

Feeding ecology and basal food sources that sustain the Paradoxal frog *Pseudis minuta*: a multiple approach combining stomach content, prey availability, and stable isotopes

Sonia Huckembeck · Daniel Loebmann ·
Edelti F. Albertoni · Sonia M. Hefler ·
Mauro C. L. M. Oliveira · Alexandre M. Garcia

Received: 25 April 2014 / Revised: 9 August 2014 / Accepted: 24 August 2014 / Published online: 31 August 2014
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Abstract In the present study, we investigated ontogenetic diet shifts, feeding strategy, prey preferences, and basal food sources that sustain the Paradoxal frog (*Pseudis minuta*) based on stomach content, prey availability, and stable isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) approaches. The feeding strategy analysis showed that the population can be considered a generalist species with each individual displaying a marked opportunism for different preys. Trophic positions estimated using nitrogen isotopic ratio ($\delta^{15}\text{N}$) revealed that tadpoles are primary consumers, but post-metamorphic individuals shifted to secondary and tertiary trophic levels as they increase in body size. A stable isotopic mixing model revealed that most of the carbon (0.61–0.72) sustaining the post-metamorphic *P. minuta* is derived from the

aquatic rather than the adjacent terrestrial environment. This finding suggests that the post-metamorphic *P. minuta* is strongly dependent on carbon sources that primarily originate in aquatic systems, regardless of the terrestrial or aquatic origins of the arthropods in its diet. Our results indicated that this species is a generalist-opportunistic predator that derives most of their carbon sources from the aquatic environment where it shows preference for aquatic preys with higher individual biomasses.

Keywords Diet · Food chain · Optimal foraging · *Pseudis minuta* · Seasonal variation

Handling editor: Lee B. Kats

S. Huckembeck (✉) · D. Loebmann
Laboratório de Vertebrados, Universidade Federal do Rio Grande (FURG), Rio Grande, Av. Itália, km 8,
Rio Grande, RS CEP 96203-900, Brazil
e-mail: sohuckembeck@yahoo.com.br

E. F. Albertoni · S. M. Hefler
Laboratório de Limnologia, Universidade Federal do Rio Grande (FURG), Rio Grande, Av. Itália, km 8,
Rio Grande, RS CEP 96203-900, Brazil

M. C. L. M. Oliveira · A. M. Garcia
Laboratório de Ictiologia, Universidade Federal do Rio Grande (FURG), Rio Grande, Av. Itália, km 8,
Rio Grande, RS CEP 96203-900, Brazil

Introduction

Prior trophic ecology studies show that amphibians, particularly anurans, have an important function in the ecosystem dynamic and play a crucial role linking the energy flow between aquatic and terrestrial environments (Kupfer et al., 2006). Although extensive literature considers the feeding ecology of anurans (e.g., Brandão et al., 2003; Maneyro et al., 2004; Maneyro & Rosa, 2004; Bonansea & Vaira, 2007; Quiroga et al., 2009), gaps in the knowledge of the trophic ecology of most species exist, especially because most data are fragmented and/or restricted to the description of food items comprising the diet (Achaval & Olmos, 2007). For instance, there is

relative little information on prey availability in studies on Amphibian trophic ecology (Toft, 1980, 1981; MacNally, 1983; Lizana et al., 1986). The absence of this crucial information could lead to erroneous interpretations on feeding strategies, diet seasonality as well as species niche amplitude (Maneyro & Rosa, 2004). Moreover, despite the fact that most amphibians inhabit the interface between aquatic and terrestrial systems, there are few studies addressing the contribution of organic matter in aquatic and terrestrial environments in supporting this group (Finlay & Vredenburg, 2007; Joseph et al., 2011). In addition, although most amphibians have a complex life-history cycle with pronounced changes on their ontogeny, few prior studies have investigated the variation on the diet from larvae to adult stages (Giarretta et al. 1998; Blackburn & Moreau, 2006; Schriver & Williams, 2013).

A relatively recent approach to investigate trophic ecology is the analysis of stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) in food web components that allows us to infer the relative contributions of food sources to consumers and their trophic position in the food chain, respectively (Peterson & Fry, 1987). This approach has been successfully applied to understand the energetic pathways that sustain populations of fish (e.g., Garcia et al., 2007; Hoeinghaus et al., 2011; Claudino et al., 2013), birds (e.g., Sydeman et al., 1997; Hobson, 1999) and mammals (e.g., Stanley & DeNiro, 1986; Kelly, 2000), but comparatively less work has been done with anurans (e.g., Gillespie, 2013).

Like other species of Paradoxal frogs (*Lysapsus/Pseudis* clade, sensu Garda et al. 2010), *Pseudis minuta* Günther, 1858, has a predominantly aquatic behavior. This frog inhabits temporary and permanent ponds, usually with vegetation in which to hide if disturbed (Huckembeck et al., 2012). This species is typical of the Pampa Biome, occurring in Northwestern Argentina, Uruguay, and Southern and Southeastern Brazil (Kwet, 2000). Despite being abundant and widespread in extreme South America, no prior study has been conducted on its feeding ecology. A prior study with a congeneric species (*P. cardosoi*, Kwet, 2000) observed several aquatic prey in the diet (Miranda et al., 2006), which seems to corroborate laboratory observations of individuals actively foraging and capturing aquatic preys such as water bugs, dragonflies, and tadpoles (Solé & Miranda, 2006).

