

How close is microhabitat and diet association in aquatic ecomorphotypes? A test with tadpoles of syntopic species

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Abstract Tadpoles are important components of food webs at aquatic ecosystems; however, their trophic roles remain understudied compared to fish. Morphology indicates general aspects of tadpole diets (e.g., food size), but a fine association between preferred microhabitats and diet composition remained to be tested. We tested whether syntopic tadpoles differed in microhabitat use and in diet composition according to food availability in different microhabitats. We also tested whether types of microhabitats were consistent in food availability or if food availability would vary among streams. Considering volumes of different food groups ingested, the diets of tadpoles could be partly explained by the microhabitats where they were sampled, but not by streams or even species.

Considering number of items of each food group, the diet of tadpoles varied according to stream, microhabitat, and species. Volumes of items of each food group differed among microhabitats, but not among streams. All the studied species could be classified as primary consumers based on the dominance of algae (especially Zygnematophyceae and Bacillariophyceae) in their diets. Patterns of food consumption of tadpoles were complex and responded to variables at a very fine spatial scale, suggesting that they may be sensitive even to small alterations in stream dynamics.

Keywords Tadpole diet · Streams · Trophic role · Habitat heterogeneity

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Introduction

Tadpoles are important components of food webs in aquatic ecosystems (Schiesari et al., 2009). They occupy different habitats and have a variety of shapes related to a multitude of important ecological functions (Altig et al., 2007; Liess et al., 2015). Tadpoles are likely to process and transform basal resources, for instance breaking ingested items into smaller particles. Tadpoles are also likely to influence population dynamics of other organisms (Seale, 1980; Osborne & McLachlan, 1985; Altig et al., 2007), and even preclude the establishment of invasive species

(Cabrera-Gúzman et al., 2013). Despite their likely important role in aquatic ecosystems, tadpoles are neglected in studies focusing on aquatic food webs if compared to other groups such as fishes and macroinvertebrates (Altig et al., 2007). In fact, even the trophic level of tadpoles remains uncertain (Altig et al., 2007), and knowing a species trophic level is fundamental to understand its role in a community (Schiesari et al., 2009).

A species diet analysis conducted in its natural habitats is fundamental to place it in the trophic web and unveil relationships among populations (Altig et al., 2007; Liess et al., 2015). Tadpoles can consume periphytic algae, filter or capture particles in the water column (Wassersug, 1972; Sousa-Filho et al., 2007), eat organic matter, pollen, decomposing leaves and animals, amphibian eggs and larvae (Duellman & Trueb, 1994; Arias et al., 2002; Dutra & Callisto, 2005), larvae of aquatic macroinvertebrates and other arthropods (Altig et al., 2007; Duellman & Trueb, 1994). Periphytic algae constitute important nutrient sources for tadpoles by being caloric and of easy access (Kupferberg, 1997). Algae regularly ingested by tadpoles vary in nutritional value and influence various aspects of growth, development, and metamorphosis, depending on the proportions ingested (Steinwacher & Travis, 1983). Animal items can have a great contribution to growth and production of freshwater omnivores, what likely also applies to tadpoles (Altig et al., 2007), who explore animal food from varied sources (Schiesari et al., 2009).

Aquatic vertebrates such as tadpoles (Altig & Johnston, 1989) and fishes (Lund et al., 2015) show morphological variations that can be associated to their potential use of the fine-grained habitat diversity (microhabitats). Such microhabitats likely offer different resources to the individuals that use them, so that realized niche can be inferred from morphological adaptations (Candioti, 2007; Lund et al., 2015). Thus, studies on the relevance of tadpole morphology and/or microhabitat use to predict diet would aid to our understanding of their trophic roles.

If tadpole ecomorphotypes really represent an association among morphology, habitat use and diet (Altig & Johnston, 1989), microhabitats could be expected to vary in food availability for tadpoles and the diet of tadpoles should reflect (at least to some extent) the resources available in the microhabitats used by them. Although many aspects of tadpole

morphology have been related to diet composition (Candioti, 2007), studies relating diet composition to microhabitat use are lacking.

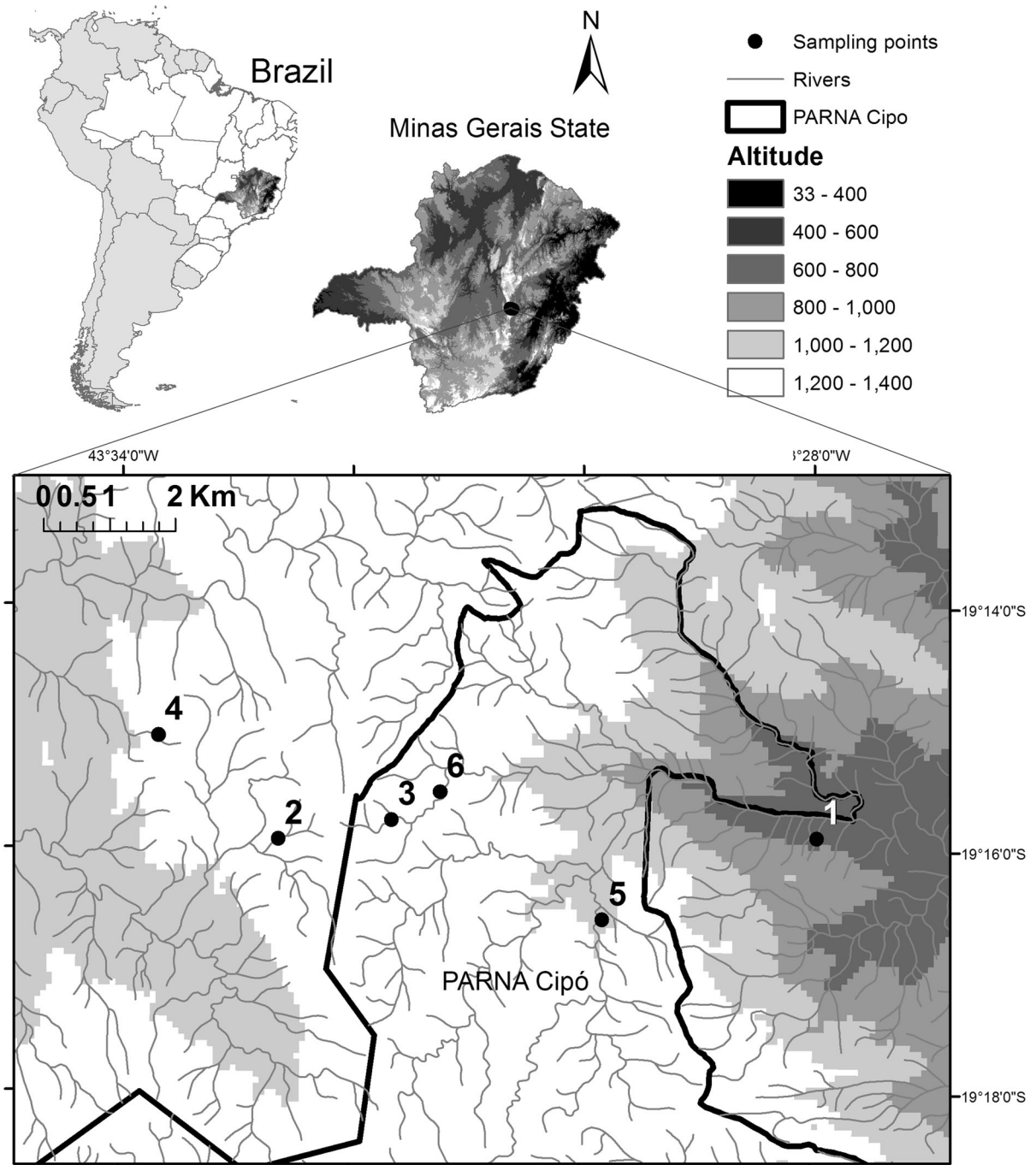
Differences in gut contents of syntopic tadpole species can result from passive foraging at different microhabitats or different food preferences (Zhou et al., 2005). In this case, foraging tadpoles could select microhabitats where their preferred food items are abundant or select specific preferred items at any microhabitat, thus not reflecting microhabitat food availability. In the latter scenario any preference for specific microhabitats could be driven by resources other than food (e.g., temperature; Wu & Kam, 2005). We explored these hypotheses using six streams and five syntopic tadpole species. Because we wanted to focus on the relationship between microhabitats and diet, we used in our study mostly species belonging to the same ecomorphotype (lotic, benthic). This way we minimized direct effects of morphology on diet composition (Candioti, 2007). If there is an association among morphology, microhabitat and diet, as previously recognized for tadpoles (Altig & Johnston, 1989), focusing on the same ecomorphotype would clarify the importance of the specific association between microhabitat and diet. Only one of the species studied has a lentic, suspension-rasping tadpole, which we expected to be the most differentiated within the group.

