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MARLUCI MÜLLER REBELATO

A HERPETOFAUNA EM BANHADOS DO SUL BRASILEIRO: ESTRATÉGIAS ALIMENTARES, FONTES DE ENERGIA E NICHO TRÓFICO

PORTO ALEGRE
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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutora em Biologia Animal.

Área de concentração: Biodiversidade

Orientadora: Prof. Dra. Laura Verrastro

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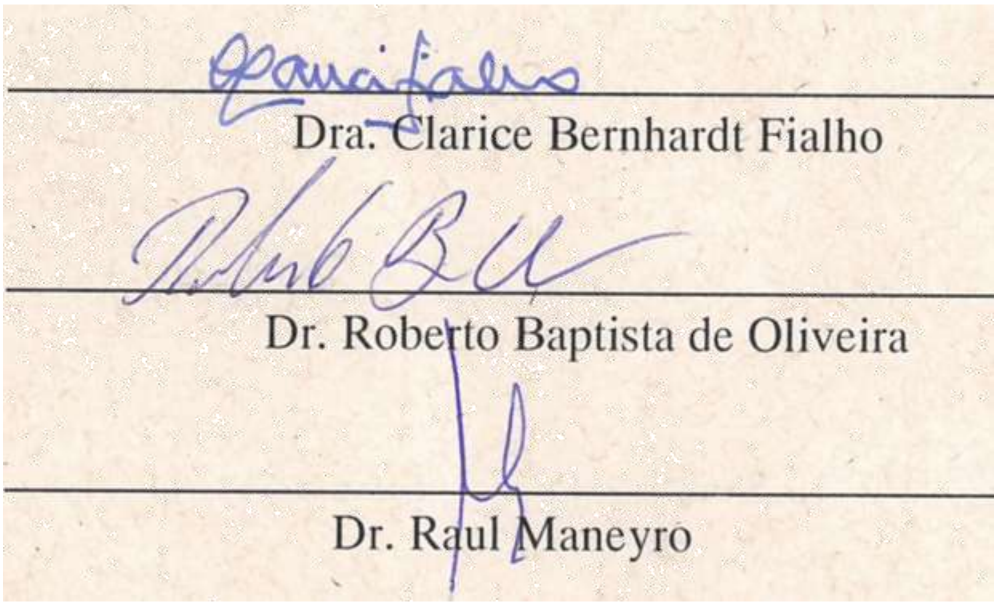

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Dr. Roberto Baptista de Oliveira

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EPÍGRAFE

“*A vida sem ciência é uma espécie de morte.*”

Sócrates

Dedico essa tese á duas mulheres mais fortes que conheço: minha mãe Véra Rosane Müller e minha avó Ortenilla Longhi Rebelato.

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O meu obrigada a todos!

APRESENTAÇÃO

Esta tese está apresentada de acordo com as normas do Programa de Pós-graduação em Biologia Animal da Universidade Federal do Rio Grande do Sul, resolução N° 37/2018, estando estruturada em cinco capítulos. O capítulo I é uma introdução geral a respeito dos assuntos abordados. Os capítulos II ao IV estão sob a forma de artigos científicos, cada qual seguindo a formatação especifica da revista para o qual será submetido. O capítulo V traz as considerações finais a respeito dos assuntos abordados.

O capítulo II, o qual se intitula "What do stable isotopes tell us about the trophic ecology of *Thamnodynastes hypoconia* (Serpentes: Dipsadidae) in southern Brazil?", será submetido à Zoology. O capítulo III intitulado "Trophic assessment and isotopic niche among coexisting anuran species in a subtropical wetland: an isotopic approach" será submetido ao Herpetological Journal. Por último, o capítulo IV é um relato de caso que aconteceu durante as atividades de campo e por se tratar de uma temática diferente dos demais manuscritos, não foi abordado na introdução geral, considerações finais e resumo da tese. Esse capítulo se intitula "Um caso de envenenamento pela serpente opistóglifa *Thamnodynastes hypoconia* (Cope, 1860) (Dipsadidae: Tachymenini) no sul do Brasil" e será submetido a revista Toxicon após a adequação do idioma inglês.

SUMÁRIO

RESUMO

Estudar a ecologia trófica dos organismos é fundamental para entender as relações de competição, predação e coexistência entre espécies e também entre indivíduos dentro de uma população. Anfíbios e répteis representam um elo muito importante dentro das teias tróficas nos banhados subtropicais, pois conectam o sistema aquático e terrestre. Isótopos estáveis de carbono e nitrogênio representam uma ferramenta útil na avaliação de nível trófico, fontes de energia assimiladas, área de nicho trófico e sobreposição entre espécies e indivíduos. Assim, nosso objetivo foi avaliar esses tópicos em uma população de serpentes, *Thamnodynastes hypoconia*, ao longo de dois anos, com o intuito de verificar se juvenis e adultos mostraram variação na estratégia alimentar. Também avaliamos esses tópicos em seis espécies de anuros coexistentes para descobrirmos se existe variação interespecífica no nicho trófico. Verificamos que tanto juvenis como adultos da serpente não difeririam quanto ao nível trófico, sendo consumidores terciários. Também não houve variação quanto aos recursos consumidos pelas duas classes etárias, apenas entre os dois anos estudados, sendo Hylidae e Leptodacylidae os anuros mais importantes. A largura de nicho isotópico foi semelhante, porém a sobreposição foi menor no primeiro ano de estudo. No estudo avaliando os anuros, foi revelada alta sobreposição de nicho entre as espécies generalistas e total segregação de nicho do único especialista, *Rhinella dorbignyi*, que inclusive revelou maior dependência de fontes como Formicidae e Isoptera. Para os generalistas, *Boana pulchella*, *Dendropsophus sanborni*, *Scinax squalirostris*, *Leptodactylus latrans* e *Pseudopaludicola falcipes*, herbívoros, Coleoptera, Araneae e Diptera foram as presas mais importantes. Ao avaliar a base da cadeia trófica, o anuro especialista apresentou maior ligação às plantas da via fotossintética C_4 e os generalistas das fontes C_3. Espécies de menor tamanho corporal apresentaram menor área de nicho. Porém o padrão contrário, de maior nicho nas espécies de maior tamanho, foi evidente somente em *B. pulchella*. Os resultados levantados nessa tese demonstram a necessidade de estudarmos as relações tróficas entre espécies coexistentes ecologicamente semelhantes, e também entre indivíduos de uma população para compreendermos a dinâmica e estruturação trófica nos ecossistemas de banhados que são ricos em biodiversidade e tão ameaçados.

Palavras chave: anfíbios, ecologia trófica, isótopos estáveis, nicho alimentar, répteis

ABSTRACT

To study the trophic ecology of organisms is fundamental to understand the relationships of competition, predation and coexistence among species and among individuals within a population. Amphibians and reptiles represent a very important link within trophic webs in subtropical wetlands as they connect the aquatic and terrestrial system. Stable isotopes of carbon and nitrogen represent a useful tool for assessing trophic level, assimilated energy sources, trophic niche area and overlap among species and individuals. Thus, our objective was to evaluate these topics in a snake population, *Thamnodynastes hypoconia*, over two years in order to verify if juveniles and adults revealed variation in feeding strategy. We also evaluated these topics in six co-existing anuran species to find out if there is interspecific variation in the trophic niche. We found that both juveniles and adults of the snake would not differ in trophic level, being tertiary consumers. There was also no variation in the resources consumed by both age groups, only between the two years studied, being Hylidae and Leptodacylidae the most important frogs. The isotopic niche width was similar, but the overlap was smaller in the first year of study. In the study evaluating anurans, high niche overlap was revealed among the generalist species and total niche segregation in the only specialist, *Rhinella dorbignyi*, which even revealed greater dependence on sources such as Formicidae and Isoptera. For the generalists, *Boana pulchella*, *Dendropsophus sanborni*, *Scinax squalirostris*, *Leptodactylus latrans* and *Pseudopaludicola falcipes*, herbivores, Coleoptera, Araneae and Diptera were the most important prey. Assessing the base of the trophic chain, the specialist anuran showed the highest connection with plants of the C_4 photosynthetic pathway and generalists with C_3 plants. Species of smaller body size had smaller niche area. However, the opposite pattern of larger niche in larger species was evident only in *B. pulchella*. The results raised in this thesis demonstrate the need to study the trophic relationships between ecologically similar coexisting species, and also between individuals of a population to understand the trophic dynamics and structuring in wetland ecosystems that are rich in biodiversity and so threatened.

Keywords**:** amphibians, trophic ecology, stable isotopes, food niche, reptiles

CAPÍTULO I

Introdução Geral

Ecologia trófica

Estudar a ecologia trófica, ou seja, a dieta de um organismo, é essencial para entender o seu papel ecológico, uma vez que as estratégias alimentares estão intimamente associadas aos demais traços da história de vida (ex., reprodução, atividade, uso do ambiente) (HUEY; PIANKA, 1981; SMITH et al., 1999; VERWAIJEN; VAN DAMME, 2007; WILDER; LE COUTEUR; SIMPSON, 2013). A maior contribuição que um organismo pode prestar ao ecossistema é através do uso, armazenamento e transferência de matéria e energia entre os diferentes níveis tróficos (HAIRSTON; HAIRSTON, 1993; LYONS et al., 2005).

Conhecer o nível trófico que as espécies ocupam na cadeia trófica é fundamental para entender como a comunidade está organizada (HAIRSTON; SMITH; SLOBODKIN, 1960). O nível trófico define o papel de cada espécie na teia e ajuda a identificar espécies chave (HEITHAUS et al., 2008). Para fins aplicáveis, o nível trófico nada mais é do que uma medida de número de transferências de energia do produtor para o consumidor dentro da teia trófica, podendo auxiliar na predição dos efeitos da perda das espécies no ecossistema (DUFFY et al., 2007; THÉBAULT; LOREAU, 2003).

Outro pilar bastante estudado dentro da ecologia trófica é avaliação do nicho trófico das espécies, o qual representa a forma como os organismos fazem uso dos recursos alimentares (CHASE; LEIBOLD, 2003; COHEN, 1977). Espécies com nichos amplo são denominadas generalistas e com nichos mais estreitos são especialistas (DENNIS et al., 2011; KASSEN, 2002). De modo geral, as espécies generalistas consomem uma gama maior de recursos alimentares sendo assim mais resilientes a distúrbios ambientais (DEVICTOR; JULLIARD, 2008; WILSON et al., 2008). Por outro lado, as especialistas utilizam uma estreita faixa de recursos e podem usar a especialização como uma estratégia de redução da sobreposição interespecífica de nicho, permitindo então a coexistência entre as espécies (WILSON; YOSHIMURA, 1994). A sobreposição interespecífica de nicho quando é alta pode causar a exclusão competitiva, ou causar a partição de nicho quando a sobreposição é baixa (PIANKA, 1972). De acordo com a teoria de partição de nicho, a coexistência de espécies competidoras em habitats com escassez de recursos só é permitida quando ocorre a partição de nicho ou que pela menos haja uma diferenciação alimentar para reduzir a sobreposição (PIANKA, 1976). Assim, a partição de nicho tem sido foco principal não só em estudos envolvendo comunidades (interespecífico), mas também entre organismos de uma mesma população (intraespecífico) (CORREA; WINEMILLER, 2014; HARDIN, 1960; SIEPIELSKI; MCPEEK, 2010).

Dessa forma, conhecer as relações tróficas intra e interespecíficas é necessário para entender como ocorre a competição, predação e coexistência (PIANKA, 1976; SIH et al., 1985; SIH; CHRISTENSEN, 2001). Indivíduos dentro de uma única população podem diferir quanto as estratégias de forrageamento (BOLNICK et al., 2007) de acordo com a idade (POLIS, 1984; SVANBÄCK; PERSSON, 2004) e sexo (SHINE et al., 1991), por exemplo. Uma das importâncias de verificar a existência de diferenças nas táticas de forrageamento entre indivíduos da mesma população é entender como ocorre a mudança no fluxo de nutrientes através dos habitats (SUBALUSKY; FITZGERALD; SMITH, 2009). Além de que essas diferenças apresentam implicações evolutivas nos atributos ecológicos do predador (BOLNICK et al., 2003; 2011) que interferem nas populações de presas, refletindo assim ao longo da comunidade e ecossistema (BOLNICK et al., 2011; POLIS, 1984; TINKER et al., 2007).

Os banhados da planície costeira do Rio Grande do Sul e a herpetofauna como modelos de estudo em ecologia trófica

A planície costeira do Rio Grande do Sul possui uma área de 37.000 km², com cerca de 640 km de extensão, da cidade de Torres até o Chuí, atingindo até 100 km de largura (TOMAZELLI; DILLENBURG, 2000). Os eventos sucessivos de transgressões e regressões marinhas ocorreram entre 400 mil e cinco mil anos atrás, dessa forma a maior parte da província situa-se sobre substrato sedimentar (TOMAZELLI; DILLENBURG, 2000). Cerca de 61% da área da planície costeira é de superfície terrestre e 39% de corpos d'água. No sentido costa oceânica para o interior, uma sequência de ambientes compõe essa região, incluindo complexos mosaicos de dunas, estuários, banhados, matas, além de um sistema de lagoas costeiras de diferentes magnitudes (CABRERA; WILLINK, 1973; SEELIGER; ODEBRECHT; CASTELLO, 1998).

A dinâmica da Planície Costeira é governada predominantemente pelos ciclos de alagamento, conforme o regime pluviométrico (SEELIGER; ODEBRECHT;

CASTELLO, 1998). Esses ciclos tem influência direta no estabelecimento de organismos que estão na base da cadeia trófica, como fitoplâncton e macrófitas aquáticas (WILLIAMS; TREXLER, 2006), influenciando na oferta de recursos (SEIGEL; GIBBONS; LYNCH, 1995; WELLBORN; SKELLY; WERNER, 1996). As áreas úmidas, chamadas aqui de banhados, são os sistemas influenciados diretamente pelos ciclos de alagamento.

Os banhados, estão entre os ecossistemas mais produtivos, fornecendo diferentes serviços ecossistêmicos (ODUM, 1989; SATHER; SMITH, 1984; ZEDLER; KERCHER, 2005). A complexidade que esse ambiente apresenta se deve a sua diversidade, que suporta teias tróficas altamente ramificadas (SABO; FINLAY; POST, 2009). Os banhados são considerados áreas prioritárias para conservação, pois exercem uma importante função reguladora do fluxo de corpos hídricos superficiais e subterrâneos (JUNK et al., 2014; ODUM, 1989; SATHER; SMITH, 1984). Além disso, esse sistema está sob fortes pressões antrópicas, principalmente devido as práticas agropecuárias e introdução de espécies exóticas, como o *Eucalyptus* (BECKER et al., 2007a; ZEDLER; KERCHER, 2005).