Other studies have reported the presence of aquatic prey in the stomach contents of other species of the genus (Duré & Kehr, 2001; Brandão et al., 2003; Teixeira et al., 2004; Vaz-Silva et al., 2005; Garda et al., 2007; Macale et al., 2008), but none has applied the stable isotope technique to investigate its trophic relationships or either conducted prey availability surveys to evaluate its prey preferences.

Here, we combined the following analytical approaches to investigate the feeding ecology and habitat use of the Paradoxal frog: (a) stomach content analysis to investigate the diet, feeding strategy, and temporal variation throughout the year, (b) field surveys of prey availability to estimate prey preferences by the predator, (c) nitrogen stable isotope ratios to evaluate ontogenetic changes in trophic position, and (d) a stable isotope mixing model to estimate the relative contribution of aquatic versus terrestrial-derived basal food sources to the species. Our integrative approach provided a more in-depth view of the trophic ecology of this Neotropical amphibian species and could be applied to anurans species elsewhere.

Materials and methods

Study area description and field work

The present study was conducted at the Lagoa do Peixe National Park (LPNP), a UNESCO Biosphere Reserve located in the coastal plain of the Rio Grande do Sul, the southernmost state in Brazil. The regional climate is classified as Subtropical humid, with mean temperatures ranging from 14.6°C in the winter to 22°C in the summer. Annual rainfall ranges from 1,150 to 1,450 mm (Seeliger et al., 1998); some years have large variations due to periods of intensive rainfall and prolonged droughts triggered by the *El Nino* Southern Oscillation (ENSO) phenomena (Garcia et al., 2003).

Fieldwork was conducted monthly from April 2008 to May 2009 in an open area with ephemeral and permanent water bodies at sea level with ca. 120 × 500 m (31°06'53" S, 50°51'40" W). The anuran assemblage that inhabits the Lagoa do Peixe National Park presents relatively low diversity, with only 14 anuran species recorded (Loebmann & Vieira, 2005). The land vegetation was dominated by Poaceae

family grasses and a few shrubs, whereas the macrophytes were primarily represented by *Salvinia herzogii*, *Azolla filiculoides*, *Eichornia crassipes*, and *Cabomba* sp. Specimens post-metamorphic were collected by hand during each field trip; after sunset, two people conducted a 2.5 h search for adult Paradoxal frogs in the study area. Tadpoles of *P. minuta* were collected in April 2009 by sweep netting in random areas of the water body in the study area. After the frogs (tadpoles and post-metamorphic) were caught, they were preserved on ice in the field and later stored in the freezer until their dissection in the laboratory. Collection permits were issued by ICM-Bio-SISBIO (license number 14523-3).

Stomach content, diet, and prey availability

The snout-vent length (mm), width of the mouth (mm), and biomass (g) of 70 specimens were measured (25 tadpoles and 45 post-metamorphic individuals) and classified into five distinct size classes (I. tadpoles, II. <25 mm, III. ≥25 mm and <30 mm, IV. ≥30 mm, and V. ≥35 mm). The tadpoles were dissected through an incision in the ventral region in order to remove their digestive tract. The content of each digestive tract was homogenized with 1 ml of distilled water and placed in a Uthermol chamber in order to be analyzed and identified under an optical microscope with 40× (Arias et al., 2002). The specimens post-metamorphic were dissected through an incision in the ventral region to remove the stomach, which was measured for total length (mm), stomach mass (full and empty) (g), and degree of repletion (DR) [empty (0), up to 25% of DR (1), up to 50% of DR (2), up to 75% of DR (3), and completely full (4)]. Stomach content of each individual was removed, stored in 70% alcohol, and subsequently identified with the help of a stereomicroscope. Food items with an advanced degree of digestion, but still with identifiable structures, such as pieces of chitinous exoskeleton and locomotor appendages or with the remaining organic matter, were classified as animal remains. Fragments of vegetation were classified as plant remains. To determine the aquatic prey availability in the study site, we calculated the average number of individuals per prey taxon and average individual dry weight. This calculation required sampling of the aquatic invertebrates associated with

the aquatic vegetation in the same location where we collected the *P. minuta* individuals. We placed a plastic container with an area of 0.045 m² over the dominant macrophytes and trapped all vegetation and associated aquatic invertebrates inside. Each sample was represented by three replicate processes conducted monthly from July 2008 to January 2009, except in September 2008. The samples were washed out in tap water over sieves with a mesh of 500 μm (Albertoni et al., 2005). All aquatic invertebrates were preserved in 80% alcohol and later identified (Borror & Delong, 1988; Costa et al., 2006) and counted with the help of a stereomicroscope. Approximately, 15 individuals from each prey taxa were placed into Petri dishes, dried in an oven at 60°C for 24 h, and weighed with a digital analytical balance (with a precision of 0.1 mg).

Stable isotope samples processing and data analyses

Each individual Paradoxal frog was thawed and dissected to extract approximately 5 g of muscle tissue for isotopic analysis. Periphyton, suspended particulate organic matter (POM), and leaves from floating, emergent and submerged macrophytes were collected monthly to determine the isotopic composition of basal food sources. Macrophytes were collected by hand, periphyton samples were obtained by carefully removing the thin upper layer of flocculent or consolidated sediment on the substrate, and POM samples were obtained by filtering water through a pre-combusted (450°C, 4 h) Whatman glass-fiber filter (porosity: 1.2 μm) with a manual pump. Each sample was inspected to ensure removal of non-target matter (e.g., bone in muscle tissue, epiphytes or detritus in macrophytes), rinsed with distilled water, placed in sterile Petri dishes, and dried in an oven at 60°C to a constant weight (minimum of 48 h). Dried samples were ground to a fine powder with a mortar and pestle and stored in clean Eppendorf tubes. Sub-samples were weighed to 6–10 g, pressed into Ultra-Pure tin capsules (Costech, Valencia, CA, USA), and sent to the Analytical Chemistry Laboratory, Institute of Ecology at the University of Georgia for measurement of stable isotope ratios (¹³C/¹²C and ¹⁵N/¹⁴N). The results are reported as parts per thousand (‰) differences from a corresponding standard:

