Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais

Variações espaciais e temporais na estrutura trófica e uso de recursos alimentares de anuros em ambientes costeiros subtropicais

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Orientador: Alexandre Miranda Garcia

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Dedico aos meus pais, Ondina Coi Huckembeck e Darcy Huckembeck
Pelo apoio e esforço para que eu realizasse meus sonhos

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RESUMO

Estudos abordando a ecologia trófica de anfíbios são de extrema importância para o entendimento da dinâmica dos sistemas ecológicos, pois estes desempenham um papel importante no fluxo de energia entre habitats. Dentre os tópicos ainda pouco compreendidos sobre a ecologia trófica de anfíbios estão a partição de recursos alimentares entre espécies e as diferenças na estrutura trófica de assembléias de anuros em ambientes distintos. O presente trabalho investiga essas questões a partir da análise da ecologia trófica dos anuros em dois habitats costeiros da região subtropical do Brasil. Para isso foram utilizados os métodos de análise de conteúdo estomacal e análise de isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$), em conjunto com informações sobre a disponibilidade de presas, uso de micro-habitats e medidas morfológicas (SVL, comprimento total do corpo e MW, largura da boca). No primeiro capítulo foram testadas duas hipóteses: (1) o anuro com maior SVL e MW, e com capacidade de usar de maneira mais ampla os micro-habitats teria uma dieta mais diversificada e (2) a diversidade da dieta de *Pseudis minuta* e *Scinax squalirostris* seria maior durante um período quente/seco, comparado a um período frio/chuvoso. Os resultados mostraram que os dois hilídeos não utilizaram os mesmos recursos alimentares devido às suas diferenças morfológicas e no uso de microhabitats. A segunda hipótese não foi corroborada pelos resultados, pois a sazonalidade não influenciou a estratégia de alimentação dos dois anuros. No segundo capítulo, a hipótese testada foi que as características contrastantes de ambientes de banhado e dunas, especialmente em relação a produtividade primária, resultaria em diferenças na estrutura trófica e no uso de recursos alimentares pelas assembleias de anuros. Os resultados mostraram que há uma tendência de maior diversidade na estrutura basal (i.e. número de fontes autotróficas contribuindo com nutrientes aos anuros) e vertical (i.e segregação de nichos tróficos ao longo da cadeia alimentar) nas dunas do que no banhado. Além disso, a hipótese sobre maior particionamento de nicho no ambiente menos produtivo (dunas) foi corroborada pela menor sobreposição de nichos tróficos entre os anuros nessa região.

Palavras-chave: anfíbios, dieta, isótopos estáveis, nicho trófico, banhados, dunas costeiras

ABSTRACT

Studies on the trophic ecology of amphibians are crucial for understanding the dynamics of ecological systems, because they play a crucial role in the energy flow between habitats. Among the topics still poorly understood on the trophic ecology of amphibians are the partitioning of food resources between species and the differences in trophic structure the anuran assemblages in distinct habitats. This work investigates these topics based on the analyzes of the trophic ecology of anurans in two coastal habitats of the subtropical region of Brazil. In order to achieve that, stomach content analysis and stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were used in conjunction with information on prey availability, use of microhabitats and morphological measures (SVL, snout-vent length and MW, mouth width). In the first chapter two hypotheses were tested: (1) the anuran with larger SVL and MW, and with the capacity to use the micro-habitats more widely, would have the diet more diversified, and (2) the dietary diversity of *Pseudis minuta* and *Scinax squalirostris* would be higher during the warm/dry period, when compared to a cold/rainy. The results showed that two hylids did not use the same resources due to differences in their morphology and microhabitats use. The second hypothesis was not corroborated by the results because the seasonality did not influence the feeding strategy of the two anurans. In the second chapter, the hypothesis was that contrasting features of wetland and sand dunes habitats, especially in terms of primary productivity, would result in differences in trophic structure and in the use of food resources by anurans assemblages. The results showed a tendency of higher diversity in basal (i.e. autotrophic sources contributing with nutrients for the anurans) and vertical (i.e. food niche segregation along the food chain) in the sand dunes than in wetland. In addition, the hypothesis on higher food niche partitioning in the less productive habitat (sand dunes) was corroborated by the lower overlap among trophic niches of anurans in this region.

