

What do stable isotopes tell us about the trophic ecology of *Thamnodynastes hypoconia* (Serpentes: Dipsadidae) in southern Brazil?

Marluci Müller Rebelato^{a,b,*}, Kirk O. Winemiller^b, Andrew M. Durso^c,
Alexandro Marques Tozetti^d, Plínio Barbosa de Camargo^e, Laura Verrastro^f

^a Programa de Pós-graduação em Biologia Animal, Laboratório de Herpetologia, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves 9500, Agronomia, 91501-970, Porto Alegre, RS, Brazil

^b Department of Ecology and Conservation Biology, Texas A&M University, College Station, TX, 77843, USA

^c Department of Biological Sciences, Florida Gulf Coast University, 10501 FGCU Blvd. S., Fort Myers, FL, 33965, USA

^d Laboratório de Ecologia de Vertebrados Terrestres, Universidade do Vale do Rio dos Sinos, Avenida Unisinos 950, 93022-000, São Leopoldo, RS, Brazil

^e Laboratório de Ecologia Isotópica, Centro de Energia Nuclear na Agricultura, Avenida Centenário 303, São Dimas, 13416-000, Piracicaba, SP, Brazil

^f Laboratório de Herpetologia, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves 9500, Agronomia, 91501-970, Porto Alegre, RS, Brazil

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ABSTRACT

Snakes are a useful model for ecological studies because they are gape-limited predators that may undergo ontogenetic changes in diet. We analyzed carbon and nitrogen stable isotope ratios to estimate percent contributions of different prey to snake biomass, trophic positions and isotopic niche width of juveniles and adults of the snake *Thamnodynastes hypoconia*. We also estimated the isotopic niche overlap between the two age categories. During eight intervals over a two-year period, we collected samples of whole blood and scales at a site in southern Brazil. Isotopic ratios of carbon and nitrogen did not differ between juveniles and adults for either tissue type, nor did mean trophic positions of juveniles and adults differ. The percent contribution of prey categories to snake biomass differed to a limited extent between the two years, with Hylidae being the most important anuran group assimilated during the first year and Leptodactylidae during the second year, for both ages. The isotopic niche occupied by adult snakes was slightly larger than that of juveniles when the analysis was based on data from whole blood samples, as expected because snakes are gape-limited. We found a reverse pattern when the analysis was based on scales, which may indicate that adult snakes have a smaller niche over the long term as they become selective foragers in certain prey. Isotopic overlap between juveniles and adults occurred during the two years, but it was bigger during the second year. We infer that, despite differences in gape size, juvenile and adult snakes in the study area exploit similar prey, with the degree of trophic similarity varying interannually.

1. Introduction

Being exclusively carnivorous and ingesting their prey whole, snakes represent a unique group among vertebrates and a useful model system for ecological research (Greene, 1997; Shine and Bonnet, 2000; Shine et al., 2002; Luiselli, 2006a). Although snakes are gape-limited predators, most species, nonetheless, ingest a wide variety of prey (Vincent and Herrel, 2007). Snakes possess a highly kinetic skull, and many species use their body to grasp and squeeze prey, while others produce venom to subdue and digest prey (Shine, 1991; Arnold, 1993;

Cundall and Greene, 2000; Luiselli, 2006b).

Ontogeny is one of the main drivers of intrapopulation variation in diet (Shine, 1991; Arnold, 1993; Bolnick et al., 2011) and evidence for ontogenetic diet shifts is especially strong for snakes (Shine and Wall, 2007). Because snakes are gape-limited predators that consume prey whole, as a snake grows, it can ingest larger prey items (Mushinsky et al., 1982; Rodríguez-Robles et al., 1999; Shine et al., 2002; Willson et al., 2010; Durso and Mullin, 2017). If ontogenetic diet shifts are sufficiently large, conspecifics may be considered distinct functional groups within the local food web (Olson, 1996). To better understand

* Corresponding author. Present address: Programa de Pós-graduação em Biologia Animal, Laboratório de Herpetologia, Departamento de Zoologia, Instituto de Biociências, Prédio 43435, Sala 102, Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves 9500, Agronomia, 91501-970, Porto Alegre, RS, Brazil.

E-mail addresses: marluci.rebelato@gmail.com, mmrebelato@yahoo.com.br (M.M. Rebelato), k-winemiller@tamu.edu (K.O. Winemiller), amdurso@gmail.com (A.M. Durso), alexandro.tozetti@gmail.com (A.M. Tozetti), pcamargo@cena.usp.br (P.B. de Camargo), lauraver@ufrgs.br (L. Verrastro).

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multiple roles of species within food webs, methods are needed to discern ontogenetic niche shifts (Bearhop et al., 2004; Newsome et al., 2007) and their potential effects on predator-prey interactions (Polis, 1984; Bolnick et al., 2003, 2011; Tinker et al., 2007) and ecosystem dynamics (Subalusky et al., 2009).

Stable isotope analysis (SIA) has proven to be a useful method to differentiate trophic niches (Peterson and Fry, 1987). The multivariate 'isotopic niche' has been compared to the n -dimensional ecological niche (Bearhop et al., 2004; Newsome et al., 2007). Carbon stable isotopic ratios ($\delta^{13}\text{C}$) are particularly useful to distinguish sources of organic matter input because ratios shift relatively little between adjacent trophic levels (Kelly, 2000; Sherwood and Rose, 2005; Caut et al., 2009). Nitrogen stable isotopic ratios ($\delta^{15}\text{N}$) undergo gradual enrichment between a food resource and consumer and therefore can be used to estimate trophic levels (DeNiro and Epstein, 1981; Kelly, 2000; Vanderklift and Ponsard, 2003; Caut et al., 2009).