Os banhados subtropicais do sul brasileiro abrigam diversas espécies da herpetofauna (DA SILVA XIMENEZ; TOZETTI, 2015; DE OLIVEIRA et al., 2013; DOS SANTOS; DE OLIVEIRA; TOZETTI, 2012; LOEBMANN, 2005; MANEYRO et al., 2017). Porém pouco se sabe sobre a ecologia trófica desses répteis e anfíbios (CORRÊA; QUINTELA; LOEBMANN, 2016; DA SILVA et al., 2018; DE OLIVEIRA; AVILA; TOZETTI, 2017; DE OLIVEIRA et al., 2015; HUCKEMBECK et al., 2014, 2018; QUINTELA; LOEBMANN, 2019; REBELATO, 2014).

Anfíbios e répteis representam um grupo de vertebrados com papel muito importante dentro das teias tróficas, pois agem como links entre os ambientes aquáticos e terrestres, distribuindo nutrientes através de diferentes níveis tróficos e cruzando a barreira dos ecossistemas (DURÉ; KEHR; SCHAEFER, 2009; PRYOR, 2003; WARINGER-LSCHENKOHL; SCHAGERL, 2001). Esse grupo, principalmente os anfíbios, geralmente ocupam níveis tróficos baixos (GIBBONS, 2003; GRIFFITHS, 1997; MAZEROLLE, 2003; WILLSON; DORCAS, 2004), estando assim mais suscetíveis aos distúrbios ambientais (ARAÚJO; THUILLER; PEARSON, 2006; BECKER et al., 2007b; HAMER et al., 2004). Qualquer problema que ocorra nos níveis tróficos inferior é refletido ao longo de toda cadeia trófica podendo levar toda a comunidade a um distúrbio (EBY et al., 2006; REGESTER; LIPS; WHILES, 2006).

<u>O uso da análise de isótopos estáveis em estudos de ecologia trófica</u>

A análise de isótopos estáveis (AIE) é uma ferramenta que vem sendo utilizada em estudos de ecologia animal pelo menos nas últimas três décadas (GANNES; O'BRIEN; MARTÍNEZ DEL RIO; 1997; PETERSON; FRY, 1987). Isótopos estáveis, cujo átomo específico isótopo é uma "espécie" de um elemento que pode ser estável, quando não emite radiação, ou radioativo, quando emite, definido então pelo seu único número de prótons (Z) e nêutrons (N) (DAWSON; BROOKS, 2001). A razão isotópica é expressa em δ (delta), que representa partes por mil (‰) de diferença da razão isotópica da amostra em relação ao padrão (PETERSON; FRY, 1987).

Os isótopos de carbono e nitrogênio são utilizados com mais frequência em estudos de ecologia trófica, pois são abundantes no ambiente físico e representam elementos fundamentais na composição dos tecidos vivos na forma de carboidratos, lipídeos, proteínas, aminoácidos e ácidos nucléicos (PETERSON; FRY, 1987). Os isótopos estáveis de carbono ($^{13}C/^{12}C$ ou $\delta^{13}C$) são utilizados principalmente para determinar as fontes de produção primária, sendo úteis na detecção da entrada destes elementos na cadeia trófica (FRY, 2006). Os isótopos de nitrogênio ($^{15}N/^{14}N$ ou $\delta^{15}N$) são indicadores de nível trófico, mostrando a posição das espécies na cadeia alimentar, já que os tecidos dos consumidores normalmente possuem valores isotópicos maiores em relação aos seus alimentos (FRY, 2006). Através dos isótopos de carbono e nitrogênio é possível estimar o 'nicho isotópico", o qual vem sendo utilizado como proxy na interpretação do nicho trófico (NEWSOME et al., 2007).

O carbono entra no sistema ecológico através da fixação atmosférica pelos produtores primários, os quais formam a base da produção de um sistema biológico e sustentam os demais níveis tróficos, ou seja, os consumidores (GANNES; O'BRIEN; MARTÍNEZ DEL RIO, 1997). As plantas apresentam diferentes razões isotópicas de carbono ($\delta^{13}C$) principalmente devido às diferenças nas enzimas envolvidas na fixação de carbono durante a fotossíntese, ou seja, existem plantas com via fotossintética do ciclo Calvin (C_3), plantas com ciclo Hatch-Slack (C_4) e as plantas que utilizam a via metabólica dos ácidos das crassuláceas (CAM) (MARSHALL; BROOKS; LAJTHA, 2007).

Apesar da AIE não ter a mesma resolução taxonômica que a análise de conteúdo estomacal (ACE) convencional, ela é uma excelente ferramenta quando já se conhece a dieta de determinada espécie pela ACE. Uma limitação da ACE é que as presas encontradas no estomago refletem apenas a ingestão recente, podendo causar a superestimação de presas, em especial daquelas de digestão mais lenta (BEARHOP et al., 1999). Além disso, a ACE não permite responder quais produtores primários são as vias de entrada de nutrientes que sustentam troficamente os consumidores de níveis tróficos superiores (LAYMAN et al., 2012; WINEMILLER; AKIN; ZEUG, 2007). Ou seja, a AIE é uma ferramenta útil na compreensão do fluxo dos nutrientes através da cadeia alimentar, fornecendo informações que integram o tempo e o espaço no entendimento da relação trófica com o organismo (LAYMAN et al., 2012). Além de que essa metodologia é extremamente usual na quantificação do nicho trófico (BEARHOP et al., 2004).

A AIE tem a vantagem de analisar tecidos que não requerem a eutanásia do animal, visto que podem ser obtidas amostras extremamente pequenas de escamas, unhas, penas e sangue (LAYMAN et al., 2012), permitindo a aplicação em espécies raras ou ameaçadas de extinção. Também tem a vantagem da praticidade de coleta de amostras em campo, não necessitando a retirada do animal do seu habitat além de requerer poucas amostras para representação de bons resultados. Essa última característica é importante dentro do grupo herpetofaunístico, principalmente para serpentes, as quais apresentam uma baixa taxa de encontro.

Justificativa e objetivo geral

Considerando que as relações tróficas que ocorrem entre as espécies (interespecífica) e entre indivíduos de uma mesma população (intrapopulacional) são necessárias para entender questões sobre coexistência, competição e predação, as quais irão refletir na organização da comunidade, o objetivo desse estudo foi verificar como uma assembleia de anuros, assim como uma população de serpentes utilizam os recursos alimentares.

Objetivos específicos:

1. Verificar se existe variação intrapopulacional nas razões isotópicas de carbono (δ^{13}C) e nitrogênio (δ^{15}N) entre as diferentes fases ontogenéticas de uma população de serpentes

(juvenil vs. adulto) de acordo com o tipo de tecido analisado (sangue vs. escama) ao longo de dois anos;

2. Estimar a largura intrapopulacional de nicho isotópico bem como calcular a área de sobreposição de nicho entre juvenis e adultos da serpente estudada;

3. Calcular a posição trófica de juvenis e adultos da serpente na teia estudada;

4. Estimar a porcentagem de contribuição de cada grupo de presas na biomassa de juvenis e adultos da serpente estudada.

5. Verificar se existe variação interespecífica nas razões isotópicas de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) da assembleia de anuros;

6. Estimar a largura interespecífica de nicho isotópico, bem como calcular a área de sobreposição de nicho entre os anuros;

7. Calcular a posição trófica de cada espécie de anuro na teia;

8. Estimar a porcentagem de contribuição de cada grupo de presas na biomassa dos anuros;

9. Verificar quais fontes da base da cadeia trófica, planta C_3 vs. C_4, estão mais propensas a serem fontes de entrada de energia no sistema onde ocorrem os anuros estudados;

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CAPÍTULO II

Manuscrito a ser submetido à revista Zoology:

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

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

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Abstract

Ontogenetic niche shifts are the major cause of intrapopulation dietary variation in snakes. Because snakes are gape-limited predators, they can consume larger prey as they grow. 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 overlap between the two age categories. During two years, we collected samples of 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 between the two years, with Hylidae being the most important anuran assimilated during the first year and Leptodactylidae during the second year. The isotopic space occupied by adult snakes was slightly larger than that of juveniles when the analysis was based on data from blood samples, but the reverse pattern was found when the analysis was based on data from scales. Niche overlap was greater during the second year. We infer that juvenile and adult snakes in the study area exploit similar food resources, with diets varying interannually based on temporal changes in the availability of alternative prey.

Keywords**:** Diet, Isotopic niche, Ontogeny, Snakes, *Thamnodynastes*, Trophic position

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 poison to subdue and digest prey (Shine, 1991; Arnold, 1993; Cundall and Greene, 2000; Luiselli, 2006b).

Ontogeny is one of the principal drivers of intrapopulation variation in trophic ecology (Shine, 1991; Arnold, 1993; Bolnick et al., 2011). Ontogenetic variation in snake diets is the norm (Shine and Wall, 2007) because, as a snake grows, it is able to ingest larger prey items (Mushinsky et al., 1982; Rodríguez-Robles et al., 1999; Shine et al., 2002; Willson et al., 2010; Brischoux et al., 2011; Durso and Mullin, 2017). If ontogenetic diet shifts are sufficiently large, then individuals of the same species may be considered distinct functional groups within the local food web (Olson, 1996). To better understand 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 (δ^{13}C) are particularly useful to distinguish sources of organic matter input, because the ratio shifts relatively little between adjacent trophic levels (Kelly, 2000; Sherwood and Rose 2005; Caut et al., 2009). Nitrogen stable isotopic ratios (δ^{15}N) undergo gradual enrichment between a food resource and consumer, and therefore can be used to estimate trophic level (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), whereby more metabolically active tissues, such as blood, plasma and liver, have more rapid assimilation rates than less active tissues, such as bone (Tieszen et al., 1983; Hobson and Clark, 1992; Dalerum and Angerbjörn, 2005). In reptiles, plasma and red blood cells reflect assimilation of food over a period of a few weeks, and scales reflect foods assimilated over several months (Pilgrim, 2005; Rosenblatt and Heithaus, 2013). Thus, SIA of different tissues allows estimation of changes in an animal's diet over different time intervals. This is especially useful for studying trophic ecology of snakes, because predation events are difficult to observe in nature and, because most snakes feed infrequently, stomach contents often are lacking or partially digested and difficult to identify.

During a two-year field study, we collected blood and scales from juvenile and adult *Thamnodynastes hypoconia,* a snake common in wetlands of southern Brazil (Carreira and Maneyro, 2013). This small, viviparous snake is nocturnal, semi-arboreal and has opistoglyphic dentition (Giraudo, 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 ontogenetic changes in diet.

We analyzed δ^{13}C and δ^{15}N of blood and scale tissues to evaluate trophic position, isotopic niche width and overlap between juveniles and adults. Percent contributions of different prey to biomass of juveniles and adults was determined using mixing models. We predicted limited variation in carbon and nitrogen isotope ratios between ages, since previous studies using SCA revealed a relatively narrow diet consisting mostly of anurans. However, given that snakes are gape-limited predators, we predicted that the isotopic niche width of juveniles would be smaller than that of adults (Werner and Gilliam, 1984; Shine, 1991; Arnold, 1993; Rodríguez-Robles et al., 1999). Better understanding about 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 was conducted 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). Climate in this region is classified as humid subtropical, with an average annual temperature of 18.8 °C. Seasons are well defined, and average annual rainfall is 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 0.5 h after sunset. Surveys were conducted over four consecutive nights during each of the eight field outings. We captured 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 squalirostris* (Hylidae), *Leptodactylus latrans* and *Pseudopaludicola falcipes* (Leptodactylidae) and *Rhinella dorbignyi* (Bufonidae). In addition, we collected samples of a fish, *Astyanax lacustris* (Characidae), that is abundant and a potential prey of *T. hypoconia*. 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 nº 50062-5; Ministry of Environment, Government of Brazil) and the Ethics Committee of Animal Use (CEUA UFRGS – 29658).

2.3. Sample processing

All samples were 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 capsules 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 ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) 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 - ‰): $\delta^{13}C$ or $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{14}N/{}^{15}N$ (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 accuracy degree of the analyses was ±0.14 for $\delta^{15}N$ and ±0.09 for $\delta^{13}C$.

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. Five individuals that measured between 269 and 299 mm SVL were 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 δ^{13}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. Because the δ^{15}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 δ^{13}C and $\boldsymbol{\delta^{15}}$N between prey species across years was tested using III ANOVA and Tukey post-hoc to test pairwise differences**.** The significance level was $p = 0.05$.

Carbon (δ^{13}C) and nitrogen (δ^{15}N) values from individual snakes and mean (±sd) values from prey were visually compared in a biplot. Assimilation of alternative prey by snakes is approximated by their positions on the X-axis (δ^{13}C), and their trophic positions were indicated by relative positions on the Y-axis (δ^{15}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 ± 1.30‰ for δ^{13}C and 2.54 ± 1.30‰ for δ^{15}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 the family. Because *B. pulchella* and *S. squalirostris* collected in 2016 differed in δ^{15}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 isotope biplot indicated they were unlikely to have contributed substantially to snake biomass (Fig. 2), 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 different time scales of element assimilation (blood reflect assimilation over a few weeks and scales 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 are 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 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 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 an undetermined ontogenetic stage were excluded from this analysis.

We estimated the trophic position (TP) of juveniles and adults in each year using the formula $TP = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{baseline}) / TDF$. λ is the trophic level of the baseline source (Vander Zanden et al., 1997), $\delta^{15}N_{consumer}$ is the nitrogen isotopic ratio of each consumer, $\delta^{15}N_{baseline}$ is the mean $\delta^{15}N$ 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 do not consume fish, only amphibian values were used for $\delta^{15}N_{baseline}$. For this analysis, we grouped prey collected during 2016 and 2017 because they revealed no between-year variation in $\delta^{15}N$ ($F_{1,58} = 0.95$; $p = 0.33$). We tested whether trophic position of snakes varied with age, tissue type and year through Kruskal-Wallis test because the data did not meet the assumption of normality. All statistical analyzes were performed using R version 3.5.3 (R Core Team, 2019).

3. Results

We were able to analyze carbon and nitrogen isotopic ratios based on 40 blood samples and 40 scale samples obtained from 41 *T. hypoconia* (Supplementary material, Table S1) plus 72 individual prey (Table S2) collected during 2016 and 2017. Overall, $\delta^{13}C$ of blood samples for juvenile snakes in both years ranged from -22.65‰ to -20.54‰ and for adults ranged from -23.03‰ to -20.54‰. $\delta^{13}C$. Values for scale samples from juveniles in both years ranged from -21.46‰ to -19.43‰ and for adults ranged from -22.62‰ to -20.08‰. $\delta^{15}N$ 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‰. $\delta^{15}N$ 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 $\delta^{13}C$ 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 variation between years **(**$F_{1,62} = 2.93$; $p = 0.09$). There was also no difference for $\delta^{15}N$ 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 $\delta^{13}C$ – $\delta^{15}N$ 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 ($\delta^{13}C$: $F_{1,70} = 1.18$; $p = 0.27$; $\delta^{15}N$: $F_{1,70} = 1.41$; $p = 0.23$, Table S2).

