32														x																x													



71									x							
72											x					
73							x		x							
74	x	x							x		x					
75									x		x					
76									x							x
77									x		x					
78					x				x							
79					x	x			x	x	x					
80														x		
81							x				x					
82									x			x	x			
83				x										x		
84					x											
85								x				x				
86				x	x										x	
87				x								x				
88														x		
89			x		x											
90			x						x							

Af= *Ameerega flavopicta*; Ba= *Bokermannohyla alvarengai*; Bn= *Bokermannohyla nanuzae*; Dj= *Dendropsophus jimi*; Dm= *Dendropsophus minutus*; Dn= *Dendropsophus nanus*; Dr= *Dendropsophus rubicundulus*; Ds= *Dendropsophus sp*; Dmu= *Dermatonotus muelleri*; Eb= *Elachistocleis bicolor*; Ec= *Elachistocleis cesarii*; Eo= *Elachistocleis ovalis*; En= *Eupemphix nattereri*; Ha= *Hypsiboas albopuncatatus*; Hl= *Hypsiboas lundii*; Hp= *Hypsiboas phaeopleurus*; Hr= *Hypsiboas raniceps*; Lc= *Leptodactylus camaquara*; Lf= *Leptodactylus furnarius*; Lfu= *Leptodactylus fuscus*; Lgl= *Leptodactylus gr labirynthicus*; Llab= *Leptodactylus labirynthicus*; Llat= *Leptodactylus latrans*; Lm= *Leptodactylus mystacinus*; Lp= *Leptodactylus podicipinus*; Ls= *Leptodactylus syphax*; Lt= *Leptodactylus troglodytes*; o.a= *Odontophrynus americanos*; Pc= *Physalaemus centralis*; Pcu= *Physalaemus cuvieri*; Pm= *Physalaemus marmoratus*; Pp= *Pseudis platensis*; Psp= *Pseudopaludicola* sp.; Sf= *Scinax fuscomarginartus*; Sfv= *Scinax fuscovarius*; Sp= *Scinax pusillus*; Sr= *Scinax ruber*; Ss= *Scinax similis*; Ssp= *Scinax* sp.; Ssq= *Scinax squalirostris*; Tm= *Thoropa megatimpano*; Tv= *Trachycephalus venulosus*.

Environmental and spatial factors affects composition and morphology of tadpoles assemblages at Cerrado

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Abstract

Environmental factors and geographic distance can significantly contribute to affect the presence and abundance of species in an ecological community. Tadpoles have a considerable morphological diversity, once tadpoles from different anuran species evolved different morphological adaptations that allowed them to occupy different microhabitats. In this study, we were interested in quantify the relative influence of environmental and spatial factors in the composition and morphological diversity of tadpoles' assemblages. We predict that assemblage composition would be strongly affected by spatial factors, while morphological diversity will be affected by environmental variables and spatial factors. We sampled 88 ponds, from which we measured environmental variables, abundance of species, spatial coordinates and collected tadpoles to measure the morphological diversity using the geometric morphometric approach. Posteriorly, we performed two analyses of variance partitioning to quantify the relative contribution of environment and spatial effects in tadpoles' assemblage composition and morphological variation. The spatial factors have significantly affected the composition of tadpoles' assemblages in Brazilian Cerrado. Tadpoles' morphological variation was also affected by spatial factors, but local

environmental factors also play a major role defining morphological variation, although to a small degree. Anurans are a group considered to have low dispersion ability and tadpoles are restricted to ponds that were thought to be previously selected by adults. Thus, a strong spatial effect was expected as a driver of the tadpoles' assemblage composition. Spatial factors can be reflected in tadpoles morphology as a response to microhabitat selection, requiring tadpoles to adapt fast to environmental imposed conditions and biotic pressures, as predation and competition.

Key Words

Amphibian; environmental filter; Geometric morphometrics; Neutral process; Variation partitioning

Introduction

Understand the process that drives the distribution and abundance of species is a central theme in community ecology (Gleason, 1917; Brown et al., 1995; Parris, 2004; Matthews & Whittaker, 2014). Environmental factors, like moisture, temperature and the habitat physical structure, can significantly contribute to control the species diversity in a local and regional scale (Parris, 2004; Both et al. 2009). Thus, we can expect that habitats with similar structure and climate are more likely to have also similar diversity patterns (Parris 2004). The availability and diversity of microhabitats, that are a consequence of habitat complexity level, can affect the number of species that can be found, or that are expected to be found, in a particular site (Eterovick & Barata, 2006; Bastazini et al., 2007). Therefore, the presence of species within a given habitat is

a result of an environmental filtering process that selects species that with better performance regarding such filters (Webb et al., 2010).

Another factor that affects the distribution and abundance of species is the geographic distance among sites. Greater geographic distance among available habitats impose several threats to individual movements, affecting the probability of a successful colonization of distant habitats, leading to an expectation that closed located habitats should have more similar species composition (Legendre & Fortin, 1989; Parris, 2004). This spatial effect can result in assemblages that are structured as a product of differences in migration and colonization ability of species in the regional pool, mediated mainly by the biotic factors such as competition, predation, disturbance and disease (McCarthy & Lindenmayer 2000), and abiotic factors that affect their distribution (Hubell 2001)

Tadpoles, the larval stage of anurans, can be found in different microhabitats like streams, temporal and permanent pond or phytotelmata (Inger, 1985; Altig & McDiarmid, 1999a,b). To occupy these different microhabitats, several kinds of morphological adaptations have evolved in different anurans lineages, resulting in a considerable morphological diversity (Orton, 1953, 1957; Altig & Johnston, 1989). Once environmental heterogeneity can be dependent of the observer's scale, local scales seems to be more important when we want to predict the morphological divergence in tadpoles, while predation and competition seems to be more important in a regional scale (Michel, 2011, 2012). For amphibian adults, abiotic factors, like moisture, temperature and availability of nutrients, seems to affect more strongly the community structure than biotic factors (Parris, 2004, Werner et al., 2007; Vasconcelos et al., 2009). However, this pattern seems to be different for tadpoles, once several studies suggest

that tadpole assemblages are more affected by biotic factors (*e.g.* predation and competition) (Inger et al., 1986; Barreto & Moreira, 1996; Kopp & Eterovick, 2006).

Here, we measure the relative influence of the environmental and spatial factors as determinant of the composition of the tadpole assemblages in the Cerrado biome. Our expectation is that the species composition in tadpoles assemblages should be more influenced by the spatial effect, once we expect that should be more responsive to biotic (*e.g.* dispersal and predation) factors than abiotic factors. Additionally, we investigate how the spatial and environmental effects affect the morphological variation of anuran species in tadpole assemblages, once morphology is correlated to resource use. Our expectation is that tadpole morphology should be better explained by environmental variables and spatial position, once tadpoles does not select the environment where they develop. As a consequence, the ability to fine-tune their phenotypes according to the realized environment should generated an increased in the importance of environmental factor as a driver of species diversity.

Material and Methods

Data acquisition

To quantify the relative contributions of environmental and spatial factors in assemblages of tadpoles, we sampled 88 ponds, throughout three years (2010-2013), in State of Goiás, Brazil. We sampled the tadpoles using a wired dip net with a mesh of 3mm and 30cm of diameter that was swiped during one hour in the ponds margins, clumps of aquatic vegetation and water with 1.5m depth. The tadpoles were anesthetized, killed, identified, and deposited in Zoological Collection of Federal University of Goiás (ZUFG). After tadpoles identification, we count the number of

individuals of each species found in each pond and organized this information in an abundance matrix (Tab. S1). The abundance matrix were then submitted by a Hellinger transformation, which homogenize the variation between species abundances (Dray & Dufour, 2007), and used in the posteriorly analysis.

Tadpoles Habitat descriptors

We used to described the ponds the following local and landscape descriptors (for the complete information, see Tab.S2): pond position (forest, open area, forest edge-inner edge, forest edge-outer edge); pond type (lentic or lotic); pond hydroperiod (permanent or temporary); pond dimensions (greatest length, greatest width and maximum sampled depth); pond margin type (cliffed, plane, sloped, excavated); pond bottom substrate (rock, stones, coarse gravel, gravel, sand, clay, mud and leaf litter); percentage of each vegetation type inside the pond margins (none, submerged, floating, upright herbaceous, shrub, tree and taboa) and percentage of each vegetation type at the pond margins (none, herbaceous undergrowth, erect herbaceous, shrub, arboreous and taboa). All percent estimative was approximated by a semi-qualitative variable to reduce the variation in the estimative of the observer as follow: 0%=0; 1-20%=0.1; 21-40%=0.3; 41-60=0.5%; 61-80%=0.7; 81-100%=0.9. These environmental variables are related to the availability of microhabitats in the ponds.

The environmental matrix were then transformed with a Principal Coordinate Analysis (PCoA) to generate independent environmental variables. We opt to include in all eigenvalues that are necessary to explain 90% of the environmental variation for the posterior statistical analysis. The analyses were performed in software R (version 3.1).