$\delta^{13}\text{C}(\text{‰}) = [(13\text{C}/12\text{C}_{\text{sample}})/(13\text{C}/12\text{C}_{\text{standard}}) - 1] \times 1,000$ and $\delta^{15}\text{N}(\text{‰}) = [(15\text{N}/14\text{N}_{\text{sample}})/(15\text{N}/14\text{N}_{\text{standard}}) - 1] \times 1,000$. To evaluate the measured values of carbon and nitrogen, we used patterns of “marine limestone fossil” and atmospheric air (Peterson & Fry, 1987) and compare the values found for ^{13}C and ^{14}C , ^{15}N and ^{14}N , respectively. Standard deviations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ replicate analyses were 0.13 and 0.14‰, respectively.

Data analyses

Air temperature ($^{\circ}\text{C}$), relative humidity (%), atmospheric pressure (hPa), wind speed (m s^{-1}), precipitation (mm^3), and solar radiation (kJ m^{-2}) between April 2008 and May 2009 were obtained from the website of the Instituto Nacional de Meteorologia (INMET, 2010). We utilized monthly average values of the above variables to conduct a cluster analysis with the Bray–Curtis similarity index to determine groups of months with similar meteorological conditions, which were later used to evaluate possible seasonal changes in diet composition.

Food items were quantified based on the frequency of occurrence (%F), which constitutes the percentage of the total number of stomachs in which a particular food item was found, and numerical abundance (%N), which represented the abundance in the percentage of a food item in relation to the total abundance of all stomachs (Hyslop, 1980). These parameters were used to estimate the importance of each food item in the diet of *P. minuta* based on the Alimentary Index (IAi) according to the formula $\text{IAi} = \%Fi \times \%Ni / \sum(\%Fi \times \%Ni) \times 100$ (Kawakami & Vazzoler, 1980). Temporal changes in diet composition (based on the IAi) were evaluated across seasons and five ontogenetic stages (I. tadpoles, II. <25 mm, III. ≥25 and <30 mm, IV. ≥30, and V. ≥35 mm). Aquatic prey availability was expressed as numerical abundance (total number of individuals per sample) and biomass (average individual dry weight) for each taxonomic group of prey. Individuals were weight in an analytical balance with a precision of 0.1 mg, and when a specimen weighted less than the capacity of the balance it was assigned a value of 0.01 mg.

We used the method proposed by Amundsen et al. (1996) to investigate feeding strategy and food niche structure in terms of between- and within-phenotypes (individual) contributions to the population’s feeding

niche. In this diagram, the dispersion of the points (one for each prey) along the vertical axis is related to the feeding strategy of the predator in terms of specialization or generalization; specialist predators have prey on the top, and generalist predators have prey at the bottom. The concentration of prey in the upper left shows the opportunistic consumption of certain prey by a few individuals within the population, whereas concentration in the upper right shows the specialization of the predator population as a whole. The niche composition does distinguish a trophic niche with a high between-phenotypes component characterized by individuals preying upon different resources or a trophic niche with a high within-phenotypes component in which most of the individuals consumed similar prey (Amundsen et al., 1996; Bolnick et al., 2003).

We employed the SIAR Bayesian stable isotope mixing model in R to estimate the relative contribution of aquatic and terrestrial-derived carbon sources to the Paradoxal frog *P. minuta* (Parnell et al. 2010). Following Phillips et al. (2005), who proposed a posteriori pooling of food sources with similar isotopic compositions to achieve higher resolution in mixing models, we considered the average value of POM, periphyton, and macrophytes (C_3) as the aquatic basal food source and terrestrial vegetation from the Cyperaceae and Poaceae families (C_4) as the terrestrial basal food source. Unlike previous approaches, the SIAR mixing model incorporates uncertainties associated with sample variability and trophic enrichment, even in undetermined systems (e.g., Isosource, Phillips & Gregg, 2003). Average and standard deviation values for aquatic and terrestrial sources used in the mixing model were $-27.72 (\pm 4.12)$ and $-19.34 (\pm 4.41)$, respectively. We used $0.47 (\pm 1.23)$ and $2.54 (\pm 0.11)$ as trophic enrichment factor (TEF) values for carbon and nitrogen isotope ratios.

Trophic position (TP) of *P. minuta* in the food chain was estimated by the following formula: $\text{TP} = 2 + (\delta^{15}\text{N}_{P. \text{minuta}} - \delta^{15}\text{N}_{\text{base}})/F$, where $\delta^{15}\text{N}_{P. \text{minuta}}$ is the individual value of nitrogen isotopic ratio for each analyzed specimen, $\delta^{15}\text{N}_{\text{base}}$ represents the nitrogen isotopic ratio value of a primary consumer (in this case, it was used the filter-feeding gastropod *Pomacea* sp.) and F is the fractionation of nitrogen by trophic level (Post, 2002). An estimative of fractionating of +2.54‰ was used (Vanderklift & Ponsard, 2003). Statistical differences among trophic position

Table 1 Alimentary Index (IAi) of the stomach content of the Paradoxal frog (*P. minuta*)

