





Trophic structure of frog assemblages in coastal habitats in southern Brazil

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Abstract We evaluated the hypothesis that contrasting environmental conditions in a coastal wetland and nearby sand dunes results in frog assemblages with divergent trophic structures. We predicted that the more productive habitat (wetland) would have higher diversity of primary producers and frogs with larger trophic niches and greater interspecific dietary overlap when compared to the less-productive dune habitat. Stomach content analysis (SCA) and stable isotope analysis (SIA) were used to compare frog trophic ecology in the two habitats. We compared trophic niche size (Shannon's index) and overlap (Pianka's index; isotopic ellipse area, SEA_c). Bayesian isotope mixing models were used to estimate relative contributions of basal production sources to frog biomass in each habitat. Estimates indicated that wetland frogs assimilated material from diverse basal sources (suspended particulate organic matter (POM), C₃ plants, periphyton), whereas frogs inhabiting dunes assimilated material mostly originating from C₃ plants. Both SCA and SIA revealed less trophic overlap among frogs from the sand dunes compared with those from the wetland. Dietary overlap was greater than expected at random for frogs in both the wetland and dunes. Mean overlap among isotopic ellipses (SEA_c) of all frogs at each habitat was higher in the wetland (0.14‰) than in the dunes (0.04‰). These findings corroborate our prediction that trophic structure in the wetland is more complex than in the sand dunes. Also, as expected, we observed greater isotopic niche segregation among frogs in the sand dunes, a potential mechanism that reduces competition for limited food resources.

Key words: amphibians, diet, Lagoa do Peixe National Park, niche overlap, stable isotopes.

INTRODUCTION

Community trophic structure often changes in response to spatial and temporal variation in environmental conditions (Winemiller 1990; Polis *et al.* 1996; Correa & Winemiller 2014; Garcia *et al.* 2017). Spatial changes in trophic structure across ecosystems may occur due to variation in factors such as composition and diversity of primary producers and consumers, primary productivity, inter- and intraspecific interactions and various abiotic environmental drivers (Polis *et al.* 1996; Catry *et al.* 2016; Garcia *et al.* 2019). Greater diversity of primary producers generally yields higher net primary production, even in oligotrophic systems (Tilman 1988; Tilman *et al.* 2014). This suggests that species packing among plants (i.e. niche compression yielding niche segregation) facilitates net productivity (McNaughton 1993). Individual consumers and community trophic structure also respond to

variation in resource availability (e.g. Oliveira *et al.* 2018). For instance, resource availability influences the number of trophic links in local food webs as well as resource and consumer contributions to energy flow within the network (Winemiller 1990; Dalerum *et al.* 2016). Interspecific interactions may also be influenced by resource availability, with low resource availability resulting in either competitive exclusion or niche partitioning and species coexistence (Pianka 1974).

Here, we compare the trophic structure of frog assemblages between contrasting coastal habitats: a productive, mesic habitat (wetland) and an adjacent semiarid habitat that is comparatively less-productive (sand dunes). The subtropical coast of southern Brazil contains wetlands and sand dunes that differ markedly in physiography, productivity and biodiversity (Ferreira *et al.* 2009; Portz *et al.* 2016). The wetlands are dominated by diverse aquatic macrophytes (at least 176 species; Rolon *et al.* 2011) and characterised by high primary production. Primary production of dominant marsh plants in wetlands in southern Brazil ranges from a mean of 2438 to

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3659 g dry weight $\text{m}^{-2} \text{year}^{-1}$ (Ferreira *et al.* 2009). This primary production sustains a great diversity of invertebrates (e.g. at least 63 families from 23 orders; Ávila *et al.* 2011; Huckembeck *et al.* 2014). In contrast, the adjacent sand dunes harbor sparse vegetation with much less diversity of macrophytes (~70 species; Seeliger *et al.* 1998). Also, this habitat is characterised by limitations in water supply and nutrients, resulting in much lower primary productivity compared to the adjacent wetland (McLachlan 1991). Mean plant productivity in sand dunes in southern Brazil ranges from 0.49 to 22.85 g dry weight $\text{m}^{-2} \text{year}^{-1}$ and supports lower diversity of invertebrates (e.g. 29 families from 12 orders; Masson & McLachlan 1990; Gianuca 1997; Seeliger *et al.* 2000).

Frogs are considered good models for trophic ecology studies because they have diverse life histories and, in many cases, play key roles in the flow of energy between aquatic and terrestrial habitats (Kupfer *et al.* 2006). Most studies of frog trophic ecology have relied on stomach contents analysis (SCA; Leivas *et al.* 2018; Oliveira *et al.* 2018). While effective, this technique also has some limitations, such as difficulty to identify prey items in advanced stages of digestion and uncertainty regarding the proportion of ingested material that actually is assimilated by the consumer (Jepsen & Winemiller 2002). Stable isotope analysis (SIA) is another technique that has become an important tool for estimating source materials assimilated by consumers and revealing major pathways in food webs (Fry 2006; Layman *et al.* 2012; Glibert *et al.* 2018). The elements most commonly analysed in trophic ecology studies are carbon ($^{13}\text{C}/^{12}\text{C}$, expressed as $\delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$, expressed as $\delta^{15}\text{N}$), which can provide information on resources supporting consumer biomass and consumer trophic position (Peterson & Fry 1987).

We used SCA to compare diets and SIA to estimate assimilation of basal production sources by anurans in a coastal wetland and an adjacent sand dunes habitat. We hypothesised that their contrasting characteristics would result in differences in food resources used by frog assemblages. The wetland is not only more productive than the dunes, but also has greater abundance of invertebrate prey (695.4 individuals h^{-1}) than the latter in the warmer period (96 individuals h^{-1} ; S. Huckembeck, unpubl. data 2010, Masson & Mc Lachlan 1990). More specifically, we predicted that (i) frogs in the more productive habitat (wetland) would have greater diet breadth and biomass supported by more diverse basal production sources and (ii) lower availability and diversity of invertebrate prey in the sand dunes would be associated with greater

trophic niche segregation (i.e. lower dietary and isotopic overlap).

MATERIALS AND METHODS

Study area and field work

Field work was carried out in the 'Lagoa do Peixe' National Park located in the state of Rio Grande do Sul, southern Brazil (31°S; Fig. 1a,b). Anurans and primary producers were collected monthly from October 2008 to May 2009 in wetland and sand dune habitats. These habitats were separated by a distance of 4.6 km (Fig. 1b). The wetland (31°06'53.00"S, 50°51'40"W; Fig. 1c) was characterised by the presence of ephemeral and permanent water bodies with floating (*Salvinia herzogii*, *Azolla filiculoides*, *Eichhornia crassipes*) and rooted aquatic macrophytes (*Cabomba* sp.). Terrestrial vegetation adjacent to the water bodies was dominated by grasses of Family Poaceae and sparsely distributed shrubs (for example *Eryngium* spp.). The sand dune (31°08'28.03"S, 50°49'30.10"W; Fig. 1d) was characterised by incipient and frontal dunes with height varying from few centimetres up to three metres. In some areas between dunes, there were ephemeral water bodies with maximum depth of 50 cm. Terrestrial vegetation adjacent to the ephemeral water bodies was sparse and dominated by *Andropogon arenarius* Hackel, *Senecio crassiflorus* (Poir.) DC, *Polygala cyparissias* A. St.- Hill. Et. Moq., *Androtrichum trigynum* (Spreng.) and *Panicum racemosum* (P. Beauv.) Spreng. The study area has a subtropical climate with an average annual temperature of 17.5°C, rainfall ranging from 1.200 to 1.500 mm year^{-1} and a warmer period from January to February and a colder period from June to July (Seeliger *et al.* 1998).