Key-words: amphibians, diet, stable isotopes, trophic niche, wetlands, sand dunes

APRESENTAÇÃO

A presente tese é composta por:

1. Introdução geral, com citações e referências bibliográficas, na qual foram expostas: (a) as principais teorias em que se baseiam as hipóteses da tese, (b) uma caracterização dos ambientes estudados, (c) a importância ecológica do modelo biológico utilizado na pesquisa e (d) um breve histórico das principais técnicas utilizadas em estudos sobre ecologia trófica. A parte introdutória da tese está formatada segundo as regras da Associação Brasileira de Normas Técnicas (ABNT).
2. O corpo central é composto por dois capítulos. O primeiro capítulo intitulado "Trophic Ecology of Two Sympatric Frogs with Contrasting Morphology and Habitat Use in a Subtropical Wetland" (HUCKEMBECK, Sônia et al., 2018), o qual foi publicado na revista "Herpetologica" (Fator de impacto 1.333; Qualis B1). Esse capitulo foi formatado segundo as normas da referida revista (vide normas de formatação em: http://www.hljournals.org/userimages/ContentEditor/1490383789680/HLInstructionsforAuthors.pdf. O segundo capítulo intitulado "Trophic structure of anuran assemblages in two coastal habitats: comparison of patterns from dietary and stable isotope analyses" foi submetido para a revista "Wetlands" (Fator de impacto 1.811; Qualis B1) e está formatada segundo as normas da revista (vide normas em: https://www.springer.com/life+sciences/ecology/journal/13157 e.
3. Considerações finais e perspectivas. Esse tópico da tese está formatado segundo as regras da Associação Brasileira de Normas Técnicas (ABNT).

SUMÁRIO

LISTA DE FIGURAS
CAPÍTULO 1

CAPÍTULO 2

LISTA DE TABELAS
CAPÍTULO 1

CAPÍTULO 2

INTRODUÇÃO GERAL

Estrutura trófica e a amplitude de nicho trófico

Em uma comunidade ecológica, a representação das relações alimentares entre predadores e presas é conhecida como teia alimentar (GIACOMINI; PETRERE, 2010). É através dessas interações que ao longo de uma teia trófica ocorre a transferência de energia dos produtores primários para os consumidores (ELTON, 1927). Estudos sobre a estrutura da teia alimentar são cruciais para entender como as interações das espécies podem influenciar no funcionamento dos ecossistemas (WINEMILLER; POLIS, 1996). Além disso, outros fatores bióticos (por exemplo, a disponibilidade de recursos) e abióticos (inundações, temperatura, perturbação do vento) também podem influenciar a estrutura das teias alimentares, a qual muda em resposta as novas condições ambientais (VANDER ZANDEN et al., 2000; SCHRIEVER et al., 2013).

A base de uma cadeia trófica é representada pelos produtores primários, dos quais a sua diversidade pode influenciar a produtividade e a estabilidade de um ecossistema (POST et al., 2002). A relação entre a produtividade e a diversidade tem sido estudada ao longo de décadas, apresentando respostas diferentes diante da quantidade de nutrientes disponíveis nos ambientes (ISBELLL et al., 2013; TILMAN et al., 2014). A maior diversidade de produtores primários, mesmo em ambientes com pouco nutrientes, resulta em uma maior produção primária (TILMAN, 1988). Alguns anos depois a hipótese da diversidade-produtividade foi proposta por MCNAUGHTON (1993), a qual sugere que comunidades de produtores primários mais diversas apresentam diferenças quanto ao uso dos recursos abióticos (por exemplo, os nutrientes), fazendo com que aumentem as taxas de produtividade líquida. A estrutura da base da cadeia trófica também influencia na estabilidade das comunidades biológicas. Os ambientes que apresentam uma alta diversidade de produtores primários são considerados mais resistentes a perturbações e se recuperam com maior eficiência (PIMM, 1984; TILMAN et al., 2014).

Os estudos sobre a estrutura de uma cadeia trófica não são limitados apenas a composição de sua base (estrutura horizontal), pois também abordam questões relacionadas com a sua estruturação vertical, a qual corresponde ao comprimento da cadeia trófica (GIACOMINI; PETRERE, 2010). O comprimento de uma cadeia trófica é mensurado pelo número de ligações que existe entre uma espécie basal e o predador de topo (WILLIAMS; MARTINEZ, 2004). Quanto ao comprimento das cadeias tróficas existe uma generalização, a qual sugere que as cadeias tróficas tendem a serem curtas (MCCANN, 2000). Isso porque segundo a hipótese energética (LINDEMAN, 1942), o comprimento de uma cadeia trófica é limitado pela ineficiência da transferência de energia ao longo da cadeia. Dessa forma, cadeias tróficas mais longas estão presentes em ambientes com maior produtividade primária (MAZUMDER et al., 2017). O comprimento da cadeia trófica também pode estar relacionado com a estabilidade do ambiente. Segundo a hipótese de estabilidade, as cadeias tróficas longas não são estáveis, pois quando ocorre flutuações na base da cadeia trófica, essas perturbações podem atingir os níveis tróficos mais altos, podendo extinguir predadores de topo (PIMM; LAWTON, 1977). Porém, apesar de vários estudos terem sido realizados abordando a influência da estabilidade dos ecossistemas sobre o comprimento da cadeia trófica, há resultados divergentes sobre essa questão (MCCANN, 2000). Além disso, estudos têm apontado que o tamanho do ambiente influencia no comprimento da cadeia trófica, sendo que ambientes com maior área proporcionam condições para uma estrutura vertical mais complexa (SCHOENER, 1989, TAKIMOTO et al., 2013).