Different tissues of the same animal have different element assimilation rates (Hobson and Clark, 1992), and more metabolically active tissues, such as blood, plasma, and liver, tend to have more rapid assimilation rates than less active tissues, such as bone, scale and claw (Tieszen et al., 1983; Hobson and Clark, 1992; Pruett et al., 2003; Dalerum and Angerbjörn, 2005). Analysis of stable isotopes from multiple tissues is particularly useful for studying trophic ecology of snakes, because predation events are difficult to observe in nature. Most snakes feed infrequently, and their stomachs usually are empty or contents are partially digested and difficult to identify (Greene, 1997). The rate of element assimilation, or turnover, besides being species-specific, is also affected by factors such as temperature and life-history stage (Bearhop et al., 2002; Witting et al., 2004). Although relatively little research has been conducted on assimilation rates of reptiles, ectotherms tend to have slower rates than endotherms of the same size (Warne et al., 2010). Hobson and Clark (1992) found that turnover rates (measured as half-life in days) varied among tissues in birds (liver ca. 2.6, blood ca. 11.4, muscle ca. 12.4, and bone collagen ca. 173.3). Consumer tissues generally are considered at equilibrium with their diet after 4–5 half-lives (Hobson and Clark, 1992). According to Vander Zanden et al. (2015), the half-life estimates for ectothermic vertebrates was 17 days for plasma, 36 days for blood, and 88 days for muscle.

Some studies with reptiles have shown that differences in isotopic turnover rates among tissues vary among species. For a crocodylian species, the half-life for nitrogen was about 62 days for plasma, 103 days for scutes, and 277 days for red blood cells (Rosenblatt and Heithaus, 2013), values that reportedly are among the slowest recorded for any animal. In contrast, a freshwater turtle was found to have a nitrogen isotope half-life of just 35 days for plasma and 38 days for whole blood (Seminoff et al., 2007). Another study with a terrestrial lizard showed that the time for carbon incorporation in plasma was about 25 days, for red blood cells it was 60 days, and for skin 94 days (Warne et al., 2010). This high variability among reptilian groups, with turnover rates often estimated for only one element and not for all tissues, makes it difficult to generalize. What is known about snakes is that, based on a controlled experiment with a ^{15}N -depleted diet that does not reflect the natural diet and using only juveniles, the turnover rate was 300 and 454 days for whole blood and muscle, respectively (Fisk et al., 2009). In other words, considering that 4–5 half-lives are necessary for the tissue to achieve equilibrium with the diet, these would represent a half-life of around 75 days for whole blood and 113 days for muscle, respectively.

Compared to many other tissues, blood has relatively high metabolic activity, with faster turnover for plasma than red blood cells. The assimilation rate of nutrients in tissues with slower turnover, such as reptile scales, is poorly documented. Fish scales have relatively slow isotopic turnover and can provide an index for long-term trophic patterns (Pruett et al., 2003). Although the tissue isotopic turnover rates for the snake species studied here are undocumented, we assume that isotopic ratios from whole blood reflect a more recent diet than those

from scales.

During a two-year field study, we collected whole blood and scale samples from juveniles and adults of *Thamnodynastes hypoconia* and samples of the anuran families that are their principal prey. This snake is common in wetlands of southern Brazil, Paraguay, Uruguay and Argentina (Carreira and Maneyro, 2013) where average snout-vent length (SVL) of adults measures from 370 to 390 mm (Bellini et al., 2013; Rebelato et al., 2016). *T. hypoconia* is viviparous, nocturnal, semi-arboreal and has opisthognathous dentition (Giraud, 2001; Bellini et al., 2013; Rebelato et al., 2016). Previous studies on the trophic ecology of this species relied on stomach contents analysis (SCA) and found its diet consists mostly of frogs and toads, with minor fractions of lizards (Moya and Maffei, 2012; Bellini et al., 2013, 2014; Rebelato, 2014; Manoel and Almeida, 2017; Canhete et al., 2018). Bellini et al. (2013) found no difference in prey consumed between males and females, but nothing is yet known about potential ontogenetic changes in diet.

We analyzed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of whole blood and scale tissues to evaluate the trophic position, isotopic niche width and overlap between juveniles and adults. Percent contributions of different prey to the biomass of juveniles and adults were determined using mixing models. We predicted minor differences in carbon and nitrogen isotope ratios between ages since previous studies using SCA revealed a relatively narrow diet consisting mostly of anurans. In most snake species for which ontogenetic variation has been observed, adults and juveniles consumed different major prey types, such as amphibians and reptiles versus birds and mammals (Shine and Wall, 2007). We therefore anticipated small isotopic differences between snakes of different ages/sizes if they consume anurans from different families. Since snakes are gape-limited predators, we predicted that the isotopic niche width of juveniles would be smaller than that of adults, because juveniles would be restricted to consume only the smallest frogs (Werner and Gilliam, 1984; Shine, 1991; Arnold, 1993; Rodríguez-Robles et al., 1999). A better understanding of the trophic ecology of herpetofauna in wetlands is needed because these organisms link food webs of aquatic and terrestrial habitats (Waringer-Löschenkohl and Schagerl, 2001; Pryor, 2003). Wetland areas in the coastal plain of southern Brazil merit urgent attention because agriculture and introduced exotic vegetation, such as *Pinus* and *Eucalyptus*, are expanding rapidly and impacting native biodiversity and ecosystem processes (Zedler and Kercher, 2005; Batzer et al., 2006; Becker et al., 2007).

2. Material and methods

2.1. Study site

The study site was located in the coastal plain of the Rio Grande do Sul state, in southern Brazil, in a wetland area of approximately 5 km² at the margin of Patos Lagoon, in Tapes municipality (Fig. 1). This area is within the system called "Butiazal de Tapes", which has already been designated as a priority area for conservation (Burger and Ramos, 2007) because it supports a locally endangered palm (*Butia odorata*). The landscape of the coastal plain is formed by a mosaic of dunes, ponds, wetlands and riparian forests (Tomazelli et al., 2000; Becker et al., 2007). The climate in this region is classified as humid subtropical, with an average annual temperature of 18.8 °C and an average annual rainfall of 1.213 mm (Maluf, 2000).