In each habitat, anurans were collected manually beginning at dusk, with survey effort lasting for 2.5 h. We performed eight surveys with a total sampling effort of 20 h. A total of five frog species were collected, three of which occurred in both habitats: *Boana pulchella* (Duméril & Bibron, 1841), *Leptodactylus latrans* (Steffen, 1815) and *Pseudis minuta* Günther 1858). *Physalaemus gracilis* (Boulenger 1883) was only collected from the wetland, and *Physalaemus biligonigerus* (Cope, 1861) was only found in the sand dunes (Loebmann & Vieira 2005; Oliveira *et al.* 2013). All of these species are abundant and widely distributed in the southern Neotropical region, and none are listed as threatened (Garcia & Vinciprova 2003; IUCN 2020). Frogs were euthanised in an ice bath and then frozen until examined in the laboratory.

Samples of the following primary producers were collected in each habitat in triplicate: periphyton (PERI), suspended particulate organic matter (POM), C_3 macrophytes and C_4 macrophytes. The leaves from C_3 and C_4 plants were collected manually by clipping with scissors (approximately 5 g each sample). PERI samples were obtained by scraping a thin upper layer of flocculent or consolidated sediment from substrates (submerged stems and leaves) using a spatula. To obtain POM samples, water samples were collected from aquatic habitats. Zooplankton were removed from the sample by pre-filtering water through

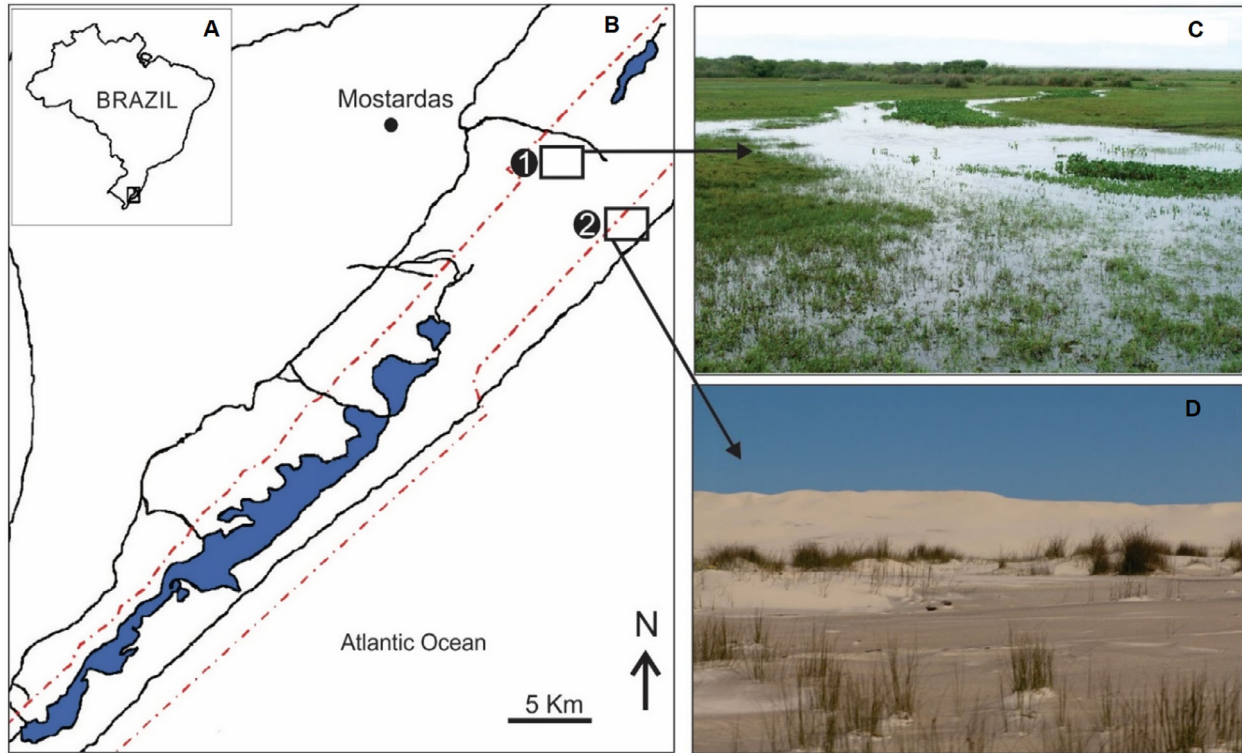


Fig. 1. Southern Brazil (a) and the location of the Lagoa do Peixe National Park (LPNP) (b; red dotted line) in the coastal plain with the location study areas in the wetland (c; 31°06'53.00"S, 50°51'40"W) and sand dunes (d; 31°08'28.03"S, 50°49'30.10"W).

200µm mesh. After pre-filtering, the POM sample was obtained by filtering water with a manual vacuum pump through a pre-combusted (450°C, 4 h) glass fibre filter (GF/F, 47 mm, porosity: 1.2 µm) on which particles were collected. All samples were kept frozen until they were processed in the laboratory. Collection permits were issued by ICMBio-SISBIO (license number 14523-3). In the wetland, *Bacopa monnieri* (L.) Wettstein, *Desmodium* cf. *adscendens* (Sw.) DC and *Enydra sessilifolia* (Ruiz & Pav.) Cabrera were classified as C₃ plants, whereas *Sporobolus virginicus* (L.) Kunth and *Kyllinga vaginata* Lam. were classified as C₄ plants. In the dune, *Achyrocline atureioides* (Lam.) DC, *Baccharis trimera* (Less.) DC, *S. crassiflorus* (Poir.) DC, *Vernonia* cf. *brevifolia* Less., *D. cf. adscendens*, *Drosera* sp.L. and *P. cyparissius* were classified as C₃ plants, whereas grass species (Poaceae species not identified) were classified as C₄ plants.

Dietary analysis

We measured the snout–vent length (SVL ± 1 mm using calliper) of each frog specimen and applied the Kruskal–Wallis test to evaluate interspecific differences within each habitat. When that result was significant, we applied the Mann–Whitney test to determine levels of statistical significance between species pairs (Hammer 2017). The stomach was removed from each specimen through an incision in the body ventrum. Food items removed from stomachs

were stored in 70% EtOH in labelled vials. Invertebrates (mostly insects) were identified up to the order level. Exoskeleton and body part fragments were classified as animal remains, and fragments of vegetation were classified as plant remains.