Items	%F	%PN	IAi
Araneae	23.30	11.72	9.60
Cladocera	2.30	0.78	0.06
Coleoptera	20.90	9.38	6.91
Díptera	9.30	6.25	2.05
Hemiptera	27.90	13.28	13.05
Hydracarina	9.30	3.13	1.02
Hymenoptera	18.60	10.94	7.17
Isopoda	7.00	3.91	0.96
Lepidoptera	2.30	0.78	0.06
Odonata	11.60	3.91	1.60
Orthoptera	4.70	1.56	0.26
Ostracoda	7.00	2.34	0.58
Gastropoda	2.30	0.78	0.06
Trichoptera	2.30	0.78	0.06
Others			
Animal remain	60.50	25.78	54.89
Plant remain	11.60	3.91	1.60
Eggs	2.30	0.78	0.06

%PN numerical percentage, %F frequency of occurrence

means (TP) both in development as size classes of *P. minuta* were evaluated through unifactorial ANOVA ($P = 0.05$) with a posteriori test of Newman–Keuls ($P = 0.05$) (Zar, 1994).

Results

A total of 25 tadpoles were analyzed, resulting in the identification of six classes of algae (Bacillariophyceae, Chlorophyceae, Coscinodiscophyceae, Euglenophyceae, Fragilariophyceae, and Zygnemaphyceae) and detritus. To facilitate the application of Index Alimentary (IAi), we consider all classes of algae as “microalgae.” Among the 45 collected post-metamorphic individuals, seven individuals had their stomachs up to 25% full, four were up to 50% full, 12 were partially full (75%), and 12 specimens had full stomachs (100%). Only two individuals had no prey in their stomachs. A total of 19 food items were found in the stomachs, with a predominance of Arthropoda, especially insects (Table 1). Hemiptera, Araneae, and Hymenoptera were the most frequent food items in the stomach contents (%F 27.9; 23.3 and 18.6,

respectively) and constituted the most important food items in the diet of *P. minuta* (IAi = 13.0, 9.6, and 7.2, respectively), Coleoptera, Diptera, Odonata, and Hydracarina were also conspicuous preys in the diet (IAi: 6.9, 2.0, 1.6, and 1.0, respectively). A significant proportion of preys found in the stomach contents could not be identified with certainty and, therefore, animal remains constituted ranked as the most important food item in the diet (IAi = 54.9) (Table 1).

Regarding seasonality, the cluster analysis revealed three periods with similar meteorological conditions: (I) May–September 2008 and May 2009 represent a period corresponding to austral fall-winter, characterized by lower values of air temperature, intensity of wind speed and radiation, and higher values of humidity, atmospheric pressure and precipitation; (II) November 2008–February 2009; and (III) one period that includes April and October 2008, March and April 2009. These last two periods corresponded to the austral spring-summer. Although periods I and II corresponded to spring-summer, period II was warmer and more humid, and most parameters (except for atmospheric pressure) were higher than group III. When analyzing diet during these periods, we observed that Araneae, Coleoptera, Hymenoptera, and Diptera were the only orders observed in the stomach content during the three periods (Table 2).

Interestingly, the dominant prey in the diet of the post-metamorphic individuals was not those one with highest numerical abundance in the study site, such as Amphipoda, Copepoda, Diptera, and Hydracarina (Fig. 1a). Rather, the dominant preys in their diet were those preys with higher individual biomass, such as Hemiptera, Coleoptera and Araneae (Fig. 1b). Hemiptera, the prey taxon with the highest average individual biomass in the aquatic habitat (41.16 mg), ranked as the most important arthropod in the diet of the post-metamorphic *P. minuta*. Other prey taxa commonly consumed by the post-metamorphic *P. minuta*, such as Coleoptera and Hymenoptera, were among the prey taxa with greater values of individual biomass (22.4 mg and 2.86, respectively). In contrast, prey taxa with higher average numerical abundance (>2,000 individuals per sample) in the study site and lower individual biomass (<0.01 mg), such as Amphipoda and Copepoda, were not important prey taxa in the diet of the post-metamorphic *P. minuta* (Fig. 1).

The feeding strategy analysis revealed that the post-metamorphic *P. minuta* has a generalist behavior in

Table 2 Alimentary Index (IAi) of the stomach contents of the Paradoxal frog (*P. minuta*)

Item	I (n = 9)			II (n = 19)			III (n = 15)		
	%F	%PN	IAi	%F	%PN	IAi	%F	%PN	IAi
Araneae	11.10	4.17	1.30	15.80	5.56	5.26	40.00	22.00	23.57
Cladocera	0.00	0.00	0.00	5.30	1.85	0.58	0.00	0.00	0.00
Coleoptera	22.20	12.50	7.79	21.10	9.26	11.7	20.00	8.00	4.29
Diptera	11.10	8.33	2.60	10.50	5.56	3.51	6.70	6.00	1.07
Hemiptera	0.00	0.00	0.00	47.40	25.93	73.68	20.00	6.00	3.21
Hydracarina	0.00	0.00	0.00	10.50	3.70	2.34	13.30	4.00	1.43
Hymenoptera	11.10	29.17	9.09	21.10	7.41	9.36	20.00	6.00	3.21
Isopoda	11.10	4.17	1.30	10.50	7.41	4.68	0.00	0.00	0.00
Lepidoptera	0.00	0.00	0.00	0.00	0.00	0.00	6.70	2.00	0.36
Odonata	0.00	0.00	0.00	15.80	5.56	5.26	13.30	4.00	1.43
Orthoptera	0.00	0.00	0.00	5.30	1.85	0.58	6.70	2.00	0.36
Ostracoda	22.20	8.33	5.19	0.00	0.00	0.00	6.70	2.00	0.36
Gastropoda	0.00	0.00	0.00	0.00	0.00	0.00	6.70	2.00	0.36
Trichoptera	0.00	0.00	0.00	0.00	0.00	0.00	6.70	2.00	0.36
Others									
Animal remain	77.80	33.33	72.73	42.10	16.67	42.11	73.30	32.00	62.86
Plant remain	0.00	0.00	0.00	26.30	9.26	14.62	0.00	0.00	0.00
Eggs	0.00	0.00	0.00	0.00	0.00	0.00	6.70	2.00	0.36