We attempted to find out whether (1) syntopic tadpoles differ in microhabitat use and diet (2) types of microhabitats used by tadpoles differ in food availability, (3) specific types of microhabitats used by tadpoles are comparable among different streams regarding food availability, and (4) tadpoles can assume different trophic roles depending on microhabitat-related dietary variations.

Methods

Study site

We conducted the study at six streams located in the montane meadows (above 800 m) of the National Park (Parque Nacional—PARNA) Serra do Cipó and the Área de Proteção Ambiental (APA) Morro da Pedreira in the southern portion of the Espinhaço Mountain Range, southeastern Brazil (Fig. 1). The Espinhaço is considered a Unesco Biosphere Reserve with unique



Author: Paula C. Eterovick. Sources: Instituto Pristino, SEMAD, Embrapa

Fig. 1 Location of six montane meadow streams where tadpoles were sampled for diet analyses at the PARNA Serra do Cipó and its surroundings (APA Morro da Pedreira), southeastern Brazil

fauna and flora and a great number of endemic species (Saadi, 1995; Davis et al., 1995; Leite et al., 2008). The climate presents strong cyclic seasonality in

relation to rainfall, with a dry season from April to September and a rainy season from October to March. Streams at the study site are considered as

oligotrophic, having 77–137% dissolved oxygen saturation, 5.5–6.4 $\mu\text{S}/\text{cm}$ conductivity, total alkalinity around 0.03 mEq/l and low nutrient concentrations (Mendes, 2003). The studied streams are permanent and belong to the Doce River Basin.

Sampling procedures

We sampled streams encompassing the spectrum of width, bottom composition (sand/sediments vs. rocks) and riparian vegetation (distribution of herbaceous, shrubby and arboreal vegetation) available at the study site. In order to compare species and to compare microhabitats among streams, we needed to collect all data within the shortest time interval possible and within the same season. Taking this into account, we were able to collect enough data for tadpoles of five species occurring simultaneously and in syntopy in six streams (Fig. 2). From these, *Bokermannohyla alvarengai* (Bokermann, 1956), *B. nanuzae* (Bokermann & Sazima, 1973), *B. saxicola* (Bokermann, 1964), and *Oloolygon machadoi* (Bokermann & Sazima, 1973) can be considered as belonging to the lotic, benthic ecomorphotype and *Pithecopus megacephalus* (Miranda-Ribeiro, 1926) can be considered as lentic, suspension-rasper (sensu Altig & McDiarmid, 1999). Although the tadpole of *P. megacephalus* occurs in streams, it usually avoids current, remaining in backwaters (JSK, PCE, pers. obs). Among the *Bokermannohyla* tadpoles included, *B. saxicola* has the most muscular tail, indicating ability to swim in flowing water, whereas the other *Bokermannohyla* tadpoles are more likely to be found in backwaters (JSK, PCE, pers. obs). We conducted the samplings from December 2015 to March 2016.

We sampled a 150-m-long section of each stream. We divided this section in ten 15-m subsections and randomized sampling order each day. We carefully inspected these sections for tadpoles and collected the first ten tadpoles in developmental stage 25 (sensu Gosner, 1960) of each of the studied species at each microhabitat for posterior analyses of gut contents. Tadpoles usually explore more than one microhabitat type, but we assumed that our sampling design would represent the most used microhabitats for each species because those are the ones where they would most likely be found. Just one person performed the searches to avoid unnecessary disturbance to tadpoles.

The water is clear at the streams and allowed us to see tadpoles from a distance and make sure they did not move before our approach. We recorded data on microhabitat use for all tadpoles found. We classified microhabitats in eight types (Fig. 2B) based on the combinations of (i) presence (V) or absence (nV) of aquatic vegetation (or vegetation from the margins submerged in the water), (ii) presence (C) or absence (nC) of current, and (iii) bottom type being either rock (R) or sand/sediment (S). We then sampled microhabitats for food availability for tadpoles scrubbing a small brush on available surfaces to dislodge periphyton within a 20×20 cm square and rapidly collecting 30 ml of the water with a syringe. At sections with water current, we were careful to perform this food availability sampling moving upstream to avoid sampling food items dislodged from one microhabitat at another. Water samples were preserved in 70% ethanol.

Tadpoles collected for analyses of gut contents were immediately killed by anesthetic overdose via immersion in lidocaine 10% and preserved in formalin (10%). Tadpole developmental stage was confirmed in a stereomicroscope before analyses. We then extracted tadpole gut and selected its anterior third corresponding to the manicoto and most of the small intestine (sensu Pryor & Bjorndal, 2005). Previous work has shown tadpole food items to be the most preserved in this portion of the gut (Kloh et al., 2018), where digestion is not complete. The material extracted was diluted in 1 ml of distilled water and placed in a Sedgewick-Rafter counting chamber. We used a microscope with $\times 400$ amplification to identify and count food items. We estimated volume of food items as the volume of the most similar geometrical solid(s) either isolated or in combinations following Sun and Liu (2003). We calculated the volume of 20 individual items of each species/food type, calculated the mean volume and then extrapolated to the total number of items recorded. Species of algae had been previously inventoried and identified (Kloh et al., 2018). We used 1 ml of each sample of water for analyses of item availability following the same procedures described for tadpole gut content analyses.