For each food item removed from each stomach, we estimated the area covered after spreading the material to a thickness no greater than 1 mm on a Petri dish with the bottom marked in a grid with mm² squares (Huckembeck *et al.* 2014). We then counted the number of grid squares covered by the material to estimate the area covered. For each food category, we estimated frequency of occurrence (%FO), which corresponds to the per cent frequency that a food item occurred across all stomachs examined, and percentage of area (%AP), which corresponds to the percentage of the total area occupied (in mm²) by that food category in relation to the total area occupied by all items removed from stomachs. For each frog species at each site, %AP values for the food categories were plotted as bar charts with different scales (absolute and percentage) to evaluate their relative contributions to diets.

We calculated the trophic niche breadth of anuran species in each habitat using Shannon's Index, $H = -\sum p_i \times (\log p_i)$, where p_i is the dietary proportion of each prey item according to %AP. Differences in mean niche breadth between habitats were evaluated by the diversity *t*-test (Hammer 2017). We used Pianka's index to calculate interspecific dietary overlap: $O_{jk} = O_{kj} = [\sum (p_{ij} \times p_{ik})] / \sqrt{(\sum p_{ij}^2 \times \sum p_{ik}^2)}$, where p_{ij} and p_{ik} are the proportions of food

item i consumed by species j and k , respectively. Statistical significance of Pianka's index was evaluated using a null model (Winemiller & Pianka 1990). We calculated this null model through 10 000 randomised simulations of the observed dietary proportions of each food item for each frog species. The P -value is based on the percentage of randomised overlap values that were greater than the observed overlap. These analyses were performed using EcoSimR 1.0 (Gotelli & Ellison 2013). We applied the parametric t -test (t) to compare the mean overlap between anurans between habitats (Hammer 2017).

Stable isotope analysis

After thawing the samples, we dissected the anurans and collected muscle samples from the posterior limb (<5 g). Muscle and primary food sources (PERI, POM, C₃ and C₄ plants) samples were rinsed with distilled water, placed in sterile Petri dishes, and then dried in an oven at 60°C for 48 h. We analysed isotopic composition of 77 anuran specimens and 16 samples of basal production sources from the wetland (PERI, $n = 4$; POM, $n = 7$; C₃ plants, $n = 3$ and C₄ plants, $n = 2$), and 76 anuran specimens and 18 production sources from the dunes (PERI, $n = 2$; POM, $n = 2$; C₃ plants, $n = 12$ and C₄ plants, $n = 2$; Table 1). Dried samples were ground to a fine powder with a mortar and pestle and stored in clean Eppendorf tubes. We weighed sub-samples (1–3 mg) into tin capsules and sent them to the Analytical Chemistry Laboratory, Institute of Ecology at the University of Georgia, for measurement of stable isotope ratios (¹³C/¹²C and ¹⁵N/¹⁴N). Differences between samples and standards (carbon: PeeDee Belemnite; nitrogen: atmospheric air) are reported as parts per thousand (‰) (Peterson & Fry 1987):

$$\delta^{13}\text{C}(\text{‰}) = \left[\left(\frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} \right) - 1 \right] \times 1000$$

$$\delta^{15}\text{N}(\text{‰}) = \left[\left(\frac{^{15}\text{N}/^{14}\text{N}_{\text{sample}}}{^{15}\text{N}/^{14}\text{N}_{\text{standard}}} \right) - 1 \right] \times 1000$$

Standard deviations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of replicate analyses of samples of the internal standard materials were 0.13% and 0.14%, respectively. $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ biplots for anurans and primary food sources were used to evaluate patterns of isotopic variation between habitats. Differences between average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of anuran muscle and production source samples were evaluated with the Mann–Whitney test (U).

Before estimating the proportional assimilation of basal production sources by frogs, we tested if the isotopic variability of frog muscle tissue fell within the isotopic mixing polygon defined by the basal resources from the corresponding habitat (Phillips *et al.* 2014). We performed this test using isotope mixing polygon simulations, which determine the limits of source values in the $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ biplot that may contribute to consumers (Smith *et al.* 2013). We used average TDF (trophic discrimination factor) values (± 0.1 SD) of 1.3 ± 0.30 for $\delta^{13}\text{C}$ and 2.9 ± 0.32 for $\delta^{15}\text{N}$ (McCutchan *et al.* 2013). TDF values were multiplied by the average trophic position (TP) of each anuran species in

order to take isotope fractionation along the food chain into account (Phillips *et al.* 2014). We used the *tRophicPosition* package in R (Quezada-Romegialli *et al.* 2018) to estimate the TP of each anuran species in each habitat, Appendix S1). Any samples located outside the 95% mixing polygon region were excluded from the mixing models, because those may not be explained by the basal resources included in the dataset (Smith *et al.* 2013; Fig. 2a–h). These analyses were computed using the *sp* (*Classes and Methods for Spatial Data* in R, Pebesma & Bivand 2005) and *splancs* packages (Bivand *et al.* 2017).

We applied Bayesian isotopic mixing models to estimate the relative contributions of basal production sources for anurans in both habitats using the SIMMR (*Stable Isotope Mixing Models* in R) package (Parnell 2016). These simulations were generated using a Dirichlet prior distribution (Parnell *et al.* 2010). We fitted these models using the Markov Chain Monte Carlo (MCMC) method, which generates simulations of source contributions to the tissue of a consumer. We ran 500 000 iterations for each model and discarded the first 50 000 as recommended by Parnell (2016).

Wetland plants classified as C₄ were grasses of the Poaceae and Cyperaceae. Samples of these plants had $\delta^{13}\text{C}$ values lower than those typical of C₄ plants (e.g. *K. vaginata* $\delta^{13}\text{C}$ was -20.37) and more characteristic of plants with C₃ metabolism. Some grasses are capable of changing their carbon fixation mechanism in response to environmental variation, such as changes in moisture and temperature. According to Bruhl and Wilson (2007), most Cyperaceae have C₄ metabolism, but there are species with intermediary metabolism and others with the ability to change their carbon fixation mechanism (e.g. some *Eleocharis* species). Species belonging to Poaceae also have variable carbon fixation mechanisms, including plants with intermediate pathways (C₃–C₄; Christin *et al.* 2009). Furthermore, variation in plant metabolism can be observed along an environmental gradient. C₄ plants often are less common in habitats prone to flooding (Wantzen *et al.* 2002). In order to reduce the number of sources for isotopic mixing model analysis and facilitate inferences about source contributions, wetland grass samples with low $\delta^{13}\text{C}$ values uncharacteristic of C₄ photosynthesis were excluded from the analysis (n samples excluded = 2).