Morphological matrix

To extract the morphological information, we selected up to five tadpoles of each species in each pond between Gosner's stages 35 to 42. We positioned the tadpoles in a petri dish with water based gel and take photographs of the lateral profile of tadpoles using a camera positioned with a tripod, that standardized the distance from the camera and the tadpole in 20 cm. From the photographs, we defined and extracted 23 landmarks for shape comparisons (modified from Van Buskirk, 2009). The landmarks are anatomical references to represent the shape of organisms and must be recognizable among all individuals sampled (Zelditch et al., 2012), being necessarily homologous to ensure biological correspondences (Sneath & Sokal, 1973). By the landmark approach, we are able to remove from shape variation any effect of size, position, and rotation (Zelditch et al., 2012), using the Procrustes transformation (Rohlf, 1990). The Procrustes transformation reduces the differences among landmarks configurations from the centroid of the average shape (*i.e.* the average distance among all landmarks from the gravity center; Zelditch et al., 2012). After applying this transformation, we obtained the partial warps scores that represent the deformation in external morphology of tadpoles. To summarize the deformation information we perform a Principal Component Analysis (PCA) and extract the eigenvectors that represent at least 90% of the total variation. We then used these eigenvectors in posteriorly statistical analysis. The landmarks were positioned with the tpsUtil (Rohlf, 2013) and tpsDig2 (Rohlf, 2013b) programs, and all the others analysis was performed in software PAST (version 3.08; Hammer et al., 2001)

Spatial matrix

To build the spatial matrix, we registered the geographical coordinates of all sites by the global positioning system (GPS; Tab. S1). The geographical information was transformed in spatial variables by the Principal Coordinates of Neighbor Matrices Method (PCNM; Borcard & Legendre, 2002), which is based in a matrix of euclidian distance among the samples. We used the positive eigenvectors with significant and higher than 0.1 Moran's coefficient in posterior statistical analysis. These eigenvectors represents the positive spatial relationships between the sampled sites (Dray et al., 2006).

Data analysis

To quantify how much of the abundance variation and the morphological variation of the species can be explained by environmental variables and spatial data we made two variance partitioning: i) we used the environmental matrix and the spatial matrix to predict the total variance found in the abundance matrix and ii) we used the environmental matrix and the spatial matrix to predict the total variance found in the morphological matrix. The method of variance partitioning (Diniz-Filho et al., 1998; Desdevises et al., 2003; Peres-Neto et al., 2006) allows the partition of the total variance into four fractions: i) the variation explained exclusively by environmental variables; ii) the variation explained exclusively by spatial variables; iii) the spatially structured environmental variation and iv) the variation that is not explained by our predictors matrices, referred as the residuals of the analysis. To test the pure fractions significance

we used a partial redundance analysis (pRDA; Legendre & Legendre, 1998). All analyses were made in R software (version 3.1).

Results

We found a total of 7,553 individuals, distributed in 45 species (Tab. S1). The environmental variables that describe tadpoles' microhabitat heterogeneity were resumed in nine eigenvectors that accounted 92% of the total variation. We found 46 partial warps that describe the total morphological variation of of 512 individuals. After the PCA analysis, we selected six eigenvectors that account for 90.7% of the total morphological variation. We selected three positive PCNM eigenvectors to represent the spatial relationships among sampled ponds (table 1). The eigenvectors matrices that represent the environmental variables, morphological variation and spatial relationship were used in variance partitioning (pRDA).

Environmental and Spatial Effects

Only the spatial effect significantly affected the amount of abundance variation among the sampled sites (Tab.2). The amount of morphological variation of the tadpoles that were explained by spatial and environmental variables is showed in table 2. Although both the spatial and environmental variables were significant to explain the morphological variance, the spatial effect was two times higher than the environmental effect (Tab. 2).

Table 1. Values of Morans'I that represents spatial factors. In bold p value that were significative

	Moran	p.value
1	0.28419	**0.001**
2	0.25948	**0.001**
3	0.05832	**0.001**
4	-0.0032	0.1
5	-0.0111	0.396
6	-0.0161	0.245
7	-0.0166	0.234
8	-0.0167	0.222
9	-0.0167	0.183
10	-0.0167	0.178
11	-0.0167	0.198
12	-0.0167	0.188
13	-0.0167	0.185

Table 2. Permutation test for Partial Redundance Analysis (pRDA). In bold significative p values.

	Df	Var.	F	N. Pe	p
Model: rda(morphology ~ spatial + environment)					
spatial	3	0.0541	3.1792	999	**0.001**
environment	9	0.06851	1.3419	999	**0.05**
residuals	75	0.42546	-	-	-
Model: rda(abundance ~ spatial + environment)					
Spatial	3	0.5693	2.9004	999	**0.001**
Environment	9	0.328	0.5571	999	0.816
Residuals	75	4.9074	-	-	-

Df= Degrees of freedom; Var= Variance; F= Statistics F; N.Pe= Number of permutations.

Discussion

We found that the species composition in tadpole's assemblages was significantly affected by spatial factors. Moreover, tadpole morphological variation was significantly affected by spatial and environmental factors. In Cerrado, one of the world's biodiversity hotspots, we can found a heterogeneous environment, with a contrasting mosaic of habitats that range from humid ciliary forests to open and dry savannah (Klink & Machado, 2005). In such conditions, we expected that sites with similar environmental structure would have similar composition in tadpole assemblages. Moreover, we also expected that ponds near each other have similar assemblages than sites that were further apart, indicating that biotic process (*e.g.* adult dispersion) could affect assemblage composition and morphological variation of tadpoles.

Tadpoles of anurans had limited ability to colonize new sites, once tadpoles were restricted to water bodies and the oviposition sites were selected by the adult form. Some studies suggest that breeding sites selections in anurans evolved to maximize tadpoles' survival (Altwegg & Reyer, 2003; Grözinger et al., 2012; Stein & Blaustein, 2015) and that adults use abiotic (*e.g.* pond depth) and biotic (*e.g.* predation) clues during the process of decision making (Van Buskirk, 2005; Grözinger et al., 2012; Stein & Blaustein, 2015). Adult choices are of great importance for tadpoles' survival. In situations that adults make poor decisions, the phenotypic plasticity (*i.e.* the capacity of a genotype to displays different phenotypes as a response to a change in the environment; Miner et al., 2005) seems to be advantageous for tadpoles, once it allow the tadpoles to adjust its morphology to the realized environmental condition. Phenotypic plasticity may result in broader niches for tadpoles and would suggest that, at least in Cerrado assemblages, adult form of anurans were not so selective when choosing oviposition sites. Environmental variables seem to act as a filter for tadpole

morphology, selecting those traits which have morphological traits that conferring higher fitness.

The spatial effects seem to be related to probabilistic events (*e.g.* random birth and death) and can generate neutral assemblages (Hubbell 2001). Neutral assemblages are independent of environmental factors and of individual traits. Conversely, tadpoles depend of adult choices for adequate developmental habitats and, when errors occur, tadpoles would be restricted to unfavorable sites. Thus, phenotypic plasticity should be advantageous for tadpoles, as a response to evaluation error of adults when selecting the developmental sites for tadpoles.

Our study demonstrated how factors that control tadpoles' assemblage composition also drives tadpole morphological diversity. Anuran is a group considered to have low dispersion and tadpoles, with rare exceptions (*e.g.* Sousa et al. 2011), are restricted to ponds previously selected by adults. The ability of tadpoles to adapt its morphology, even slightly, in response to realized environmental (both biotic and abiotic) traits can be a response to evaluate error in oviposition site choice by adults, and should interact with spatial factors to control tadpoles assemblage.

Acknowledgments

We thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for fellowships. We are grateful to Girinos do Brasil (SISBIOTA: grants CNPq 563075/2010-4 and FAPESP 2010/52321-7) for the financial support.

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Supplementary information

Table S1. Abundance data of 45 species in 88 ponds.

Pond	af	ba	bc	bn	dj	dm	dn	dr	ds1	ds2	dmu	ec	en	ha	hl	hp	hr	lf	la	lla	lm	lp	ls	lt	lc	lgl	o_a	pc	pcu	pm	psp	pcr	ps1	ps2	ps3	pp	sf	sfv	ss	ssq	sp	s1	s2	tm	tv
1	0	0	0	0	0	4	0	2	0	0	0	9	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65	0	0	0	18	0	0	0	6	0	24	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	8	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	24	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	8	0	0	0	0	107	414	0	0	0	0	0	0
4	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	72	0	0	0	1	0	0	0	166	1	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	19	0	0	0	5	0	0	0	128	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	4	0	0	0	56	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	31	0	0	0	0	0	5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	83	0	0	0	0	0	0	0	23	23	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	12	0	0	0	1	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	81	0	0	0	0	0	0	0	20	1	0	0	0	0	0	0	0
11	0	0	0	0	0	1	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0
12	39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	13	16	0	0	15	0	1	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	5	10	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	1	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0
17	0	0	0	0	0	5	39	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	40	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	4	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	35	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	0	0	0	0	0
26	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	2	2	0	0	4	0	0	0	0	0	0	0	0
29	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	2	0	0	0	0	0	0	0	0
30	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	23	0	0	0	0	0	0	0	0