Statistical analyses

We analyzed diet data based on number of individual items and volume consumed separately to account for

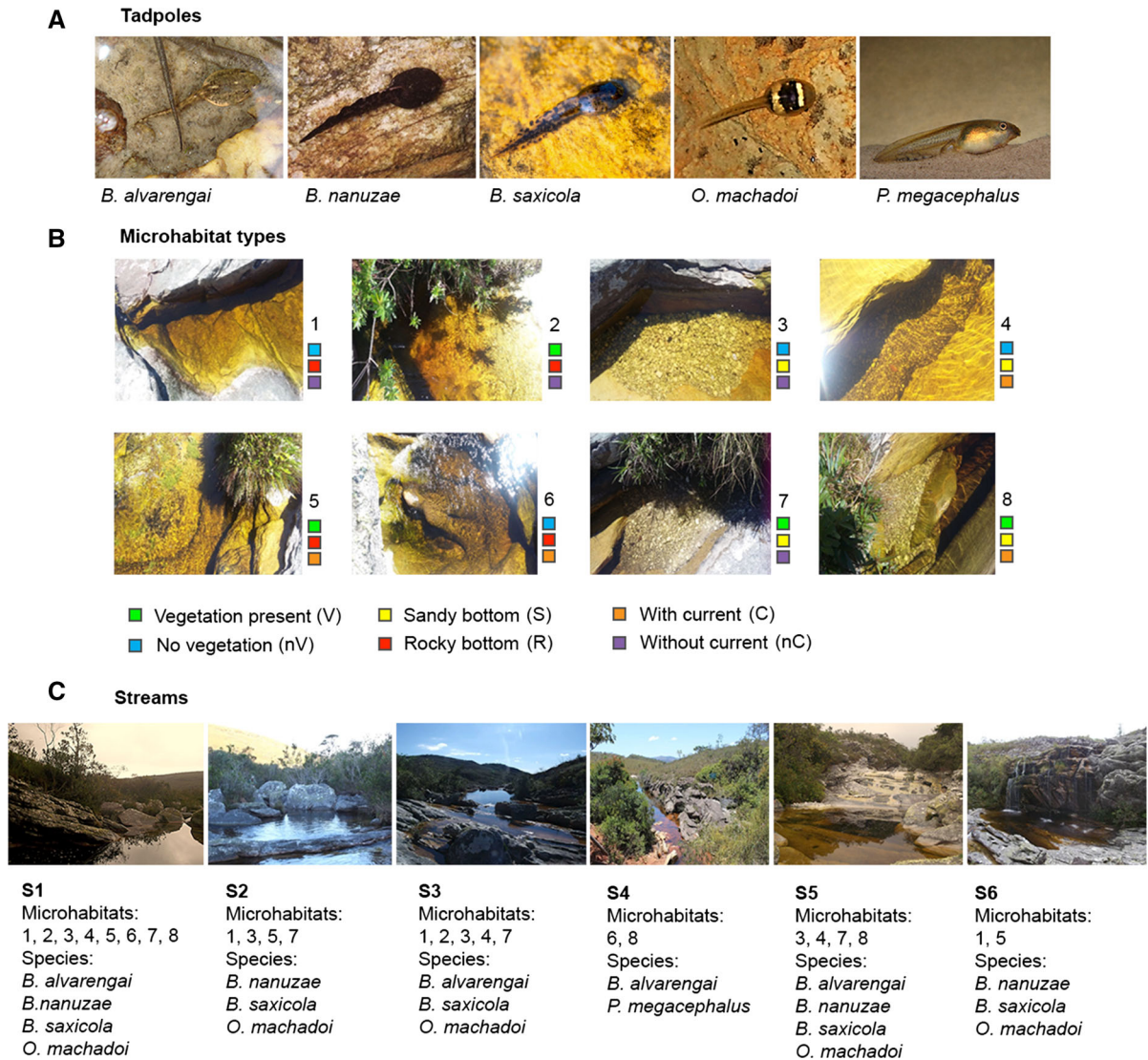


Fig. 2 Study system showing **A** tadpoles of five species, **B** eight microhabitat types according to our classification system and **C** six streams with the lists of microhabitat types and species of tadpoles recorded in each one of them

the effects of size variation among the seven groups of food items considered: filamentous and unicellular diatoms (Bacillariophyceae), Cyanophyceae (Cyanobacteria), pollen, Tecamoeba, filamentous and unicellular green algae (Zygnematomyceae). For each of these two approaches, we had a total of 58 entries representing each species at each microhabitat in each stream (that is, the recorded combinations of five species, eight microhabitats, and six streams). Each entry included seven values corresponding to the mean values of either volume or number of items of each food group ingested by the set

of 10 tadpoles of a given species at a specific microhabitat in a specific stream. We wanted to know the effects of microhabitats and streams on variations in diet composition (food types and their abundances) and how they differed among species. Thus, in order to represent this complex variable (tadpole diet), we first compared tadpoles of different species to test whether they differed regarding food consumption. We represented these data in a Non-Metric Multidimensional Scaling (NMDS) using metaMDS function in the package MASS (Venables & Ripley, 2002) for R (R Core Team, 2015). We used the same data to conduct a

PERMANOVA in the package Vegan (Oksanen et al., 2016) using species as explanatory variable for diet composition. We then compared diets of tadpoles based on microhabitats used, regardless of species. If microhabitat determines tadpole diet, we expected tadpoles using the same microhabitat type(s) to have the most similar diets. For this purpose, we conducted another PERMANOVA using microhabitat as explanatory variable. Finally, we compared diets of tadpoles among streams, to test whether stream would be the most important determinant of tadpole diet (this could happen if streams differed markedly in food availability, regardless of available microhabitats). For this, we conducted another PERMANOVA with stream as explanatory variable. In order to account for possible effects of interactions among variables and to identify what specific species, microhabitats and streams diverged more in terms of tadpole diet composition, we calculated vector lengths based on 1000 simulations in the R package RRPP (Collyer & Adams, 2018). We used Chi-square transformation and residual randomization in order to fit our high-dimensional data (diet data) to linear models.

Next, we analyzed variations in the availability of potential tadpole food items. We represented data on item quantification at each sampled microhabitat in another NMDS. In this case, we had 25 entries representing the microhabitats available in each stream (the existing combinations of eight microhabitats and six streams). Each entry had the mean values for each food group at a given microhabitat in a given stream. We tested whether microhabitat type or stream best explained the observed differences with two PERMANOVAs using microhabitat type and stream as explanatory variables, respectively. We adopted the level of significance of $P \leq 0.05$.