Focando nas relações interespecíficas de uma cadeia trófica, ELTON (1927) foi um dos pioneiros em usar o termo nicho trófico para descrever o papel funcional dos organismos em um ambiente, o qual definiu como a soma de todas as interações que ligam uma espécie a outra em um ecossistema. Um dos aspectos estudados sobre nicho trófico é a sua amplitude, a qual pode variar de acordo com a estratégia alimentar das espécies (GIACOMINI; PETRERE, 2010). Espécies que utilizam recursos alimentares de maneira restrita (estratégia alimentar especialista) apresentam um nicho trófico estreito, enquanto espécies que apresentam uma dieta diversificada (estratégia alimentar generalista) são caracterizadas por apresentarem um nicho trófico amplo (AMUNDSEN et al.,1996).

A amplitude do nicho trófico pode ser influenciada por uma gama de fatores como, por exemplo, a disponibilidade de presas e a competição entre as espécies (SCHRIEVER et al., 2013). As mudanças na disponibilidade de recursos podem ser induzidas por variações dos fatores abióticos. Por exemplo, as espécies de macroinvertebrados aquáticos em banhados subtropicais apresentam sua riqueza, composição e densidade influenciadas pelos pulsos de inundação (MORAES et al., 2014). Similarmente, em áreas com pouca disponibilidade de água e baixa produtividade (regiões áridas e desertos), as variações ambientais também influenciam nas interações tróficas, ocorrendo maior disponibilidade de presas em períodos com maior precipitação e inundação (PIANKA, 1975; HAGEN; SABO, 2012; HAGEN; SABO, 2014). Consequentemente, a dieta de espécies como, por exemplo, de anfíbios (dieta composta principalmente por invertebrados), podem sofrer alterações em sua composição (HUCKEMBECK et al., 2014). Essas mudanças na composição da dieta podem interferir nas relações entre espécies com nichos tróficos semelhantes. Segundo a teoria do particionamento de nicho, a coexistência estável de espécies competidoras é atingida através da diferenciação do nicho que reduz a sobreposição entre as espécies (PIANKA, 1974). Assim, quando há uma escassez de recursos (disponibilidade de presas), as espécies competidoras restringem suas dietas para reduzir a sobreposição do nicho trófico.

Além disso, a amplitude do nicho trófico pode sofrer variações devido ao uso do micro-habitat (MANEYRO; ROSA, 2004) porque a estruturação de um habitat pode alterar o sucesso de forrageamento das espécies devido as mudanças, por exemplo, na acessibilidade as presas (HOWARD et al., 2003). A utilização de um micro-habitat também está relacionada com as características morfológicas das espécies, que podem ampliar a utilização de diferentes micro-habitats. Segundo HUCKEMBECK et al. (2012), diferente de outras espécies de hilídeos, *Pseudis minuta* possui membranas interdigitais bem desenvolvidas nos membros posteriores e olhos posicionados dorso-lateralmente. Essas características permitem que a espécie realize o forrageio tanto nos ambientes aquáticos, quanto nas áreas adjacentes aos corpos d’água. Outro exemplo é a correlação existente entre a morfologia de peixes e o tipo de micro-habitat utilizado pelos mesmos, os quais quando são bentônicos com maior mobilidade apresentam corpo arredondado e comprimido, nadadeiras peitorais alongadas e caudal bifurcada, olhos laterais e boca sub-terminal (MOTTA et al, 1995).