The isotopic space based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of each anuran species from each habitat was calculated using the Bayesian standardised ellipse area corrected for small samples (SEAc). SEAc is an isotopic metric unaffected by differences in sample sizes among groups that can be obtained using the program SIBER (*Stable Isotope Bayesian Ellipses* in R) (Jackson *et al.* 2011). An isotopic baseline correction is required to compare isotopic niches of consumers from different habitats containing basal production sources that vary in their isotopic composition (Newsome *et al.* 2007; Hoeninghaus & Zeug 2008). We applied the standardisation method suggested by Catry *et al.* (2016) that subtracts the mean value of basal sources (C₃, C₄, POM, PERI) from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of each consumer from a given site (wetland and dunes) and then divides by the range value of the same sources. We calculated the pairwise overlap of isotopic spaces for anuran species in each habitat using an asymmetrical overlap metric (Albernaz *et al.* 2016). Average interspecific isotopic overlap of each anuran

Table 1. Number of samples (*n*) and mean values (\pm SD) of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios (in parts per thousand, ‰) of anurans and basal sources collected in the wetland and dunes

			$\delta^{13}\text{C}$ (‰)						$\delta^{15}\text{N}$ (‰)					
	Wetland <i>n</i>	Dunes <i>n</i>	Wetland		Dunes		<i>P</i> -value	Wetland		Dunes		<i>P</i> -value		
			Mean	SD (\pm)	Mean	SD (\pm)		Mean	SD (\pm)	Mean	SD (\pm)			
<i>Boana pulchella</i>	15	4	-22.8	2.4	-20.3	1.3	0.06	6.2	0.5	3.7	1.5	0.01		
<i>Leptodactylus latrans</i>	17	6	-21.6	2.7	-20.6	1.0	0.24	5.6	0.8	6.0	0.9	0.42		
<i>Physalaemus biligonigerus</i>		22			-17.4	1.5				2.9	0.8			
<i>Physalaemus gracilis</i>	27		-17.9	2.6				5.5	0.4					
<i>Pseudis minuta</i>	18	44	-23.3	0.9	-22.6	2.0	0.16	5.4	0.8	6.6	0.9	<0.001		
POM	7	2	-26.0	1.9	-22.7	3.5	0.01	1.0	0.6	-0.5	0.6	0.04		
PERI	4	2	-36.9	1.5	-17.0	3.7	0.01	0.7	0.8	1.0	1.1	0.24		
C ₃ plants	3	12	-29.3	1.5	-29.1	1.4	0.95	-1.7	0.8	-2.7	1.4	0.09		
<i>Bacopa monnieri</i>	1		-27.8					-1.4						
<i>Desmodium cf. adscendens</i>	1	2	-30.7		-29.0			-2.6		-2.8				
<i>Enydra sessilifolia</i>	1		-29.5					-1.0						
<i>Achyrocline satereioides</i>	1			-27.9					-3.2					
<i>Baccharis trimera</i>	1			-31.2					-5.5					
<i>Drosera</i> sp.	1			-27.0					-1.5					
<i>Polygala cyparissius</i>	1			-28.5					-2.2					
<i>Margynicarpus pinnatus</i>	1			-30.1					-1.2					
<i>Senecio crassiflorus</i>	3			-29.4					-2.4					
<i>Vernonia cf. brevifolia</i>	2			-29.1					-3.3					
C ₄ plants	2	2	-12.2	2.4	-12.3	0.1	0.69	-0.3	0.2	-5.3	3.0	0.24		
<i>Sporobolus virginicus</i>	1	1	-13.9	0.03										
<i>Kyllinga vaginata</i>	1	1	-10.5	-0.30										
Poaceae		2			-12.3	0.1				-5.3	3.0			

Sources: POM (particulate organic matter), PERI (periphyton), C₃ plants (average values of C₃ plants), C₄ plants (average values of C₄ plants).

assemblage was estimated, which correspond to the average of all overlaps involving the anuran species for each habitat. We applied the non-parametric Kruskal–Wallis test (KW) to compare the mean overlap between anurans between habitats (Hammer 2017).

RESULTS

Dietary patterns

Two hundred frog specimens were collected, including 97 from the wetland and 103 from the sand dunes (Appendix S2). The mean encounter rate was similar in both habitats: 4.85 anurans h⁻¹ (one average, one anuran every 20 min) in the wetlands and 5.15 anurans h⁻¹ (an average of one anuran every 19 min) in the sand dunes. *Leptodactylus latrans* occurred in both habitats and was the largest species (wetland: SVL = 37.60 \pm 13.90 mm, *n* = 24; dunes; 46.50 \pm 7.77 mm, *n* = 11) (Appendix S2). *Boana pulchella* occurred in both habitats and tended to be larger in the wetland than in the sand dunes (wetland: 37.27 \pm 2.91 mm, *n* = 23; dunes; 23.88 \pm 2.84 mm, *n* = 12; *U* = 0; *P* < 0.001; Appendix S2). *Physalaemus gracilis*

was only collected from the wetland where it was the smallest species (25.71 \pm 2.61 mm, *n* = 25) (Appendix S2). *Boana pulchella* occurred in both habitats, and it was the smallest species in the dunes (23.88 \pm 2.84 mm, *n* = 12; Appendix S2). *Physalaemus biligonigerus* (*n* = 22) was only captured from dunes and had an average SVL of 28.59 \pm 2.72 mm (*U* = 107; *P* < 0.001) (Appendix S2). *Pseudis minuta* was common in both habitats, and samples revealed no statistically significant size difference (wetland: 28.97 \pm 5.08 mm, *n* = 25; dunes: 31.30 \pm 3.34 mm, *n* = 58; *U* = 293; *P* = 0.08; Appendix S2).

Sixteen and 18 food categories were identified in stomach contents of anurans from the wetland and dunes, respectively (Appendix S3). Intraspecific comparison of diets in the two habitats for the three species that occurred in both (*B. pulchella*, *L. latrans* and *P. minuta*) revealed differences in the average relative abundance (%AP) of their dominant prey (Fig. 3a,b). Hemiptera (%AP = 21.3), Coleoptera (%AP = 19.7) and Lepidoptera (%AP = 15.3) showed greater contribution in the anurans' diets in the wetland, whereas Coleoptera (%AP = 35.3), Odonata (%AP = 28.0), Hymenoptera (%AP = 10.6) were the most abundant food items in the dunes (Fig. 3a,b).