Mixing model results were very similar for juveniles and adults and for both tissues, with a small difference in percent contribution between the two years studied (Table 1). MM using blood and scale samples in 2016 showed that the most important item in the diet of juveniles and adults were anurans belonging to Hylidae family (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-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 with 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 (χ^2 = 3.25; p = 0.07). TP did not differ between years (χ^2 = 0.21; p = 0.64) and tissue type (χ^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*. δ^{13}C values of juveniles and adults were similar for both tissues, indicating 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 no difference 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 δ^{13}C in *T. hypoconia* of all sizes suggests the species is anuran specialist.

The two age groups also had similar δ^{15}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), including those inhabiting wetlands (Willson and Winne, 2016). The anurans consumed by this snake mainly feed on arthropods, and therefore are 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, 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 they are more likely to be released from the body as nitrogenous waste. Given that offspring tissue derives from maternal resources, the nitrogen isotopic ratio in newborns should be 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 δ^{15}N of blood samples between juveniles and adults, and this reinforces the inference that individuals of both ages consume prey at similar trophic levels.

Mixing model results were similar for juveniles and adults, reinforcing that both ages consume the same prey types or, at least, some portion of their diets derive from the same primary production sources. Although *T. hypoconia* is known as specialist on anurans, the percent contribution of the different anuran groups may have varied between years. In 2016, MM estimates from blood and scale samples indicated that both age groups were specialized on Hylidae. Hylids were the most frequently items in stomachs of *T. hypoconia* populations in Argentina, Uruguay and Paraguay (Bellini et al., 2013) and a population in the 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 stomachs of *T. hypoconia* (Rebelato, 2014). In our study, snake δ^{13}C values were higher than those of *B. pulchella*, indicating that this anuran likely comprised a minor component of the snake's diet.

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). *T. hypoconia* is semi-arboreal and is commonly seen foraging at night among shrubs (e.g., *Eryngium* sp.) where hylids shelter (M. M. Rebelato, personal observation; Langone, 1994). Among these hylids, *B. pulchella* is the largest and occupies a greater range of microhabitats (da Rosa et al., 2006; Achaval and Olmos, 2007; da Rosa et al., 2011; Santos et al., 2016).

Mixing model results for the second year (2017) inferred that Leptodactylidae was the family with the highest percent 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). 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. In tropical areas, precipitation has a large influence on frog population dynamics (Ficetola and Maiorano, 2016), and the studied wetlands are ephemeral.

Bufonidae 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 by *Thamnodynastes* snakes, indicating that they may be resistant to certain anuran toxins.

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 (Arnold, 1993; Bellini et al., 2015). For example, *T. strigatus*, a larger congener, consumes larger prey, including fish and rodent mammals (Bernarde et al., 2000; Giraudo, 2001; Ruffato et al., 2003; Bortolanza-Filho et al., 2019). Interestingly, adults *T. hypoconia* did not have a broader isotopic niche than juveniles did. Although there is some anecdotal information about *T. hypoconia* consuming fish, the species is not considered strongly aquatic (Bellini et al., 2014). The δ^{13}C value of an abundant fish in the wetland, *Astyanax*, (c.a. -25‰) was different than values recorded for the snake (-22 to -20‰), suggesting relatively little contribution of this fish to snake biomass.

Isotopic ellipses during 2017 were slightly larger than those estimated during 2016, when δ^{13}C values were lower for adults than juveniles. In the 2017, isotopic niche overlap was higher when the analysis was based on blood samples, indicating that juveniles and adults likely had consumed similar prey over the short term. Adults were estimated to have a broader niche when the analysis was based on scale data, which integrates food assimilation over a longer time interval.

5. Conclusion

We examined the influence of age and tissue type on the isotopic ratios of carbon and nitrogen in *Thamnodynastes hypoconia*, a snake in a wetland area in southern Brazil. Mixing models estimated that Hylidae and Leptodactylidae anurans were the most important prey groups exploited by snakes during the two years of the study. Both juveniles and adults occupied the fourth trophic level. Bayesian ellipses revealed little difference in isotopic niches of juveniles and adults during both years. When we used blood samples, the adult niche was slightly smaller than that of juveniles, and when we used scale samples, we obtained an opposite pattern. Isotopic niche overlap between juveniles and adults was smaller in the first year compared to the second year. This emphasizes the importance of analyzing tissues with different turnover rates to better understand the temporal dynamics of trophic ecology.

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TABLES AND FIGURES

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		Blood	Scale	Blood	Scale
	B. pulchella	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**
	Bufonidae	8.1 ± 4.7	15.1 ± 7.9	22.5 ± 13.2	27.9 ± 16.5
Adult					
	B. pulchella	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**
	Bufonidae	8.5 ± 5.3	10.7 ± 6.9	21.5 ± 12.8	23.4 ± 15.0
Undefined					
	B. pulchella	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
	Bufonidae	17.6 ± 13.1	25.5 ± 16.3	26.2 ± 16.4	**31.5 ± 20.4**

Table 2. Indicators of isotopic niche width based on C and N isotopic ratios of blood and scale samples from 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	**Blood**		**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

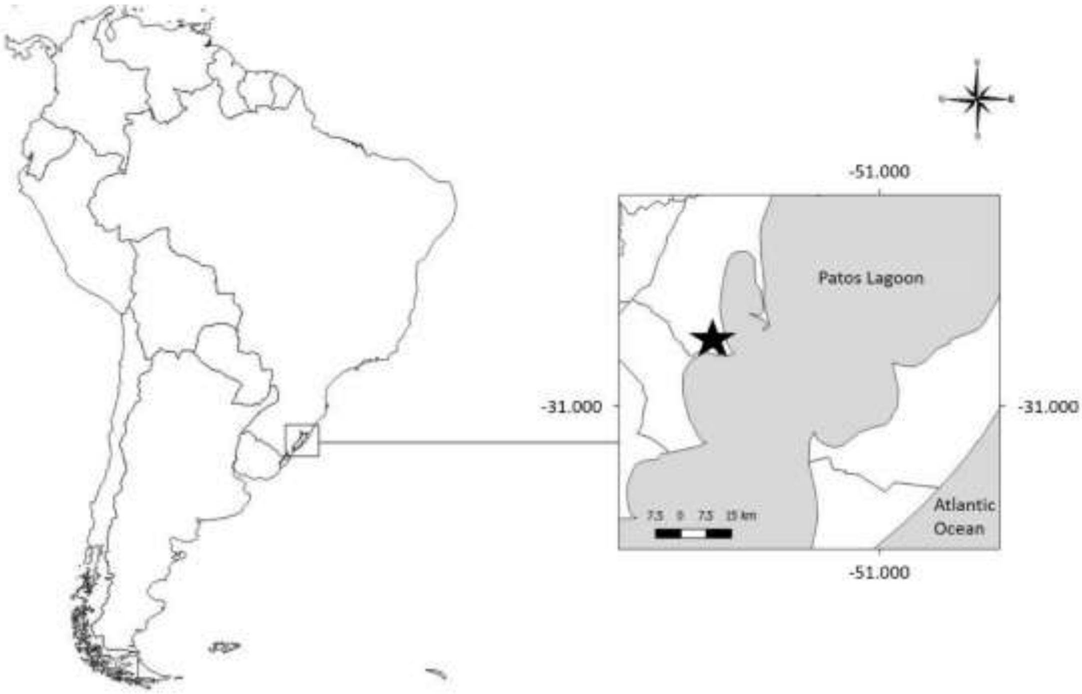

Figure 1. Map showing the study area in Rio Grande do Sul state in southern Brazil. The black star indicates the location of the study area on the shore of Patos Lagoon (30°52'7.15"S and 51°23'41.92"W).

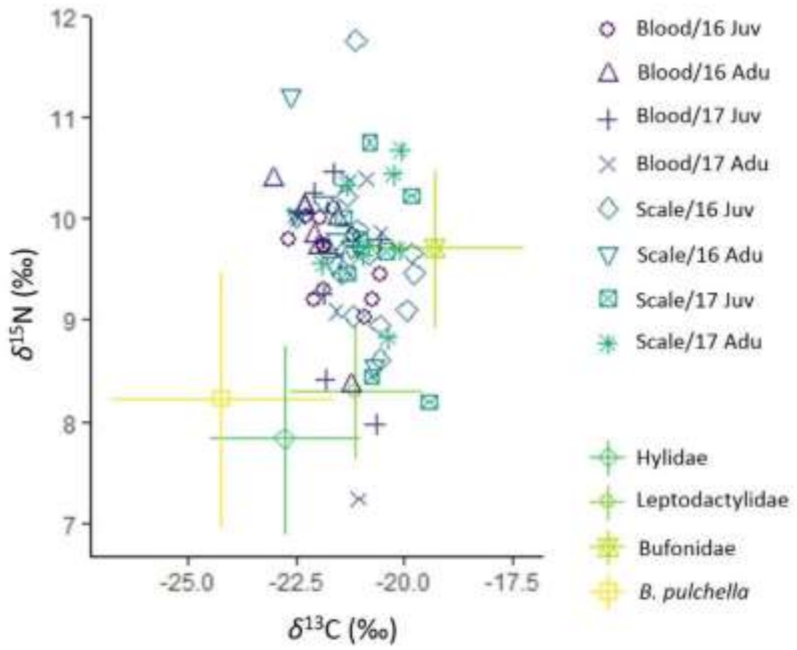

Figure 2. Individual δ^{13}C and δ^{15}N values of *Thamnodynastes hypoconia* according to age, tissue and year, and the mean (± sd) of the prey groups collected in southern Brazil.

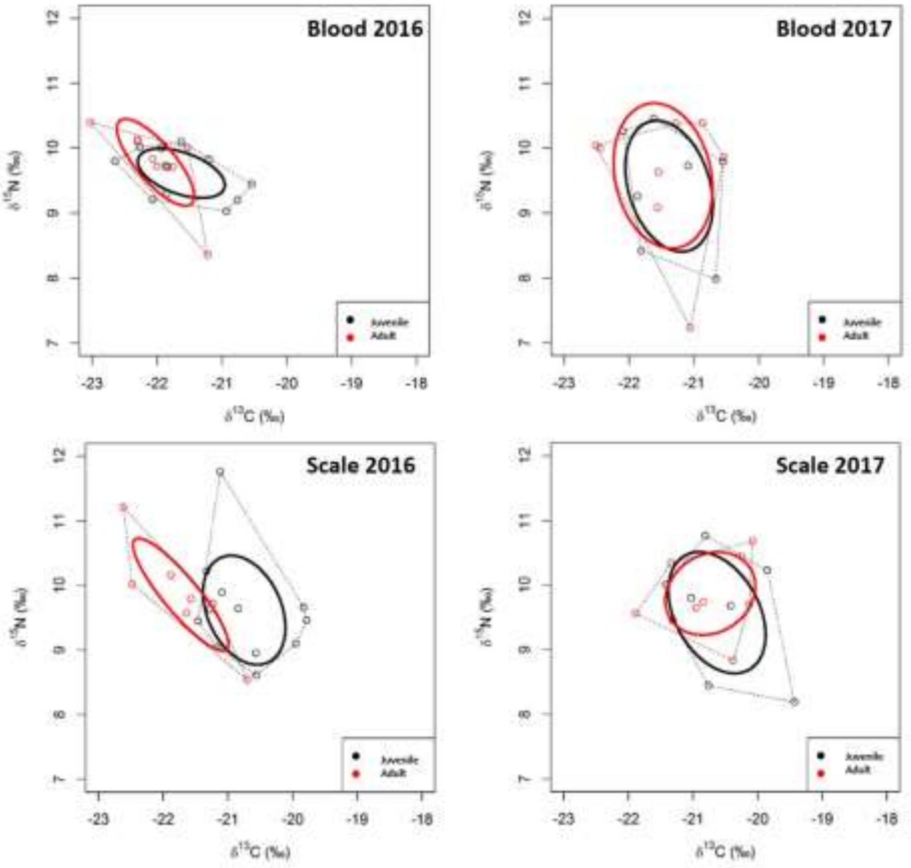

Figure 3. Isotopic niches based on blood and scale samples for juveniles and adults of *Thamnodynastes hypoconia* collected in 2016 and 2017 in southern Brazil. Corrected Standard Ellipse Areas (SEA$_C$) showing the areas of isotopic niches (‰^{2}) are represented by solid bold lines (ellipses). The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

Supplementary material

Table S1. Mean ± standard deviation (sd) of stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) for blood and scales of *Thamnodynastes hypoconia* according to age group collected in 2016 and 2017 in southern Brazil. Sample sizes are shown in parentheses.

Age	Tissue type	**Carbon (δ^{13}C) ± sd**		**Nitrogen (δ^{15}N) ± sd**	
		2016	2017	2016	2017
Adult (31)	Blood (16)	-22.02 ± 0.55 (8)	-21.47 ± 0.70 (8)	9.78 ± 0.61 (8)	9.57 ± 1.03 (8)
	Scale (15)	-21.73 ± 0.67 (7)	-20.73 ± 0.64 (8)	9.85 ± 0.79 (7)	9.86 ± 0.59 (8)
Juvenile (39)	Blood (19)	-21.62 ± 0.63 (12)	-21.38 ± 0.61 (7)	9.61 ± 0.36 (12)	9.41 ± 0.92 (7)
	Scale (20)	-20.74 ± 0.60 (12)	-20.63 ± 0.69 (8)	9.61 ± 0.80 (12)	9.57 ± 0.87 (8)
Undefined (10)	Blood (5)	-21.83 (1)	-21.09 ± 0.33 (4)	10.14 (1)	8.77 ± 0.84 (4)
	Scale (5)	-20.21 (1)	-20.05 ± 0.28 (4)	10.70 (1)	8.98 ± 0.65 (4)

Table S2. Mean ± standard deviation (sd) of stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for muscle of prey collected in 2016 and 2017 in southern Brazil. Sample sizes are shown in parentheses.