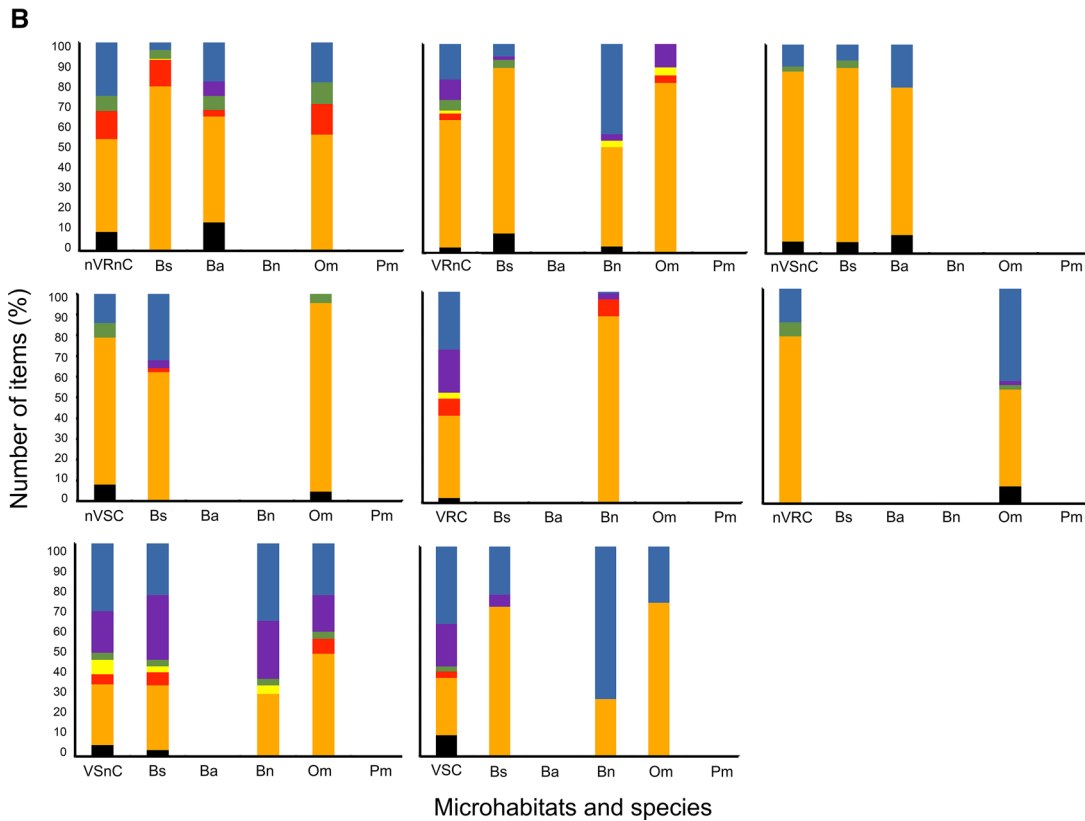
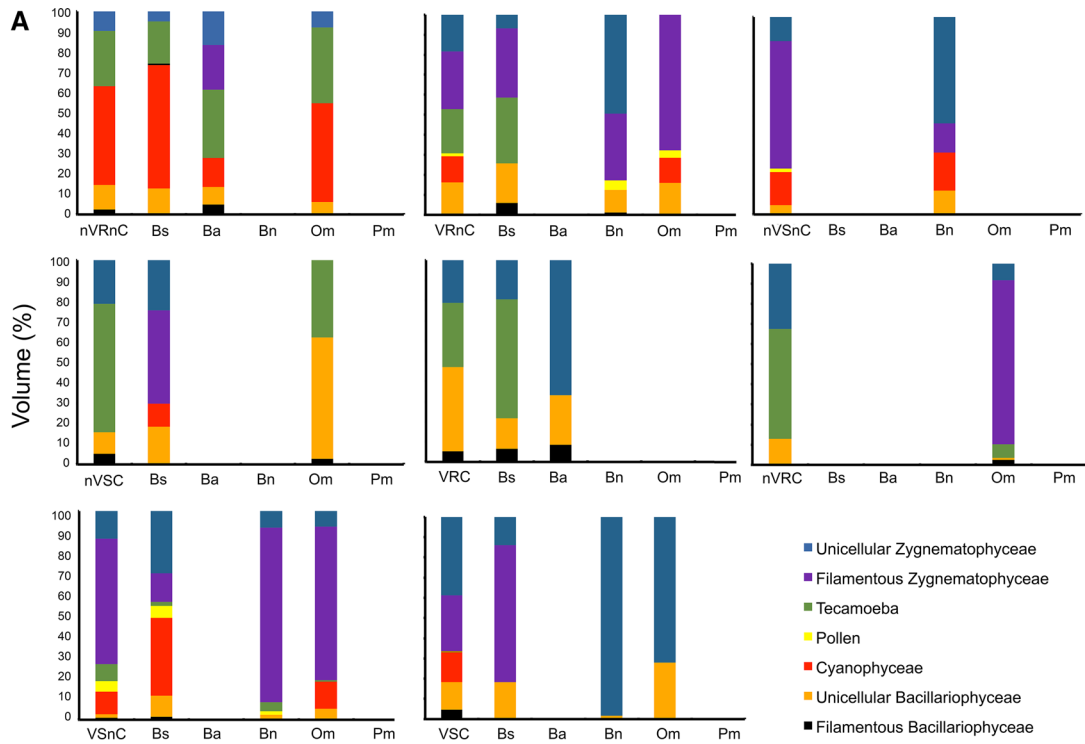
Results

The same food groups that were recorded in the microhabitats examined were also present in the diets of tadpoles (Figs. 3, S1–S5). Although Zygnematomyxaceae (Charophyta) and Bacillariophyceae (Bacillariophyta) were the most consumed groups independently of the quantification unit (individuals or volume), the results for other items were generally different depending on the unit. There is no consensus

Fig. 3 Relative composition of tadpole diets and available food items at eight microhabitats in Stream 1 based on **A** volume of food groups and **B** number of items. Results for the other streams had similar patterns and are presented as supplementary files. Bs, *Bokermannohyla saxicola*; Ba, *B. alvarengai*; Bn, *B. nanuzae*; Om, *Ololygon machadoi*; Pm, *Pithecopus megacephalus*. Microhabitats are defined as in Fig. 2 according to combinations of vegetation presence (V) or absence (nV), rocky (R) or sandy/silty (S) bottoms, and current presence (C) or absence (nC)

on whether number of items or volume is the best to describe diet; thus we present both data here.

Based on volume, the PERMANOVA indicated tadpole diets not to differ among species ($MS = 0.209$, $F = 1.13$, $df = 4$, $R^2 = 0.078$, $P = 0.312$; Fig. 4A) or among streams ($MS = 0.242$, $F = 1.33$, $df = 5$, $R^2 = 0.113$, $P = 0.160$; Fig. 4E), but to differ among microhabitat types ($MS = 0.331$, $F = 1.96$, $df = 7$, $R^2 = 0.216$, $P = 0.010$; Fig. 4C). Analyses of transformed residuals, however, showed isolated effects of species ($P = 0.158$), microhabitats ($P = 0.066$), and streams ($P = 0.061$) not to be significant, but the interactions among them were all significant in explaining tadpole diet variation (species \times stream: $P = 0.005$; stream \times microhabitat: $P = 0.003$; species \times microhabitat: $P = 0.011$). *Bokermannohyla saxicola* ($d = 0.791$, $P = 0.001$) and *Ololygon machadoi* ($d = 0.721$, $P = 0.005$) had the most divergent diets (largest residuals compared to estimated values). The diets of tadpoles occupying microhabitats nVRC, VSnC, and VSC were the most divergent ($d = 1.515$, $P = 0.038$; $d = 0.800$, $P = 0.032$; $d = 1.143$, $P = 0.003$; respectively). Diets of tadpoles in streams 2, 3, 5, and 6 were the most divergent ($d = 0.939$, $P = 0.002$; $d = 0.774$, $P = 0.004$; $d = 0.785$, $P = 0.004$; $d = 0.921$, $P = 0.048$; respectively). Larger volumes of filamentous Bacillariophyceae were consumed at microhabitats VRnC, nVSnC, and nVRC (see Fig. 2 for microhabitats), and larger volumes of unicellular Bacillariophyceae were consumed at microhabitats VRnC, nVSC, and nVSnC. Larger volumes of Cyanophyceae were consumed at microhabitats nVRnC, VSnC, and nVSnC, and there was no consumption at microhabitat nVRC. Pollen was mostly consumed at microhabitats VRC, VSnC, and VSC, and not consumed at microhabitat nVSC. *Tecamoeba* were mostly consumed at microhabitats nVRnC and VRnC. Filamentous



Zygnematophyceae were mostly consumed at microhabitats VSnC, nVRC, and VSC, and unicellular Zygnematophyceae were consumed in similar amounts among microhabitats in comparison with the other food groups (Fig. 4A, Table 1).