2.2. Sample collection

Snakes and potential prey were sampled during eight field outings between May 2015 and September 2017. Snakes were located by searching with spotlights in microhabitats commonly used by these animals, such as shrubs (Campbell and Christman, 1982). Surveys were always conducted by three to four experienced biologists for 5 h and beginning at 0.5 h after sunset. Surveys were conducted over four consecutive nights during each of eight field outings. We captured

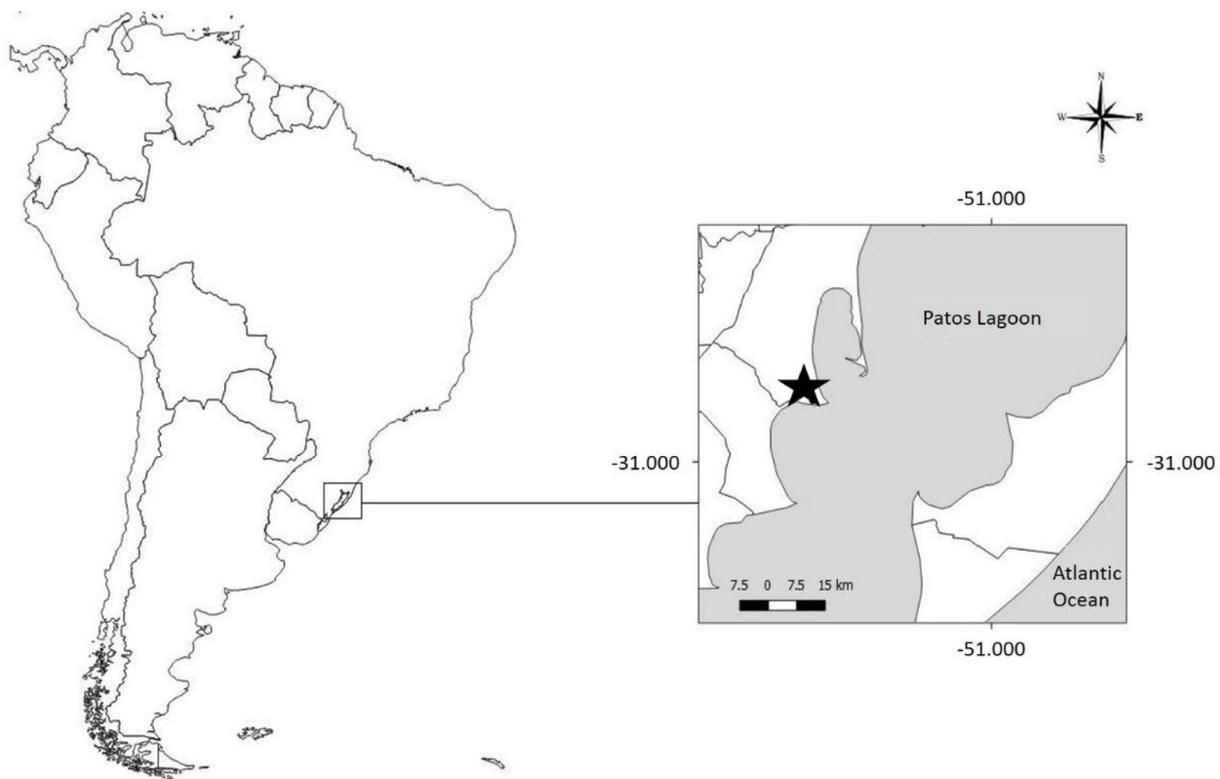


Fig. 1. Map showing the study area in Rio Grande do Sul state in southern Brazil. The black star indicates the location of the study area on the shore of Patos Lagoon (30°52'7.15"S and 51°23'41.92"W).

snakes by hand using gloves and measured snout-vent length (SVL, mm) using a measuring tape (accuracy of 1 mm). We collected ventral scale clips from two to four scales from the mid-body (see Willson et al., 2010). We collected blood from the caudal vein using a syringe (see Brischoux et al., 2011), and blood and scale samples were placed separately in microcentrifuge tubes. Specimens were returned alive to the locations where they were collected. Tissue samples were kept on ice until placed in a freezer in the laboratory.

We also collected muscle samples of potential prey of *T. hypoconia*. We targeted amphibians that were the most common prey found in snake stomach contents during previous investigations (Carreira, 2002; Pombal, 2007; Sawaya et al., 2008; Maffei et al., 2011; Moya and Maffei, 2012; Bellini et al., 2013, 2014; Dorigo et al., 2014; Rebelato, 2014; Manoel and Almeida, 2017; Canhete et al., 2018). These also were the most abundant species/families in the study area of the present study: *Boana pulchella*, *Dendropsophus sanborni* and *Scinax squarir-ostri* (Hylidae); *Leptodactylus latrans* and *Pseudopaludicola falcipes* (Leptodactylidae); and *Rhinella dorbignyi* (Bufonidae). Although some studies have found that lizards are occasionally consumed by *T. hypoconia* (Bellini et al., 2013; Rebelato, 2014), we were unable to capture and obtain tissue samples from the common lizard at the site, *Cercosaura schreibersii*. Because there are anecdotal accounts of *T. hypoconia* feeding on fish, we collected samples of the most common fish in the wetlands, *Astyanax lacustris* (Characidae). Anurans and fish were euthanized with a lethal dose of anesthetic (lidocaine 4%), taken to the laboratory and kept frozen until processing to obtain tissue samples. All samples were collected with permits from the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio; SISBio n° 50062-5; Ministry of Environment, Government of Brazil) and the Ethics Committee of Animal Use (CEUA UFRGS – 29658).