32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
33	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
35	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	11	22	0	0	0	0	0	0	2	0	1	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	6	33	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	2	87	0	0	0	0	0	0
41	0	0	0	0	0	2	0	0	0	0	0	5	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
44	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	7	0	0	0	3	15	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	15	0	0	0	14	0	0	0	1	0	0	0	0	0	0	0
46	0	0	0	0	0	47	0	0	0	0	0	0	0	13	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	3	37	0	0	0	0	0	0
47	0	0	0	0	0	44	0	0	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	14	0	0	0	10	91	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	11	0	0	0	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	6	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	85	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	166	161	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
57	0	0	0	0	7	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0
59	0	0	0	0	0	1	0	2	0	0	0	0	13	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	138	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0	109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	53	0	0	0	0	0
61	0	0	0	0	0	94	0	0	1	0	0	6	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	0	0	0	0	0	0	0	0	2	723	0	0	0	0	13
62	0	0	0	0	1	0	0	0	0	0	0	2	0	31	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	11	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	0	0	0	0	0	0	0	25	2	1	0	0	0	0	0
66	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	52	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0	6	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0	0	0	0	0	0	0	21	0	0	0	1	0	0	0

69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0
70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	21	0	0	0	0	0	66	0	0	0	0	44	0	0	0	0	0	0	0	0	0	6	0	14	0
71	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	1008	0	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
75	0	0	0	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0
76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	43	0	6	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
77	0	67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
78	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	1	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	16	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	28	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	33	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	5	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	2	19	3	0	0	0	0	0	0
83	0	0	0	0	0	0	0	23	0	0	16	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	183	0	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	104	0	0	0	0	0	0	0
85	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	9	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Af= *Ameerega flavopicta*; Ba= *Bokermannohyla alvarengai*; Bc= *Bokermannohyla circumdata*; Bn= *Bokermannohyla nanuzae*; Dj= *Dendropsophus jimi*; Dm= *Dendropsophus minutus*; Dn= *Dendropsophus nanus*; Dr= *Dendropsophus rubicundulus*; Ds1= *Dendropsophus sp1*; Ds2= *Dendropsophus sp2;* Dmu= *Dermatonotus muelleri*;; Ec= *Elachistocleis cesarii*; En= *Eupemphix nattereri*; Ha= *Hypsiboas albopuncatatus*; Hl= *Hypsiboas lundii*; Hp= *Hypsiboas phaeopleurus*; Hr= *Hypsiboas raniceps*; Lc= *Leptodactylus camaquara*; Lf= *Leptodactylus fuscus*; Lgl= *Leptodactylus gr labirynthicus*; La= *Leptodactylus labirynthicus*; Lla= *Leptodactylus latrans*; Lm= *Leptodactylus mystacinus*; Lp= *Leptodactylus podicipinus*; Ls= *Leptodactylus syphax*; Lt= *Leptodactylus troglodytes*; o.a= *Odontophrynus americanos*; Pc= *Physalaemus centralis*; Pcu= *Physalaemus cuvieri*; Pm= *Physalaemus marmoratus*; Pcr= *Proceratophrys cururu*; Psp= *Physalaemus sp.;* Pp= *Pseudis platensis*; Psp1= *Pseudopaludicola* sp1.; Psp2= *Pseudopaludicola* sp2.; Psp3= *Pseudopaludicola* sp3.; Sf= *Scinax fuscomarginartus*; Sfv= *Scinax fuscovarius*; Sp= *Scinax pusillus*;; Ss= *Scinax similis*; Ssp1= *Scinax* sp1.; Ssp2= *Scinax* sp2; Ssq= *Scinax squalirostris*; Tm= *Thoropa megatimpano*; Tv= *Trachycephalus venulosus*.

Table S2. Environmental variables measured in ponds

				Pond dimensions			Pond margins type					
Locality	**Pond type**	**Pond position**	**Pond hydroperiod**	**Length**	**Width**	**Depth**	**cliffed**	**plane**	**sloped**	**excavated**	**rock**	**stones**
RESEX Lago do Cedro	lentic	open area	permanent	100.00	100.00	1.50	0.0	0.5	0.5	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	permanent	150.00	50.00	0.50	0.0	0.5	0.5	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	15.00	10.00	0.30	0.0	0.9	0.0	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	forest	unknown	20.00	15.00	0.60	0.0	0.3	0.5	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	2.00	1.50	0.15	0.0	0.0	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	25.00	15.00	0.30	0.0	0.9	0.0	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	permanent	400.00	150.00	0.50	0.0	0.3	0.5	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	128.00	40.00	0.40	0.0	0.0	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	permanent	100.00	20.00	0.03	0.0	0.0	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	forest/edge-outer edge	temporary	400.00	200.00	0.60	0.0	0.0	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	100.00	50.00	0.12	0.0	0.9	0.0	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	50.00	20.00	0.07	0.0	0.9	0.0	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	60.00	15.00	0.33	0.9	0.0	0.7	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	permanent	50.00	25.00	0.36	0.0	0.0	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	100.00	25.00	0.39	0.0	0.0	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	forest/edge-outer edge	temporary	200.00	50.00	0.12	0.0	0.9	0.0	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	permanent	400.00	200.00	0.41	0.5	0.9	0.5	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	forest/edge-outer edge	temporary	50.00	200.00	0.21	0.0	0.0	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	permanent	400.00	25.00	2.00	0.3	0.9	0.3	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	40.00	25.00	0.10	0.0	0.9	0.0	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	forest/edge-outer edge	permanent	400.00	50.00	2.00	0.0	0.3	0.5	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	100.00	100.00	0.80	0.0	0.9	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	permanent	25.00	60.00	1.50	0.0	0.3	0.5	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	permanent	70.00	5.00	0.40	0.0	0.0	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	50.00	30.00	0.50	0.0	0.9	0.0	0.0	0.0	0.0
RESEX Lago do Cedro	lentic	open area	temporary	20.00	50.00	0.50	0.0	0.9	0.0	0.0	0.0	0.0
Britânia	lentic	open area	permanent	62.00	37.00	0.35	0.3	0.7	0.0	0.0	0.0	0.0
Britânia	lentic	open area	permanent	100	26	50	0.2	0.0	0.9	0.0	0.0	0.0
Sao Joao da Alianca	lentic	forest/edge-outer edge	permanent	120	50	0.15	0.0	0.0	0.9	0.0	0.0	0.0
Sao Joao da Alianca	lentic	open area	permanent	114	100	0.16	0.3	0.5	0.5	0.0	0.0	0.0
Sao Joao da Alianca	lentic	open area	permanent	40	11	1.12	0.9	0.0	0.7	0.9	0.0	0.9
Sao Joao da Alianca	lentic	open area	permanent	80	38	1.5	0.9	0.7	0.9	0.0	0.0	0.0

Sao Joao da Alianca	lentic	forest/edge-inner edge	permanent	90	35	1.2	0.5	0.3	0.3	0.0	0.0	0.0
Sao Joao da Alianca	lentic	open area	permanent	100	5	0.3	0.0	0.9	0.9	0.0	0.0	0.7
Sao Joao da Alianca	lentic	forest/edge-inner edge	permanent	80	25	1.5	0.7	0.0	0.9	0.5	0.0	0.0
Alto Paraíso de Goiás	lotic	areaaberta	permanent	250	50	1.5	0.3	0.9	0.7	0.0	0.0	0.0
Alto Paraíso de Goiás	lentic	forest/edge-outer edge	permanent	70	15	1.3	0.5	0.3	0.3	0.0	0.0	0.0
Alto Paraíso de Goiás	lentic	areaaberta	permanent	30	6	1.2	0.0	0.0	0.7	0.9	0.0	0.0
Alto Paraíso de Goiás	lentic	edge-inner edge	permanent	100	50	1.5	0.0	0.9	0.9	0.0	0.0	0.0
Alto Paraíso de Goiás	lentic	open area	temporary	20	15	0.3	0.3	0.5	0.9	0.0	0.0	0.0
Alto Paraíso de Goiás	lentic	open area	temporary	25	20	1.5	0.9	0.0	0.9	0.0	0.0	0.0
Pires do Rio	lentic	open area	permanent	36	30	0.8	0.3	0.0	0.7	0.0	0.0	0.0
Pires do Rio	lentic	open area	permanent	50.51	26.25	0.37	0.9	0.5	0.5	0.0	0.0	0.0
Pires do Rio	lentic	open area	unknown	23	31	0.49	0.7	0.0	0.3	0.0	0.0	0.0
Pires do Rio	lentic	open area	permanent	140	42	0.5	0.9	0.0	0.9	0.0	0.0	0.0
Pires do Rio	lentic	open area	permanent	44.37	50.68	0.55	0.30	0.0	0.7	0.0	0.0	0.0
Pires do Rio	lentic	open area	permanent	52.16	91.44	2.00	0.0	0.0	0.9	0.0	0.0	0.0
Parna Emas	-	-	-	100	60	0.7	20	-	80	-	-	-
Parna Emas	-	-	-	100	40	2	30	-	70	-	-	-
Parna Emas	-	-	-	110	100	1	10	-	90	-	-	-
Parna Emas	-	-	-	100	100	5	50	-	50	-	-	-
Parna Emas	-	-	-	70	30	0.6	-	-	100	-	-	-
Pontalina	lentic	forest/edge-outer edge	permanent	79.61	42	0.4	0.3	0.0	0.9	0.0	0.0	0.0
Pontalina	lentic	open area	permanent	41	28	0.6	0.9	0.0	0.0	0.0	0.0	0.0
Pontalina	lentic	open area	permanent	265	76	-	0.9	0.0	0.0	0.0	0.0	0.0
Pontalina	lentic	open area	permanent	38	20	0.5	0.3	0.0	0.7	0.0	0.0	0.0
Pontalina	lentic	forest/edge-inner edge	temporary	31	19	0.5	0.9	0.0	0.0	0.0	0.0	0.0
Pontalina	lentic	open area	permanent	100	20	0.5	0.9	0.0	0.0	0.0	0.0	0.0
Pontalina	lentic	forest/edge-inner edge	temporary	20	10	42	0.7	0.0	0.1	0.0	0.0	0.0
Parna Sempre Vivas	lentic	open area	temporary	1.5	1	0.05	0.7	0.0	0.3	0.9	0.9	0.0
Parna Sempre Vivas	lotic	open area	temporary	-	-	-	0.5	0.0	0.3	0.9	0.7	0.3
Parna Sempre Vivas	lentic	open area	temporary	100	1	0.3	0.5	0.0	0.5	0.0	0.0	0.0
Parna Sempre Vivas	lotic	forest/edge-outer edge	permanete	25	3	0.3	0.0	0.0	0.9	0.0	0.9	0.0
Parna Sempre Vivas	lotic	open area	permanete	50	5	2	0.9	0.0	0.0	0.9	0.9	0.0
Parna Sempre Vivas	lotic	open area	permanete	20	1	0.3	0.5	0.0	0.0	0.9	0.9	0.0
Parna Sempre Vivas	lentic	open area	temporary	3	2	0.3	0.0	0.0	0.9	0.0	0.0	0.0
Parna Sempre Vivas	lotic	open area	unknown	20	2	0.5	0.9	0.0	0.9	0.0	0.5	0.0
Parna Sempre Vivas	lentic	open area	temporary	25	15	0.15	0.0	0.9	0.0	0.0	0.0	0.0
Parna Sempre Vivas	lentic	open area	temporary	20	3	0.3	0.0	0.9	0.0	0.0	0.9	0.0