Based on number of items, tadpole diets differed among species (MS = 0.268, $F = 2.31$, $df = 4$, $R^2 = 0.148$, $P = 0.013$; Fig. 4B), microhabitat types (MS = 0.347, $F = 3.62$, $df = 7$, $R^2 = 0.336$, $P = 0.001$; Fig. 4D), and streams (MS = 0.263, $F = 2.45$, $df = 5$, $R^2 = 0.193$, $P = 0.007$; Fig. 4F). The effect of species ($P = 0.001$), microhabitat ($P = 0.001$) and stream ($P = 0.001$) were important by themselves to explain variations in tadpole diet composition. Interactions of species with microhabitats ($P = 0.001$), species with streams ($P = 0.001$), and microhabitats with streams ($P = 0.001$) were also all significant. *Bokermannohyla saxicola* ($d = 0.221$, $P = 0.004$), *Ololygon machadoi* ($d = 0.156$, $P = 0.045$), and *Pithecopus megacephalus* ($d = 0.198$, $p = 0.012$) had the most divergent diets (largest residuals compared to estimated values). The diets of tadpoles occupying microhabitats nVRnC, nVRC, VSnC, and VSC were the most divergent ($d = 0.244$, $P = 0.017$; $d = 0.286$, $P = 0.005$; $d = 0.237$, $P = 0.016$; $d = 0.214$, $P = 0.036$; respectively). Diets of tadpoles in streams 2, 3, 4, 5, and 6 were divergent ($d = 0.233$, $P = 0.006$; $d = 0.211$, $P = 0.012$; $d = 0.388$, $P = 0.001$; $d = 0.175$, $P = 0.052$; $d = 0.308$, $P = 0.001$; respectively). *Bokermannohyla alvarengai* ate the largest number of unicellular Bacillariophyceae (Figs. 3B, S2B–S4B). This species occurred in Streams 1, 3, 4, and 5, and used microhabitats nVRnC, nVSnC, nVSC, VSnC, and VSC. *Bokermannohyla nanuzae* ate a remarkably greater amount of pollen than the other species, as well as the largest number of unicellular Zygnematophyceae (Figs. 3B, S1B, S4B, S5B). This species occurred in Streams 1, 2, 5, and 6 and used microhabitats nVRnC, VRnC, VRC, VSnC, and VSC. *Bokermannohyla saxicola* ate the smallest amount of unicellular Zygnematophyceae (Figs. 3B, S1B, S2B, S4B, S5B). This species occurred in all studied streams but Stream 4 and used all microhabitat types except for microhabitat nVRC. *Ololygon machadoi* ate the smallest number of filamentous Zygnematophyceae and Tecamoeba (Figs. 3B, S1B, S2B, S5B). This species occurred in all streams except for Stream 4, and used all microhabitat types.

Fig. 4 NMDSSs showing tadpole diet composition based on volume (A, C, E) and number (B, D, F) of food items. The shapes shown in gray connect the points representing different species (A, B), different microhabitat types (C, D), and different streams (E, F) in the dataset. The asterisks (**) indicate variables that explained a significant amount of the variation in tadpole diet. Species and streams are represented like in Fig. 2. Microhabitats are defined as in Fig. 2 according to combinations of vegetation presence (V) or absence (nV), rocky (R) or sandy/silty (S) bottoms, and current presence (C) or absence (nC). *UB* unicellular Bacillariophyceae, *FB* filamentous Bacillariophyceae, *CY* Cyanophyceae, *PO* pollen, *TE* Tecamoeba, *FZ* filamentous Zygnematophyceae, *UZ* unicellular Zygnematophyceae

Pithecopus megacephalus had the most divergent diet, with markedly larger consumption of Tecamoeba, filamentous Bacillariophyceae and Zygnematophyceae than the other species (Fig. S3B). We only found this species in microhabitat nVRC of Stream 4 (see Fig. 2), where these items were available although in small amounts in the case of filamentous Bacillariophyceae. On the other hand, it did not eat unicellular Bacillariophyceae and Cyanophyceae (the latter not found in this microhabitat).

Based on volume, availability of potential tadpole food items varied among microhabitat types (MS = 0.334, $F = 2.30$, $df = 7$, $R^2 = 0.486$, $P = 0.004$; Fig. 5A) but not among streams (MS = 0.230, $F = 1.19$, $df = 5$, $R^2 = 0.239$, $P = 0.266$; Fig. 5C). We recorded large volumes of filamentous Bacillariophyceae at microhabitats nVRnC, nVSnC, nVSC, and VSC (see Fig. 2 for microhabitat types). Unicellular Bacillariophyceae were always present and occurred in large volumes in microhabitats nVRnC, VRnC, nVSnC, and VSC. We did not record Cyanophyceae at microhabitats nVSC and nVRC, but recorded large volumes at microhabitats nVRnC, VRnC, VRC, VSnC, and VSC. Microhabitats nVRnC, nVSC, and nVRC presented no pollen. Tecamoeba occurred in large volumes at microhabitats nVRnC, VRnC, and nVRC. We recorded large volumes of filamentous Zygnematophyceae at microhabitats VRnC, VRC, VSnC, and VSC. Unicellular Zygnematophyceae were ubiquitous and abundances did not vary greatly among microhabitats (Fig. 5A, Table 1). The variation in numbers of items was explained by stream (MS = 0.119, $F = 1.98$, $df = 5$, $R^2 = 0.342$, $P = 0.023$; Fig. 5A) but not by microhabitat type, although differences among microhabitat types