2.3. Sample processing

After defrosting samples, they were washed in deionized water,

placed separately in Petri dishes and dried in an oven at 60 °C for 48 h. We ground the samples into a fine homogeneous powder using a mortar and pestle and stored them in microcentrifuge tubes. We placed sub-samples (2-5 mg) into a tin capsule and sent them for isotopic analysis at the Laboratory of Isotope Ecology, which is linked to the Center for Nuclear Energy in Agriculture (Centro de Energia Nuclear na Agricultura - CENA) at the University of São Paulo (Universidade de São Paulo - USP).

The isotopic ratio of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) of samples was measured via mass spectrometry (Continuous-flow-Isotope Ratio Mass Spectrometry - CF-IRMS) using a Carlo Erba elemental analyzer (CHN 1110) coupled to a Delta Plus mass spectrometer from Thermo Scientific. Isotopic ratios were expressed in δ (per mil - ‰): $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{14}\text{N}/{}^{15}\text{N}$ (Peterson and Fry, 1987; Barrie and Prosser, 1996; Post, 2002). Pee Dee Belemnite (PDB: 0.0112372) and atmospheric nitrogen (N_2 : 0.0036765) were used as international standards for carbon and nitrogen, respectively. The standard deviations of these samples indicate that the accuracy degree of the analyses was ± 0.14 for $\delta^{15}\text{N}$ and ± 0.09 for $\delta^{13}\text{C}$. We did not extract lipid from the samples, nor did we perform mathematical correction of isotope values for lipids. This was because all C:N values were < 4 ; according to Post et al. (2007), when C:N is < 4 for terrestrial animal tissue, mathematical correction has little influences on $\delta^{13}\text{C}$.

2.4. Statistical analyses

Because we did not determine the sex of snakes, we followed Rebelato et al. (2016) to categorize specimens as juvenile or adult; females mature at 300 mm SVL and males at 270 mm. Individuals that measured between 269 and 299 mm SVL were classified as undefined, and we, therefore, analyzed two age categories: adults (SVL > 300 mm) and juveniles (SVL < 270 mm).

We tested the normality and homogeneity of the isotopic data using the Shapiro-Wilk test and Levene test, respectively. We used three-way

analysis of variance (III ANOVA) to test whether $\delta^{13}\text{C}$ of snakes varied with age (juvenile vs. adult); tissue type (blood vs. scale); and year (2016 vs. 2017) through the function 'Anova' in package 'car' in R environment (R Core Team, 2019). Because the $\delta^{15}\text{N}$ data for snake tissues were not normally distributed even after natural logarithm or square-root transformation, we used the Kruskal-Wallis test to compare groups. Variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between prey species across years was tested using III ANOVA and Tukey posthoc to test pairwise differences. We set the significance level at $p = 0.05$ (Zar, 2010).

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) values from individual snakes and mean (\pm sd) values from prey were visually compared in a biplot. The assimilation of alternative prey by snakes is approximated by their positions on the X-axis ($\delta^{13}\text{C}$), and their trophic positions were indicated by relative positions on the Y-axis ($\delta^{15}\text{N}$; Peterson and Fry, 1987). Mixing models (MM) were used to estimate the relative contributions of alternative prey to the biomass of juvenile and adult snakes. We used the Stable Isotope Mixing Models package in R (SIMMR, Version 0.4.0), which uses a Bayesian statistical framework (Parnell and Inger, 2016) and Markov Chain Monte Carlo (MCMC) methods to estimate the parameters (Parnell et al., 2010). A Bayesian approach allows the incorporation of uncertainty in trophic discrimination factors (TDF), sources and estimated mixtures (Parnell et al., 2010). TDF values, i.e. the difference between isotopic values of the consumer and potential sources, were $0.40 \pm 1.30\text{‰}$ for $\delta^{13}\text{C}$ and $2.54 \pm 1.30\text{‰}$ for $\delta^{15}\text{N}$ (Post, 2002).

To achieve better MM resolution, prey with similar isotopic values were pooled together as suggested by Phillips et al. (2014). Consequently, amphibian species were grouped according to family. Because *B. pulchella* and *S. squalirostris* collected in 2016 differed in $\delta^{15}\text{N}$ (Tukey HSD, $p = 0.01$), we kept *B. pulchella* separate from *S. squalirostris* and *D. sanborni*, and the latter two species were grouped as "Hylidae". Given that fish were never found in snake stomachs and the relative positions of snakes and fish in the isotopic biplot indicated they were unlikely to have contributed substantially to snake biomass, fish were excluded from the MM. Mixing models were run separately for each tissue type, and comparison of results from the two tissues allowed us to evaluate prey contributions to snake biomass according to a different rate of element assimilation (under the assumption that blood likely reflects assimilation over a few weeks, and scales likely reflect assimilation over multiple months).

Isotopic niches of juveniles and adults were quantified from standard ellipse areas (SEA, expressed in ‰^2) using the Stable Isotope Bayesian Ellipses calculated by SIBER (Jackson et al., 2011) in the SIAR package in R (Parnell et al., 2010). We report the corrected standard ellipse area (SEA_C) for small sample sizes. SEA_C is a bivariate measure of the distribution of individuals in trophic space, where each ellipse encloses 40% of the data regardless of sample size. We also calculated the Bayesian approximation of the standard ellipse area (SEA_B) and the convex hull area (TA; Layman et al., 2007) as a measurement of the area of the isotopic niche (Jackson et al., 2011). The TA included all samples of each age group in the δ_{space} , representing total niche space occupied. SEA_C and SEA_B calculations allowed us to measure the trophic niche and indicate the degree of niche overlap (‰^2) among the age groups (Jackson et al., 2011, 2012). Like MM, SEA was calculated separately for each tissue and year. Snakes of undefined ontogenetic stage were excluded from this analysis.