Serranópolis	lentic	open area	temporary	11.35	7.70	1.50	0.0	0.7	0.9	0.0	0.0	0.0
Serranópolis	lentic	forest/edge-outer edge	permanent	133.00	68.00	0.39	0.9	0.9	0.7	0.0	0.0	0.0
Serranópolis	lentic	forest/edge-outer edge	permanent	85.92	74.96	2.00	0.5	0.9	0.3	0.0	0.0	0.0
Serranópolis	lentic	open area	temporary	47.11	30.44	0..6	0.9	0.9	0.9	0.9	0.0	0.0
Serranópolis	lentic	open area	permanent	59.00	47.00	0.67	0.9	0.5	0.3	0.0	0.0	0.0
Serranópolis	lentic	forest/edge-outer edge	permanent	90.00	40.00	2.00	0.3	0.9	0.7	0.0	0.0	0.0
Serranópolis	lentic	open area	permanent	31.00	29.00	1.00	0.0	0.0	0.9	0.0	0.0	0.0
Serranópolis	lentic	forest/edge-outer edge	permanent	113.26	64.61	3.00	0.9	0.9	0.7	0.0	0.0	0.0
Caiapônia	lentic	open area	permanent	70.00	45.00	NA	0.3	0.0	0.7	0.0	0.0	0.0
Caiapônia	lentic	open area	temporary	37.40	212.00	1.00	0.9	0.0	0.7	0.0	0.0	0.0
Caiapônia	lentic	open area	temporary	65.68	27.20	0.70	0.0	0.9	0.9	0.0	0.0	0.0
Caiapônia	lentic	open area	permanent	127.66	114.90	NA	0.9	0.0	0.0	0.0	0.0	0.0
Chapadão do Céu	lentic	open area	temporary	59.3	42	0.29	0.0	0.9	0.0	0.0	0.0	0.0
Chapadão do Céu	lentic	open area	temporary	55.7	32.3	0.57	0.0	0.5	0.5	0.0	0.0	0.0
Chapadão do Céu	lentic	forest/edge-inner edge	permanent	176.14	142	4	0.1	0.0	0.7	0.0	0.0	0.1
Chapadão do Céu	lentic	forest	permanent	40	20	0.4	0.9	0.0	0.0	0.0	0.0	0.1
Chapadão do Céu	lentic	open area	temporary	90	45	0.27	0.0	0.9	0.1	0.0	0.0	0.0
Chapadão do Céu	lentic	open area	temporary	37.4	14.2	0.45	0.0	0.5	0.3	0.1	0.0	0.0
Chapadão do Céu	lentic	open area	temporary	28.4	20.6	0.52	0.0	0.7	0.0	0.3	0.0	0.0

Table S2. Table continuation of environmental variables measured in ponds.

	Pond bottom substrate						Percentage of each vegetation type inside the pond margin							Percentage of each vegetation type at the pond margin					
Locality	coarse gravel	gravel	sand	clay	mud	leaf litter	none	submerged	floating	upright h.	shrub	tree	taboa	none	H. undergrowth	erect h.	shrub	arboreous	taboa
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.3	0.9	0.0	0.0	0.0	0.0	0.3	0.3	0.7	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.9	0.7	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	0.0	0.0	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.9	0.9	0.9	0.0	0.0	0.0	0.7	0.9	0.0	0.9	0.0	0.0	0.7	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.9	0.3	0.5	0.0	0.0	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.0	0.7	0.9	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.5	0.3	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.9	0.0	0.0	0.0	0.3	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.7	0.3	0.3	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.9	0.0	0.7	0.7	0.3	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.9	0.9	0.3	0.0	0.0	0.9	0.9	0.7	0.0	0.0	0.0	0.9	0.0	0.0	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.5	0.9	0.0	0.0	0.0	0.0	0.7	0.3	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.7	0.3	0.9	0.0	0.0	0.0	0.9	0.9	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.9	0.0	0.0	0.3	0.0	0.9	0.3	0.0	0.0	0.0	0.0	0.0	0.7	0.9	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.5	0.0	0.7	0.0	0.3	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.9	0.0	0.7	0.9	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.9	0.0	0.3	0.0	0.0	0.0	0.9	0.7	0.0	0.5	0.0	0.3	0.9	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.0	0.9	0.0	0.9	0.0	0.0	0.3	0.3	0.9	0.0	0.9	0.0	0.3	0.3	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.9	0.0	0.0	0.5	0.3	0.0	0.0	0.3	0.3	0.3	0.0	0.5	0.0	0.5	0.3	0.3	0.0
RESEX Lago do Cedro	0.0	0.0	0.9	0.0	0.0	0.0	0.5	0.0	0.0	0.3	0.9	0.0	0.0	0.5	0.0	0.5	0.3	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.9	0.0	0.0	0.9	0.5	0.0	0.0	0.7	0.0	0.0	0.0	0.9	0.0	0.7	0.3	0.3	0.0
RESEX Lago do Cedro	0.0	0.0	0.9	0.0	0.0	0.9	0.3	0.0	0.0	0.9	0.5	0.0	0.0	0.7	0.0	0.3	0.7	0.9	0.0
RESEX Lago do Cedro	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.9	0.0	0.7	0.3	0.0	0.0
RESEX Lago do Cedro	0.0	0.0	0.9	0.0	0.0	0.5	0.5	0.0	0.0	0.9	0.9	0.9	0.0	0.5	0.0	0.9	0.3	0.3	0.0
RESEX Lago do Cedro	0.0	0.9	0.9	0.0	0.0	0.0	0.7	0.0	0.0	0.3	0.0	0.0	0.0	0.7	0.0	0.3	0.9	0.0	0.0
RESEX Lago do Cedro	0.0	0.0	0.9	0.9	0.0	0.7	0.5	0.0	0.0	0.5	0.3	0.3	0.0	0.0	0.0	0.9	0.5	0.3	0.0
RESEX Lago do Cedro	0.0	0.0	0.0	0.9	0.0	0.5	0.9	0.7	0.0	0.7	0.3	0.0	0.0	0.0	0.0	0.7	0.5	0.9	0.0
Britânia	0.0	0.0	0.5	0.5	0.0	0.0	0.5	0.0	0.0	0.5	0.3	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0
Britânia	0.0	0.0	0.5	0.5	0.0	0.0	0.2	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0
Sao Joao da Alianca	0.0	0.0	0.0	0.9	0.0	0.0	0.9	0.3	0.0	0.9	0.3	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0
Sao Joao da Alianca	0.0	0.0	0.0	0.0	0.9	0.7	0.9	0.5	0.0	0.5	0.5	0.9	0.0	0.0	0.7	0.7	0.9	0.7	0.0
Sao Joao da Alianca	0.0	0.0	0.0	0.0	0.9	0.0	0.7	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.7	0.7	0.3	0.0	0.0
Sao Joao da Alianca	0.0	0.0	0.0	0.5	0.9	0.0	0.9	0.5	0.0	0.3	0.0	0.0	0.9	0.0	0.7	0.0	0.9	0.0	0.9
Sao Joao da Alianca	0.0	0.0	0.0	0.5	0.5	0.9	0.9	0.0	0.5	0.5	0.7	0.9	0.0	0.0	0.7	0.7	0.9	0.9	0.0
Sao Joao da Alianca	0.5	0.9	0.0	0.0	0.0	0.0	0.3	0.7	0.0	0.7	0.0	0.0	0.0	0.0	0.9	0.5	0.5	0.9	0.0