Algumas características morfológicas, como o tamanho do corpo e largura da boca do predador, também podem influenciar na capacidade de ingestão de presas (HIRAI, 2002; MANEYRO et al., 2004; COSTA-PEREIRA et al., 2018). Geralmente, espécies ou indivíduos com tamanho corpóreo maior são capazes de explorar mais amplamente presas de diferentes tamanhos (MUÑOZ-GUERRERO et al., 2007), além de poder apresentar uma posição trófica mais elevada (HUCKEMBECK et al., 2014). Por exemplo, espécies que passam por um processo de metamorfose, como algumas espécies de anuros, podem explorar recursos diferentes durante o processo ontogenético. Nesses casos, ao longo do seu desenvolvimento, alguns anuros podem exercer tantas funções ecológicas de consumidor primário (girinos), quanto de consumidor terciário após a metamorfose (HOCKING; BABBITT, 2014). O contrário, com relação as funções ecológicas, pode ser observada para algumas espécies de Diptera, os quais em sua fase larval aquática são predadores de outros invertebrados e após a metamorfose, se alimentam de néctar (PINDER, 1986).

Os ambientes costeiros e as espécies de anuros

A planície costeira do Rio Grande do Sul (RS) é formada por várias lagoas, estuários, áreas alagadas (banhados) e dunas, as quais apresentam grande importância ecológica, pois abrigam inúmeras espécies ameaçadas, migratórias e de valor econômico (SILVEIRA-PEREIRA; POERSCHKE, 2010; CORRÊA et al., 2013; TAGLIANI, 2018). Dentre os ambientes continentais, os banhados e as dunas estão entre os ambientes com características mais contrastantes em vários aspectos. Os banhados são considerados como áreas de transição entre os ambientes aquáticos e terrestres, com vegetação predominantemente aquática (~ 176 espécies, ROLON et al., 2011), que podem apresentar pulsos de inundação, os quais promovem o fluxo de matéria orgânica entre esses ambientes (JUNK et al., 1989). Além disso, são reconhecidos por apresentarem uma alta produção primária, a qual sustenta uma grande diversidade de espécies (por exemplo, 23 ordens de macroinvertebrados registrados por HUCKEMBECK et al., 2014) e que subsidia ambientes adjacentes (MITSCH; GOSSELINK, 2000). Por exemplo, a produção primária média de macrófitas emergentes em áreas úmidas no sul do Brasil varia por ano de 2.438 a 3.599 g/m^{-2} (FERREIRA et al., 2009). Diferentemente, as dunas costeiras que se localizam paralelamente aos banhados da planície costeira, apresentam uma taxa de produtividade baixa de 0,49 a 22,85 g/m^{-2} (SEELIGER et al., 2000). Por apresentarem uma cobertura vegetal esparsa, a qual é representada por cerca de 71 espécies (SEELIGER et al., 1998), as dunas costeiras são suscetíveis a ação dos ventos, a qual contribui para a constante mudança do seu substrato (CALLIARI et al., 2005). Além disso, esse habitat é submetido a uma variação térmica diária. Quanto as condições hídricas, em partes mais baixas eventualmente há formação de corpos d'água efêmeros e o substrato apresenta uma capacidade alta de drenagem (CALLIARI; KLEIN, 1993).

Devido ao grande aporte de recursos hídricos e vastas áreas de campos, há o desenvolvimento de inúmeras atividades antrópicas ao longo da planície costeira do RS, como monoculturas de espécies exóticas e pecuária (TAGLIANI, 2018). Portanto, com o intuito de preservar parte dessa área costeira do crescente impacto humano foi criado em 1986, pelo Governo Federal, o Parque Nacional da Lagoa do Peixe (PNLP). Este parque abrange uma área com grande variedade de hábitats e uma alta biodiversidade, apresentando a ocorrência de espécies ameaçadas de extinção. Por este motivo, o PNLP foi incluído no programa Ramsar (*Wetlands of International Importance*), além de ser considerado uma Reserva da Biosfera pela UNESCO e identificado pela *Birdlife International* como uma importante área de alimentação e descanso para aves migratórias (BUGONI et al., 2005; LOEBMANN; VIEIRA, 2006).