We estimated the trophic position (TP) of juveniles and adults in each year using the formula $\text{TP} = \lambda + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}) / \text{TDF}$. λ is the trophic level of the baseline source (Vander Zanden et al., 1997), $\delta^{15}\text{N}_{\text{consumer}}$ is the nitrogen isotopic ratio of each consumer, $\delta^{15}\text{N}_{\text{baseline}}$ is the mean $\delta^{15}\text{N}$ of the baseline, and TDF is the trophic discrimination factor of nitrogen per trophic level (2.54‰; Vander Zanden et al., 1997; Post, 2002). Because these snakes apparently consume fish very rarely or not eat, only amphibian values were used for $\delta^{15}\text{N}_{\text{baseline}}$. For this analysis, we grouped prey collected during 2016 and 2017 because they revealed no between-year variation in

$\delta^{15}\text{N}$ ($F_{1,58} = 0.95$; $p = 0.33$). We tested whether the trophic position of snakes varied with age, tissue type and year through the Kruskal-Wallis test because the data did not meet the assumption of normality. All statistical analyses were performed using R version 3.5.3 (R Core Team, 2019).

3. Results

We collected samples from 41 individuals of *T. hypoconia*. The average SVL was 341.47 mm (sd = 26.97 mm; range = 300.50 – 390 mm; n = 16) for adults, 213.95 mm (sd = 31.34 mm; range = 150.50 – 260 mm; n = 20) for juveniles, and 284.18 mm (sd = 5.32 mm; range = 280 – 290; n = 5) for undefined specimens. Sample size varied from 1 – 3 individuals per size interval. We were able to analyze carbon and nitrogen isotopic ratios based on 40 blood samples and 40 scale samples (Supplementary material, Table S1) plus 72 individual prey (Table S2) collected during 2016 and 2017. Overall, $\delta^{13}\text{C}$ of blood samples for juvenile snakes during both years ranged from -22.65‰ to -20.54‰ and for adults ranged from -23.03‰ to -20.54‰. $\delta^{13}\text{C}$ values of scale samples for juveniles in both years ranged from -21.46‰ to -19.43‰ and for adults ranged from -22.62‰ to -20.08‰. $\delta^{15}\text{N}$ of blood samples for juveniles in both years ranged from 7.98‰ to 10.46‰ and for adults ranged from 7.23‰ to 10.40‰. $\delta^{15}\text{N}$ of scale samples for juveniles in both years ranged from 8.19‰ to 11.76‰ and for adults ranged from 8.54‰ to 11.21‰ (Fig. 2). Analysis of variance showed no significant difference in $\delta^{13}\text{C}$ between juveniles and adults ($F_{1,62} = 1.84$; $p = 0.17$) and tissue types ($F_{1,62} = 0.78$; $p = 0.37$), with little, but not statistically significant, variation between years ($F_{1,62} = 2.93$; $p = 0.09$). There was also no difference for $\delta^{15}\text{N}$ between years ($\chi^2 = 0.18$; $p = 0.67$), tissues ($\chi^2 = 0.04$; $p = 0.82$), and very small and statistically insignificant variation between ages ($\chi^2 = 3.25$; $p = 0.07$).

The $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ biplot revealed little segregation between snake ontogenetic stages, with consistent patterns for both tissues during both years (Fig. 2; Supplementary material, Table S1). Isotopic ratios of potential prey were not significantly different between years ($\delta^{13}\text{C}$: $F_{1,70} = 1.18$; $p = 0.27$; $\delta^{15}\text{N}$: $F_{1,70} = 1.41$; $p = 0.23$, Table S2).

Mixing model results were very similar for juveniles and adults and both tissues, with a small difference in percent contribution between the two studied years (Table 1). MM using blood and scale samples in 2016 showed the most important item in the diet of juveniles and adults were anurans belonging to family Hylidae (from 43.4% to 53.9%). Conversely, in 2017, MM analysis for both tissues showed that Leptodactylidae contributed the most to juvenile and adult biomass (from 33.8% to 40.6%). MM analysis of the five specimens classified as age-

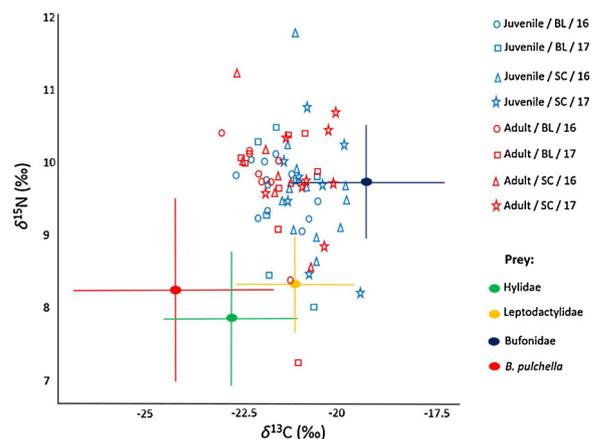


Fig. 2. Individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *Thamnodynastes hypoconia* according to age, tissue and year, and the mean (\pm sd) of the prey groups collected in southern Brazil. Legend: BL/16 and BL/17 = blood samples collected in 2016 and 2017, respectively; SC/16 and SC/17 = scale samples collected in 2016 and 2017, respectively.

Table 1

Mean ± standard deviation (sd) of estimated percent contributions of prey categories to biomass of juvenile, adult and undefined *Thamnodynastes hypoconia* collected in 2016 and 2017 in southern Brazil.