Prey category	**Carbon ($\delta^{13}C$) ± sd**		**Nitrogen ($\delta^{15}N$) ± sd**	
	2016	2017	2016	2017
AMPHIBIAN (60)				
Hylidae (31)	**-22.69 ± 1.61 (18)**	**-24.01 ± 2.51 (13)**	**8.01 ± 1.02 (18)**	**7.90 ± 1.11 (13)**
Boana pulchella (10)	-23.44 ± 2.22 (6)	-25.40 ± 2.90 (4)	8.69 ± 0.43 (6)	7.54 ± 1.84 (4)
Dendropsophus sanborni (12)	-22.42 ± 1.57 (6)	-23.93 ± 2.37 (6)	8.42 ± 0.64 (6)	8.42 ± 0.40 (6)
Scinax squalirostris (9)	-22.21 ± 0.62 (6)	-22.34 ± 0.56 (3)	6.91 ± 0.86 (6)	7.33 ± 0.56 (3)
Leptodactylidae (18)	**-21.07 ± 1.61 (12)**	**-21.29 ± 1.72 (6)**	**8.15 ± 0.73 (12)**	**8.60 ± 0.41 (6)**
Leptodactylus latrans (11)	-20.81 ± 1.96 (6)	-20.70 ± 1.05 (5)	8.29 ± 0.42 (6)	8.66 ± 0.44 (5)
Pseudopaludicola falcipes (7)	-21.34 ± 0.93 (6)	-24.24 (1)	8.01 ± 0.97 (6)	8.32 (1)
Bufonidae (11)	**-18.70 ± 2.30 (5)**	**-19.78 ± 1.78 (6)**	**9.48 ± 0.63 (5)**	**9.90 ± 0.89 (6)**
Rhinella dorbignyi (11)	-18.70 ± 2.30 (5)	-19.78 ± 1.78 (6)	9.48 ± 0.63 (5)	9.90 ± 0.89 (6)
FISH (12)				
Astyanax lacustris (12)	-25.87 ± 0.95 (6)	-25.23 ± 2.67 (6)	8.96 ± 0.58 (6)	9.18 ± 0.74 (6)

CAPÍTULO III

Manuscrito a ser submetido ao Herpetological Journal:

Trophic assessment and isotopic niche among coexisting anuran species in a subtropical wetland: an isotopic approach

Trophic assessment and isotopic niche among coexisting anuran species in a subtropical wetland: an isotopic approach

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Abstract

Trophic niche partitioning may be a strategy adopted by ecologically and morphologically similar coexisting species to avoid competitive exclusion. We analyzed questions around the trophic niche of six coexisting anuran species in subtropical wetlands in southern Brazil. For this, carbon and nitrogen isotopic ratios of anurans, potential prey and primary producers were analyzed. We estimated percent contributions of different prey to anuran biomass, trophic levels, isotopic niche width and we also estimated niche overlap among species. *Rhinella dorbignyi*, the only specialist anuran, revealed no overlap with any other generalist anuran. It also revealed higher trophic level and greater dependence on Formicidae and Isoptera sources, as well as greater proximity to the C_4 plants chain. Regarding to generalists, the highest contribution estimates were herbivores, Araneae, Coleoptera and Diptera, as well as greater similarity with the C_3 chain. *Boana pulchella* and *Leptodactylus latrans*, which represent the largest generalists, revealed the largest and smallest niche areas, respectively. The estimates for *L. latrans* may contain bias because we could not capture larger individuals. *B. pulchella* revealed niche overlap with all generalists. *Dendropsophus sanborni*, *Scinax squalirostris* and *Pseudopaludicola falcipes*, which are the smallest generalists, seem to have revealed some degree of niche partitioning due to the great similarity and occurrence in the same microhabitat (*D. sanborni* vs. *S. squalirostris*) and the possibility of niche expansion when high overlap occurs (*P. falcipes*). We suggest further studies addressing the morphology and habitat use associated with trophic niche assessment to better understand the mechanisms that allow species to coexist.

***Keywords*:** Bufonidae, Hylidae, Leptodactylidae, Niche partitioning, Stable isotope, Trophic niche

1. Introduction

Studies on trophic ecology of organisms are important within the ecological niche context (Arditi & Ginzburg, 2012). These studies bring knowledge about the relationships of competition, predation and coexistence among species (Pianka, 1981; Sih et al., 1985; Sih & Christensen, 2001) and are fundamental to understand how interspecific interaction influences the structure and trophic dynamics of the community and ecosystem (Hairston & Hairston, 1993; Jepsen & Winemiller, 2002; McGill et al., 2006; Wilson, 2010). For ecologically and morphologically similar species to coexist some strategies must be adopted to avoid competition (Kuzmin, 1995; Schoener, 1974; Toft, 1985), because competition for the same resource may lead to competitive exclusion (Hardin, 1960; Pianka, 2000). When food resources are limited, one alternative is the trophic niche partitioning (Duré, Kehr, & Dure, 2001; Martin & Martin, 2001; Murray et al., 2016; Schoener, 1974; Toft, 1980).

Studies investigating trophic niche of coexisting amphibian species have become more evident in recent decades (Arribas et al., 2018; Cloyed & Eason, 2017; Costa-Pereira et al., 2018; Denton & Beebee, 1994; Vignoli & Luiselli, 2012; Toft, 1985), mainly since the global decline (Alford & Richards, 1999; Becker et al., 2007a; Green, 2003). This group of vertebrates play a very important role within food webs, acting as links between aquatic and terrestrial environments and distributing nutrients among different trophic levels and across ecosystem borders (Waringer-Lschenkohl & Schagerl 2001; Pryor 2003; Duré et al. 2001). Amphibians usually have low mobility and occupy low trophic levels (Gibbons, 2003; Griffiths, 1997; Willson & Dorcas, 2004), which make them susceptible to environmental disturbances (Araujo et al., 2006; Hamer et al., 2004). Any problem that occurs at these lower trophic levels may be reflected along the entire trophic web, leading to disruption of the community (Eby et al. 2006; Regester et al. 2006).

In subtropical wetlands of southern Brazil many species of anuran amphibians coexist (Loebmann, 2005; Maneyro et al., 2017; Oliveira et al., 2013; Ximenez et al., 2014; Ximenez & Tozetti, 2015) and little is yet known about trophic niche of the main anuran species (Oliveira et al., 2017; Huckembeck et al., 2018, 2014; Silva et al., 2018). Wetlands are interesting systems to study because they have high productivity and support branched food webs (Batzer et al., 2006; Gibbs, 2000; Sather & Smith, 1984; Zedler, 2000). Due to great biodiversity they harbor and the threats they face, wetland ecosystems are priority areas for conservation (Junk et al., 2014), whereas in southern Brazil the main threats are conversion to practice agriculture, livestock and introduction of exotic species (Becker et al., 2007b; Zedler & Kercher, 2005).

Stable isotope analysis has been a very useful tool to evaluate trophic niche of coexisting species (Bearhop et al., 2004; Jackson, et al., 2011; Swanson et al., 2015). Natural stable isotopes of carbon and nitrogen are abundant in physical environment, as they are fundamental elements involved in the composition of living tissues (Kupfer et al., 2006; Peterson & Fry, 1987). These isotopes are commonly used to examine contribution of different primary production sources, importance of different prey to predators, trophic level of organisms, niche width and interspecific niche overlap (Jackson et al., 2011; Newsome et al., 2007). The term "*isotopic niche*" has been used to illustrate some aspects of the current trophic niche (Newsome et al., 2007), which can be understood as the measurement of the sum of the trophic interactions that connect species in an ecosystem, which is assessed through the usage of food resources (Bearhop et al., 2004; Ingram et al., 2009). Carbon isotope ratio ($^{13}C/^{12}C$ or $\delta^{13}C$) is mainly used to distinguish the origin of organic matter and to determine the point of entry of this element into the food chain (Kelly, 2000; Sherwood & Rose, 2005). Carbon isotope ratios vary according to the photosynthetic mechanism adopted by the producer: C_3 (Calvin cycle), C_4 (Hatch-Slack cycle) or CAM (Metabolic pathway of crassulacean acids; Gannes et al., 1997; Marshall et al., 2007). These differences in the primary sources of production will be reflected in herbivorous animals and pass on to carnivores until them reach the top of the animal food chain. Nitrogen isotope ratio ($^{15}N/^{14}N$ or $\delta^{15}N$) is an indicator of trophic level. It shows the position of a species in the food chain, since the tissues of a consumer usually have higher isotopic values than their food (Kelly, 2000).

In this study, we analyzed trophic niche and other questions about the trophic ecology of six anuran species that coexist in southern Brazilian wetlands in order to verify if there is food resource partition. We evaluated the importance of different dietary sources, trophic position, niche width and interspecific overlap of isotopic niche. For this we analyze carbon and nitrogen stable isotope ratios in primary producers, prey and anuran tissues. The studied species belong to three families, Hylidae: *Boana pulchella* (Duméril and Bibron, 1841), *Dendropsophus sanborni* (Schmidt, 1944) and *Scinax squalirostris* (Lutz, 1925); Leptodactylidae: *Leptodactylus latrans* (Steffen, 1815) and

Pseudopaludicola falcipes (Hensel, 1867) and; Bufonidae: *Rhinella dorbignyi* (Duméril and Bibron, 1841).

All these Hylidae and Leptodactylidae species are mentioned in the literature as generalist consumers, and the Bufonidae frog, *R. dorbignyi*, is ant specialist (da Rosa et al., 2002; França et al., 2004; Isacch & Barg, 2002; Kittel & Solé, 2015; Maneyro et al., 2004; Maneyro & Rosa, 2004; Menin et al., 2005; Silva et al., 2018). Therefore, our prediction is that there will be no overlap between the generalists and the specialist frog, and it will have a narrower niche. Within the Hylidae family, *B. pulchella* is a larger body species than *D. sanborni* and *S. squalirostris*, which could lead to the consumption of a greater diversity of prey. The two leptodactylids, *L. latrans* and *P. falcipes*, are anurans of large and small size, respectively. *L. latrans* is a very generalist species in terms of environmental use and prey consumption, including anurans (França et al., 2004; Sanabria, 2005). These peculiarities will help to address various hypotheses about trophic niche. Our prediction is that larger species such as *B. pulchella* and *L. latrans* will reveal more niche area and greater overlap with other species. Conversely, smaller species (*D. sanborni*, *S. squalirostris* and *P. falcipes*) will reveal smaller niche areas. Thus, it is expected that these variations will also influence the position occupied in the trophic web. We further predict that *R. dorbignyi* will reveal Formicidae as the most important prey group as well as the generalist species will present greater contribution similar of all prey categories.

2. Material and methods

2.1. Study area

The study was conducted 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). The landscape of the coastal plain is formed by a mosaic of dunes, ponds, wetlands and riparian forests (Becker et al., 2007b; Tomazelli & Dillenburg, 2000). Climate in this region is classified as humid subtropical, with an average annual temperature of 18.8 °C. Seasons are well defined and the average annual rainfall is 1.213 mm (Maluf, 2000).

2.2. Sampling

We collected samples from consumers (anurans), prey (invertebrates) and primary producers in two outings throughout the anuran-breeding season during the spring/summer 2016. Each outing lasted four consecutive days, always conducted by three to four samplers. All samples were collected with permits from the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio; SISBio nº 50062-5; Ministry of Environment, Brazilian Government) and the Ethics Committee of Animal Use of the University Federal of Rio Grande do Sul (CEUA UFRGS – 29658).

Anurans: The searches took place at night, half an hour after sunset, for five consecutive hours. Anurans were visually found through active searching in commonly used microhabitats, such as in vegetation and near water bodies (Campbell & Christman, 1982). We only collect post-metamorphosed anurans, that is, part of the life cycle that occurs in the terrestrial environment. Tadpoles and froglets, which still have tail remnants (aquatic phase), were not included. Anurans were collected, euthanized with a lethal dose of anesthetic (Lidocaine 4%) applied on the skin and immediately placed in ice until taken to the laboratory where they were kept frozen until processing.

Invertebrates: We obtain invertebrate samples by dissecting and removing prey found in the stomach of the collected anurans. These samples were based only on invertebrates in good condition, indicating recent ingestion and could be identified to order level (see de Carvalho et al., 2017; Grey et al., 2002; Nolan & Britton, 2018).

Producers: In order to select samples of the primary producer species, we used the criteria recommended through similar isotope studies (de Oliveira et al., 2014; Fry, 2006; Garcia et al., 2007). We selected plants that constituted most of the biomass in the different environments (i.e., terrestrial and aquatic), as well as the plants which had different isotopic ratios and therefore different photosynthetic pathways (C_3 and C_4). Here, we remember that carbon is conserved along the trophic web and indicates if an animal is linked to a C_3 or C_4 food chain, for example (Peterson & Fry, 1987; Fry, 2006). The following plant groups were collected: C_4 terrestrial – *Aristida* sp. and *Axonopus* sp.; C_3 terrestrial – *Eryngium pandanifolium* and *Sebastiania commersoniana*; C_3 aquatic – *Eichhornia* sp., *Pistia stratiotes*, *Schoenoplectus californicus* and *Salvinia herzogii*. To obtain plant samples leaves were cut with scissors and placed in identified plastic bags. In addition, we collected biofilm carefully scraping a thin layer adhered to solid surfaces within the aquatic environment, which consists of various microorganisms and debris. Suspended samples of particulate organic matter (POM) in the water and sediment organic matter (SOM) were also collected. POM samples were obtained by filtering 400 mL of water through Whatman glass fiber filter (1.2 µm pore size). SOM samples were collected in the bottom of permanent wetlands with a spoon and putting in plastic bottle (Garcia et al., 2007; Jardine et al., 2003). All producer samples were stored in plastic bags and frozen until processing.

2.3. Sample processing

After defrosting, each anuran had the snout-vent length (SVL, mm) measured with digital caliper (± 0.01 mm) and the stomach removed through an incision in the abdomen to collect prey. Invertebrates found in good condition were collected, identified and washed in deionized water. Small muscle fractions (< 5 g) of the anuran thighs were collected and placed separately in Petri dishes, just as invertebrates. Plant samples were washed under running water to remove any adhered material and rinsed in deionized water. Like other producers, consumers and prey, plant samples were placed in Petri dishes and then dried in an oven set to 60 °C for 48 hours. 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 for isotopic analysis at the Laboratory of Isotope Ecology, which is linked to the Center for Nuclear Energy in Agriculture at the University of São Paulo. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios 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 - ‰): $\delta^{13}C$ or $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where $R = {}^{13}C/{}^{12}C$ or ${}^{14}N/{}^{15}N$ (Peterson and Fry, 1987; 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 accuracy degree of the analyses was ±0.14 for $\delta^{15}N$ and ±0.09 for $\delta^{13}C$.

2.4. Statistical analyses

To visualize the isotopic variation patterns among species we constructed a bi-plot with the individual values of δ^{13}C and δ^{15}N of anurans and mean (± sd) of prey and producers. The relative assimilation of various carbon sources by anurans and their potential prey were indicated by their positions on the X-axis (δ^{13}C), and their trophic positions were indicated by relative positions on the Y-axis (δ^{15}N; Peterson & Fry, 1987).

To verify the assumption of normality and homogeneity of data we used Shapiro-Wilk test and Levene test, respectively. We used ANOVA test followed by Tukey HSD post-hoc test when assumptions were met. If even after natural logarithm or square transformation the data did not normalize, we then used Kruskal-Wallis test with pairwise Wilcoxon tests with Bonferroni-adjusted *p*-values. Thus, variation in δ^{13}C and δ^{15}N among anurans and among invertebrate groups were tested by ANOVA, and variation in δ^{13}C and δ^{15}N among producer groups and variation in SVL among anurans by Kruskal-Wallis test. To examine the relationship between isotope types and SVL for each species we used linear models (LM), with carbon or nitrogen isotopic values being the dependent variable. The significance level adopted was $p = 0.05$.