Dentre a fauna ocorrente no PNLP, estão os anfíbios anuros, dos quais foram registradas o total de 13 espécies (LOEBMANN; VIEIRA, 2005). Os anfíbios são considerados bons modelos para estudos de ecologia trófica, pois algumas espécies apresentam um ciclo de vida dependente dos ambientes aquáticos (fase pré-metamorfose) e terrestres (fase pós-metamórfica), representando um papel fundamental no fluxo de energia entre diferentes habitats (KUPFER et al., 2006). Em regiões litorâneas, como a planície costeira do RS, as espécies de anfíbios existentes apresentam uma alta plasticidade ecológica quanto à utilização dos habitats (OLIVEIRA et al., 2013). No presente estudo foram utilizadas seis espécies de anuros abundantes na região:

1) *Boana pulchella* (Duméril & Bibron, 1841) (Anexo 1): essa espécie está distribuída pela região pampiana (RS, Uruguai e Argentina), além do estado do Paraná e sul do Paraguai. Geralmente *B. pulchella* é encontrada ativa durante o período crepuscular e a noite, associada a vegetação nas áreas de banhado e campos alagados. É uma espécie relativamente pequena (2 – 5 cm), com dieta generalista composta principalmente por aracnídeos, dípteros, himenópteros e coleópteros (MANEYRO; ROSA, 2004).
2) *Scinax squalirostris* (Lutz, 1925) (Anexo 2): é uma espécie de distribuição ampla no Brasil (registrada do sul da Bahia até o RS), ocorrendo também em parte da Argentina e Paraguai e em todo o Uruguai. Essa espécie pode ser encontrada em atividade nos períodos diurno e noturno, associada a vegetação de campos e áreas alagadas (OLIVEIRA; ETEROVICK, 2010). É uma espécie pequena (1,9 – 2,5 cm) que apresenta a dieta baseada em artrópodes (KITTEL; SOLÉ, 2015).
3) *Pseudis minuta* Günther 1858 (Anexo 3): essa espécie está distribuída na região pampiana do RS, em todo o território uruguaio e parte da Argentina. Quanto ao uso dos micro-habitats, *P. minuta* é uma espécie predominantemente com hábito aquático, associada as macrófitas aquáticas flutuantes e enraizadas e nas margens dos corpos d'água (HUCKEMBECK et al., 2012). É um anuro generalista, com sua dieta composta por artrópodes (insetos e aracnídeos) (HUCKEMBECK et al., 2014), podendo ocorrer a ingestão de pequenos vertebrados (MANEYRO; CARREIRA, 2012). Em espécimes pós metamórficos, o comprimento rostro - cloacal pode variar entre 2 e 5 cm (MELCHIORS et al., 2004).
4) *Physalaemus biligonigerus* (Cope, 1861) (Anexo 4): essa espécie também apresenta uma distribuição ampla, ocorrendo no sudeste da Bolívia, parte do centro-oeste ao sul do Brasil, Paraguai e partes da Argentina e Uruguai. Geralmente *P. biligonigerus* pode ser encontrada em campos e áreas alagadas, vocalizando no período noturno sobre o

substrato ou entre gramíneas e juncos (CONTE; ROSSA-FERES, 2007). Os espécimes pós metamórficos possuem pequeno porte, variando entre 2,5 e 4 cm de comprimento rostro-cloacal. A alimentação é composta principalmente por presas terrestres como isópteros e himenópteros (ATTADEMO et al., 2007).

5) *Physalaemus gracilis* (Boulenger 1883) (Anexo 5): é uma espécie de ampla distribuição, ocorrendo do sudeste ao sul do Brasil e em partes do Uruguai e Argentina. Apresenta uma grande plasticidade quanto ao uso dos ambientes, sendo encontrada vocalizando durante os períodos crepuscular e noturno em áreas de campos, banhados e áreas urbanas (CONTE; ROSSA-FERES, 2007). É uma espécie pequena (3 – 3,5 cm), com os itens alimentares mais importantes representados por himenópteros e coleópteros (OLIVEIRA et al., 2015).
6) *Leptodactylus latrans* (Steffen, 1815) (Anexo 6): é uma espécie com ampla distribuição, ocorrendo da Venezuela até o sul da Argentina. Com relação a utilização de habitats, *L. latrans* se destaca pela amplitude no uso dos habitats, pois pode viver em áreas de campos, banhados plantações e áreas urbanas (CONTE; ROSSA-FERES, 2007). Os indivíduos pós metamórficos apresentam comprimento rostro-cloacal variando entre 9 e 14 cm. A composição da dieta de *L. latrans* é diversificada, sendo composta por artrópodos e vertebrados (peixes, anfíbios e pequenos mamíferos) (MANEYRO et al., 2004).