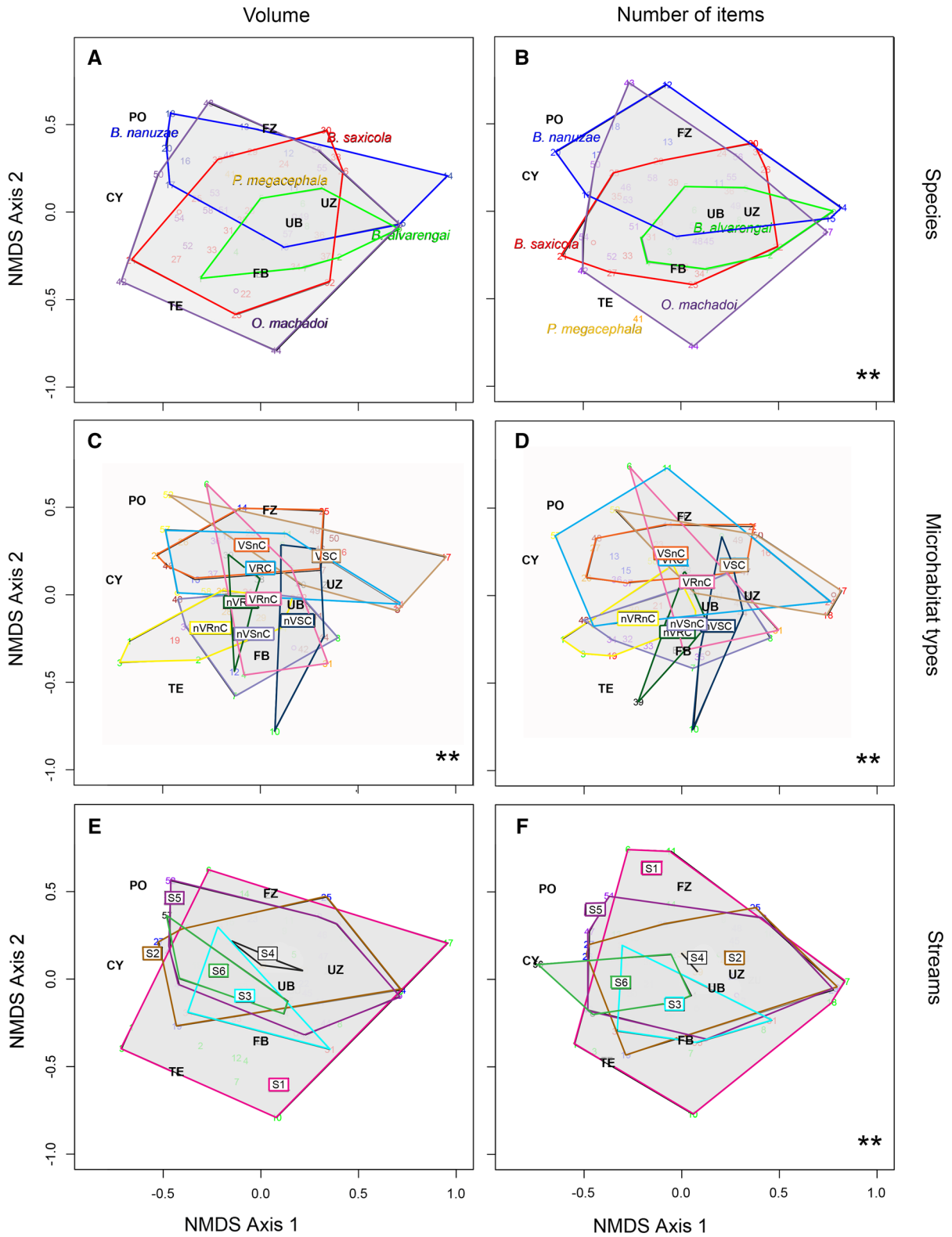


Table 1 Means \pm SD of number of items and volumes of types of food consumed by tadpoles and recorded at each of eight stream microhabitats (identified as in Fig. 2)

| Microhabitats | nVRnC | | | nVSnC | | | nVSC | | |
|-------------------------------|-------------------------|---------------------|------------------------|-----------------------|-----------------------|-----------------------|----------------------|-----------------------|-----------------------|
| | Available | Consumed | Volume | Available | Consumed | Volume | Available | Consumed | Volume |
| Number of items | | | | | | | | | |
| Filamentous Bacillariophyceae | 145 \pm 107 | 212 \pm 199 | 40 \pm 56 | 40 \pm 43 | 40 \pm 43 | 140 \pm 78 | 124 \pm 85 | 106 \pm 70 | 96 \pm 115 |
| Unicellular Bacillariophyceae | 1215 \pm 172 | 2429 \pm 1089 | 1540 \pm 480 | 640 \pm 164 | 640 \pm 164 | 1415 \pm 774 | 1316 \pm 658 | 1086 \pm 450 | 1233 \pm 563 |
| Cyanophyceae | 217 \pm 145 | 132 \pm 177 | 70 \pm 42 | 5 \pm 10 | 5 \pm 10 | 40 \pm 80 | 60 \pm 92 | 0 | 3 \pm 8 |
| Pollen | 0 | 20 \pm 36 | 50 \pm 14 | 15 \pm 19 | 15 \pm 19 | 20 \pm 28 | 22 \pm 57 | 0 | 0 |
| Tecamoeba | 115 \pm 75 | 154 \pm 89 | 100 \pm 84 | 10 \pm 20 | 10 \pm 20 | 55 \pm 19 | 80 \pm 80 | 113 \pm 41 | 23 \pm 39 |
| Filamentous Zygnematophyceae | 20 \pm 23 | 152 \pm 179 | 240 \pm 84 | 30 \pm 26 | 30 \pm 26 | 55 \pm 110 | 72 \pm 146 | 6 \pm 11 | 60 \pm 82 |
| Unicellular Zygnematophyceae | 405 \pm 298 | 680 \pm 370 | 390 \pm 183 | 195 \pm 252 | 195 \pm 252 | 285 \pm 165 | 364 \pm 173 | 253 \pm 70 | 333 \pm 209 |
| Volume | | | | | | | | | |
| Filamentous Bacillariophyceae | 26,970 \pm 20004 | 7096 \pm 6140 | 7440 \pm 10,521 | 14,920 \pm 16,981 | 14,920 \pm 16,981 | 26,040 \pm 14,566 | 9083 \pm 5962 | 19,840 \pm 13,064 | 5497 \pm 6065 |
| Unicellular Bacillariophyceae | 158,499 \pm 68,377 | 47,335 \pm 21,751 | 225,410 \pm 84,584 | 62,672 \pm 21,098 | 62,672 \pm 21,098 | 172,906 \pm 74,085 | 50,143 \pm 19,568 | 76,041 \pm 19,568 | 52,739 \pm 24,997 |
| Cyanophyceae | 519,117 \pm 351,280 | 60,960 \pm 84,141 | 169,015 \pm 102,438 | 12,072 \pm 24,145 | 12,072 \pm 24,145 | 96,580 \pm 193,160 | 54,970 \pm 84,445 | 0 | 8048 \pm 19,714 |
| Pollen | 0 | 3246 \pm 6753 | 37,725 \pm 10,670 | 7545 \pm 8712 | 7545 \pm 8712 | 15,090 \pm 21,340 | 4401 \pm 10,853 | 0 | 0 |
| Tecamoeba | 156,414.5 \pm 271,917 | 31,445 \pm 41,908 | 210,270 \pm 282,868 | 25,643 \pm 51,286 | 25,643 \pm 51,286 | 60,256 \pm 96,621 | 16,143 \pm 30,886 | 152,140 \pm 223,576 | 10,185 \pm 20,408 |
| Filamentous Zygnematophyceae | 84,048 \pm 105,357 | 67,142 \pm 80,120 | 556,609.5 \pm 34,025 | 119,977 \pm 107,523 | 119,977 \pm 107,523 | 174,636 \pm 349,273 | 77,323 \pm 158,437 | 2906 \pm 5034 | 101,531 \pm 125,493 |
| Unicellular Zygnematophyceae | 115,514 \pm 73,588 | 59,373 \pm 51,949 | 248,639 \pm 130,917 | 57,948 \pm 72,038 | 57,948 \pm 72,038 | 107,512 \pm 54,764 | 51,559 \pm 18,333 | 87,525 \pm 46,952 | 54,812 \pm 38,529 |
| Microhabitats | | | | | | | | | |
| | VRC | | | NVRC | | | VSC | | |
| Food items | Available | Consumed | Volume | Available | Consumed | Volume | Available | Consumed | Volume |
| Number of items | | | | | | | | | |
| Filamentous Bacillariophyceae | 46 \pm 41 | 53 \pm 65 | 20 \pm 28 | 340 \pm 383 | 340 \pm 383 | 70 \pm 82 | 65 \pm 114 | 200 \pm 163 | 14 \pm 25 |
| Unicellular Bacillariophyceae | 980 \pm 419 | 1203 \pm 574 | 1390 \pm 410 | 1013 \pm 1391 | 1013 \pm 1391 | 570 \pm 205 | 654 \pm 456 | 840 \pm 214 | 634 \pm 392 |
| Cyanophyceae | 126 \pm 141 | 110 \pm 123 | 0 | 0 | 0 | 115 \pm 30 | 114 \pm 161 | 126 \pm 25 | 20 \pm 53 |
| Pollen | 80 \pm 20 | 46 \pm 45 | 0 | 26 \pm 23 | 26 \pm 23 | 130 \pm 105 | 112 \pm 217 | 73 \pm 103 | 111 \pm 294 |
| Tecamoeba | 40 \pm 69 | 63 \pm 145 | 90 \pm 70 | 126 \pm 184 | 126 \pm 184 | 60 \pm 59 | 43 \pm 71 | 60 \pm 43 | 17 \pm 45 |
| Filamentous Zygnematophyceae | 293 \pm 363 | 20 \pm 18 | 50 \pm 70 | 300 \pm 242 | 300 \pm 242 | 295 \pm 198 | 220 \pm 149 | 426 \pm 263 | 180 \pm 286 |
| Unicellular Zygnematophyceae | 393 \pm 475 | 317 \pm 354 | 300 \pm 56 | 406 \pm 41 | 406 \pm 41 | 515 \pm 332 | 434 \pm 307 | 746 \pm 512 | 568 \pm 446 |
| Volume | | | | | | | | | |
| Filamentous Bacillariophyceae | 17,360 \pm 18,723 | 3487 \pm 5225 | 3720 \pm 5260 | 8184 \pm 9498 | 8184 \pm 9498 | 13,020 \pm 15,337 | 2970 \pm 4616 | 59,520 \pm 29,526 | 531 \pm 932 |
| Unicellular Bacillariophyceae | 87,958 \pm 74,610 | 37,197 \pm 15,510 | 89,142 \pm 6491 | 19,391 \pm 14,452 | 19,391 \pm 14,452 | 49,757 \pm 15,907 | 27,018 \pm 11,322 | 160,549 \pm 106,571 | 31,993 \pm 20,318 |
| Cyanophyceae | 305,836 \pm 342,598 | 44,265 \pm 40,880 | 0 | 0 | 0 | 277,667 \pm 72,435 | 71,081 \pm 58,614 | 305,836 \pm 73,764 | 9658 \pm 25,552 |