Mixing models (MM) were used to determine the relative contribution of different prey to anuran biomass. We used Stable Isotope Mixing Models package in R (SIMMR, Version 0.4.0), which uses a Bayesian statistical framework (Parnell & Inger, 2016). The SIMMR package uses Markov Chain Monte Carlo (MCMC) methods to estimate the parameters (Parnell et al., 2010). Bayesian approach allows the incorporation of uncertainty in trophic discrimination factors (TDF), sources and mixtures (Parnell et al., 2010). TDF values, i.e., difference between the isotopic values of the consumer and their diet, were 0.40 ± 1.30‰ to δ^{13}C and 2.54 ± 1.30‰ to δ^{15}N (Post, 2002). For better resolution of MM, some prey groups were pooled in trophic guilds, as in the case of herbivores, as suggested by (Phillips et al., 2014).

As some prey groups presented great variability (sd) around the average δ^{13}C due to the species diversity that make up the group, we divided into two groups, one enriched (E) and another depleted (D) (see Huckembeck et al., 2018). As on the basis of δ^{13}C- δ^{15}N bi-plot it was possible to visualize the spatial trend of the isotopic ratios of each anuran species, we consider as sources the following prey for each anuran species in the mixture models: *B. pulchella*, *D. sanborni* and *S. squalirostris*: Araneae, Diptera, Coleoptera enriched and herbivores; *L. latrans*: Araneae, Coleoptera enriched, herbivores, Formicidae and Isoptera; *P. falcipes*: Araneae, Diptera, herbivores and Isoptera; and *R. dorbignyi*: Araneae, herbivores, Formicidae and Isoptera. We emphasize that Formicidae was the only prey group identified at family level, the others only at order level.

Isotopic niche width of anurans were quantified from standard ellipse areas (SEA_B, expressed in ‰^{2}) using 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 are 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 total area (TA; i.e., the convex hull; Layman et al., 2007) which is a measure of area represented by a polygon drawing connecting the most extreme points of each species in the δ_{space} (Jackson et al., 2011), representing total niche space occupied. SEA_B and SEA_C calculations allowed us to measure trophic niche and indicate the degree of niche overlap (‰^{2}) among species (Jackson et al., 2011; 2012).

We estimated the trophic position (TP) of anuran species using the formula $TP = \lambda + (\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{prey}}) / TDF$. λ is the trophic level of the prey species (Zanden et al., 1997), $\delta^{15}N_{\text{consumer}}$ is the nitrogen isotopic ratio of each consumer, $\delta^{15}N_{\text{prey}}$ is the mean $\delta^{15}N$ of prey, and TDF is the trophic discrimination factor of nitrogen per trophic level (2.54‰; Post, 2002; Zanden et al., 1997). We tested whether trophic position differed among species by ANOVA test. All statistical analyzes were performed using the statistical environment R version 3.5.3 (R Core Team, 2019).

Results

Morphology and Isotopic variability

We collected samples from 91 consumers (anurans), 32 prey (invertebrates) and 88 producers (C_3 and C_4 plants, biofilm, POM and SOM; Table 1). Anuran SVL varied from 13.05 mm (*P. falcipes*) to 100 mm (*R. dorbignyi*) with significant difference among species (H (χ^2) = 72.10; $p < 0.001$; Table 1; supplementary material, Fig. S1). Carbon isotopic ratio ($\delta^{13}C$) of anurans ranged from -28.88‰ (*B. pulchella*) to -16.46‰ (*R. dorbignyi*; Table 1; Fig. 2a; Fig. 3). Analysis of variance showed significant difference among species ($F_{5,85} = 14.72$; $p < 0.001$; Fig. 2a). Nitrogen isotopic ratio ($\delta^{15}N$) ranged from 5.70‰ (*S. squalirostris*) to 10.70‰ (*R. dorbignyi*; Table 1; Fig. 2b; Fig. 3) and also differed significantly among species ($F_{5,85} = 11.67$; $p < 0.001$; Fig. 2b). Linear models do not show a statistically significant effect of the snout-vent length (SVL) on $\delta^{13}C$ and $\delta^{15}N$ values of anurans (Fig. 4; Supplementary material, Table S1).

Carbon isotopic ratio ($\delta^{13}C$) of invertebrates ranged from -31.44‰ (Coleoptera) to -13.82‰ (Formicidae; Table 1; Fig. 3). ANOVA showed significant difference among invertebrates ($F_{9,22} = 7.76$; $p < 0.001$). Nitrogen isotopic ratio ($\delta^{15}N$) ranged from 2.83‰ (Larva Lepidoptera) to 10.69‰ (Formicidae; Table 1; Fig. 3) and invertebrates were statistically different from each other ($F_{9,22} = 4.61$; $p = 0.001$). Carbon isotopic ratio ($\delta^{13}C$) of producers ranged from -33.55‰ (biofilm) to -11.94‰ (C_4 terrestrial; Table 1; Fig. 3), where the majority of groups were statistically different (H (χ^2) = 62.18; $p < 0.001$), except biofilm vs. C_3 terrestrial, C_3 aquatic and POM (Table 1). Nitrogen ($\delta^{15}N$) ranged from -0.37‰ (C_4 terrestrial) to 9.79‰ (C_3 aquatic; Table 1; Fig. 3), where the majority of groups were statistically different (H (χ^2) = 35.26; $p < 0.001$), except between pairs C_3 terrestrial vs. biofilm, POM and SOM; biofilm vs. C_3 aquatic, POM and SOM; and POM vs. SOM (Table 1).

Food assimilation, Isotopic niche and Trophic position

Mixing model results showed a large contribution of herbivorous invertebrates (i.e., lepidoptera, hemiptera and orthoptera larvae) to all anuran species except for *R. dorbignyi*, which showed greater assimilation of the Formicidae (29.5%) and Isoptera (27.9%) groups. Contribution of herbivores varied from 54.4% (*S. squalirostris*) to 33.5% (*D. sanborni*). Coleoptera was the second most important prey group for four amphibian species, ranging from 32.8% (*B. pulchella*) to 18.2% (*L. latrans*; Fig. 5; supplementary material, Table S2).

Results of the corrected standard ellipse area (SEA_C) showed that anuran isotopic niches varied from 8.83‰² **(***B. pulchella*) to 2.39‰² (*L. latrans*; Fig. 6; Table 2). The largest niche overlaps occurred between *B. pulchella* and *D. sanborni*, and between *S. squalirostris* and *P. falcipes* (Table 2). Besides *B. pulchella* being the species with the largest niche, it was also the species that most overlapped the isotopic niche area with the other species, except *R. dorbignyi*, which there was no overlap. In fact, *R. dorbignyi* was the only species that presented zero or practically zero overlap with the other species (Fig. 6; Table 2).

The average trophic position of the species ranged from 2.5 ± 0.28 (*S. squalirostris*) to 3.4 ± 0.31 (*R. dorbignyi*) and was significantly different among species ($F_{5,85} = 11.63$; $p < 0.001$; Table 2; supplementary material, Fig. S2).

Discussion

Our results revealed different trophic niche width and overlap among the six coexisting anuran species studied in a wetland area in southern Brazil. Through isotopic ellipses analysis we could notice species with total separation such as *R. dorbignyi*, until species that share the trophic niche with several others, such as *B. pulchella*. This result corroborated our prediction that the isotopic niche of the specialist *R. dorbignyi* does not overlap with the generalist species. This may happen because specialization can act to reduce interspecific resource overlap (Wilson & Yoshimura, 1994). On the other hand, the generalist *B. pulchella* presented niche overlap with all species, except *R. dorbignyi*, and the largest overlaps were with the other hylids (*D. sanborni* and *S. squalirostris*). However, these hylids, which are small and very morphologically similar, seem to present isotopic niche partitioning. We speculate that may be a strategy that allows them to coexist in the same microhabitat. Both species use the habitat more restrictively than *B. pulchella*, and are often seen in plants such as those of the genus *Eryngium* in wetlands, which accumulate water and form phytothelma, where they can forage (Achaval & Olmos, 2007; Huckembeck et al., 2018). Similar to *B. pulchella*, *P. falcipes* overlapped with all species (except *R. dorbignyi*). Although the niche area was narrower than *B. pulchella*, it showed a wide range on the δ^{15}N axis, indicating that this small leptodactylid consumes prey of different trophic levels. In contrast, some studies have shown that small leptodactylids may be more specialized (de Oliveira et al., 2015; Maneyro & Carreira, 2012; Van Sluys & Rocha, 1998). Consumption of a wider range of prey may be associated with niche expansion strategies to avoid competition (Lister, 1976).

According to our prediction, the smallest species **(***D. sanborni*, *S. squalirostris* and *P. falcips*) revealed the smallest isotopic niche areas (SEA_C, ‰^{2}). The only exception was *L. latrans*, which is a medium to large anuran and had the smallest elliptical area among all species, and many studies point to it as a generalist consumer (da Rosa et al., 2002; Maneyro et al., 2004; Pazinato, Trindade, Oliveira, & Capellarri, 2011; Solé et al., 2009; Teixeira & Vrcibradic, 2003). This may be because our sample did not present larger individuals that are able to consume a greater variety of prey, including preying on other anurans and even practicing cannibalism (França et al., 2004; Sanabria, 2005). We found individuals with an average 60 mm of SVL, but this species can reach up to 120 mm (Achaval & Olmos, 2007) and that probably caused a bias about the real isotopic niche size. *B. pulchella* revealed the largest isotopic niche width and it has been mentioned as generalist in stomach content studies (Basso, 1990; da Rosa et al., 2002; Maneyro & Rosa, 2004). As it has a larger body size compared to the other hylids and it is also generalist in habitat use, occurring in natural and anthropogenic environments, in arboreal strata, on the ground and even in floating vegetation, this favors the consumption of a wide range of prey (Achaval & Olmos, 2007; da Rosa et al., 2006; Santos et al., 2016). Although *R. dorbignyi* presented the highest average SVL among the species, it was also the anuran classified as specialist (da Rosa et al., 2002; Isacch & Barg, 2002), so our prediction that this species would reveal a small isotopic niche was not corroborated. *R. dorbignyi* revealed the second largest niche, indicating that it is not so specialist and may be opportunistic, which has already been recorded for other congeners (Batista et al., 2011; Evans & Lampo, 1996; Maia-Carneiro et al., 2013).

Regarding to trophic position, larger species could be expected to present higher nitrogen isotopic ratios, however our results showed no relationship between body size (SVL) and δ^{15}N, as Cloyed & Eason, (2017) found for four of the five species studied. Thus, Bufonidae occupied the largest level, and Hylidae and Leptodacylidae, although some different between pairs of species, occupied intermediate and lower levels. Calculations estimated secondary consumers like *S. squalirostris*, and tertiary or quaternary consumers like *R. dorbignyi*. This result agrees with mixing model, since herbivores were more important for *S. squalirostris* and ants for *R. dorbignyi*. We also noticed ecologically and morphologically similar species such as *S. squalirostris* and *D. sanborni* occupying different trophic levels. Mixing models corroborate that the higher consumption of Coleoptera and Araneae, which are detritivores and carnivores, was responsible for the superior position of the *D. sanborni* in relation to *S. squalirostris*. The trophic position for *P. falcipes* was similar to those of the previously mentioned hylids, but did not differ from the larger anurans (*B. pulchella* and *L. latrans*). This may have happened because *P. falcipes* prey on different trophic levels. In the case of *R. dorbignyi*, a predominantly ant diet may be responsible for the high δ^{15}N values**,** because some ant species are carnivorous and occupy high levels in the food chain (Tillberg et al., 2007; Verburg et al., 2007; Vogt et al., 2002). Similarly, the presence of termites (Isoptera) in the diet also increases the predator δ^{15}N, because according to Bourguignon et al. (2009), termites with δ^{15}N between 6‰ and 16.5‰ were recorded in their study. Termites are decomposers that feed on humid organic matter and thus have δ^{15}N as high as predators (Bourguignon et al., 2009). A number of mechanisms are still not fully understood, however humic soils, like wetlands, are rich in organic matter, deeper and older, and are therefore more enriched in nitrogen (Billings & Richter, 2006; Martinelli et al., 1999).

Mixing model estimates for *R. dorbignyi* corroborated with the expected, showing Formicidae (ants) as the group that contributed the most, followed by Isoptera. Similar result was found for *R. granulosa* (Duré et al., 2009) and *R. scitula* (Maragno & Souza, 2011). In addition, Araneae and herbivores seem to be a very important item, which makes *R. dorbignyi* not so specialist on ants, at least in the study area. As we can see, the isotopic ratios of anurans and prey in the biplot (Fig. 2), Formicidae and Isoptera were not important food resource in the diet of the other species, seeming to be occasionally consumed by leptodactylids. Mixing models inferred greater contribution to Hylidae and Leptodactylidae from the group of prey we call herbivores (Orthoptera, Hemiptera and Lepidoptera larvae). However, we emphasize that this importance may be overestimated due to the large δ^{13}C range from which these invertebrates may be derived carbon. When we look at Fig. 2, we realize that Araneae, Coleoptera and Diptera are the most important prey for these two anuran families, similar to what is known from many dietary studies (Kittel & Solé, 2015; Maneyro et al., 2004; Maneyro & Rosa, 2004; Menin et al., 2005; Rosa et al., 2011; Van Sluys & Rocha, 1998)**.**

The isotopic space formed by carbon ratios of anurans and carbon ratios of different primary producers - C_3 vs. C_4 chain - revealed that anurans were in the middle of these two basal food chains, but a little closer to the C_3 chain. We noticed species such as *R. dorbignyi* more linked to C_4 chain and *B. pulchella* to C_3 chain. C_4 plants are considered lower in nutritional quality than C_3 plants (Barbehenn et al., 2004a, b), therefore many herbivores avoid C_4 plants due to their higher concentrations of nondigestible structural carbons such as lignin (Throop & Archer, 2009; Vanderbilt et al., 2008). This low nutritional quality is due to their higher carbon content and lower nitrogen content (Barbehenn et al., 2004a, b). In addition, the leaves of C_4 plants present larger fiber bundles and silica phytoliths, causing a greater foliar hardening than in C_3 plants (Boutton et al., 1978; Massey et al.,, 2007). Although C_4 plants are considered as sources of low energy, Adis & Victoria, (2001) showed that they were important sources for terrestrial arthropods. This corroborates the findings of Magnusson et al. (1999), who showed Bufonidae highly dependent on the C_4 food chain. In addition, termites that were items consumed by Bufonidae have already been observed obtaining energy from the C_4 trophic pathway (Symes & Woodborne, 2011).