Periods: I. Autumn–Winter, II. Spring–Summer 1 and III. Spring–Summer 2. Values inside parentheses indicate the number of specimens analyzed
%PN numerical percentage, %F frequency of occurrence

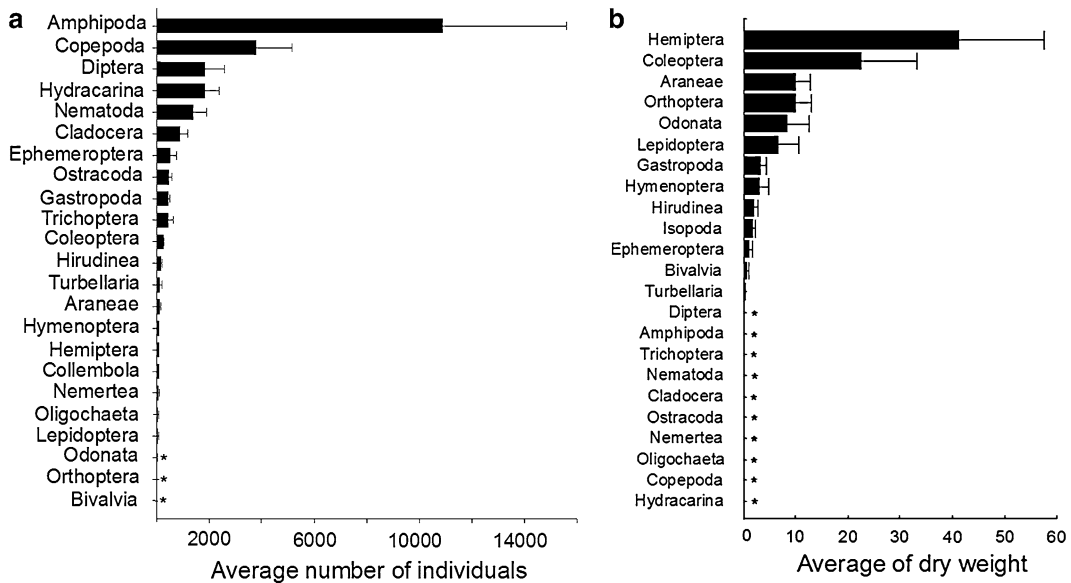


Fig. 1 Average number of individuals (+SD) (a) and average individual dry weight (mg) (+SD) (b) of aquatic invertebrates associated with aquatic vegetation in the study area. Asterisks denote values lower than 0.01 mg

the consumption of some prey, particularly Araneae and Coleoptera, which were preyed upon in moderate abundance (Pi: 32.25 and 37.26, respectively) and

frequency (both with %F 0.23) (Fig. 2). Preys such as Orthoptera, eggs from frog’s spawning, and Trichoptera were consumed in high abundance (Pi > 90) by

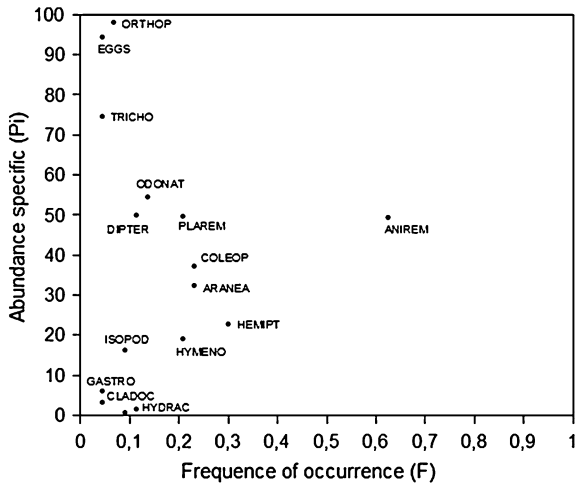


Fig. 2 Graphic representation (Amundsen’s method) of the feeding strategy of *Pseudis minuta*. Food item abbreviations: Odonata (ODONAT), Orthoptera (ORTHOP), Coleoptera (COLEOP), Trichoptera (TRICHO), plants remains (PLAREM), Isopoda (ISOPOD), Araneae (ARANEA), animal remains (ANIREM), Hemiptera (HEMIPT), Hymenoptera (HYMENO), Egg (spawning), and Diptera (DIPTER)

fewer post-metamorphic individuals ($%F < 0.1$), highlighting their opportunistic feeding behavior. Hence, the feeding strategy of *P. minuta* can be classified as generalist-opportunist with a broad feeding niche comprised of many individuals in the population sharing the same resources (e.g., Araneae and Coleoptera), but with some individuals consuming occasionally other preys (e.g., Orthoptera, frog’s eggs, and Trichoptera) in high quantities (Fig. 2).