Age	Prey category	Prey contribution percentage (mean ± sd)			
		2016		2017	
Juvenile	<i>B. pulchella</i>	14.9 ± 8.5	11.3 ± 7.4	18.7 ± 11.3	13.5 ± 9.4
	Hylidae	53.9 ± 13.5	43.4 ± 16.3	24.9 ± 15.4	19.9 ± 13.8
	Leptodactylidae	23.1 ± 12.2	30.3 ± 15.8	33.8 ± 17.6	38.7 ± 20.4
	Bufoidea	8.1 ± 4.7	15.1 ± 7.9	22.5 ± 13.2	27.9 ± 16.5
Adult	<i>B. pulchella</i>	20.5 ± 11.9	19.9 ± 12.2	18.9 ± 10.9	14.6 ± 9.7
	Hylidae	50.2 ± 16.7	45.6 ± 18.0	25.7 ± 15.5	21.4 ± 14.7
	Leptodactylidae	20.9 ± 13.5	23.8 ± 15.4	34.0 ± 17.8	40.6 ± 20.1
	Bufoidea	8.5 ± 5.3	10.7 ± 6.9	21.5 ± 12.8	23.4 ± 15.0
Undefined	<i>B. pulchella</i>	25.4 ± 17.3	22.5 ± 16.3	18.8 ± 13.6	16.5 ± 13.9
	Hylidae	30.9 ± 19.9	24.5 ± 16.9	23.7 ± 16.6	20.7 ± 16.4
	Leptodactylidae	26.2 ± 18.4	27.5 ± 18.0	31.2 ± 19.3	31.2 ± 20.8
	Bufoidea	17.6 ± 13.1	25.5 ± 16.3	26.2 ± 16.4	31.5 ± 20.4

undefined indicated substantial contributions from all prey groups.

Corrected standard ellipse area (SEA_C) indicated that isotopic niche widths were similar for juveniles and adults (Fig. 3, Table 2). Analyses using blood samples (indicating assimilation over the short term) showed that the adult isotopic niche was slightly larger than the juvenile niche during both years. When this analysis was based on scale samples (indicating assimilation over a longer-term), the juvenile isotopic niche was larger than the adult niche during both years. For both tissue types, SEA_C was greater and with a higher overlap between juveniles and adults during 2017 (Fig. 3, Table 2).

Trophic positions (TP) of adults tended to be higher than those of juveniles, but this difference was not statistically significant ($\chi^2 = 3.25$; $p = 0.07$). TP did not differ between years ($\chi^2 = 0.21$; $p = 0.64$) and tissue type ($\chi^2 = 0.05$; $p = 0.80$; Table 2).

4. Discussion

Although ontogenetic shifts are well known for snakes (Shine and Wall, 2007), we found little evidence for this in *T. hypoconia*. $\delta^{13}C$ values of juveniles and adults were similar for both tissues, indicating

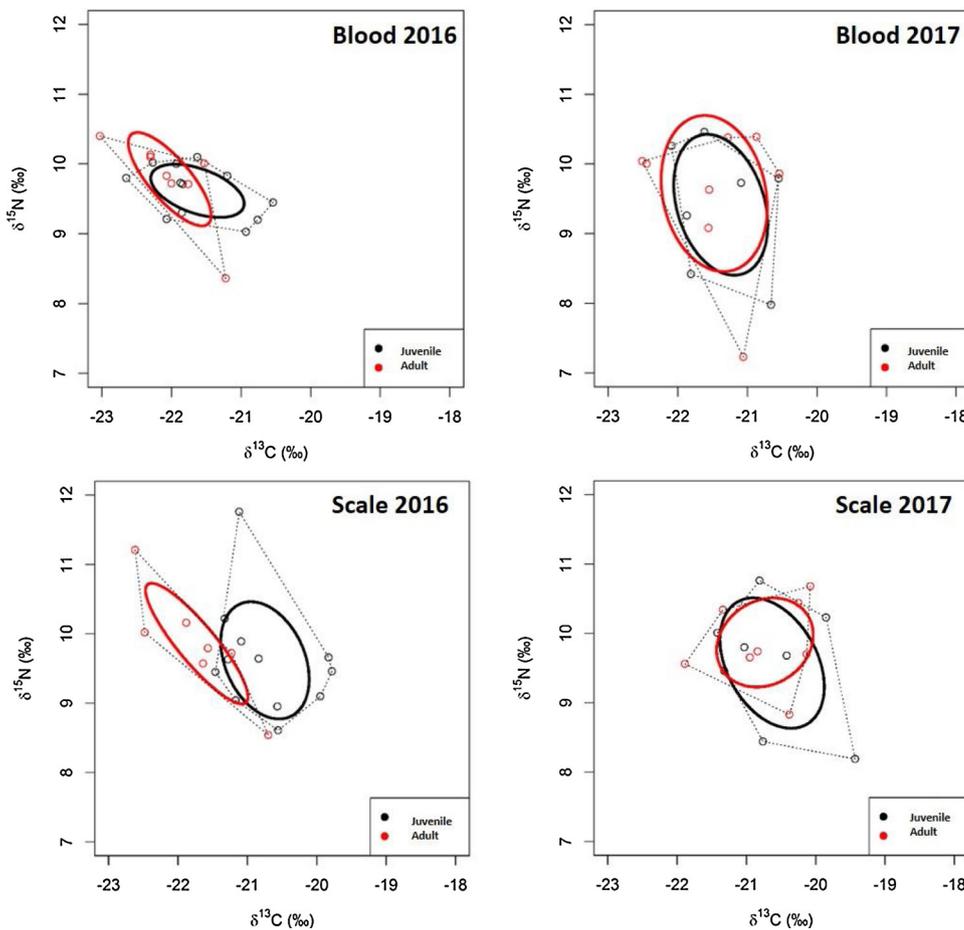


Fig. 3. Isotopic niches based on blood and scale samples for juveniles and adults of *Thamnodynastes hypoconia* collected in 2016 and 2017 in southern Brazil. Corrected Standard Ellipse Areas (SEA_C) showing the areas of isotopic niches (‰²) are represented by solid bold lines (ellipses). The Layman metric of the convex hull (total area; TA) for all individuals is represented by black dotted lines. Black = juvenile; Red = adult.