Table 1 continued

| Microhabitats | VRC | | NVRC | | VSnC | | VSC | |
|------------------|---------------------|------------------|-------------------|-------------------|---------------------|-------------------|-------------------|-------------------|
| | Available | Consumed | Available | Consumed | Available | Consumed | Available | Consumed |
| Pollen | 45,270 ± 30,180 | 15,404 ± 13,623 | 0 | 3018 ± 3018 | 98,085 ± 79,372 | 17,284 ± 21,377 | 55,330 ± 95,834 | 16,814 ± 44,487 |
| Tecamoeba | 6834 ± 11,837 | 4698 ± 9229 | 184,627 ± 246,603 | 20,001 ± 27,297 | 73,078 ± 122,972 | 9042 ± 15,431 | 11,960 ± 10,670 | 878 ± 2324 |
| Filamentous | 912,449 ± 1,475,949 | 95,118 ± 136,524 | 122,237 ± 172,870 | 317,011 ± 284,535 | 1,208,327 ± 496,326 | 400,493 ± 333,066 | 778,639 ± 461,248 | 246,865 ± 322,810 |
| Zygnematophyceae | | | | | | | | |
| Unicellular | 273,098 ± 222,800 | 68,456 ± 57,357 | 144,199 ± 97,330 | 37,853 ± 24,142 | 216,789 ± 141,521 | 73,932 ± 38,173 | 364,936 ± 354,367 | 83,149 ± 47,791 |
| Zygnematophyceae | | | | | | | | |

approached the level of significance we adopted ($MS = 0.098$, $F = 1.58$, $df = 7$, $R^2 = 0.394$, $P = 0.068$; Fig. 5A). There was a large amount of variation among microhabitats within streams but, in general, filamentous Bacillariophyceae were more abundant in Streams 1, 5, and 6 (see Fig. 2 for pictures and microhabitats found in each stream). Cyanophyceae were more abundant in Streams 1 and 6. Pollen was more abundant in Stream 5, and Tecamoeba, filamentous, and unicellular Zygnematophyceae were more abundant in Stream 1.

Discussion

Food ingestion by tadpoles differed among species, microhabitats, and streams considering the number of items ingested. However, considering volume of different food types, only differences among microhabitats remained significant in a broad perspective, corroborating the intimate association between diet composition and microhabitat use proposed by Altig & Johnston (1989). The availability of food items also differed among microhabitats considering volume, but not among streams, indicating that there is an association between microhabitats used by tadpoles and food availability/consumption. The food items with the greatest volume recorded at specific microhabitats were frequently also consumed in large volumes by tadpoles sampled at those microhabitats. For instance, filamentous Bacillariophyceae at microhabitat nVSnC, unicellular Bacillariophyceae at microhabitats VRnC and nVSnC, Cyanophyceae at microhabitats nVRnC and VSnC, Tecamoeba at microhabitats nVRnC and VRnC, filamentous Zygnematophyceae at microhabitats VSnC and VSC. On the other hand, sometimes a low availability (or no detection) of a certain food item reflected in no consumption (e.g., pollen at microhabitat nVSC), and similar availabilities resulted in similar consumption of unicellular Zygnematophyceae among microhabitats (see Table 1). Such variation in food availability may respond to physical and chemical features of specific microhabitats, for instance, phosphorus availability. Phosphorus availability is important in freshwater systems (Brezonik & Pollman, 1999) either limiting or triggering algal growth as it becomes available in larger amounts (Reynolds & Davies, 2001).