Trophic position (TP) of tadpoles and post-metamorphic adults estimated by nitrogen stable isotope ratios revealed a clear difference between tadpoles and adults ($F(4,39) = 11.48; P < 0.000$) (Fig. 3). Tadpoles were positioned in the base of the food chain (TP mean: 1.9), whereas adults shifted to higher trophic position along their increment in body size. Comparisons by size classes of post-metamorphic adults showed a tendency of increase at the trophic position in those classes with higher SVL values, but significant differences were observed only in classes II (< 25 mm) and V (≥ 35 mm) (Newman–Keuls post hoc test; $P < 0.05$) (Fig. 3). These results seem to corroborate those revealed by stomach content analysis, which showed that tadpoles fed upon mainly on microalgae at the base of the food chain, whereas post-metamorphic adults preyed upon animal preys (Fig. 4). This analysis also showed differences in diet

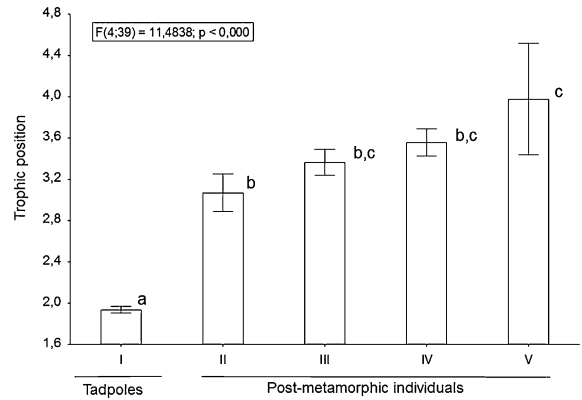


Fig. 3 Average trophic position (\pm standard deviation) along five ontogenetic classes of *Pseudis minuta*: I (tadpoles), II (< 25 mm), III (≥ 25 and < 30 mm), IV (≥ 30 mm), and V (≥ 35 mm). Letters (a–d) denote statistical significant differences considering a probably level of 5%

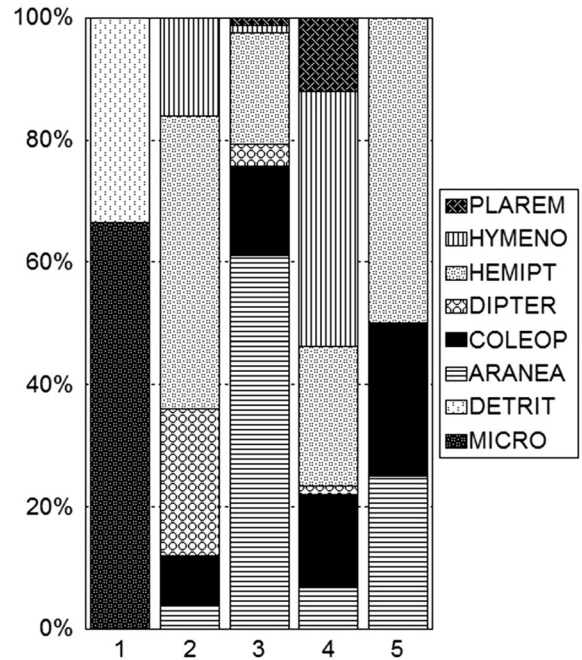


Fig. 4 Alimentary Index (IAi) for food items found in the stomach content of *Pseudis minuta* along five ontogenetic classes of *P. minuta*. Code for food items as follows: Araneae (ARANEA), Coleoptera (COLEOP), rest of vegetable (PLAREM), Odonata (ODONAT), Homoptera (HOMOPT), Diptera (DIPTER), Hemiptera (HEMIPT), detritus (DETRIT), and microalgae (MICROA)

composition among body size classes II to V (adults), which could be associated with the shift in trophic position revealed by the stable isotope analysis. For

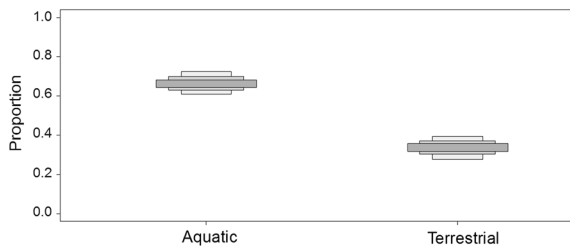


Fig. 5 Relative contribution of aquatic and terrestrial-derived basal food sources to the Paradoxal frog *Pseudis minuta*. Each plot shows the 50% (darker gray), 75% (lighter gray), and 95% (white). Bayesian credible intervals of the feasible contributions of each basal production source

Table 3 Sample number (n) and mean values (± 1 SD) of carbon and nitrogen isotope ratios of basal production sources collected in the study area

	n	$\delta^{15}\text{N} \pm 1$ SD	$\delta^{13}\text{C} \pm 1$ SD
Aquatic sources			
<i>Enydra sessilifolia</i>	1	-0.1	-29.5
<i>Cabomba</i> sp.	1	3.3	-28.0
<i>Myriophyllum aquaticum</i>	1	1.4	-33.4
<i>Bacopa monnieri</i>	1	-1.4	-27.8
<i>Polygonum</i> sp.	1	3.3	-30.6
<i>Eichornia crassipes</i>	3	2.6 ± 1.5	-28.3 ± 1.8
<i>Potamogeton polygonus</i>	1	4.0	-31.1
<i>Potamogeton</i> sp.	2	2.6 ± 0.6	-31.3 ± 4.4
<i>Salvinia herzogii</i>	3	1.3 ± 0.6	-29.7 ± 0.2
<i>Micranthemum umbrosum</i>	1	2.8	-33.3
Periphyton	7	0.9 ± 1.4	-33.0 ± 3.6
POM	14	2.2 ± 2.1	-24.8 ± 3.7
Terrestrial sources			
<i>Kyllinga vaginata</i>	2	1.1 ± 1.9	-10.9 ± 0.6
<i>Paspalum vaginatum</i>	3	4.2 ± 1.8	-13.3 ± 1.1
<i>Sporobolus virginicus</i>	2	0.3 ± 0.4	-13.5 ± 0.6