Table 2

Indicators of isotopic niche width based on C and N isotopic ratios of blood and scale samples for juvenile (J) and adult (A) *Thamnodynastes hypoconia* collected in 2016 and 2017 in southern Brazil. Indicators are Bayesian approximation of the Standard Ellipse Area (SEA_B, ‰²), sample size-corrected Standard Ellipse Area (SEA_C, ‰²) and Layman's metric of the convex hull, i.e., total area (TA, ‰²). Niche overlap (‰²) between age groups and tissues during each year was calculated using the SEA_C. Vertical trophic position was calculated using N isotopic data.

PARAMETERS	YEAR	2016				2017			
		TISSUE		Scale		Blood		Scale	
	AGE	J	A	J	A	J	A	J	A
SEA _B (‰ ²)		0.64	0.67	1.45	0.79	1.71	2.26	1.77	1.18
SEA _C (‰ ²)		0.70	0.78	1.60	0.94	2.05	2.64	2.07	1.38
TA (‰ ²)		1.34	1.17	2.85	1.38	2.68	3.19	3.23	2.00
SEA _C Overlap (‰ ²)		0.39		0.10		1.96		1.23	
Trophic position		3.98	4.05	3.98	4.08	3.90	3.97	3.97	4.08

that there was little difference in sources assimilated in both the short- and long-term diets. Interannual variation in isotopic values was minimal. We predicted there would be relatively small differences in carbon and nitrogen isotope ratios between juveniles and adults since prior studies analyzing stomach contents revealed that *T. hypoconia* mostly feeds on anuran prey, and no studies have analyzed differences between age classes (Carreira, 2002; Bellini et al., 2013, 2014; Rebelato, 2014). There are anecdotal reports that *T. hypoconia* sometimes consumes invertebrates, fish and eggs of other reptiles; however, our finding of low variation in $\delta^{13}\text{C}$ in *T. hypoconia* of all sizes suggests the species is typically an anuran specialist.

The two age groups also had similar $\delta^{15}\text{N}$ values, and both juveniles and adults were estimated to feed at trophic level 4 during both years. Most snakes are classified as secondary or tertiary carnivores (Greene, 1997), and snakes may be among the top predators in some wetlands (Willson and Winne, 2015). The anurans consumed by this snake mainly feed on arthropods and therefore are themselves secondary or tertiary consumers (Stewart and Woolbright, 1996; Brito, 2008; Huckembeck et al., 2014).

Scales, a tissue with relatively slow isotopic turnover, from juveniles and adults had similar isotopic ratios in both juveniles and adults, which could have been influenced by maternal contributions to juvenile biomass (Pilgrim, 2005, 2007) in this viviparous species (Bellini et al., 2013; Rebelato et al., 2016). Lighter nitrogen isotopes are more reactive within organisms because of their lighter mass and lower adhesion strength; therefore, these are more likely to be released as nitrogenous waste. Given that offspring tissue derives from maternal resources, the nitrogen isotopic ratio in newborns should be ^{15}N enriched relative to the parent (Power et al., 2002; Olive et al., 2003; Vanderklift and Ponsard, 2003). However, because there also was no significant difference in $\delta^{15}\text{N}$ of blood samples between juveniles and adults, this reinforces the inference that individuals of both ages consumed prey at similar trophic levels.

Mixing model (MM) results were similar for juveniles and adults, reinforcing that both ages consume the same prey types or, at least, some portions of their diets are derived from the same primary production sources. Although *T. hypoconia* is known as a specialist on anurans, the percent contributions from various anuran families may have varied between years. In 2016, mixing model estimates from blood and scale samples indicated that both age groups consumed mostly Hylidae. Hylids were the most frequent items in the stomachs of *T. hypoconia* in Argentina, Uruguay and Paraguay (Bellini et al., 2013) and those from a population in southern Brazil (Rebelato, 2014). Bellini et al. (2013) showed that the most frequently consumed hylids were species of *Scinax* and *Dendropsophus*. In southern Brazil, *B. pulchella* was the hylid

most often found in the stomachs of *T. hypoconia* (Rebelato, 2014). In our study, snake $\delta^{13}\text{C}$ values were lower than those of *B. pulchella*, indicating that this anuran likely comprised a minor component of the snake's diet.

Thamnodynastes hypoconia is semi-arboreal and is commonly seen foraging at night in shrubs (e.g., *Eryngium* sp.) that accumulate water and form phytotelma where hylids forage and shelter (Langone, 1994; Achaval and Olmos, 2007; Huckembeck et al., 2018). Commonly known as treefrogs, hylids occupy multiple vegetation layers in wetlands of our study region (Pombal, 1997; Kwet and Di-Bernardo, 1999; Achaval and Olmos, 2007; Borges-Martins et al., 2007; Ximenez and Tozetti, 2015; Santos et al., 2016). Among the hylids evaluated here, *B. pulchella* is larger than *D. sanborni* and *S. squalirostris*, occupies a greater range of microhabitats (da Rosa et al., 2006; Achaval and Olmos, 2007; da Rosa et al., 2011; Santos et al., 2016), and has a broader diet (da Rosa et al., 2002; Maneyro and da Rosa, 2004). The larger size of *B. pulchella* might have been responsible for lower predation by *T. hypoconia* compared to other hylids in the wetland.