Sao Joao da Alianca	0.9	0.9	0.9	0.0	0.9	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.7	0.7	0.7	0.9	0.0
Alto Paraíso de Goiás	0.0	0.0	0.0	0.9	0.0	0.7	0.9	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.9	0.5	0.3	0.3	0.0
Alto Paraíso de Goiás	0.0	0.0	0.0	0.5	0.5	0.9	0.5	0.9	0.0	0.5	0.9	0.0	0.0	0.0	0.9	0.7	0.5	0.5	0.0
Alto Paraíso de Goiás	0.0	0.9	0.0	0.0	0.7	0.0	0.9	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.7	0.0	0.9	0.9	0.0
Alto Paraíso de Goiás	0.0	0.9	0.0	0.9	0.0	0.9	0.9	0.0	0.0	0.9	0.9	0.9	0.0	0.9	0.7	0.5	0.7	0.7	0.0
Alto Paraíso de Goiás	0.0	0.9	0.0	0.0	0.0	0.7	0.7	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.9	0.9	0.9	0.0	0.0
Alto Paraíso de Goiás	0.0	0.9	0.0	0.0	0.0	0.7	0.9	0.0	0.0	0.9	0.0	0.0	0.0	0.9	0.7	0.9	0.9	0.9	0.0
Pires do Rio	0.0	0.0	0.0	0.0	0.9	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.3	0.0
Pires do Rio	0.0	0.0	0.0	0.0	0.9	0.9	0.3	0.0	0.9	0.0	0.9	0.0	0.7	0.0	0.9	0.9	0.0	0.0	0.0
Pires do Rio	0.0	0.0	0.0	0.9	0.0	0.0	0.3	0.0	0.5	0.5	0.9	0.0	0.0	0.5	0.0	0.3	0.3	0.9	0.0
Pires do Rio	0.0	0.0	0.0	0.9	0.0	0.0	0.9	0.0	0.9	0.9	0.0	0.0	0.0	0.3	0.0	0.7	0.5	0.9	0.0
Pires do Rio	0.0	0.0	0.9	0.0	0.0	0.0	0.7	0.0	0.9	0.3	0.9	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0
Pires do Rio	0.0	0.0	0.0	0.9	0.0	0.0	0.7	0.0	0.0	0.3	0.9	0.0	0.0	0.0	0.0	0.7	0.9	0.9	0.0
Parna Emas	-	-	-	-	0.9	0.7	-	-	-	0.5	0.5	0.3	-	0.9	-	0.9	0.9	0.5	-
Parna Emas	-	-	-	-	0.9	-	-	-	-	0.9	0.3	0.1	-	0.9	-	0.7	0.5	0.5	-
Parna Emas	-	-	-	-	0.9	-	-	-	-	0.9	0.3	-	-	0.9	-	0.9	-	-	-
Parna Emas	-	-	-	-	0.7	0.3	-	-	-	0.5	0.3	0.1	-	0.9	-	0.9	0.3	0.8	-
Parna Emas	-	-	-	-	0.9	-	-	-	-	0.9	-	-	-	0.1	-	100	-	-	-
Pontalina	0.0	0.0	0.0	0.5	0.5	0.9	0.7	0.0	0.0	0.9	0.0	0.9	0.9	0.5	0.0	0.3	0.9	0.9	0.0
Pontalina	0.0	0.0	0.0	0.7	0.5	0.0	0.3	0.0	0.0	0.3	0.9	0.0	0.0	0.0	0.7	0.0	0.9	0.9	0.0
Pontalina	0.0	0.0	0.0	0.7	0.5	0.0	0.9	0.0	0.0	0.9	0.9	0.0	0.0	0.9	0.3	0.7	0.3	0.9	0.0
Pontalina	0.0	0.0	0.0	0	0.9	0.0	0.7	0.0	0.0	0.3	0.0	0.0	0.0	0.5	0.0	0.9	0.9	0.9	0.0
Pontalina	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.7	0.0
Pontalina	0.0	0.0	0.0	0.5	0.5	0.0	0.5	0.0	0.0	0.9	0.0	0.9	0.3	0.0	0.0	0.9	0.3	0.9	0.0
Pontalina	0.0	0.0	0.0	0.0	0.7	0.3	0.3	0.0	0.1	0.3	0.7	0.0	0.0	0.0	0.0	0.3	0.7	0.3	0.0
Parna Sempre Vivas	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	-	-
Parna Sempre Vivas	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.0	0.0	-	-
Parna Sempre Vivas	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	-	-
Parna Sempre Vivas	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.3	0.9	0.0	-	-
Parna Sempre Vivas	0.7	0.0	0.9	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.7	0.5	0.0	0.0	0.0	-	-
Parna Sempre Vivas	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.9	0.9	0.9	0.0	-	-
Parna Sempre Vivas	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.7	0.9	0.0	0.0	-	-
Parna Sempre Vivas	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.9	0.3	0.0	0.0	0.0	-	-
Parna Sempre Vivas	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.5	0.9	0.0	0.0	0.0	-	-
Parna Sempre Vivas	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9	0.9	0.0	0.0	-	-
Serranópolis	0.0	0.0	0.9	0.0	0.7	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0
Serranópolis	0.0	0.9	0.3	0.0	0.7	0.0	0.9	0.9	0.9	0.9	0.3	0.3	0.0	0.9	0.7	0.3	0.9	0.9	0.0

Serranópolis	0.0	0.9	0.5	0.0	0.9	0.9	0.9	0.9	0.0	0.3	0.3	0.9	0.0	0.9	0.9	0.5	0.9	0.9	0.0
Serranópolis	0.0	0.0	0.9	0.0	0.0	0.0	0.9	0.0	0.0	0.9	0.9	0.0	0.0	0.9	0.0	0.9	0.9	0.0	0.0
Serranópolis	0.0	0.0	0.7	0.0	0.0	0.3	0.9	0.3	0.0	0.3	0.3	0.0	0.0	0.0	0.9	0.3	0.7	0.0	0.0
Serranópolis	0.0	0.0	0.9	0.0	0.0	0.9	0.0	0.9	0.9	0.9	0.9	0.0	0.0	0.9	0.9	0.7	0.9	0.9	0.0
Serranópolis	0.0	0.0	0.9	0.0	0.0	0.9	0.9	0.7	0.0	0.3	0.9	0.0	0.0	0.9	0.9	0.7	0.9	0.0	0.0
Serranópolis	0.0	0.9	0.7	0.0	0.0	0.3	0.3	0.3	0.9	0.3	0.9	0.0	0.0	0.0	0.9	0.3	0.9	0.3	0.0
Caiapônia	0.0	0.0	0.0	0.0	0.7	0.9	0.7	0.0	0.0	0.9	0.0	0.0	0.0	0.9	0.5	0.0	0.3	0.0	0.0
Caiapônia	0.0	0.9	0.3	0.0	0.7	0.9	0.9	0.0	0.0	0.9	0.0	0.0	0.0	0.9	0.7	0.9	0.0	0.0	0.0
Caiapônia	0.0	0.0	0.0	0.0	0.9	0.9	0.9	0.9	0.0	0.7	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0
Caiapônia	0.0	0.0	0.0	0.0	0.5	0.5	0.0	0.9	0.0	0.9	0.0	0.0	0.0	0.9	0.5	0.0	0.9	0.0	0.0
Chapadão do Céu	0.0	0.1	0.9	0.0	0.0	0.0	0.1	0.3	0.0	0.5	0.0	0.0	0.0	0.0	0.1	0.7	0.0	0.0	0.0
Chapadão do Céu	0.0	0.0	0.9	0.0	0.0	0.0	0.9	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.9	0.0	0.0	0.0	0.0
Chapadão do Céu	0.0	0.0	0.9	0.0	0.0	0.1	0.5	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.3	0.3	0.1	0.0
Chapadão do Céu	0.0	0.0	0.1	0.0	0.0	0.7	0.0	0.0	0.1	0.7	0.0	0.3	0.0	0.0	0.0	0.3	0.3	0.3	0.0
Chapadão do Céu	0.0	0.1	0.9	0.0	0.0	0.1	0.0	0.0	0.0	0.7	0.5	0.0	0.0	0.0	0.0	0.7	0.3	0.1	0.0
Chapadão do Céu	0.0	0.0	0.9	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.0	0.0	0.0	0.1	0.0	0.9	0.0	0.0	0.0
Chapadão do Céu	0.0	0.0	0.9	0.0	0.1	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.9	0.0	0.0	0.0	0.0