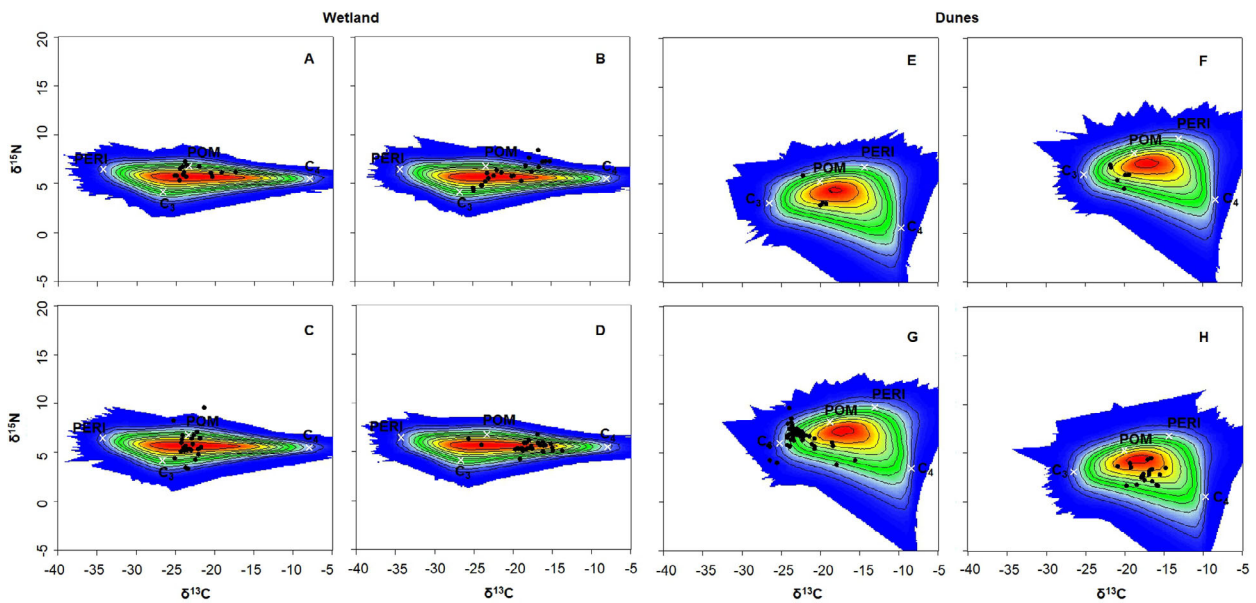


Fig. 2. Biplots of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios with simulated polygons for frogs based on their estimated trophic levels and trophic isotopic fractionation of available basal sources; frog positions are represented by black dots and basal sources are represented by white crosses. Probability contours are at the 5% level (outermost contour) and 10% level. Anurans: wetland (a) *Boana pulchella*; (b) *Leptodactylus latrans*; (c) *Pseudis minuta* and (d) *Physalaemus gracilis*; dunes (e) *Boana pulchella*; (f) *Leptodactylus latrans*; (g) *Pseudis minuta* and (h) *Physalaemus biligonigerus*. Sources: POM (particulate organic matter), PERI (periphyton), C_3 (C_3 plant), C_4 (C_4 plant). TDF values: 1.3 ± 0.30 for $\delta^{13}\text{C}$ and 2.9 ± 0.32 for $\delta^{15}\text{N}$ (McCutchan *et al.* 2013).

Anurans had greater dietary breadth in the wetland than in the dunes (Wetland, $H' = 1.62$; Dunes, $H' = 1.16$; $t_{177.6} = 3.44$, $P < 0.05$). Observed dietary overlap was greater than expected by chance for anurans in the wetland (observed: 0.50; expected: 0.29; $P < 0.01$) and dunes (observed: 0.32; expected: 0.12; $P < 0.02$). Average dietary overlap was lower among frogs from the sand dunes than those from the wetland ($t = -47.65$; $P < 0.05$).

Stable isotope patterns

The range in average $\delta^{13}\text{C}$ values of basal production sources was greater in the wetland (-38.7‰ to -10.5‰) compared to the dunes (-31.6‰ to -12.2‰ ; Fig. 4a,b), but mean $\delta^{13}\text{C}$ for frog assemblages of the two habitats were not significantly different ($U = 114$; $P > 0.45$). In contrast, average $\delta^{15}\text{N}$ values of basal production sources were greater in the dunes (-7.4‰ to 1.8‰) than in the wetland (-2.6‰ to 1.8‰), and the average $\delta^{15}\text{N}$ of basal sources was lower in dunes ($U = 37$; $P < 0.001$). Between-habitat comparisons of average $\delta^{13}\text{C}$ values for each basal production source revealed statistically significant differences for POM ($P < 0.01$) and PERI ($P < 0.01$), with both sources showing lower average values in the wetland (Table 1). Average $\delta^{15}\text{N}$ values showed

statistically significant differences only for POM ($P < 0.04$), which had lower average value in the dunes (Table 1).

Anuran assemblages in both habitats revealed similar variation in $\delta^{13}\text{C}$, with values ranging from -25.7‰ to -13.8‰ in the wetland and from -26.5‰ to -14.8‰ in the dunes (Fig. 4a,b). There was slightly greater variation in $\delta^{15}\text{N}$ values in the dunes ($1.6\text{--}8.1\text{‰}$) than in the wetland ($3.4\text{--}7.3\text{‰}$; Fig. 4a,b). Among anurans that occurred in both habitats, between-habitat differences in $\delta^{15}\text{N}$ were significantly different for *B. pulchella* ($P < 0.01$) and *P. minuta* ($P < 0.001$), with the former showing higher average value in the wetland than in the dunes and the latter the opposite pattern (Table 1).

In general, wetland frogs assimilated material from a greater variety of basal production sources compared to frogs from the dunes (Fig. 5a–h). C_3 plants were the dominant production source supporting most species in the dunes (Fig. 5e–h). The basal sources contribution varied among frog biomass in the wetland were as follows: *B. pulchella* (POM: 10–78%), *P. minuta* (C_3 plants: 21–53%) and *P. gracilis* (C_4 plants: 40–58%) (Fig. 5a–d). Estimates of proportional assimilated of material by *L. latrans* were similar for the four major sources (Fig. 5b). C_3 plants were estimated to have the greatest contribution for three of the four frog species in the dunes