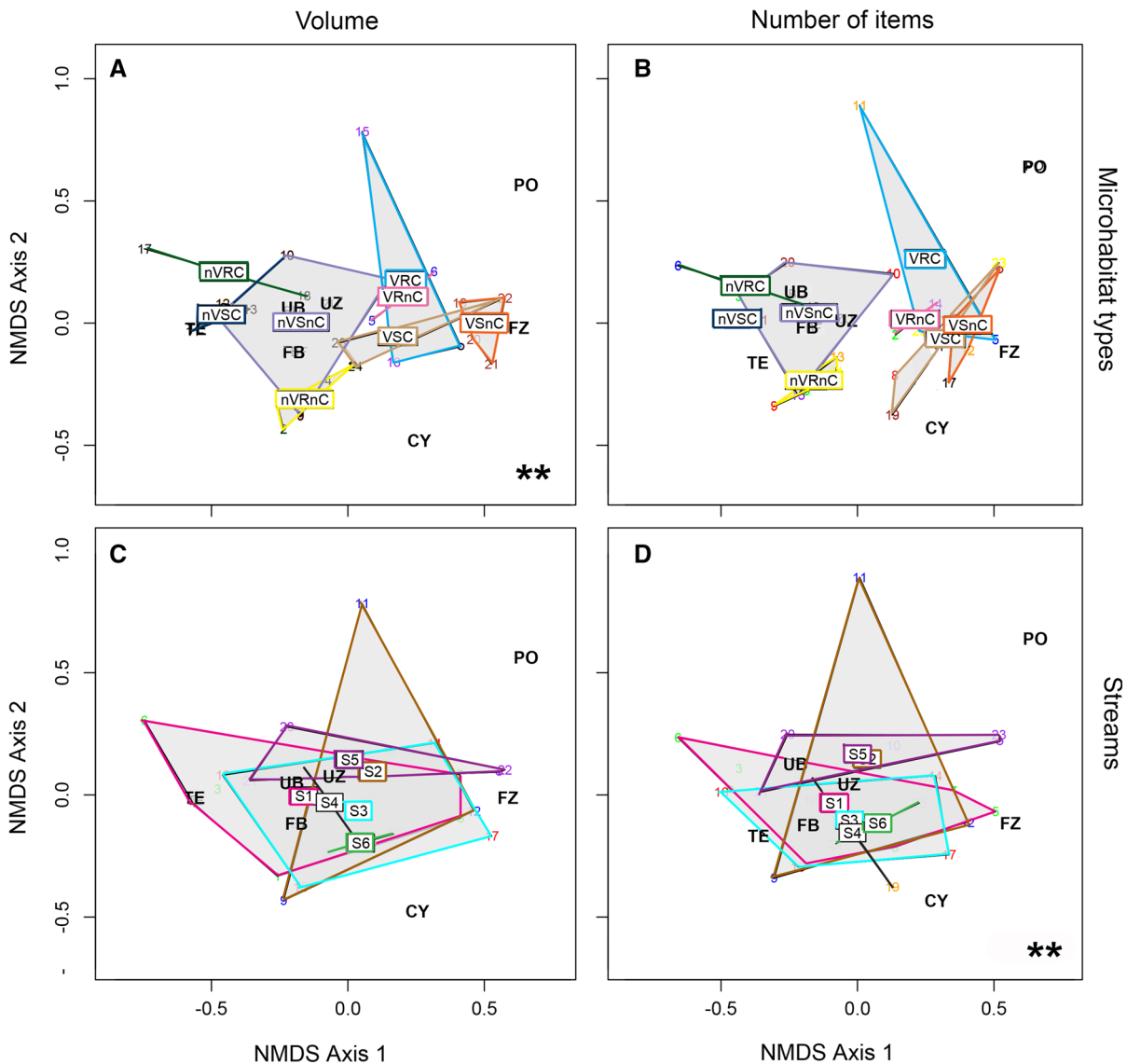


Fig. 5 NMDSs showing availability of potential tadpole food items in eight microhabitat types in six streams based on volume (A, C) and number (B, D) of food items. The shapes shown in gray connect the points representing different microhabitat types (A, B), and different streams (C, D) in the dataset. The asterisks (**) indicate variables that explained a significant amount of the variation in availability of potential food items. Microhabitats and streams are represented like in Fig. 2.

Microhabitats are defined according to combinations of vegetation presence (V) or absence (nV), rocky (R) or sandy/silty (S) bottoms, and current presence (C) or absence (nC). *UB* unicellular Bacillariophyceae, *FB* filamentous Bacillariophyceae, *CY* Cyanophyceae, *PO* pollen, *TE* Tecamoeba, *FZ* filamentous Zygnematophyceae, *UZ* unicellular Zygnematophyceae

When considering number of items instead, streams differed among them but not microhabitats. In this case, the general pattern seemed to reflect variations in the abundance of the highly dominant unicellular Bacillariophyceae (see Fig. 3B, S1B–S5B). Tadpoles tended to keep their guts completely filled, so that

species that ingested larger items ate smaller numbers of them compared to other species that ingested greater amounts of small items. Based on this and the dominance of very abundant small items in tadpole guts and in their microhabitats, we believe focusing on volume of food types is a more accurate approach to

understand dynamics of tadpoles and their consumed items.

The bulk of the diet of tadpoles of all species analyzed was composed by algae, characterizing them as consumers of aquatic primary producers. However, they were also able to explore other food items such as tecamoeba (*Arcella* sp) and pollen. The algae classes Zygnematophyceae and Bacillariophyceae, the most consumed by the tadpoles, have a high energetic content and are sources of carbohydrates, proteins, and lipids that influence production of thyroid hormones responsible for metamorphosis (Sousa-Filho et al., 2007). In fact, tadpoles fed diets enriched with Bacillariophyceae and Zygnematophyceae under laboratory conditions developed faster and metamorphosed earlier than tadpoles fed diets composed by blue-green algae (*Nostoc*) and detritus (Kupferberg et al., 1994).

Tadpoles of *Lithobates* species were shown to have different diets, and also to diversify their diets among lakes with different food availabilities in Michigan (USA; Schiesari et al., 2009). In this case, however, tadpoles were maintained in enclosures and the role of natural microhabitat use on tadpole diet could not be evaluated. In our study, tadpole diets reflected to some extent the availability of food items in the microhabitats used by them (see Figs. 3, S1–S5), but there were also items consumed in proportionally smaller or larger volumes than available. This means that tadpoles can diversify their diets either selecting preferred food items in the microhabitats occupied or exploring different microhabitats to complement their nutritional needs by consuming different food items available. Some level of food selection was also reported for *Lithobates* tadpoles (Schiesari et al., 2009).

Tadpoles represent an important connection between aquatic and terrestrial systems; they take the biomass consumed from water to land when they metamorphose (Sousa-Filho et al., 2007). Thus, even subtle alterations in stream microhabitats (amounts of sediment and nutrients) are likely to affect the biomass and dynamics of the periphyton (Pellegrini & Ferragut, 2012). Consequently, success in tadpole growth and development can be compromised, ultimately affecting terrestrial foodwebs through decreased numbers of successfully metamorphosed froglets. The intimate association observed here between tadpoles and their microhabitats indicate how complex aquatic food webs may be. Several human activities cause

changes in aquatic systems that are likely to be detrimental to aquatic species even before major habitat alterations can be observed. For instance, even moderate siltation can cause microhabitat loss, not to mention pollutants that are frequently washed to lotic systems inhibiting or triggering the growth of different groups of aquatic organisms (Tundisi et al., 2006; Zhu et al., 2008).

In this study, we showed that tadpole diets, considering volume of ingested food types, vary among stream microhabitat types used by them, which also differ in food availability. Similar studies in other systems including other tadpole ecomorphotypes and food types (e.g., animal food) are recommended to further understand the role of microhabitats in tadpole diet. We could observe a close association between microhabitat and diet composition even among tadpoles within a narrow spectrum of morphological variation represented by the species studied here. These results call attention to the complexity of trophic webs in lotic systems. At the community level, tadpoles are likely to regulate populations of algae, reducing their biomass at significant levels (Rosenfeld, 1997). On the other hand, changing amounts of sediment and nutrients can affect periphyton dynamics and biomass as well (Pellegrini & Ferragut, 2012), likely reflecting on growth and development of tadpoles. We observed a fine spatial variation in algae availability and consumption, so that even small alterations in these systems can potentially have marked effects throughout the foodweb and the surrounding terrestrial ecosystems.

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