Métodos para o estudo de ecologia trófica: análise do conteúdo estomacal e isótopos estáveis

Ao longo de décadas a análise do conteúdo estomacal (ACE) tem sido utilizada como ferramenta para o estudo de ecologia trófica das espécies (HYSLOP, 1980). Através da identificação e quantificação do conteúdo estomacal pode se determinar a composição da dieta, a importância dos itens alimentares, a amplitude do nicho trófico, bem como caracterizar a estrutura de uma teia alimentar (MOSER et al., 2017; OLIVEIRA et al., 2018). Contudo, este método apresenta limitações, pois em alguns casos o conteúdo estomacal encontra-se em um grau elevado de digestão o que dificulta a identificação do recurso alimentar (SOLÉ et al., 2005). Outra questão é a impossibilidade de determinar o valor nutricional dos itens alimentares, pois algumas partes do conteúdo estomacal podem ser material refratário, o qual não é assimilado pelo consumidor (WINEMILLER et al., 2007).

Nas últimas décadas, a análise de isótopos estáveis (AIE) vem sendo utilizada de modo crescente como ferramenta nos estudos de ecologia trófica (PETERSON; FRY, 1987; LAYMAN et al., 2012). Isótopos estáveis são átomos de um mesmo elemento que possuem diferente número de nêutrons, mas que ao contrário dos isótopos radioativos, não emitem radiação e podem ser manipulados sem perigo (FRY, 2006). Através da análise dos isótopos estáveis é possível determinar qual material foi realmente assimilado nos tecidos do consumidor (POST, 2002). Porém, essa técnica também apresenta desvantagens, representada pela limitação em relação à determinação taxonômicas específicas dos itens (PETERSON; FRY, 1987; LAYMAN et al., 2012).

Em estudos ecológicos os isótopos estáveis mais empregados são do elemento carbono (^{12}C, ^{13}C) e nitrogênio (^{14}N, ^{15}N), pois são abundantes no ambiente físico e representam elementos fundamentais na composição da estrutura dos tecidos vivos. Assim, a abundância da forma isotópica mais pesada e mais rara (^{13}C, ^{15}N), em relação à forma isotópica mais leve e mais comum (^{12}C, ^{14}N), pode ser medida nos tecidos dos organismos com grande precisão através de um espectrômetro de massas de razão isotópica. A razão isotópica do nitrogênio (representando pela notação delta: $\delta^{15}N$) sofre um fracionamento (enriquecimento) do isótopo pesado em relação ao leve entre o recurso e o consumidor geralmente de 3 a 4 ‰ (permil) (PETERSON; FRY 1987). Em contrapartida, a razão isotópica do carbono ($\delta^{13}C$) tende a ter um fracionamento isotópico menor (entre 0,5 e 1,0‰) durante o processo de assimilação do alimento pelo consumidor (PETERSON; FRY 1987). Dessa forma, partindo do pressuposto que os consumidores refletem a composição isotópicas dos recursos assimilados, as razões isotópicas de $\delta^{13}C$ e $\delta^{15}N$ fornecem informações sobre as fontes alimentares que sustentam uma determinada cadeia trófica e sobre a posição trófica de um consumidor, respectivamente (PETERSON; FRY,1987). Portanto, dada as limitações inerentes em cada abordagem, a combinação dos métodos de ACE e AIE pode fornecer maior resolução e capacidade de entendimento dos processos envolvidos na ecologia trófica de uma espécie (WINEMILLER et al., 2007).

Análises estatísticas utilizando isótopos estáveis tem sido aplicadas para fazer inferências sobre amplitude de nicho trófico (BEARHOP et al., 2004; JACKSON et al., 2011; CLOYED; EASON, 2017), sobreposição de nichos (SWANSON et al., 2015), posição trófica (QUEZADA et al., 2018) e estimativas da assimilação das fontes alimentares pelos consumidores (PARNELL, 2016). Além dessas análises, um conjunto de métricas baseadas na variabilidade e no posicionamento dos valores de $\delta^{13}C$ e $\delta^{15}N$ no espaço isotópico foram desenvolvidas para caracterizar a estrutura trófica de comunidades biológicas (LAYMAN et al., 2007). Essas métricas têm sido empregadas como indicadores (*proxies*) para inferir a diversidade trófica, por exemplo, pela amplitude do carbono ($\delta^{13}C$ *range*, CR) e do nitrogênio ($\delta^{15}N$ *range*, NR), área total (TA) e distância média do centroide (CD). Em contraste, a redundância trófica (i.e. maior similaridade nos nichos tróficos das espécies) de uma determinada comunidade poderia ser avaliada pela média da distância ao ponto mais próximo (*mean nearest neighbour distance*, MNND) e pelo desvio padrão da distância ao ponto mais próximo (*standard deviation of nearest neighbour distance*, SDNND).