instance, individuals with body size less than 25 mm (class II) had a diet more diversified than those ones with body size greater than 35 mm (class V), which preyed predominantly on carnivorous preys, such as Hemiptera, Coleoptera and Araneae, which could partially explain why the largest *P. minuta* individuals (class V) had the highest trophic levels (Fig. 5).

The carbon stable isotope ratios ($\delta^{13}\text{C}$) of the basal production sources from terrestrial (grasses from the Cyperaceae and Poaceae family, $n = 7$) and aquatic

origin $n = 37$ (macrophytes, 15; periphyton, 8 and POM, 14) are shown on Table 3. $\delta^{13}\text{C}$ values of terrestrial food sources ranged from -10.52 to -14.52‰ , with an average of -12.70‰ , whereas aquatic production sources samples ranged from -13.70 to -38.66‰ , with an average of -29.19‰ . $\delta^{13}\text{C}$ values of the *Pseudis minuta* ($n = 38$) ranged from -26.64 to -21.39‰ , with an average of -23.48‰ . The stable isotopic mixing model indicated that aquatic-derived production sources had approximately three times greater nutritional contribution to the *Pseudis minuta* (0.61–0.72) than terrestrial-derived food sources (0.28–0.39) (Fig. 5).

Discussion

Our findings gleaned from a multiple analytical approach combining prey availability, stomach content, and stable isotope ratios demonstrated that, in its early development, *P. minuta* shows a diet based on algae and detritus. After metamorphosis, the species becomes an aquatic predator employing a generalist feeding strategy to forage on prey with greater individual biomasses. Among the seventeen prey taxa available in its aquatic habitat, the post-metamorphic specimens feed mainly on prey taxa with higher individual biomasses, mainly hemipterans and arachnids, in detriment of highly abundant, but much smaller prey taxa such as amphipods and copepods. A similar result was obtained by Lima & Moreira (1993), who found that *Colostethus stephensi* tended to eat larger prey with increasing size of the predator. Aside the relation with increasing predator's body size, ingestion of prey with greater biomass may also be related with microhabitat use and foraging strategy. Post-metamorphic individuals of *P. minuta* exhibit foraging behavior known as sit-and-wait, being the specimens observed floating on the surface of the water on aquatic plants and in the margins of water bodies (Huckembeck et al., 2012). Invertebrates in the diet of adults of *P. minuta* (e.g., spiders, beetles, and hemipterans) originated from terrestrial environment and also post-metamorphic aquatic insects are abundant in these microhabitats, becoming susceptible to predation. Furthermore, studies on the foraging behavior show that species exhibiting sit-and-wait predatory behavior have a more diverse diet and tend to feed on larger prey (Huey & Pianka, 1981; Lima &

Moreira, 1993). Such preference for prey taxa with higher biomasses could be explained in the context of an optimal forage theory predicting that predatory foraging behavior tends to maximize net energy intake per unit of time (MacArthur & Pianka, 1966).

The observed temporal changes in the diet of post-metamorphic *P. minuta* (more diverse in the warmer months) should be interpreted with caution because the number of individuals analyzed in each period varied considerably, i.e., the larger number of stomachs analyzed in the warmer months could simply result in a higher probability of finding more different food items in this period. Nevertheless, seasonal variations in diet composition have been recorded in several anuran species at tropical latitudes (Santos et al., 2004; Vaz-Silva et al., 2005; Brasileiro et al., 2010) and subtropical/temperate regions (Maneyro & Rosa, 2004; Miranda et al., 2006). Diet changes throughout the year closely relate to seasonal fluctuations in prey abundance (Vaz-Silva et al., 2005) and with seasonal activity of the species (Brasileiro et al., 2010). These factors can act separately or in combination, depending on habitat type or species biology. Additionally, some studies suggest that variation in the diet of some amphibian species is related to reproductive activity. For example, Kovács et al. (2007) in a study of the diet of *Hyla arborea* found that most of the stomachs of specimens analyzed were empty, presumably because of decreased foraging activity for the benefit of energy demand involved with reproductive activity. Further studies are needed to confirm the observed patterns and disentangle these controlling factors.

The method to evaluate the feeding strategy revealed that analyzed post-metamorphic individuals can be considered overall feeding generalists. However, the intra-specific analysis of the diet revealed individual marked opportunism for consuming different preys. Therefore, it is possible to conclude that the post-metamorphic specimens have a broad food niche formed by the sum of relatively narrow food niches of groups of individuals within the population, which opportunistically feed on distinct food resources. Prior studies of congeneric species have reported diverse and omnivorous diets for the genus *Pseudis* (Basso, 1990; Duré & Kehr, 2001; Texeira et al., 2004; Vaz-Silva et al., 2005; Miranda et al., 2006), but few studies addressed the relative importance of individuals to the feeding strategy and food niche of a frog

population (Bolnick et al., 2003; Da Rosa, et al., 2011). Bolnick et al. (2003) previously noted that diet variation between individuals is much greater in the animal population than previously supposed. Future research should evaluate individual-level food niche variation in amphibians and influences on conspecific competition and resource use patterns.