Mixing model results for the second year (2017) inferred that Leptodactylidae was the family with the highest percentage contribution to snake biomass, although Hylidae was still important. Unlike treefrogs, leptodactylids are found on the ground (Heyer, 1969) near bodies of water where they make foam nests (Van Sluys and Rocha, 1998; Borges-Martins et al., 2007; Fonte et al., 2013). Bellini et al. (2013) registered Leptodactylidae as the second most consumed frog family, representing about 16% of the frequency of occurrence compared to the 34% represented by Hylidae. The difference in MM estimates between the two years could have been associated with differences in the relative availability of hylids and leptodactylids in the wetland. Precipitation is one of the main environmental factors that could influence on frog population dynamics, mainly in tropical areas (Ficetola and Maiorano, 2016), as the studied wetlands are ephemeral and highly influenced by rainfall (Deil, 2005; Junk et al., 2014).

Bufoidea was estimated to contribute the lowest proportion to snake biomass, which was expected because the genus *Rhinella* was rare in stomach contents (Bellini et al., 2013; Rebelato, 2014). Bufonids are terrestrial and usually dig burrows for shelter (Achaval and Olmos, 2007), and they also have toxic skin secretions. Bufonids have been recorded in the diet of a congeneric snake, *T. strigatus* (Bernarde et al., 2000; Ruffato et al., 2003; Bellini et al., 2014; Winkler et al., 2011), suggesting that the toxic secretions produced by the skin do not prevent them from being preyed on *Thamnodynastes* snakes, indicating that they may be resistant to certain anuran toxins. Even snakes that do not specialize on toads may possess mutations that confer resistance to toad toxins (Mohammadi et al., 2016).

The narrow diet breadth of *T. hypoconia* may be associated with its relatively small size. Larger snakes are able to consume a greater range of prey sizes, which should result in wider trophic niches (Henderson, 1982; Bellini et al., 2015). For example, *T. strigatus*, a larger congener, consumes larger prey that include fish and rodents (Bernarde et al., 2000; Giraudo, 2001; Ruffato et al., 2003; Bortolanza-Filho et al., 2019). Although there is some anecdotal information about *T. hypoconia* consuming fish, the species is not considered strongly aquatic (Bellini et al., 2014). We obtained tissue samples from a fish (*Astyanax lacustris*) that was abundant in shallow waters that should be accessible to snakes; however, the fish $\delta^{13}\text{C}$ was significantly lower (c.a. -25‰) than values recorded for the snake (-22 to -20‰), suggesting little or no contribution of this fish to snake biomass.

Based on analysis of blood samples, the isotopic niche of adults was larger than that of juveniles, as expected, and this was the case during both years. Interestingly, adults of *T. hypoconia* did not have a broader isotopic niche than juveniles when the analysis was based on scale samples in both years. When adult snakes learn that certain prey are more profitable, they may become selective foragers with a trophic niche breadth that is smaller than that of juveniles despite the relatively larger mouth gape of the former (Miller and Mushinsky, 1990; Brito,

2004). Between-individual variation can sometimes comprise the majority of a population's niche width (Bolnick et al., 2002, 2003), and snakes that inhabit isolated wetlands may have very different diets in wetlands with differing hydroperiods (Durso et al., 2013).

Isotopic ellipses were slightly smaller in 2016 than 2017 for both juveniles and adults. In 2016, $\delta^{13}\text{C}$ values were lower for adults than juveniles, resulting in low overlap. In the second year, isotopic overlap was greater when the analysis was based on blood samples, indicating that juveniles and adults may have consumed similar prey over the short term. The highly synchronized nature of amphibian breeding in some isolated wetlands, representing a short-term pulse of resources (Willson et al., 2010), could explain the convergence of multiple size classes of snake on the same diet (see Durso et al., 2013 for a similar example among snake species).

Neither of the theoretical models proposed by Arnold (1993) seems to be entirely consistent with trophic interpretations of isotopic patterns for *T. hypoconia* in our study. His ontogenetic telescope model predicts that larger snakes consume larger prey but continue to prey on smaller prey items as well, which would result in adult snakes having broader trophic niches than juveniles. In our study, adults occupied only slightly larger isotopic spaces than juveniles. Arnold's ontogenetic shift model applies when larger snakes consume larger prey without retaining small prey in their diet. In this case, there might be little difference in trophic niche breadth of adults and juveniles, but diet composition would diverge. Although limited isotopic overlap between adult and juvenile of the *T. hypoconia* seems consistent with this hypothesis, our mixing model results suggested similar diet composition for adults and juveniles. Arnold's framework was developed at a time when almost every detailed snake dietary study was of a relatively large colubrine, natricine, or elapid; detailed dietary data on small, semi-aquatic, or dip-sadine snakes was and is still largely lacking. At 39 cm maximum, *T. hypoconia* is smaller than all but one of the species reviewed by Arnold (1993) and the only dipsadine in Arnold's review is the arboreal species *Uromacer frenatus*. However, Carter (2015) found that diets of adult *Farancia abacura* (a North American aquatic dipsadine that may reach > 200 cm SVL) were less specialized those of juveniles. Differences in evolutionary history, habitat/ecoregion, or body size, or combinations thereof, may influence how isotopic niche breadth changes with age.

Our findings suggest that *Thamnodynastes hypoconia* in the studied wetland has very limited ontogenetic dietary variation, despite the fact that snakes are gape-limited predator. High niche overlap among age groups might reflect a lack of resource limitation and intraspecific competition. Wetlands are highly productive and often support large frog populations, the main prey for this snake. Snake populations might be controlled by raptors and other snake predators that are common in the studied wetlands. Our results also suggest that there may be inter-annual variation in the types of prey exploited by snakes of both age groups. Our study examined two tissue types collected from snakes over two years, and longer-term studies that include more diverse tissues are needed to reveal patterns on the feeding ecology of species. To infer mechanisms driving patterns of dietary and isotopic variation, these long-term studies should track prey availability as well as abiotic factors such as precipitation. Also needed are laboratory experiments with snakes that estimate rates of elemental and isotopic turnover of different tissues.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.zool.2020.125812>.

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