Table S3. Spatial matrix of 88 ponds

City	Pond	Latitude	Longitude
Alto Paraíso de Goiás	1	-14.28311	-47.53267
Alto Paraíso de Goiás	2	-14.29228	-47.62933
Alto Paraíso de Goiás	3	-14.41583	-47.52192
Alto Paraíso de Goiás	4	-14.50039	-47.51744
Alto Paraíso de Goiás	5	-14.48328	-47.59186
Alto Paraíso de Goiás	6	-14.47872	-47.57814
Sao Joao da Alianca	7	-14.73281	-47.56314
Sao Joao da Alianca	8	-14.72767	-47.55364
Sao Joao da Alianca	9	-14.61281	-47.54106
Sao Joao da Alianca	10	-14.65942	-47.52642
Sao Joao da Alianca	11	-14.58983	-47.64247
Sao Joao da Alianca	12	-14.55567	-47.62864
Sao Joao da Alianca	13	-14.66200	-47.61694
Pontalina	14	-17.36844	-49.32308
Pontalina	15	-17.42067	-49.34783
Pontalina	16	-17.43722	-49.47578
Pontalina	17	-17.432639	-49.343861
Pontalina	18	-17.47481	-49.36353
RESEX Lago do Cedro	19	-14.81950	-51.02256
RESEX Lago do Cedro	20	-14.80461	-51.01589
RESEX Lago do Cedro	21	-14.77994	-51.02394
RESEX Lago do Cedro	22	-14.73919	-51.02156
RESEX Lago do Cedro	23	-14.87206	-51.06839
RESEX Lago do Cedro	24	-14.88897	-51.07581

RESEX Lago do Cedro	25	-14.86747	-51.04156
RESEX Lago do Cedro	26	-14.92092	-51.00069
RESEX Lago do Cedro	27	-14.87550	-50.98183
RESEX Lago do Cedro	28	-14.93686	-51.01292
RESEX Lago do Cedro	29	-14.89706	-50.91275
RESEX Lago do Cedro	30	-14.67114	-50.91275
RESEX Lago do Cedro	31	-14.68308	-50.91078
RESEX Lago do Cedro	32	-14.69419	-50.91192
RESEX Lago do Cedro	33	-14.76358	-50.96386
RESEX Lago do Cedro	34	-14.81922	-50.97678
RESEX Lago do Cedro	35	-14.94911	-51.04225
RESEX Lago do Cedro	36	-14.85725	-50.97950
RESEX Lago do Cedro	37	-14.83167	-51.04433
RESEX Lago do Cedro	38	-14.71903	-50.98869
RESEX Lago do Cedro	39	-14.75278	-51.02239
RESEX Lago do Cedro	40	-14.82606	-51.04197
RESEX Lago do Cedro	41	-14.80156	-51.03114
RESEX Lago do Cedro	42	-14.83142	-51.04342
RESEX Lago do Cedro	43	-14.82658	-51.02747
RESEX Lago do Cedro	44	-14.81506	-51.02106
Caiapônia	45	-17.24072	-51.46756
Caiapônia	46	-17.28936	-51.96222
Caiapônia	47	-17.21556	-51.44256
Caiapônia	48	-17.21067	-51.46839
Pires do Rio	49	-17.44069	-48.40858
Pires do Rio	50	-17.32714	-48.45597
Pires do Rio	51	-17.32286	-48.43756
Pires do Rio	52	-17.40161	-48.35697

Pires do Rio	53	-17.40372	-48.38328
Chapadão do Céu	54	-18.23622	-52.61333
Chapadão do Céu	55	-18.23494	-52.60622
Chapadão do Céu	56	-18.05433	-52.63689
Chapadão do Céu	57	-18.11688	-52.59325
Chapadão do Céu	58	-18.00459	-52.72456
Chapadão do Céu	59	-18.23321	-52.57968
Chapadão do Céu	60	-18.04381	-52.66135
Serranópolis	61	-18.45033	-52.08223
Serranópolis	62	-18.45033	-52.08223
Serranópolis	63	-18.45593	-52.07349
Serranópolis	64	-18.46906	-52.23453
Serranópolis	65	-18.32908	-52.16260
Serranópolis	66	-18.31311	-52.15761
Serranópolis	67	-18.35858	-52.00864
Serranópolis	68	-18.42683	-52.18658
PARNA Sempre Vivas	69	-17.89525	-43.76806
PARNA Sempre Vivas	70	-17.91672	-43.78363
PARNA Sempre Vivas	71	-17.92217	-43.76475
PARNA Sempre Vivas	72	-17.89111	-43.80706
PARNA Sempre Vivas	73	-17.88925	-43.80525
PARNA Sempre Vivas	74	-17.96217	-43.78575
PARNA Sempre Vivas	75	-17.95731	-43.78344
PARNA Sempre Vivas	76	-17.91353	-43.78639
PARNA Sempre Vivas	77	-17.86292	-43.76000
PARNA Sempre Vivas	78	-17.86292	-43.76000
Pontalina	79	-17.493194	-49.276611
Pontalina	80	-17.425703	-49.370136

Pires do Rio	81	-17.302722	-48.308894
Britania	82	-15.366222	-51.123833
Britania	83	-15.455389	-51.070139
PARNA Emas	84	-17.887444	-53.152694
PARNA Emas	85	-17.919389	-52.967694
PARNA Emas	86	-17.921583	-52.967694
PARNA Emas	87	-17.919389	-52.967056
PARNA Emas	88	-17.937611	-53.081861

Phenotype-environment interaction in tadpoles: phenotypic plasticity in ecomorphological guilds

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Abstract

Tadpoles were classified into ecomorphological guilds according to their general morphology, feeding behavior and ecology. Benthic, nektonic and intermediate benthic-nektonic are three of the most common tadpoles ecomorphological guilds and are easily found in lentic and lotic habitats. Individuals that belong to these three ecomorphological guilds have distinguished morphological traits, but they can also adapt its morphology in response to changes in environment through phenotypic plasticity. However, we hypothesized that the degree of morphological variation, in response to environmental changes, would not be equal for different guilds, once these tadpoles were not found in the same microhabitat in the pond. We can predict that individuals from ecomorphological guild that are limited to heterogeneous environment (benthic tadpoles) have higher levels of phenotypic plasticity than individuals that are limited to less heterogeneous environment (nektonic tadpoles). We sampled 35 ponds and collected a total of 46 individuals that belongs to benthic, nektonic and intermediate benthic-nektonic guilds, and five environmental variables of the ponds. We described the morphological variation of tadpoles

using the geometric morphometric approach. To assess the relationship between morphological variation of tadpoles and environmental variables we perform a redundancy analysis (RDA) for each ecomorphological guild. Only for benthic tadpoles we were able to detect a phenotype-environment association supporting our hypothesis. Based on our results, we suggest that ecomorphological guilds of tadpoles are exposed to different levels of environmental heterogeneity, with nektonic and intermediate benthic-nektonic guilds actively selecting the ponds patches that maximized its efficiency. Conversely, once benthic tadpoles are restricted to the bottom of the ponds, these tadpoles were exposed to an more unpredictable environment and have a strong phenotype-environment association, that reflect in a larger morphological variation.

Key Words

Benthic; Habitat selection; Nektonic; Morphological variation; Phenotypic plasticity

Introduction

Orton (1953) was the first researcher to formalize the concept of ecomorphological types and, in her view, the tadpoles types were derived from a generalised lentic-benthic tadpole form. After this seminal work of Orton (1953), several authors tried to organize the morphological diversity of tadpoles according to different classifications based on ecological, geographic, and morphological characters (Van Dijk 1972, Lamotte & Lescure 1989, Altig & Johnston 1989). The most known tadpoles classification was made by Altig & Johnston (1989) and take in

account developmental modes, general morphology (body and tail, eye position, oral apparatus), feeding behavior and ecology.

The benthic ecomorphological guild is probably the largest tadpole guild. This tadpole type can be found in different anuran families, occur in lentic and/or lotic sites, usually occupying the bottom of pools and backwaters in lotic habitats (Altig & Johnston 1989). The general morphology of benthic tadpoles is recognizable by a depressed body, dorsal eyes, low dorsal and ventral fins, with rounded or slightly pointed tip, and keratinized mouthparts present (McDiarmid & Altig 1999). Another larger ecomorphological guild is the nektonic guild that assembles tadpoles that are also found in lentic and lotic habitat, but occupy the water column. These tadpoles have a compressed body, lateral eyes, high fins with pointed tip that can be used as an equilibrium structure named flagellum (McDiarmid & Altig 1999).

Although tadpoles included in one of these two ecomorphological guilds are easily recognizable by distinguished morphological traits, tadpoles in general are known to display a large amount of phenotypic plasticity (Relyea 2002, Miner *et al* 2005). Phenotypic plasticity is a major mechanism of adaptation to environmental heterogeneity (Bradshaw & Hardwick, 1989), and is defined as the capacity of a given genotype to displays different phenotypes in response to environmental changes (Miner *et al.*, 2005). Given the particularities of tadpole's ecomorphological guilds about their ecology, distribution and general morphology, we can expect that individuals which belong to different ecomorphological guilds have different levels of phenotype-environment associations, according a gradient of behavior specialization. We can predict that individuals from ecomorphological guild that are limited to heterogeneous environment have higher levels of phenotypic plasticity than individuals that are limited to less heterogeneous environment. Thus, we expected to find different phenotype-environment

associations, where benthic tadpoles, that are restricted to the bottom of the ponds, should have higher correlation between morphology and environment variation, while nektonic and intermediate benthic-nektonic tadpoles, that uses the water column should have less correlation. To test this hypothesis, we choose three species of the hylidae family, *Hypsiboas albopunctatus*, *Dendropsophus minutus* and *Scinax fuscomarginatus* that belong to benthic, nektonic and intermediate benthic-nektonic ecomorphological guilds, respectively.