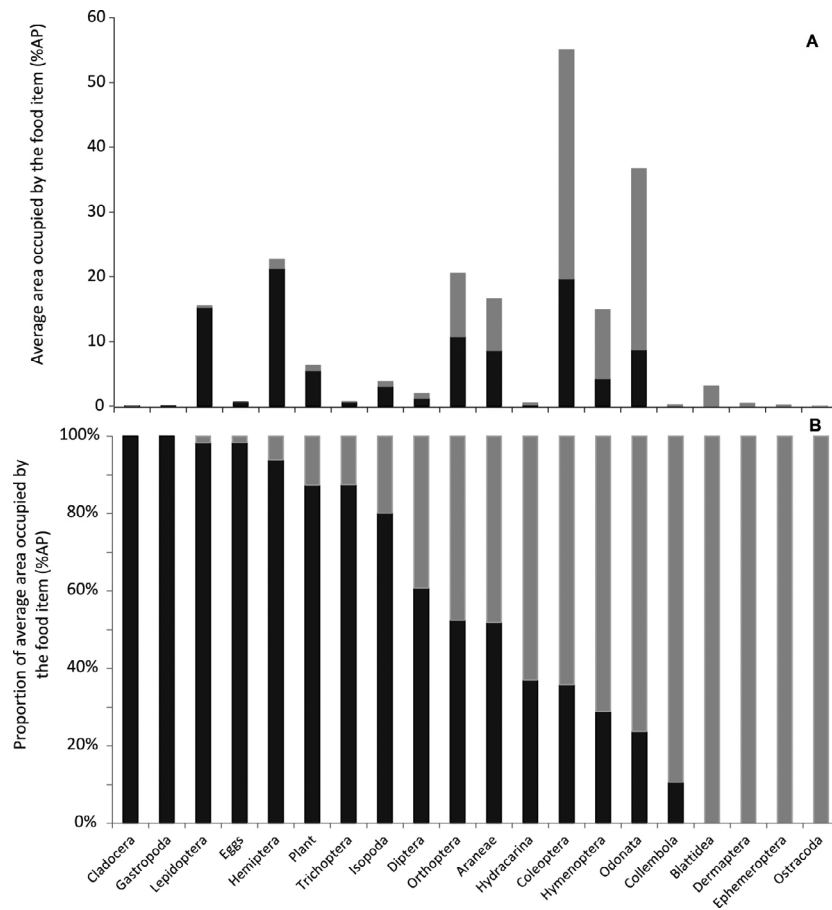


Fig. 3. Average area occupied by the food item (%AP) (a) and the proportion of average area occupied by the food item (b) from frog species in the wetland (black bars) and sand dunes (grey bars).

(*B. pulchella*: 9–63%, *L. latrans*: 44–72%, *P. minuta*: 60–78%; Fig. 5e–g), and carbon from C₄ plants (30–42%) contributed most to the biomass of *P. biliginigerus* (Fig. 5h). Only 11 individuals (5 *L. latrans* and 2 *P. minuta* in the wetland (Fig. 2b,c), and 4 *P. minuta* in the dunes (Fig. 2g) were excluded from the mixing model analyses because their isotopic variation plotted outside the isotopic mixing polygon defined by the basal production sources.

Isotopic ellipse sizes (SEAc) and their degree of overlap varied among species within habitats, for the same species between habitats, and between anuran assemblages from the two habitats (Fig. 6a,b). At the species level, *B. pulchella* and *L. latrans* had larger isotopic ellipses in the wetland (4.27‰ and 4.29‰, respectively) than in the dunes (2.56‰ and 2.86‰, respectively), whereas *P. minuta* showed the opposite pattern (2.43‰ – wetland, 4.96‰ – dunes; Fig. 6a, b). Mean interspecific overlap among isotopic ellipses (SEAc) was significantly higher in the wetland (0.14‰) than in the dunes (0.04‰; KW-H (1;24) = 4.2551; *P* = 0.0391). In the wetland, all species had some degree of isotopic overlap with the

other species, highest average overlap values were recorded for *P. minuta* (0.26 ± 0.27‰) and *L. latrans* (0.18 ± 0.12‰; Fig. 6c). In the dunes, only two species (*L. latrans*: 0.12 ± 0.20‰; and *P. minuta*: 0.07 ± 0.12‰) had isotopic ellipses that overlapped with other frog species (Fig. 6d).

DISCUSSION

The findings corroborated our prediction that frogs inhabiting the wetland would have broader trophic niches and be supported by more diverse basal production sources than frogs from the less-productive sand dunes. Isotopic mixing models estimated that the anuran biomass in the wetland is supported by POM and variable fractions from C₃ and C₄ plants, and frogs in the sand dunes were supported mostly by food chains originating from C₃ plants. Other studies have revealed multiple primary producers supporting food webs of coastal ecosystems, including other wetlands in southern Brazil (e.g. Garcia *et al.* 2017). For example, Catry *et al.* (2016) found

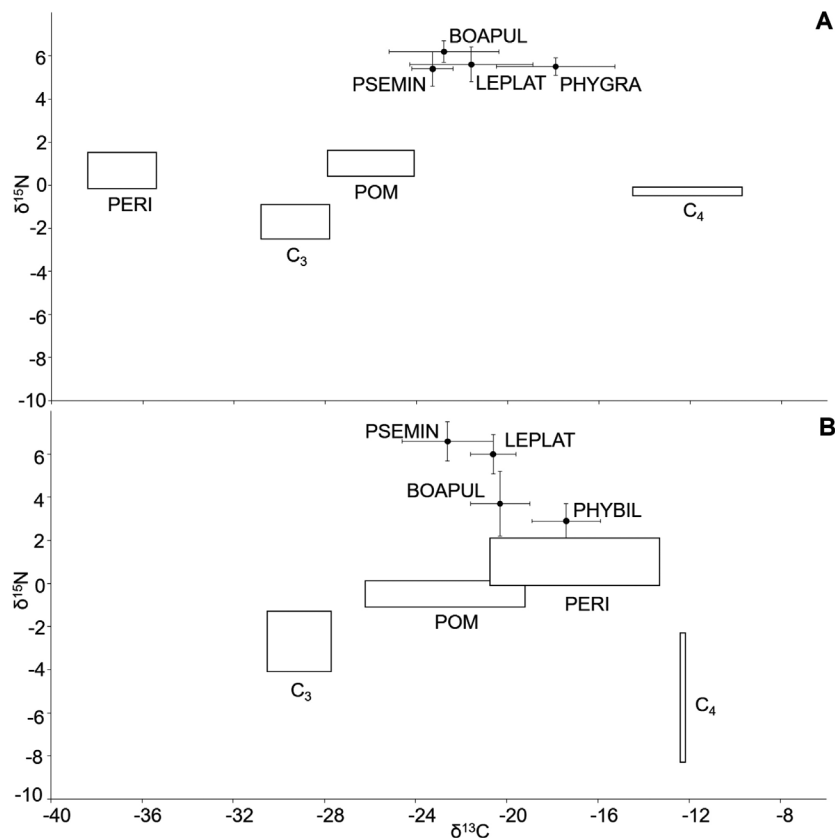


Fig. 4. Average (\pm SD) values of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios of frog species (black dots) and primary sources (open boxes) from the wetland (a) and dunes (b). Anurans: BOAPUL (*Boana pulchella*), LEPLAT (*Leptodactylus latrans*), PHYBIL (*Physalaemus biligonigerus*), PHYGRA (*Physalaemus gracilis*), PSEMIN (*Pseudis minuta*). Sources: POM (particulate organic matter), PERI (periphyton), C₃ (C₃ plant), C₄ (C₄ plant).

that productive coastal ecosystems with diverse basal production sources were associated with relatively low trophic redundancy among consumers. In our study, wetland frogs appeared to be supported by food chains originating from at least three basal sources, but frogs living in the sand dunes were supported by food chains with C₃ plants at the base. Thus, frogs in the more productive habitat seem to be supported by more diverse sources of basal production.