Conclusions

Our study brings some inferences about how some anuran species can coexist through the trophic peculiarities that each species has. We observed a generalist species such as *B. pulchella* revealing the largest trophic niche area as well as the highest overlap. Conversely, *L. latrans*, another large and generalist species, revealed the smallest isotopic niche, which may have been a sample bias because we could not capture the largest individuals in the population. We also observed two ecologically and morphologically very similar species, *D. sanborni* and *S. squalirostris*, with segregated isotopic niches and occupied distinct trophic levels in the food web, which may be a niche partitioning strategy that allow the coexistence of them in the same microhabitat. *P. falcipes* revealed a high niche overlap with other hylids and leptodactylids and although the niche area was narrow, it was wide on the nitrogen axis, suggesting prey consumption of different levels. The specialist, *R. dorbignyi*, despite showing a slightly larger niche area than expected for its foraging strategy, revealed a fully segregated isotopic niche without overlapping with any other anuran. Mixing models estimated a greater importance of ants and termites for *R. dorbignyi*, which may have resulted in their greater trophic position. For the other anurans, herbivores, Araneae, Coleoptera and Diptera were the most important estimated items. C_3 plants chain seemed to represent a highly important trophic pathway for these generalist anurans and C4 chain for the specialist *R. dorbignyi*. We emphasize that Brazilian wetlands are environments that have been influenced by anthropic activities that besides interfering in the natural levels of nitrogen, also are responsible for the conversion of the wetlands (Junk et al., 2014). These changes are reflected throughout the entire wetland community, but mainly in species such as anurans that are highly dependent on water, destabilizing the entire food chain (Regester et al. 2006).

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TABLES AND FIGURES

Table 1. Mean ± standard deviation (sd) of stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for anurans, invertebrates and producers in southern Brazil (Tapes, Rio Grande do Sul state). For anurans, the column next to isotopic values contains values for snout-vent length measurements **(**SVL, mm). Sample sizes are shown in parentheses.

Group	**$\delta^{13}C$ ± sd (‰)**	**$\delta^{15}N$ ± sd (‰)**	
AMPHIBIANS (91)			**SVL (mm)**
Hylidae			
Boana pulchella (16)	-24.16 ± 2.14	8.20 ± 1.35	41.73 ± 15.01
Dendropsophus sanborni (24)	-22.78 ± 1.67	8.61 ± 0.54	19.03 ± 1.27
Scinax squalirostris (16)	-22.12 ± 1.37	7.32 ± 0.71	21.06 ± 2.88
Leptodactylidae			
Leptodactylus latrans (12)	-20.70 ± 1.48	8.40 ± 0.47	59.92 ± 22.26
Pseudopaludicola falcipes (9)	-21.58 ± 1.38	7.81 ± 0.95	19.55 ± 8.87
Bufonidae			
Rhinella dorbignyi (14)	-19.17 ± 2.04	9.53 ± 0.78	82.83 ± 20.03
INVERTEBRATES (32)			
Predators (17)	**-22.58 ± 3.60**	**8.46 ± 1.50**	
Araneae (5)	-22.55 ± 1.59	7.33 ± 1.39	
Diptera (3)	-24.75 ± 0.63	8.26 ± 1.34	
Formicidae E (3)	-16.33 ± 2.84	8.38 ± 0.50	
Formicidae D (6)	-24.66 ± 2.18	9.35 ± 1.61	
Detritivors (10)	**-24.32 ± 4.87**	**6.70 ± 1.29**	
Coleoptera E (4)	-24.42 ± 1.31	6.49 ± 1.17	
Coleoptera D (3)	-29.66 ± 1.84	5.86 ± 1.54	
Isoptera (3)	-18.87 ± 3.72	7.82 ± 0.14	
Herbivores (5)	**-23.26 ± 4.33**	**4.70 ± 1.52**	
Lepidoptera larvae (2)	-19.96 ± 5.43	4.03 ± 1.69	
Orthoptera (1)	-27.33	5.25	
Hemiptera (2)	-24.52 ± 2.24	5.11 ± 2.21	
PRODUCERS (88)			
C_4 terrestrial	**-12.43 ± 0.26**	**1.92 ± 1.77**	
Aristida sp. (8)	-12.55 ± 0.23	0.90 ± 1.24	
Axonopus sp. (8)	-12.31 ± 0.23	2.93 ± 1.70	
C_3 terrestrial	**-30.49 ± 1.56**	**4.01 ± 2.22**	
Eryngium pandanifolium (8)	-29.57 ± 1.45	2.17 ± 0.48	
Sebastiania commersoniana (8)	-31.41 ± 1.08	5.84 ± 1.64	
C_3 aquatic	**-29.09 ± 1.18**	**5.86 ± 2.09**	

Eichhornia sp. (8)	-29.58 ± 0.60	3.85 ± 2.96
Pistia stratiotes (8)	-29.66 ± 0.85	7.10 ± 1.56
Salvinia herzogii (8)	-29.81 ± 0.19	6.54 ± 0.92
Schoenoplectus californicus (8)	-27.32 ± 0.55	5.97 ± 0.61
Other		
Biofilm (8)	-27.47 ± 5.58	5.73 ± 2.15
POM (8)	-25.89 ± 0.80	4.37 ± 1.09
SOM (8)	-21.78 ± 3.12	5.11 ± 0.69

Table 2. Trophic position of anuran species and isotopic niche width given as Bayesian approximation of the Standard Ellipse Area (SEA$_B$, ‰^{2}), sample size-corrected Standard Ellipse Area (SEA$_C$, ‰^{2}) and Layman metric of the convex hull area (TA, ‰^{2}). Niche overlap (‰^{2}) among species was calculated using the SEA$_C$.

Species	**Trophic position ± sd**	**SEA$_B$ (‰^{2})**	**SEA$_C$ (‰^{2})**	**TA (‰^{2})**	**Area of Overlap (‰^{2})**					
					BP	**DS**	**SS**	**LL**	**PF**	**RD**
B. pulchella (BP)	2.8 ± 0.53	8.24	8.83	22.62	-	2.17	0.57	0.08	0.80	0
D. sanborni (DS)	3.0 ± 0.22	2.66	2.78	9.46	-	-	< 0.01	0.46	0.80	< 0.01
S. squalirostris (SS)	2.5 ± 0.28	2.82	3.02	7.75	-	-	-	< 0.01	1.23	< 0.01
L. latrans (LL)	2.9 ± 0.19	2.17	2.39	4.40	-	-	-	-	0.60	< 0.01
P. falcipes (PF)	2.7 ± 0.38	3.01	3.45	5.22	-	-	-	-	-	< 0.01
R. dorbignyi (RD)	3.4 ± 0.31	5.00	5.42	10.07	-	-	-	-	-	-

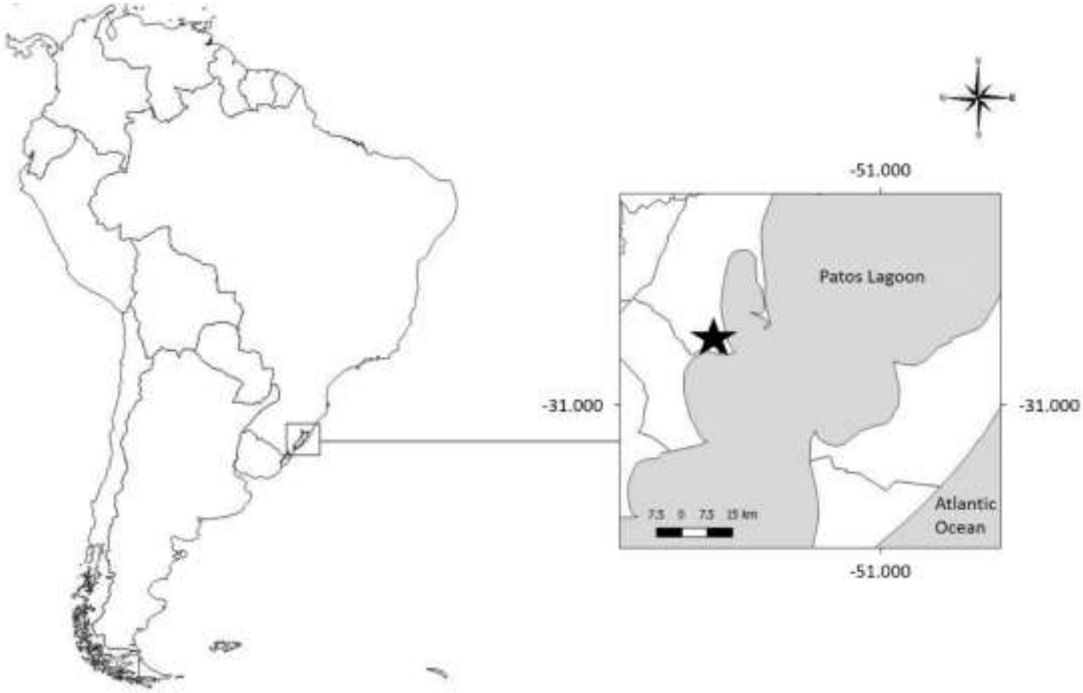

Figure 1. Map showing the study area in Rio Grande do Sul state, in southern Brazil. The black star indicates the location of the study area on the shore of Patos Lagoon (30°52'7.15"S and 51°23'41.92"W).

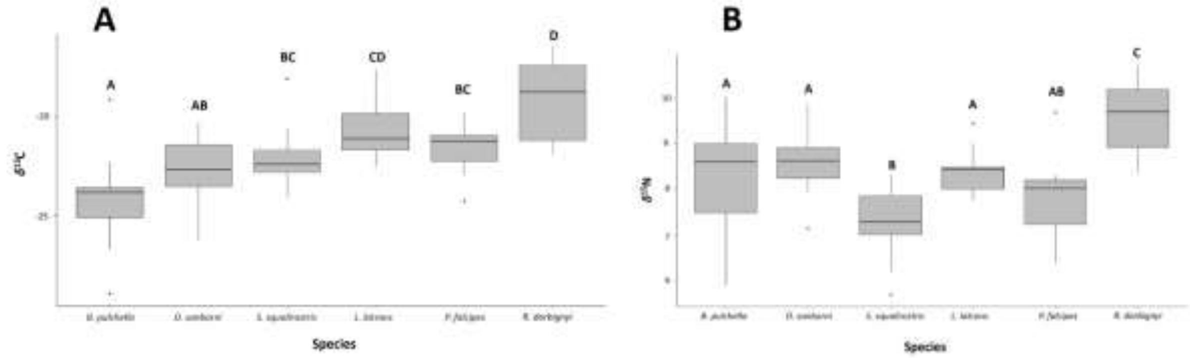

Figure 2. A) Carbon isotopic ratios (δ^{13}C) and; B) Nitrogen isotopic ratios (δ^{15}N) of anuran species collected in southern Brazil (Tapes, Rio Grande do Sul state). Different letters indicate significant difference in means according Tukey test ($p < 0.05$). Box: 25th and 75th percentiles; whiskers: 5th and 95th percentiles; horizontal line: median; dots: outliers.

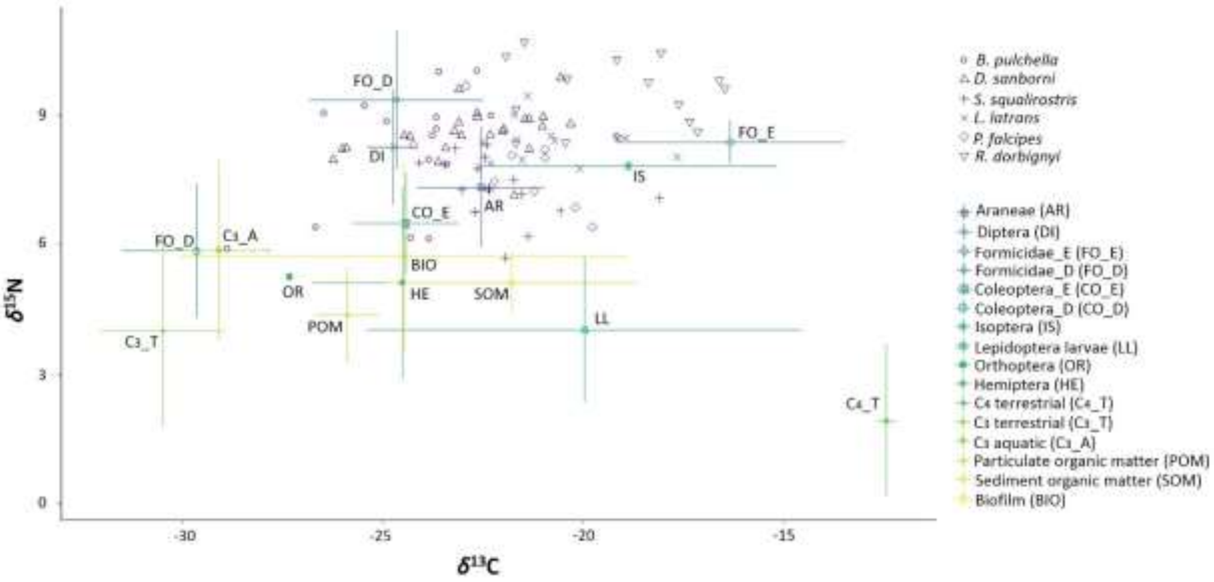

Figure 3. Individual $\delta^{13}C$ and $\delta^{15}N$ values for anuran species and mean value (± sd) for prey (invertebrate) and basal food sources collected in southern Brazil (Tapes, Rio Grande do Sul state).

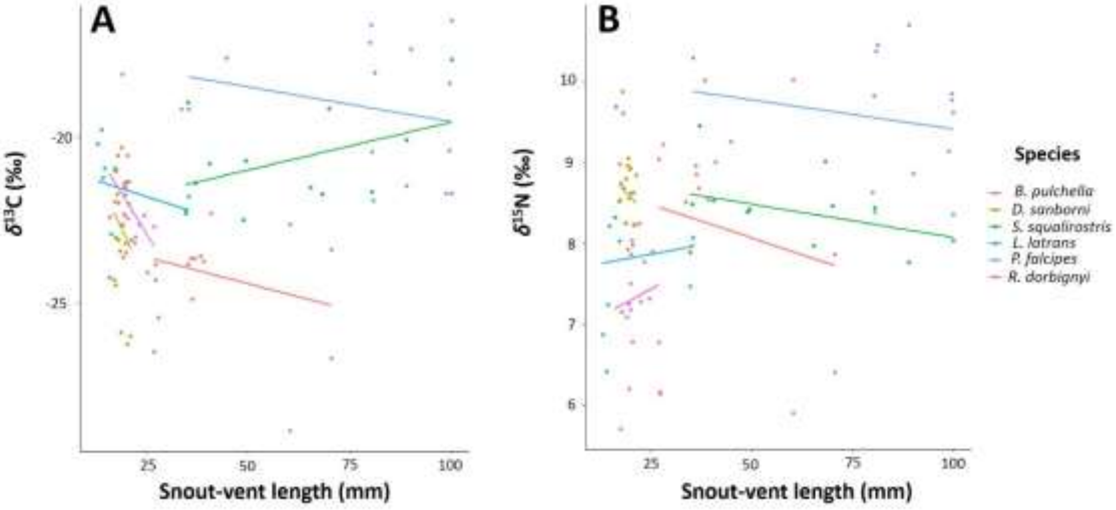

Figure 4. A) Carbon isotopic ratio ($\delta^{13}C$) and; B) nitrogen ($\delta^{15}N$) versus snout-vent length (SVL) for each anuran species. Lines represent the linear model, with different color for each species according to the legend.