Material and Methods

Data acquisition

To test our hypothesis, we sampled 35 ponds, throughout the rainy seasons of 2010/2011, 2012/2013 and 2013/2014, in the State of Goiás, Brazil (Tab.1). We collected tadpoles using a wired dip net with a mesh of 3 mm and 30 cm of diameter that was swiped during one hour in the ponds margins, clumps of aquatic vegetation and water with 1.5m maximum depth. Tadpoles collected were anesthetized, killed and identified in laboratory using taxonomic keys (Rossa-Feres & Nomura, 2006), and deposited in the Zoological Collection of the Federal University of Goiás (ZUFG). The species chosen belong to the same family to avoid the phylogenetic effects that can have played a certain role in determining the evolution of morphological characteristics (*e.g.* Marques & Nomura 2015).

Morphological matrix

To extract the morphological information of tadpoles we choose up to five individual of each species of each pond between Gosner's stages 35 and 42. Then, we positioned the tadpoles in a petri dish, with ultrasound gel, in lateral view and take photographs using a camera positioned with a tripod, to standardize the distance from the camera to the tadpole in 20 cm. From the photographs, we defined and extracted 23 landmarks for shape comparisons (modified from Van Buskirk 2009). Landmarks are anatomical references that represent the shape of organisms and must be recognizable in all individuals sampled (Zelditch *et al.* 2012) and be homologous to ensure biological correspondences (Sneath & Sokal 1973). By the landmark approach, we are able to remove from shape variation any effect of size, position, and rotation (Zelditch et al., 2012), using the Procrustes transformation (Rohlf, 1990). The Procrustes transformation reduces the differences among landmarks configurations from the centroid of the average shape (*i.e.* the average distance among all landmarks from the gravity center; Zelditch et al., 2012). After applying this transformation, we obtained the partial warps scores that represent the deformation in external morphology of tadpoles. To summarize the deformation information, we perform a Principal Component Analysis (PCA) and extract the eigenvectors that represent at least 90% of the total variation. We then used these eigenvectors in posteriorly statistical analysis. The landmarks were digitized with the tpsUtil (Rohlf, 2013) and tpsDig2 (Rohlf, 2013b) programs, and all the others analysis was performed in software PAST (version 3.08; Hammer et al. 2001).

Tadpole habitat descriptors

We described the ponds where the tadpoles were sampled based on local and landscape descriptors: pond margin type (cliffed, plane, sloped, excavated); pond bottom substrate (rock, stones, coarse gravel, gravel, sand, clay, mud and leaf litter); percentage of each vegetation type inside the pond margins (none, submerged, floating, upright herbaceous, shrub, tree and taboa); percentage of each vegetations type at the pond margins (none, herbaceous undergrowth, erect herbaceous, shrub, arboreous and taboa) and percentage of land use within 500m of ponds perimeter (nature vegetation, pasture and crops). All percent estimative was approximated by an semi-qualitative variable to reduce the variation in the estimative of the observer as follow: 0%=0; 1-20%=0.1; 21-40%=0.3; 41-60=0.5%; 61-80%=0.7; 81-100%=0.9. These environmental variables can reflect the pond heterogeneity, the availability of microhabitats and the land change of landscape.

Using the environment descriptors, we created five independent indexes (Tab. 1) based on the Nessiman *et al.* (2008) approach, but adapted for tadpoles as a measure of habitat heterogeneity (Costa and Nomura 2016). To calculate these indexes, for each pond trait (*i.e.* pond marginal type, pond bottom substrate, percentage of each vegetation type inside the pond margins, percentage of each vegetation type at the pond margins and percentage of land use within 500m perimeter of ponds) we assigned a score, that was posteriorly weighted for maximum scores (*i.e.* all features has the same weight in the analysis). Then, we obtained, for each pond trait, an average of all analyzed parameters resulting in a value that range between 0 to 1.

Table 1. Values of environmental index of 44 sites for species. Pond MT= pond marginal type; Pond BS= pond bottom substrate; Veg. IM= vegetation inside pond margins; Veg. PM= vegetation at pond margins.

Sites	Species	Land Use	Pond MT	Pond BS	Veg. IM	Veg. PM
1	*H.albopunctatus*	0.00	0.40	0.23	0.47	0.55
2	*H.albopunctatus*	0.00	0.35	0.28	0.57	0.59
3	*H.albopunctatus*	0.50	0.35	0.28	0.43	0.44
4	*H.albopunctatus*	0.40	0.30	0.13	0.40	0.24
5	*H.albopunctatus*	0.50	0.30	0.13	0.07	0.60
6	*H.albopunctatus*	0.30	0.25	0.15	0.23	0.32
7	*H.albopunctatus*	0.90	0.30	0.23	0.17	0.10
8	*H.albopunctatus*	0.40	0.45	0.40	0.17	0.23
9	*H.albopunctatus*	0.10	0.25	0.15	0.10	0.20
10	*H.albopunctatus*	0.30	0.25	0.20	0.27	0.00
11	*H.albopunctatus*	0.20	0.30	0.18	0.23	0.28
12	*H.albopunctatus*	0.40	0.70	0.28	0.63	0.03
13	*S. fuscomarginatus*	0.50	0.25	0.13	0.13	0.32
14	*S. fuscomarginatus*	0.50	0.25	0.13	0.33	0.40
15	*S. fuscomarginatus*	0.40	0.25	0.13	0.07	0.40
16	*S. fuscomarginatus*	0.50	0.25	0.25	0.47	0.20
17	*S. fuscomarginatus*	0.50	0.45	0.13	0.23	0.56
18	*S. fuscomarginatus*	0.50	0.25	0.13	0.13	0.56
19	*S. fuscomarginatus*	0.50	0.25	0.13	0.30	0.48
20	*S. fuscomarginatus*	0.50	0.40	0.13	0.30	0.36
21	*S. fuscomarginatus*	0.50	0.25	0.20	0.20	0.28
22	*S. fuscomarginatus*	0.40	0.25	0.23	0.23	0.40
23	*S. fuscomarginatus*	1.00	0.70	0.20	0.33	0.43
24	*S. fuscomarginatus*	0.80	0.55	0.23	0.07	0.32
25	*S. fuscomarginatus*	0.60	0.50	0.38	0.50	0.47
26	*S. fuscomarginatus*	0.50	0.50	0.45	0.47	0.56
27	*S. fuscomarginatus*	0.50	0.50	0.15	0.20	0.28
28	*S. fuscomarginatus*	0.00	0.55	0.25	0.67	0.60
29	*S. fuscomarginatus*	0.50	0.70	0.28	0.47	0.40
8	*S. fuscomarginatus*	0.40	0.45	0.40	0.17	0.23
30	*S. fuscomarginatus*	0.50	0.25	0.15	0.33	0.22
31	*S. fuscomarginatus*	0.40	0.25	0.28	0.27	0.01
32	*S. fuscomarginatus*	0.40	0.25	0.13	0.20	0.04
33	*D. minutus*	0.50	0.50	0.13	0.33	0.24
34	*D. minutus*	0.30	0.25	0.20	0.50	0.36
35	*D. minutus*	0.50	0.25	0.13	0.33	0.24
6	*D. minutus*	0.30	0.25	0.15	0.23	0.32
36	*D. minutus*	0.50	0.45	0.29	0.33	0.04

37	*D. minutus*	0.50	0.28	0.13	0.20	0.04
38	*D. minutus*	0.70	0.55	0.20	0.27	0.01
39	*D. minutus*	0.70	0.55	0.20	0.27	0.01
40	*D. minutus*	0.50	0.35	0.25	0.20	0.04
41	*D. minutus*	0.50	0.25	0.10	0.07	0.00
42	*D. minutus*	0.50	0.30	0.13	0.03	0.01
43	*D. minutus*	0.00	0.30	0.15	0.20	0.00
44	*D. minutus*	0.00	0.30	0.28	0.20	0.00

Data analysis

To assess the relationship between morphological variation (phenotypic plasticity) of tadpoles and environmental variables we perform a redundance analysis (RDA) for each species separately. RDA is a constrained ordination analysis that first relates the response variable (morphology) to the explanatory variable (environmental index) by regression. Thereafter, RDA performs a constrained ordination on the predicted values of the response variable (Legendre & Legendre 1998). The significance of the RDA was tested by Monte Carlo permutations. The analyses were made in software R (version 3.0).

Results

We collected a total 12 individuals of *Hypsiboas albopunctatus,* 17 of *Scinax fuscomarginatus* and 13 of *Dendropsophus minutus* in the 35 ponds sampled. The amount of shape variation was described by 46 partial warps, for each species. From these partial warps, we extracted three eigenvectors that accounted for 91.8% of total morphological variation for *Hypsiboas albopunctatus*, four eigenvectors that accounted for 91.7% of total morphological

variation for *Scinax fuscomarginatus*, and seven eigenvectors that accounted for 91.6% of total morphological variation for *Dendropsophus minutus*.

Redundance analysis

The association between morphology and environment was different for each species (Tab.2), with only the benthic tadpole, *Hypsiboas albopunctatus*, presenting a morphological-environment interaction (75% of variation in morphology explained by environment, p=0.009). In *S. fuscomarginatus* (benthic-nektonic) and *D. minutus* (nektonic) the association was not significantly (Tab. 3).

Morphological variation of *H. albopunctatus* had strong association with margin type of the pond and land use (Fig. 1). Vegetation inside pond margins is correlated with RDA axis 2 and pond bottom substrate with RDA axis 1. Vegetation at pond margins is the variable that is less correlated with morphology.