The assimilation of material derived from mostly from C₃ plants by frogs in the sand dunes was somewhat surprising considering that the plant community was dominated by species that use C₄ and CAM (Crassulacean Acid Metabolism) photosynthetic pathways (Seeliger *et al.* 2000; Seeliger & Kjerfve 2013). These plants are adapted for environments with water stress and strong solar irradiance (Lane *et al.* 2008). C₄ and CAM plants tend to be more enriched in ^{13}C than C₃ plants (average $\delta^{13}\text{C}$: -14‰ and -27‰ , respectively), mainly due to differences in the enzymes responsible for carbon fixation during photosynthesis (Marshall *et al.* 2007). Therefore, we expected that frogs from the sand dunes would have

comparatively higher $\delta^{13}\text{C}$ values due to assimilation of prey supported by food chains originating from dominant C₄ and CAM plants. Instead, most sand dune frogs had relatively low $\delta^{13}\text{C}$ reflecting assimilation of material originating from C₃ plants. A possible explanation for this result is that a substantial contribution of C₃ plant material to anuran biomass in the sand dunes results from a spatial food web subsidy from the wetlands dominated by C₃ plants (Rolon *et al.* 2011). Spatial food web subsidies are especially important when connected ecosystems have asymmetric productivity, with the energy flowing from a highly productive (donor) to less-productive (receiver) system (Polis *et al.* 1997; Koshino *et al.* 2013; Garcia *et al.* 2017). These contrasting habitat features, and the inferred direction of energy and biomass flow are consistent with our study system and findings. Among the potential biological vectors able to transfer C₃ plant-derived material from the wetland to the dunes are insects, such as dragonflies (Odonata) and flies (Diptera), that have an aquatic life stage. Upon emergence as winged adults, these insects disperse and subsidise consumers in terrestrial habitats (Gladyshev *et al.* 2011;

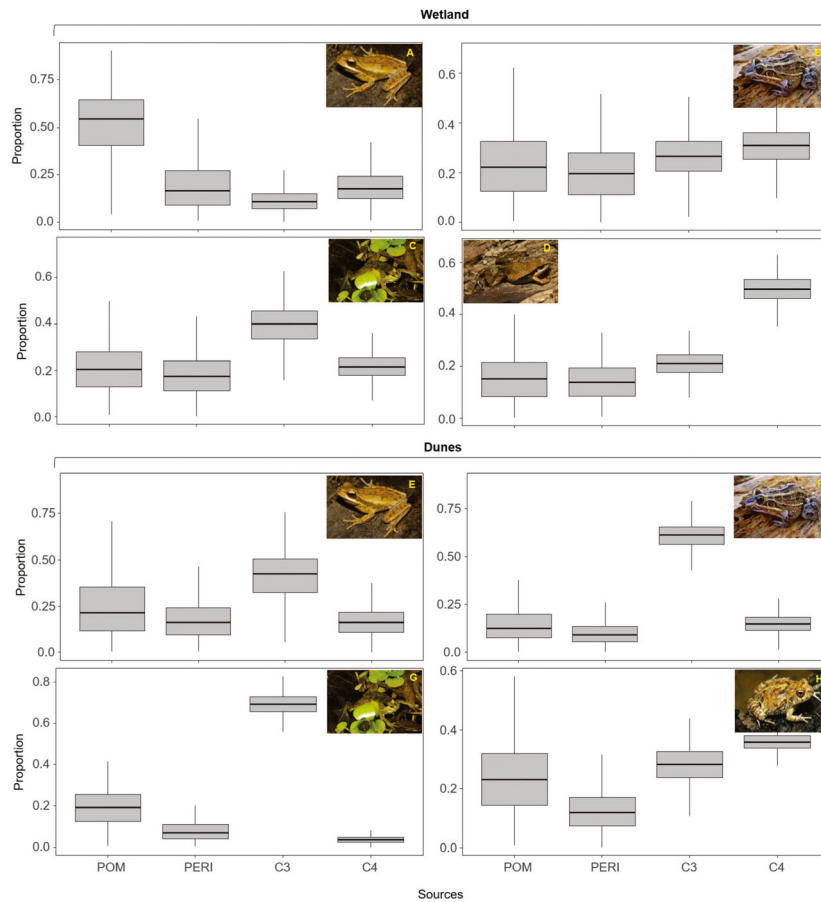


Fig. 5. Comparison of basal source contributions to frog biomass in the wetland and sand dunes (95% credible interval values from the SIMMR Bayesian isotopic mixing model). Anurans: wetland (a) *Boana pulchella*; (b) *Leptodactylus latrans*; (c) *Pseudis minuta* and (d) *Physalaemus gracilis*; dunes (e) *Boana pulchella*; (f) *Leptodactylus latrans*; (g) *Pseudis minuta* and (h) *Physalaemus biligonigerus*. Photographs: Daniel Loebmann. Sources: POM (particulate organic matter), PERI (periphyton), C₃ (C₃ plant), C₄ (C₄ plant).

Ciss *et al.* 2013). Although there are ephemeral water bodies in the dunes that might support dragonfly nymphs (Arthington & Watson 1982), these small, shallow pools probably produce a small fraction of the dragonflies produced by nearby wetlands (Malchik *et al.* 2010). Dragonflies are important prey for several groups of aquatic vertebrates (Huckembeck *et al.* 2014; Gonçalves *et al.* 2018), including several of the frog species that inhabit the sand dunes. Odonata was the most common prey of *B. pulchella* (% AP = 58.82) and *P. minuta* (%AP = 52.97) in the dunes. The magnitude and timing of spatial food web subsidies involving aquatic insects and frogs, including their influence on frog population dynamics, could be estimated via manipulative field experiments and modelling.

We also observed a difference in $\delta^{15}\text{N}$ values of primary producers between the wetland and the sand dunes, with the sand dunes having greater variability in these values. The physiology of C₃ and C₄ plants largely determines variation in their $\delta^{13}\text{C}$; however,

plant $\delta^{15}\text{N}$ values may vary due to the influence of several factors, such as the nutrient availability, fire exposure and hydric stress (Bustamante *et al.*, 2006). Therefore, depleted $\delta^{15}\text{N}$ values found among plants inhabiting sand dunes may be related to the low availability of nutrients in the environment (mainly nitrogen). When nitrogen is limiting in the environment, plants increase its retention in tissues resulting in lower isotopic fractionation. For example, many plants in the Brazilian Cerrado, a dry region, have negative $\delta^{15}\text{N}$ values (Bustamante *et al.* 2004). Nitrification and denitrification rates are low in this system, and N isotopic fractionation in plant tissues also is low (Bustamante *et al.* 2004). Plants growing in the sand dunes may have similar ecophysiological strategies affecting N isotopic fractionation, and research is needed to reveal factors that influence $\delta^{15}\text{N}$ variation in plants of subtropical coastal dunes.