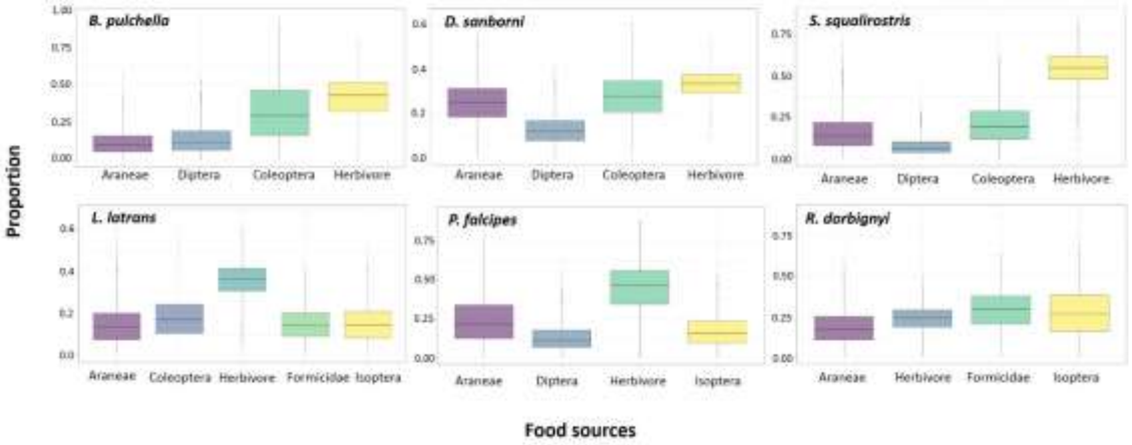

Figure 5. Mixing models showing percent contribution of different prey to anuran species in southern Brazil (Tapes, Rio Grande do Sul state).

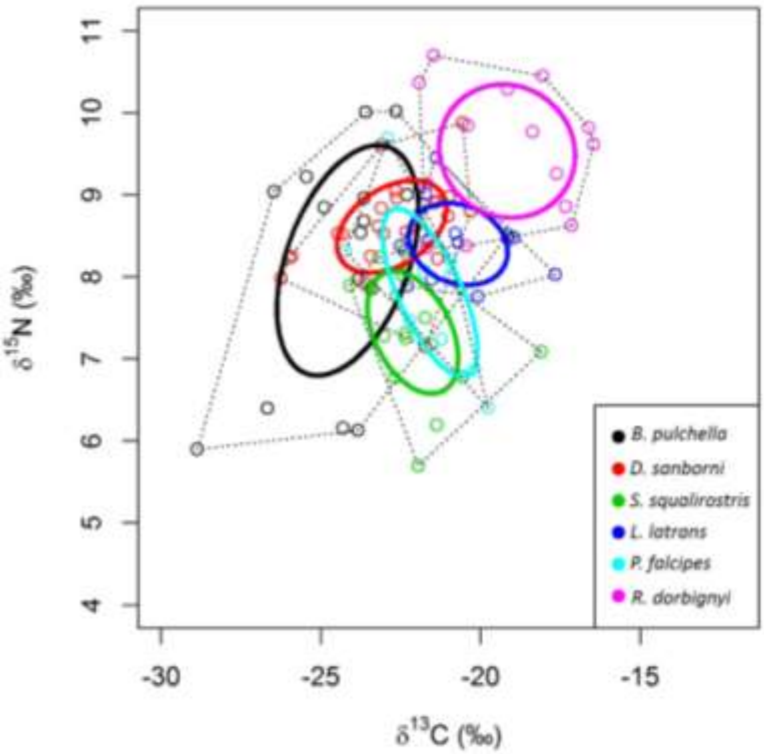

Figure 6. Isotopic niches for anuran species in southern Brazil (Tapes, Rio Grande do Sul state). Corrected Standard Ellipse Areas (SEA_C) showing the area of the isotopic

niches (‰^{2}) are represented by the solid bold lines (ellipses). The Layman metric of the convex hull area (TA) for all individuals is represented by the black dotted lines.

Supplementary material

Table S1. Statistical values for linear models (LM) testing whether carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic values vary by snout-vent length (SVL).

	t	**d.f.**	***p*-value**
δ^{13}C			
B. pulchella	-0.863	1,14	0.403
D. sanborni	-0.950	1,22	0.352
S. squalirostris	-1.711	1,14	0.109
L. latrans	1.533	1,10	0.156
P. falcipes	-0.688	1,7	0.514
R. dorbignyi	-0.733	1,12	0.478
δ^{15}N			
B. pulchella	-0.700	1,14	0.496
D. sanborni	-0.588	1,22	0.563
S. squalirostris	0.413	1,14	0.685
L. latrans	-1.317	1,10	0.217
P. falcipes	0.226	1,7	0.827
R. dorbignyi	-0.652	1,12	0.527

Table S2. Mean ± standard deviation (sd) of percent contribution (%) of prey to anuran species in southern Brazil (Tapes, Rio Grande do Sul state).

ANURANS	*B. pulchella*	*D. sanborni*	*S. squalirostris*	*L. latrans*	*P. falcipes*	*R. dorbignyi*
PREY						
Araneae	12.1 ± 8.6	25.3 ± 9.3	16.5 ± 11	14.9 ± 9.5	24.8 ± 16	18.7 ± 10.1
Diptera	14 ± 9.6	13.1 ± 6.6	7.8 ± 5.2	-	13.1 ± 8.7	-
Coleoptera_E	32.8 ± 19.9	28.1 ± 10.2	21.3 ± 11.7	18.2 ± 9.6	-	-
Herbivores	41.2 ± 14.6	33.5 ± 6.2	54.4 ± 10. 7	36.1 ± 8.6	44.9 ± 15.9	24 ± 7.6
Formicidae_E	-	-	-	15.1 ±7.6	-	29.5 ± 11.9
Isoptera	-	-	-	15.6 ± 8.9	17.2 ± 9.8	27. 9 ± 14.5

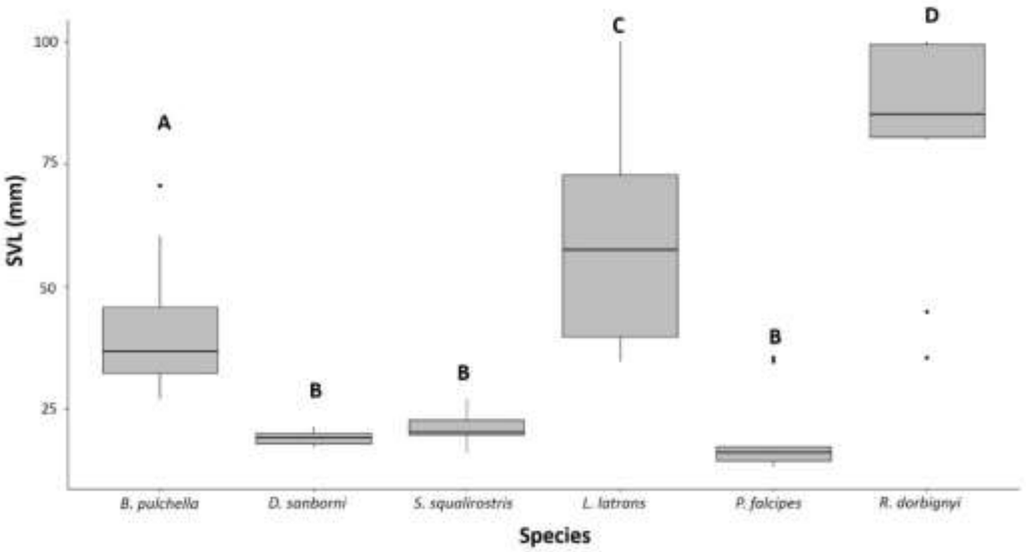

Figure S1. Snout-vent length (SVL, mm) of anuran species in southern Brazil (Tapes, Rio Grande do Sul state). Different letters indicate significant difference in means according Tukey test ($p < 0.05$). Box: 25th and 75th percentiles; whiskers: 5th and 95th percentiles; horizontal line: median; dots: outliers.

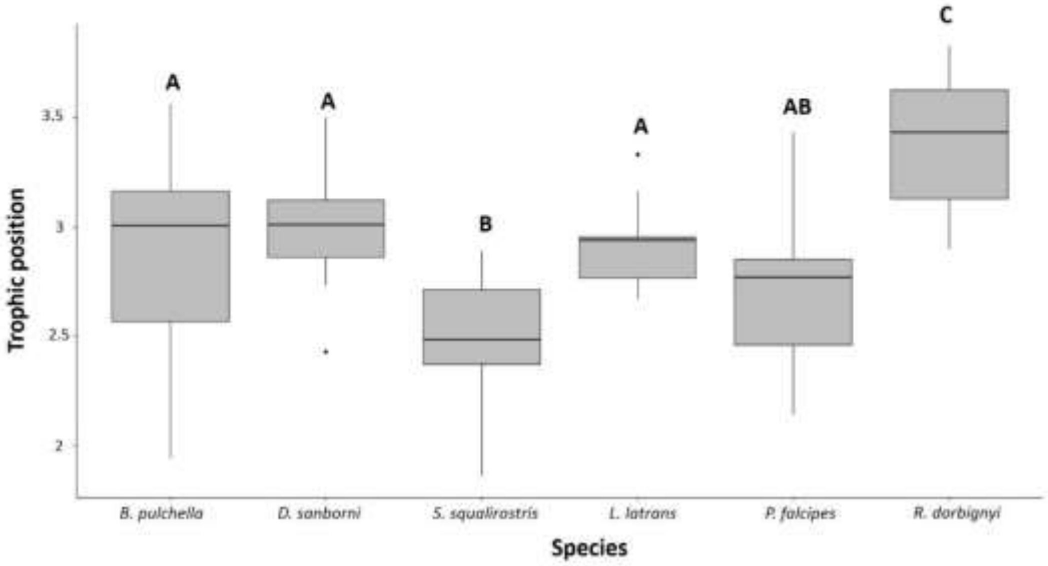

Figure S2. Trophic position of anuran species in southern Brazil (Tapes, Rio Grande do Sul state). Different letters indicate significant difference in means according Tukey test ($p < 0.05$). Box: 25th and 75th percentiles; whiskers: 5th and 95th percentiles; horizontal line: median; dots: outliers.

CAPÍTULO IV

Manuscrito a ser submetido à revista Toxicon:

Um caso de envenenamento pela serpente opistóglifa *Thamnodynastes hypoconia* (Cope, 1860) (Dipsadidae: Tachymenini) no sul do Brasil

Um caso de envenenamento pela serpente opistóglifa *Thamnodynastes hypoconia* (Cope, 1860) (Dipsadidae: Tachymenini) no sul do Brasil

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Resumo

Esse é um relato de caso de um acidente humano com a serpente opistóglifa da família Dipsadidae, *Thamnodynastes hypoconia* (Cope, 1860), no município de Tapes, Rio Grande do Sul, Brasil. A mordida foi no pulso do braço esquerdo durante o manuseio da serpente em uma atividade de campo. Não houve dor no momento da mordida e após 20 minutos observou-se edema ao longo da mão e antebraço, leve sensação de dormência e dor leve ao movimentar os dedos. Após 15 horas também ocorreu eritema, parestesia e sensação de temperatura bastante alta no local da picada. Após 30 horas do acidente ocorreu equimose nos dedos e antebraço, porém o edema começou a diminuir. Passadas 70 horas ainda havia equimose, coceira e dor moderada no momento da coceira. Somente no sétimo dia que não foi registrado mais nenhum sintoma. Esse é o primeiro registro de caso de acidente com *T. hypoconia* no Brasil.

Palavras chave: Acidente ofídico; Colubridae; Picada de serpente; Veneno

Introdução

O número anual de pessoas picadas por serpentes no Brasil pode chegar próximo a 30 mil, sendo que em 2017, num total de 28 mil casos, 23 mil (~ 81%) foram causados por serpentes peçonhentas (Viperidae e Elapidae), 1.890 (~ 7%) por serpentes não peçonhentas e 3.245 por serpentes não identificadas (~ 12%; SINAN, 2019). Possivelmente o número de acidentes com serpentes não peçonhentas estar subestimado, pois grande parte desse número de serpentes não identificadas pode ser de serpentes não peçonhentas. Os acidentes com serpentes não peçonhentas são causados principalmente por espécies das famílias Colubridae e Dipsadidae (Salomão et al., 2003).

Diferentemente de Viperidae e Elapidae que possuem dentição solenóglifa e proteróglifa, respectivamente, os representantes das famílias Colubridae e Dipsadidae (“colubrídeos”) apresentam dentição áglifa ou opistóglifa, as quais não são consideradas perigosas para os humanos devido a ineficiência de injetar veneno (Gans e Elliott, 1968; Weinstein e Kardong, 1994). No entanto, os colubrídeos, chamados de “não peçonhentos”, possuem glândula de Duvernoy que produzem toxinas usadas para subjugar presas (Kardong, 2002), sendo considerada homóloga as glândulas de veneno dos viperídeos e elapídeos (Jackson et al., 2017). Muito pouco ainda se sabe sobre a caracterização dessas toxinas e atividade nos tecidos vivos (Ching et al., 2012, 2006; Fry et al., 2003; Peichoto et al., 2012, 2011). De modo geral, os sintomas dos acidentes causados por essas serpentes “não peçonhentas” são dor local, edema e equimose (Prado-Franceschi e Hyslop, 2002).