Justificativas

Apesar de existirem inúmeros trabalhos sobre a ecologia trófica de anfíbios (e.g. LE et al., 2018), existem poucas informações em latitudes subtropicais sobre o efeito da sazonalidade e de condições ambientais contrastantes (e.g. encontradas entre banhados e dunas costeiras) sobre a ecologia trófica da assembleia de anfíbios. Além disso, são escassos os estudos que abordam a influência de fatores como restrições morfológicas, uso de micro-habitats e disponibilidade de presas na amplitude do nicho trófico de anuros (MANEYRO; ROSA, 2004; MUÑOZ-GUERRERO et al., 2007; LÓPEZ et al., 2009).

Portanto, o presente trabalho contribui com novas informações sobre fatores que podem influenciar a estruturação trófica de assembleias de anuros em dois tipos de ambientes característicos na planície costeira do sul do Brasil. Além disso, a presente Tese contribui para o avanço do conhecimento sobre funcionamento dos ambientes costeiros, podendo servir de subsídio para tomadas de decisões sobre o manejo e conservação dos banhados e dunas costeiras.

Objetivos e hipóteses

Com base nas informações apresentadas acima, o presente trabalho tem como objetivo geral avaliar a ecologia trófica dos anuros em dois habitats costeiros da região subtropical do Brasil, utilizando como ferramentas as análises de conteúdo estomacal e isótopos estáveis.

No capítulo 1, foi investigada a influência da sazonalidade, de restrições morfológicas e do uso de micro-habitats sobre a dieta e sobreposição do nicho trófico de duas espécies simpátricas de anuros em uma área de banhado. As hipóteses avaliadas foram as seguintes: (i) a espécie com maior tamanho e que usa de maneira mais ampla os micro-habitats tem uma dieta mais diversa e (ii) a composição da dieta dos anuros é mais diversificada durante o período mais quente e seco, quando há maior disponibilidade de presas e as taxas metabólicas dos anuros são mais altas.

No capítulo 2, foi comparada quantitativamente a estrutura trófica das assembleias de anuros numa região de banhado e dunas costeiras, bem como o particionamento dos nichos tróficos entre as espécies de anuros nesses dois ambientes. As hipóteses avaliadas foram: (i) as condições ambientais contrastantes (e.g. especialmente a maior produtividade no banhado comparado as dunas) estão associadas com diferenças na estrutura trófica das assembleias de anuros em cada habitat. Mais especificamente, o habitat com maior produtividade (banhado) apresentará uma estrutura trófica basal mais diversificada e menor redundância trófica (i.e. espécies com ecologias tróficas semelhantes); (ii) a menor disponibilidade de recursos alimentares nas dunas e os efeitos restritivos da competição interespecífica entre os anuros levarão a uma menor sobreposição de nicho trófico do que no banhado.

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CAPÍTULO 1

Manuscrito publicado na revista *Herpetologica.*

Trophic Ecology of Two Sympatric Frogs with Contrasting Morphology and Habitat Use in a Subtropical Wetland

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RRH: HUCKEMBECK ET AL.—TROPHIC ECOLOGY OF SYMPATRIC HYLIDS

ABSTRACT: Frog diets are influenced by multiple factors, including morphological constraints, habitat use, and seasonal variation in environmental conditions and food availability. This study combined stomach content analysis (SCA), stable isotope analysis (SIA) and estimates of prey availability to investigate the influence of body size and microhabitat use on seasonal variation the trophic ecology of two sympatric hylids (*Pseudis minuta* and *Scinax squalirostris*). We evaluated two hypotheses: (1) the species with larger body and mouth sizes and/or broader use of microhabitats will have greater diet breadth, and (2) regardless of differences in morphological traits and microhabitat use, diet breadth of both species will be greater during the warmer of two periods. *Pseudis minuta* exhibited larger body size and mouth width and revealed broader use of microhabitats (mostly within and near major water bodies), whereas *S. squalirostris* had smaller body size and mouth gape and was found exclusively within or near phytotelmata (plant-held water bodies). SCA revealed that *P. minuta* had a more diverse diet than *S. squalirostris*. Only *P. minuta* showed temporal dietary differences, but these findings did not corroborate our prediction of greater diet diversity during the warmer and drier period when prey densities were higher. The two species had distinct carbon and nitrogen stable isotope ratios, indicating assimilation of different resources, except during the colder wetter season when their isotope spaces overlapped partially. We concluded that the two hylids did not use the same food resources on account of their differences in morphology and microhabitat use, and environmental seasonality did not influence their feeding strategies.