Analyses of stomach contents and stable isotope data revealed lower interspecific trophic overlap among frogs from the dunes, thus corroborating our

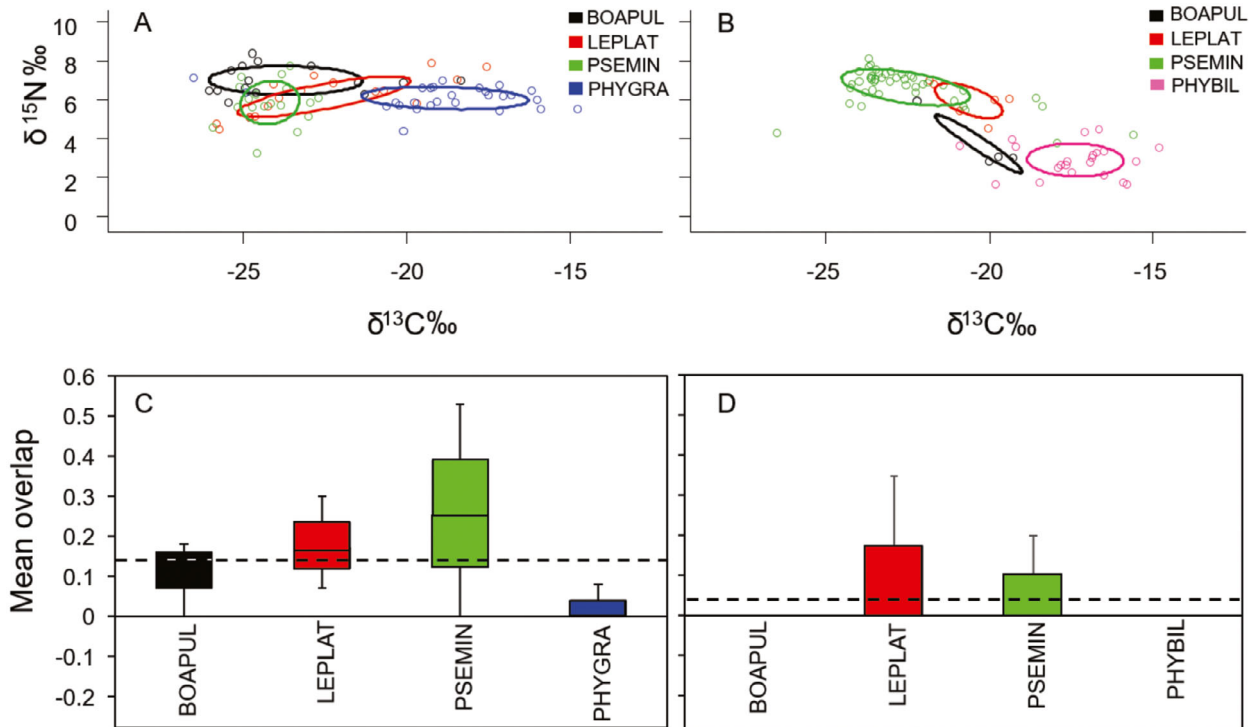


Fig. 6. Plots of isotopic spaces occupied by frog species in the wetland (a) and dunes (b) within Lagoa do Peixe National Park based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The lower graphs show the variation of isotopic overlap for each species (\pm SE) in the wetland (c) and in the sand dunes (d); the mean overlap for the entire assemblage is shown by a horizontal line. Anurans: BOAPUL (*Boana pulchella*), LEPLAT (*Leptodactylus latrans*), PHYBIL (*Physalaemus biligonigerus*), PHYGRA (*Physalaemus gracilis*), PSEMIN (*Pseudis minuta*).

second hypothesis (Fig. 3a,b,6a–d). This pattern could reflect trophic niche partitioning in response to competition for relatively scarce invertebrate prey in dune habitat (Pianka 1974; Oliveira *et al.* 2018). Previous studies in subtropical coastal regions found that the numerical abundance of invertebrate prey was approximately 16-fold lower in sand dunes compared with wetlands (Masson & McLachlan 1990; Huckembeck *et al.* 2018). A recent study that compared trophic niches of frogs from the Caatinga (dry region) with frogs from the Atlantic Forest (mesic region) of Brazil similarly found lower prey availability and lower niche overlap in the dry region (Caldas *et al.* 2019). In our study, trophic niche overlap was higher for frogs in the wetland where primary productivity and prey availability were greater, which implies a lack of competition for food resources in the coastal wetland (Oliveira *et al.* 2019).

We observed highest isotopic overlap between *L. latrans* and *P. minuta* in both habitats. This is probably due to their use of diverse microhabitats and ability to ingest larger prey compared to the other frog species in these habitats. For example, *P. minuta* inhabits water bodies but feeds in riparian areas (Huckembeck *et al.* 2012). *L. latrans* commonly feeds on insects within terrestrial habitats

(Huckembeck, unpublished data), but also consumes aquatic invertebrates. In the wetland, *B. pulchella* had low dietary overlap with *P. minuta* and *L. latrans*, and no overlap with frogs inhabiting the sand dunes. *B. pulchella* usually was captured from phytotelmata, aquatic macrophytes, grasses and shrubs within the wetland; in the dunes, this species was associated only with terrestrial vegetation. Because *P. gracilis* and *L. latrans* use similar microhabitats (e.g. grasslands and ephemeral water bodies) for foraging, we expected these species to have high isotopic overlap. Their relatively low isotopic overlap in the wetland might be explained, at least in part, by differences in average body size (*P. gracilis* 25.7, *L. latrans* 37.3 mm). Smaller frogs may be more restricted in their use of microhabitats for foraging (Huckembeck *et al.* 2018). Several recent studies also concluded that frog diets were strongly influenced by microhabitat use (e.g. Cloyd & Eason 2017; Huckembeck *et al.* 2018). Finally, *P. biligonigerus* was the only species that had no isotopic overlap with other species. This species is the most terrestrial among frog species in the region and was only captured from sand substrate during our study.

In conclusion, coastal habitats with strongly contrasting features were associated with predictable

differences in the trophic structure of anuran assemblages. Frog biomass in the more productive habitat (wetland) was supported by food chains sustained by a greater diversity of basal production sources. Frogs in the less-productive sand dunes were supported largely by food chains originating from C_3 plants. Analysis of dietary and stable isotope data corroborated our prediction of greater niche partitioning among frogs inhabiting sand dunes. Dietary and isotopic data also indicated a potential spatial food web subsidy between wetland and nearby dune habitats, a topic that merits further research using experimental and modelling approaches.

Protection of coastal wetlands will be important for conservation of frog diversity in southern Brazil. Some of the region's wetlands already have been and drained for agriculture and silviculture. This impacts frog populations not only directly by reducing habitat, but also indirectly by the decreased availability of insect prey originating from wetlands that may be consumed by frogs and other animals resident in adjacent areas.

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AUTHOR CONTRIBUTIONS

Sônia Huckembeck: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); writing-original draft (equal); writing-review & editing (equal). **Kirk O. Winemiller:** Formal analysis (equal); methodology (equal); supervision (equal); writing-original draft (equal); writing-review & editing (equal). **Daniel Loebmann:** Writing-review & editing (equal). **Alexandre Miranda Garcia:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); supervision (lead); writing-

original draft (equal); writing-review & editing (equal).

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SUPPORTING INFORMATION

Additional supporting information may/can be found online in the supporting information tab for this article.

Appendix S1. Average in estimated TP (\pm SD) of anuran in the wetland and in the dunes and the credible interval (95%).

Appendix S2. Mean snout–vent length (\pm SD) and the Mann–Whitney *P*-values among anuran species.

Appendix S3. Area percentage (%AP) of the stomach content of the anurans in the wetland and dunes.