Alguns dos gêneros responsáveis pelos acidentes relatados no Brasil são *Clelia*, *Philodryas*, *Helicops*, *Spilotes*, *Erythrolamprus* (*Liophis*), *Simophis*, *Oxyrhopus*, *Thamnodynastes*, *Xenodon* (*Lystrophis*), *Sibynomorphus*, *Oxybelis* (Araújo et al., 2018; Prado-Franceschi e Hyslop, 2002; Quintela, 2010; Salomão et al., 2003; Silva et al., 2019; Silveria e Nishioka, 1992). *Thamnodynastes* é um gênero Neotropical da tribo Tachymenini pertence à família Dipsadidae (Zaher et al., 2009). A espécie *Thamnodynastes hypoconia* (Cope 1860) (Fig. 1) ocorre no nordeste, centro-oeste, sudeste e sul do Brasil, Paraguai, Uruguai e Argentina (Franco, 1999; Giraudo, 2001). É uma serpente opistóglifa, de pequeno porte, atingindo cerca de 700 mm de comprimento total, que apresenta comportamento bastante agressivo quando ameaçada (Giraudo, 2001). Possui atividade noturna, é vivípara e alimenta-se preferencialmente de anuros

(Bellini et al., 2013, 2014; Rebelato et al., 2016). Apresentamos aqui o relato de um caso de envenenamento humano ocasionado por *Thamnodynastes hypoconia*.

Figura 1. Espécime de *Thamnodynastes hypoconia,* Tapes, Rio Grande do Sul, Brasil. Foto: Natalia Dallagnol Vargas.

Relato de caso

O acidente ocorreu no dia 15 de fevereiro de 2016 às 20h45, com uma mulher de 27 anos, 1.83 m de altura, 68 kg, durante uma saída de campo para coleta de dados da serpente *T. hypoconia* em uma área de banhado na beira da Lagoa dos Patos, no município de Tapes, Rio Grande do Sul, Brasil (30°52'7.15"S e 51°23'41.92"W). A serpente mordeu no pulso do braço esquerdo da pesquisadora, logo abaixo da luva, durante a manipulação para medição de comprimento do exemplar (Fig. 2A). A serpente precisou ser removida manualmente, forçando a abertura da mandíbula por elevação da maxila. A mordida não causou dor imediata, e segundos após o acontecimento, foram observadas duas perfurações com leve sangramento causadas pela dentição opistóglifa, circundadas por edema leve local (Fig. 2B). Passados 20 minutos, o edema se estendeu pela mão e antebraço, com leve sensação de dormência e dor ao mexer os dedos (Fig. 2C). Após 3 horas do incidente, a mão esquerda, a qual foi mordida, apresentava bastante edema em relação a mão direita (Fig. 2D) e a pesquisadora foi levada até o Centro Municipal de Saúde de Arambaré, RS, para ser avaliada por um médico. Os seguintes sinais vitais foram medidos: temperatura corporal: 36.7 ºC, pressão arterial: 110/60 mm e frequência cardíaca: 85 bpm. O medicamento Celestamine® (maleato de dexclorfeniramina – 2 mg + betametasona – 0.25 mg) foi receitado para a paciente, na dosagem de um comprimido a cada oito horas.

Durante o atendimento o profissional de saúde solicitou que a paciente se dirigisse para o Hospital Nossa Senhora Aparecida (HNSA), um centro de saúde maior e mais especializado no município de Camaquã, pois acreditava que a espécie causadora do acidente se tratasse de uma serpente peçonhenta. Durante o atendimento a pesquisadora mencionou que a espécie em questão não era uma serpente de interesse médico. Os responsáveis pelo atendimento no HNSA acreditavam se tratar de uma picada de serpente peçonhenta do grupo das jararacas (gênero *Bothrops*), mesmo sem terem visto o animal, e recomendaram que a paciente fosse transferida para Porto Alegre, município localizado a 130 km de Camaquã e local mais próximo com disponibilidade de soro antibotrópico. A paciente novamente afirmou conhecer a espécie e salientou que não era necessário a aplicação de nenhum soro antiofídico. A equipe do hospital contatou o Centro de Informações Toxicológicas do Rio Grande do Sul (CIT), o qual assessora e orienta frente à ocorrência de acidentes tóxicos no Estado, e mencionou a espécie em questão. O CIT confirmou que a *T. hypoconia* não era de interesse médico e que a paciente poderia ser liberada. Antes de liberarem, a paciente teve o local da picada higienizado com iodo e coberto com um curativo (Fig. 2E). Também foi receitada a administração de Paracetamol 500 mg e Ibuprofeno 400 mg como analgésico e anti-inflamatório, respectivamente, a cada seis horas, porém a paciente fez uso apenas do Ibuprofeno.

No segundo dia, após 15 horas da picada a mão e o antebraço apresentavam edema, eritema, parestesia e sensação de temperatura bastante alta no local da picada com dor leve (Fig. 3A). Após 20 horas do acidente foram medidas a temperatura corporal (37.4 ºC), a pressão arterial (130/80 mm) e a frequência cardíaca (97 bpm). No terceiro dia, após 30 horas do incidente todos os dedos e parte interna do antebraço apresentavam equimose e dor moderada ao realizar qualquer esforço muscular (Fig. 3B-C), porém o edema começou a diminuir. No quarto dia, passadas 70 horas da mordida, a mão e o antebraço não apresentavam mais edema e a equimose passou de uma coloração roxa para uma coloração verde amarelada, com bastante coceira e dor leve (Fig. 3D-E). No quinto dia a coloração verde amarelada praticamente era imperceptível e não havia mais dor ao realizar qualquer esforço físico, sendo que no sétimo dia nenhum sintoma era visível.

A serpente era adulta, porém não teve o sexo determinado. A mesma apresentou 320.40 mm de comprimento rostro cloacal e 18.30 g de massa total. A serpente foi coletada sobre licença do SISBio (Nº 50062-5) e tombada na coleção herpetológica do Laboratório de Ecologia de Vertebrados Terrestres (LEVERT) da Universidade do Vale do Rio dos Sinos sobre o número tombo CHLEVT330.

Figura 2. A) Espécime de *Thamnodynastes hypoconia* no momento do acidente; B) Marcas da dentição opistóglifa após a serpente ser removida; C) Edema após 20 minutos do acidente; D) Edema na mão após 3 horas; E) Curativo na mão após atendimento médico.

Figura 3. A) Edema e eritema após 15 horas após o acidente; B-C) Equimose na mão e antebraço após 30 horas; D-E) Equimose mudando de coloração arroxeada para verde amarelada após 70 horas.

Discussão

Esse é o primeiro relato de acidente ofídico causado pela serpente opistóglifa “não peçonhenta” *Thamnodynastes hypoconia*. Apesar de haverem outros registros para o gênero (Araújo et al., 2018; Diaz et al., 2004; Hoge, 1952; Lema, 1978; Prado-Franceschi e Hyslop, 2002; Salomão et al., 2003), esse é o primeiro para a espécie. Os dois relatos de caso com a congênere *T. pallidus*, um no nordeste brasileiro (Araújo et al., 2018) e o outro na Venezuela (Diaz et al., 2004), e com *T. strigatus* (Hoge, 1852) também foram com pesquisadores atingidos nas mãos durante a manipulação, o que demonstra o comportamento agressivo das espécies do gênero *Thamnodynastes*. Salomão et al. (2003) destacam que as mãos são as partes mais atingidas por serpentes “não peçonhentas”. Os relatos acima mencionados com *T. pallidus* também citaram edema, equimose, dor leve e alta temperatura no local da mordida. No acidente registrado por Araújo et al. (2018), os sintomas apresentaram-se mais leves e passaram após 36 horas sem necessidade de medicação. Já no caso relatado por Diaz et al. (2004), além dos sintomas acima descritos, também foi registrado salivação excessiva com gosto metálico e forte dor de cabeça. Nesse caso o paciente foi medicado com hidrocortisona e analgésico, sendo que após 36 horas não apresentava edema. No caso que aqui relatamos, o edema também foi diminuindo após 30 horas do acidente, porém a equimose ainda era visível no antebraço após terem passadas 70 horas do acidente e somente após uma semana todos os sintomas desapareceram.

Acidentes com *T. strigatus* não são muito bem documentados, apesar da espécie ser associado a um grande número de acidentes no Brasil (Hoge, 1852; Puorto e França, 2003). Porém, um estudo sobre o perfil venômico revelou metaloproteinases com atividade proteolítica que já foram identificadas em outras serpentes da mesma família (Assakura et al., 1994; Lemoine et al., 2004), além de proteínas novas que ainda não se sabe a função (Ching et al., 2012). Ressaltamos aqui a importância de conhecer o perfil das toxinas das serpentes "não peçonhentas", dado o enorme potencial farmacológico que essas substâncias podem apresentar (Georgieva et al., 2008). Um experimento realizado com veneno de *T. strigilis* inoculado em ratos mostrou sintomas neurotóxicos, edema, necrose local e hemorragia local e sistêmica (Lemoine et al., 2004). Dentre os "colubrídeos", o veneno das serpentes do gênero *Philodryas* merecem maior atenção devido ao grande número de casos (Medeiros et al., 2010) e ao risco em potencial, inclusive *P. olfersii* já foi responsável pelo óbito de uma criança (Salomão e Di-Bernardo, 1995).

Destacamos aqui a problemática que pode ocorrer quando o profissional de saúde confunde os sintomas de acidentes com serpentes "não peçonhentas" (Colubridae e Dipsadidae) com os de peçonhentas, principalmente do gênero *Bothrops* (Viperidae). Quando a vítima não consegue identificar a espécie ou levar o exemplar até o centro de saúde mais próximo, o falso diagnóstico pode levar a aplicação de soro antiofídico de forma desnecessária causando danos ainda maiores à vítima (Bernarde, 2014; Silveria e Nishioka, 1992). É perceptível a necessidade da padronização de protocolos de atendimento para diagnóstico correto e aplicação adequada do tratamento em vítimas de serpentes "não peçonhentas", ou seja, áglifas e opistóglifas. Os relatos mostram que os sintomas desaparecem em poucos dias com o uso de analgésicos e anti-inflamatórios, sendo que em alguns casos não há necessidade de medicamento, apenas aplicação de gelo para redução do edema e observação. Após o acidente é aconselhável que a vítima se acalme e lave o local com água e sabão ou algum antisséptico, pois a boca das serpentes possui uma microbiota que pode causar infecções secundárias (Silva et al., 2016; Puorto e França, 2003). Nenhum medicamento ou substância deve ser ingerida sem consulta de um profissional médico, o paciente deve apenas manter-se hidratado. Quando a vítima não consegue levar com segurança o animal até o centro de saúde, o máximo de características devem ser observadas para o diagnóstico correto (veja FUNASA 2001; Bernarde, 2014). Nosso relato de caso reforça a precaução que se deve ter ao manusear serpentes da família Colubridae e Dipsadidae, inclusive pesquisador que estejam acostumados. Aqui mostramos que *Thamnodynastes hypoconia* é uma serpente considerada "não peçonhenta", mas que merece precaução ao manuseio pois as toxinas presentes no seu veneno são de interesse médico, devido aos sintomas de envenenamento aqui registrados. Serpentes classificadas como "não peçonhentas" devem ser revisadas e não serem tratadas de forma negligente, pois esses relatos mostram que essas serpentes também representam um problema de saúde pública.

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CAPÍTULO V

Considerações Finais

As principais conclusões obtidas a partir desse trabalho de tese avaliando as relações tróficas da herpetofauna em banhados subtropicais do sul brasileiro, de acordo com cada capítulo, foram:

Capítulo II – Nesse capítulo avaliamos a variação intrapopulacional no uso dos recursos alimentares em juvenis e adultos de *Thamnodynastes hypoconia*. Ambas idades ocupam o quarto nível trófico na teia alimentar onde ocorrem, padrão mantido ao analisar diferentes tecidos (sangue vs. escama) e diferentes anos (2016 vs. 2017). Os modelos de mistura estimaram uma maior contribuição. Esse resultado corroborou as estimativas dos modelos de mistura, os quais estimaram os anfíbios da família Hylidae como as presas mais importantes no primeiro ano, e Leptodactylidae no segundo ano. Adultos relevaram nicho isotópico maior do que os juvenis ao analisar o sangue, na menor escala temporal da dieta, em ambos os anos. O padrão contrário foi revelado em ambos os anos quando foi usado escama, ou seja, a dieta em uma maior escala temporal. Houve sobreposição de nicho isotópico entre as duas faixas etárias ao analisar as duas escalas temporais, porém no primeiro ano a sobreposição foi menor. De modo geral, juvenis e adultos parecem utilizar os mesmos recursos alimentares na área estudada, porém pequenas oscilações foram percebidas ao analisar os dois tecidos diferentes, sangue e escama, mostrando a importância de levar essa questão em consideração em trabalhos que utilizem a análise de isótopos estáveis.

Capítulo III – Avaliamos nesse capítulo as relações tróficas entre seis espécies de anuros coexistentes. Os nichos isotópicos mostraram que essas espécies devam utilizar diferentes estratégias de forrageamento. Como no caso de *Rhinella dorbignyi*, o único especialista, que apresentou nicho segregado das demais espécies e maior nível na teia trófica. Além de formigas serem as presas com maiores estimativas de contribuição para *R. dorbignyi*, parece que essa espécie também faz uso de outras presas o que acaba por aumentar sua área de nicho. Para as demais espécies, as quais são generalistas, invertebrados herbívoros, Araneae, Coleoptera e Diptera foram as presas com maiores estimativas de contribuição energética. Dentre os dois anuros generalistas de médio a grande porte corporal, apenas *Boana pulchella* revelou um grande nicho isotópico, contrariamente de *Leptodactylus latrans*. Assim como *Boana pulchella* apresentou o maior nicho, também revelou uma alta sobreposição com as demais espécies. As duas pererecas arborícolas de pequeno porte, *Dendropsophus sanborni* e *Scinax squalirostris*, as quais pertencem à mesma família e costumam usar o mesmo micro-habitat, além de apresentarem nichos de tamanho pequeno, também revelaram partição de nicho isotópico entre elas. Houve sobreposição com espécies mais distintas morfo e ecologicamente. Por outro lado, a pequena rã terrícola, *Pseudopaludicola falcipes*, a qual apresentou alta sobreposição com a maioria das espécies, apesar de possuir um nicho isotópico estreito, esse ocupou uma gama maior no eixo do nitrogênio, indicando que essa rã consome espécies de diferentes níveis tróficos. Essa pode ser uma estratégia de expansão de nicho trófico para evitar a competição. De modo geral, as espécies generalistas mostraram maior similaridade com as fontes primárias da cadeia C_3 e contrariamente, *R. dorbignyi* parece estar mais ligado as plantas da cadeia C_4.

Por último, enfatizamos que os banhados do sul brasileiro são ecossistemas afetados por atividades antrópicas, principalmente a conversão de áreas para a implementação agropecuária e plantação de espécies exóticas como o *Eucalyptus*. Essas mudanças interferem diretamente na concentração natural de elementos como carbono, visto que muda a cobertura vegetal, e também no nitrogênio, devido ao uso de adubação artificial e as excretas dos animais criados. Essas mudanças podem refletir ao longo da cadeia trófica, podendo desestabilizar todo o ecossistema. Dessa forma, entender as relações tróficas de anfíbios e repteis em área de banhado é extremamente fundamental para compreender a dinâmica do ecossistema como um todo.