Table 2. Summary of RDA analysis for each species.

	Species	Inertia	Proportion
Total	*H. albopunctatus*	0.5415	1
Constrained		0.1756	0.3244
Unconstrained		0.3658	0.6756
Total	*S. fuscomarginatus*	0.46112	1
Constrained		0.36613	0.794
Unconstrained		0.09499	0.206
Total	*D. minutus*	0.4093	1
Constrained		0.2015	0.4922
Unconstrained		0.2078	0.5078

Table 3. Summary of permutation test for RDA analysis for each species, in bold the result that is statistically significant.

	Df	Variance	F	Pr(>F)
H. albopunctatus	5	0.36613	4.6254	**0.009**
S. fuscomarginatus	5	0.17564	1.4404	0.166
D. minutus	5	0.20145	1.3572	0.176

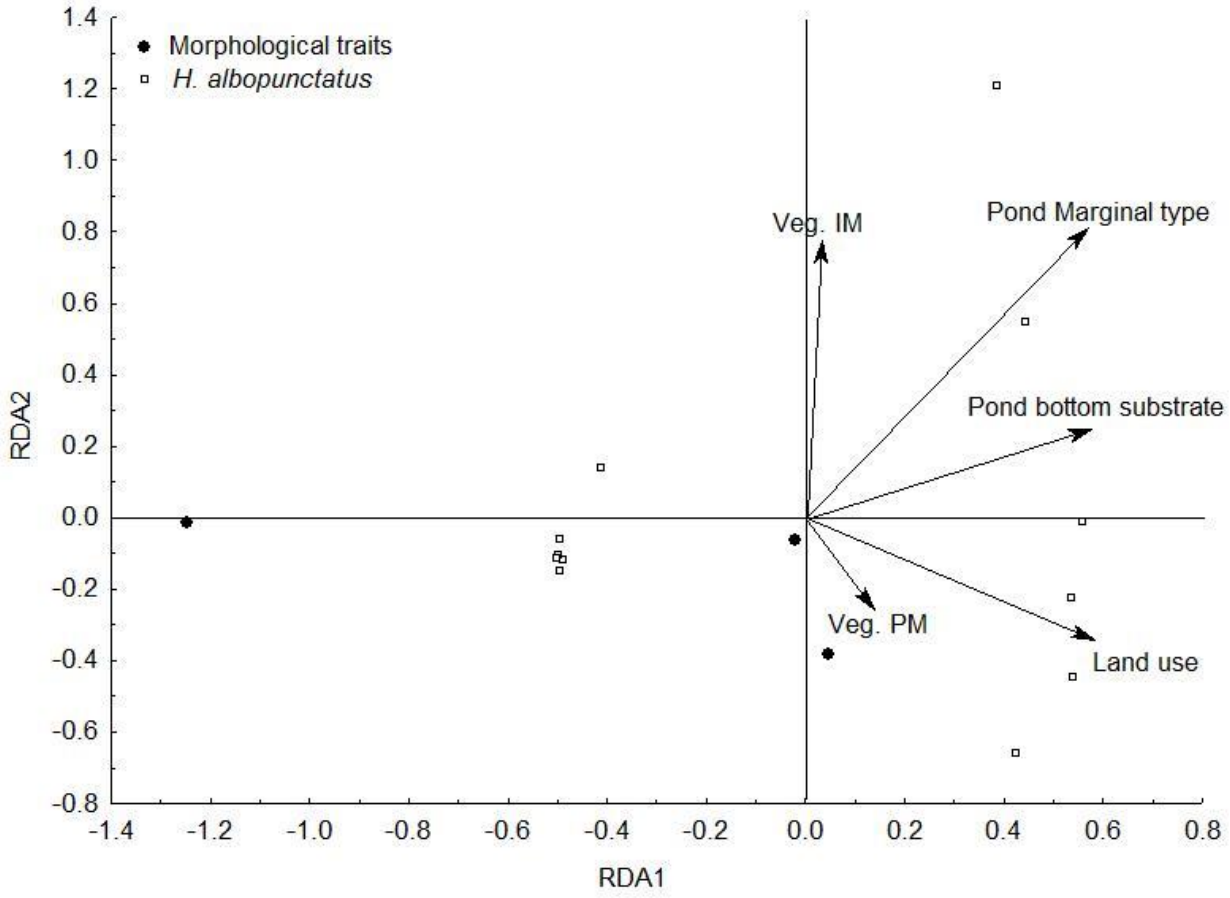

Figure 1. First two axes of a redundance analysis (RDA) to demonstrate the relationship among the morphology of *Hypsiboas albopunctatus*, a benthic species, in relation to five environmental variables

Discussion

We could confirm our hypothesis about different levels of phenotype-environment interaction among tadpoles from different ecomorphological guilds. We found that only the benthic tadpoles of *H. albopunctatus* have a significantly environment-morphology interaction. Nektonic (D. *minutus*) and intermediate benthic-nektonic (*S. fuscomarginatus*) tadpoles does not have a significantly phenotype-environment interaction.

Phenotypic plasticity is a major means of adaptation to environmental heterogeneity and biotic pressures (*e.g.* predation and competition; Miner *et al.* 2005). Tadpoles is known to have high levels of phenotypic plasticity and is an important mechanism to adaptation to different microhabitats. Our results show that tadpoles of *Hypsiboas albopunctatus* have morphology-environment interactions and they are found generally among the marginal vegetation and, in a lower proportion, in lentic ponds (Rossa-Feres & Nomura 2006). Vegetation inside pond margin and pond marginal type had the higher contribution to explain *H. albopunctatus* morphological variation. These traits increase microhabitat complexity and provide feeding opportunity and refuge from predators. Benthic tadpoles spend long time periods dwelling in the bottom of the pond, and some tadpoles of anuran species could display behavioral (*e.g.* substrate color selection) mechanisms to evaluate the substrate of the pond's bottom (*e.g. Physalaemus gracilis*; Ximenes *et al.* 2012) for selecting suitable microhabitats. In such context, the ability to perform morphological changes to fine-tune their phenotypes to the environmental conditions could prevent decisions errors by tadpoles and adults. Another factor that had a correlation with morphology of benthic tadpoles was land use around pond's perimeter. In this case, these modifications could be related to allochthones contaminants originating from pasture and short cycle crops. Herbicides and pesticides directly impact aquatic organisms like tadpoles, which can

have morphological changes induced by these agrochemicals (Costa & Nomura 2015). However, we are unable to quantify and differentiate the morphological changes that were resultant of local adaptation to pond's traits from changes induced by agrochemicals.

All of these environmental descriptors are directly related to microhabitat heterogeneity and reflect the structural complexity of the ponds in the vertical strata. Environmental heterogeneity in microhabitat presents a fundamental challenge for tadpoles and there are several ways for species to respond to environmental heterogeneity including phenotypic plasticity, habitat selection and ecological generalization. According to our results, seems that benthic tadpoles, as *H. albopunctatus*, respond through phenotypic plasticity and we can found phenotype-environment association in those species. Nektonic and intermediate benthic-nektonic tadpoles may respond to environmental heterogeneity through habitat selection, once they live in water column and dense vegetation can provides site refuges and feeding opportunities. However, dense vegetation can imposes barrier to swimming performance and difficult to escape from predators.

Our data suggest that ecomorphological guilds of tadpoles have different ways to respond to environmental heterogeneity, which is expected, once they have distinct requirements. *Hypsiboas albopunctatus* tadpoles are more generalist and we were able to detect a phenotype-environment association. *Dendropsophus minutus* and *Scinax fuscomarginatus* tadpoles are more habitat specialists and habitat selection is playing a role in respond to environmental heterogeneity.

Acknowledgments

We thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for fellowships. We are grateful to Girinos do Brasil (SISBIOTA: grants CNPq 563075/2010-4 and FAPESP 2010/52321-7) for the financial support.

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CONCLUSÃO GERAL

Nosso estudo apresenta uma contribuição ao entendimento de como a ocorrência e a composição de espécies podem interagir com as variáveis abióticas do hábitat e também sua relação com a morfologia dos indivíduos. Girinos de anuros são conhecidos por sua alta plasticidade fenotípica, permitindo a eles se adaptarem as mais diversas mudanças no meio. Pressões bióticas, como predação e competição, são as mais conhecidas de promoverem mudanças induzidas na morfologia dos indivíduos. Nosso estudo mostra que fatores abióticos, por exemplo presença de vegetação e uso da terra ao redor da poça, também podem estar agindo mudando o fenótipo dos indivíduos e determinando a ocorrência e a composição das assembleias.

Fatores espaciais também são determinantes em assembleias de girinos, sendo um grupo que possui baixa capacidade de dispersão. Girinos estão, em geral, limitados a poça que foi previamente escolhida pelo adulto, e processos neutros como nascimentos e mortes aleatórias na população podem operar nas assembleias. Abordamos as guildas ecomorfológicas dos girinos, uma proposta de agrupamento das espécies com base em semelhanças morfológicas, ecológicas e comportamentais, e sugerimos que em guildas maiores pode haver variação morfológica atreladas as variáveis ambientais, enquanto nas menores mudanças no comportamento de forrageio e na seleção de hábitat são mais comuns.