Through the stable isotope approach, it was possible to observe an increased in trophic position of *P. minuta* in the food chain during its development, especially a marked shift between tadpoles that were ranked as primary consumers and post-metamorphic stages that were classified as tertiary consumers. Most studies on the diet of tadpoles shows that the main food items are algae and detritus and emphasize the importance of ingestion of organisms of the periphyton, which have an important role in fixing elements that serve as nutrients for the development of tadpoles. However, little is known about the real contribution and assimilation of such food items (Altig et al., 2007). In fact, a preliminary analysis of tadpoles of *P. minuta* revealed that the most frequent items on its diet were algae, including diatoms, euglenas, chlorophytes algae, besides detritus (S. Huckembeck, unpubl. data). This corroborates with our findings in this study which rank tadpoles as primary consumers. Changes in the food chain levels seem to be a tendency for most Amphibian species being confirmed in others studies with stable isotopes (e.g., Whiles et al., 2006; Trakimas et al., 2011). Among post-metamorphic individuals are also possible to observe significant changes in TP between II (<25 mm) and V (>35 mm) classes. Probably, the higher values found for large individuals can be attributed to their capacity to prey upon a more diverse spectrum of animals, including those ones with trophic position more elevated. Similar results were found for *Ichthyophis* cf. *kohtaoensis* (Kupfer et al., 2006).

As previously mentioned, in a prior study on the activity patterns and microhabitat utilization of the post-metamorphic *P. minuta* in this same study site revealed that most individuals remain partially submerged in the water and commonly rest on aquatic vegetation (Huckembeck et al., 2012). This behavior seems consistent with the species' well-developed interdigital membranes on the hind feet and dorso-laterally positioned eyes (Maneyro & Carreira, 2012). Given this prior evidence, a plausible assumption suggests that the hemipterans and arachnids observed

in the stomachs of the post-metamorphic individuals were captured in the aquatic vegetation of the study site and not in the terrestrial environment.

We found difficult to determine if the hemipterans and arachnid preys consumed by the post-metamorphic *P. minuta* were primarily originated in terrestrial or aquatic habitats and therefore would represent allochthonous and autochthonous resources sustaining this aquatic frog. Insects (adult forms) and arachnids were frequent and abundant in the margins of the studied wetland (S. Huckembeck, unpublished data) and could be carried by the wind into water and over the aquatic vegetation. Hence, the stomach content analysis alone did not allow us to ascertain if the prey taxa consumed by the studied predator were from terrestrial or aquatic environments. However, the stable isotope carbon ratio analysis did allow us to infer from which environment the post-metamorphic *P. minuta* derived its main primary carbon sources. The stable isotopic mixing model revealed that most of the carbon (0.61–0.72) sustaining the post-metamorphic *P. minuta* is derived from the aquatic rather than terrestrial environment.

Other authors have noted that terrestrial material plays a crucial role in the aquatic environment. Uieda & Kikuchi (1995) pointed out that the availability of allochthonous materials as plant remains and terrestrial invertebrates in oligotrophic water bodies from temperate regions are essential to the diet of aquatic organisms. For instance, observations indicate that not only preys associated with aquatic vegetation, but insects that fall on the surface of water bodies make an important contribution to the diet composition of *P. bolbodactyla*, *P. paradoxa*, and *L. limellus* (Duré & Kehr, 2001; Texeira et al., 2004). In contrast with these findings, our results suggest that some amphibians, such as the Paradoxal frog, are strongly dependent on carbon sources originating in aquatic systems, regardless of the terrestrial or aquatic origins of the arthropods in their diet. This crucial trophic link could be in jeopardy in the case of the Paradoxal frog because ongoing anthropogenic impacts such as cattle grazing and farming pose a threat to the future maintenance of their aquatic habitats. A similar pattern could be expected for other congeneric species of the genus *Pseudis* that are also well-adapted to the aquatic environment, but we do not anticipate this pattern for other sympatric species that mainly forage in the terrestrial environment.

Conclusions

The present study concludes that *P. minuta* in the initial phase of its life cycle may be considered as a primary consumer. After metamorphosis, however, the species changes its diet, becomes a predator generalist with a marked opportunism for prey such as Orthoptera, eggs and Trichoptera. This predator forages on preys with higher individual biomasses and derives most of its carbon primary sources from the aquatic environment, regardless of the terrestrial or aquatic origins of the arthropods in its diet. We predict that amphibian species with similar life cycle of *P. minuta* also play a crucial ecological role integrating aquatic and terrestrial systems in subtropical wetlands throughout their feeding behavior.

Acknowledgments The authors are grateful to Fabiano Corrêa, Marlucy Claudino Coelho, Rodrigo Ferreira Bastos, and Mario Vinicius Condini, who assisted in the field, and Milene Matos Stangler, who identified the invertebrates; and to the ‘Conselho Nacional de Desenvolvimento Científico e Tecnológico’ (CNPq; Universal Grant: 482920/2007-6) and the International Foundation of Science (IFS Grant: A/4419-1) for funding. SH and DL (Grant 338632/2010) give thanks for fellowships provided by the ‘Coordenação de Aperfeiçoamento de Pessoal de Nível Superior’ (CAPES). AMG acknowledges fellowship support from CNPq (305888/2012-9). This is a contribution of the research group “Grupo de Análises de Isótopos Estáveis em Ambientes Aquáticos” (GAIA/CNPq).

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