Key words: Anuran; Isotopic space; Prey availability; *Pseudis*; *Scinax*; Stable isotope analysis; Stomach content analysis

GIVEN their diverse life histories, feeding strategies and microhabitats, frogs are useful for studying trophic ecology in wetlands (Hocking and Babbitt 2014). Frogs play different ecological roles throughout their ontogeny, with aquatic tadpoles consuming benthic algae and detritus, with potential to have top-down effects on aquatic primary production (Ranvestel et al. 2004). Most adult frogs use both aquatic and terrestrial habitats and consume invertebrates, and therefore might influence the flow of matter and energy between aquatic and terrestrial compartments of wetland food webs (Huckembeck et al. 2014). Wetlands tend to have high productivity and rapid nutrient cycling that support essential ecosystems services and high biodiversity, including herpetofauna (Zedler 2000). At subtropical and tropical latitudes, wetlands are influenced by rainfall and, to a lesser degree, temperature (Simioni et al. 2017) that directly affect environmental conditions, productivity and community dynamics. During periods of low rainfall, the area of water bodies and flooded zones is reduced and aquatic organism density increases, which might increase predator–prey encounter rates and foraging success of aquatic consumers (Maltchik et al. 2007).

In addition to the influence of these extrinsic environmental factors, intrinsic factors, such as body size, morphology and behavior, affect frog feeding (Lima and Magnusson 1998; Maneyro and Rosa 2004). Morphological constraints on diet can influence microhabitat use and competitive interactions. For example, the presence of well-developed interdigital membranes on the hind feet and eyes positioned dorso-laterally are common features of adult anurans that forage in flooded areas (Huckembeck et al. 2012). Prior studies inferred high dietary similarity between frogs coexisting in the same habitat (Moser et al. 2017), but few studies have evaluated frog dietary overlap in relation to food availability (e.g., Toft 1980; Huckembeck et al. 2014). Even fewer studies have investigated how seasonal changes in frog trophic ecology are associated with morphology and microhabitat use (but see Gutiérrez-Cárdenas et al. 2016; Moser et al. 2017; Ordoñez-Ifarraguerri et al. 2017).

We investigated the trophic ecology of two hylids sympatric in a subtropical wetland to assess relationships of seasonal environmental variation and morphology with dietary diversity and interspecific overlap. Hylidae is the most diverse anuran family (~982 species) with the widest geographic distribution (distributed on all continents, except the poles; Frost 2017). Most treefrogs are arboreal, but some species are aquatic, semi-aquatic or fossorial (Macale et al. 2008). In the southern Neotropical region, *Pseudis minuta* and *Scinax squalirostris* often inhabit wetlands in sympatry, but with contrasting microhabitat use (Huckembeck et al. 2012). *Pseudis minuta* forages at the water–land interface, whereas *S. squalirostris* is more often associated with shrubs and phytotelmata in plants (Huckembeck et al. 2012; Kittel and Solé 2015). Both species are trophic generalists with diets dominated by insects and spiders (Huckembeck et al. 2014; Kittel and Solé 2015). These species differ morphologically (e.g., body size, mouth width) and anatomically (e.g., *P. minuta* has interdigital membranes that should enhance swimming; Macale et al. 2008; Kittel and Solé 2015).

We studied trophic ecology using stomach content analysis (SCA), stable isotope analysis (SIA), and field estimates of prey availability. During recent decades, SIA has become an important tool for investigating trophic ecology (Layman et al. 2012; Phillips et al. 2014), including studies of anurans (Araújo et al. 2007; Trakimas et al. 2011; Carvalho-Rocha et al. 2017). Ratios of heavier and lighter isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) often used to estimate the proportional assimilation of food resources into consumer tissues as well as the consumer's vertical trophic position (Peterson and Fry 1987). Stable isotope data have been used to make inferences about trophic ecology, including niche width (Bearhop et al. 2004; Jackson et al. 2011; Cloyed and Eason 2017) and interspecific niche overlap (Swanson et al. 2015). SIA has become more effective when combined with SCA, which allows a more precise and detailed description of a consumer's diet (Winemiller et al. 2011; Condini et al. 2015).

Using two sympatric hylids (*P. minuta* and *S. squalirostris*), we investigated the following questions: (1) Are there differences in diet composition and food assimilation between coexisting frog species with contrasting morphology and microhabitat use; and (2) do dietary and assimilation patterns in each species change with seasons? We hypothesized that the species with the larger body and mouth sizes and broader use of microhabitats would have a more diverse diet, and that both species would undergo seasonal shifts in diet and food assimilation. More specifically, we hypothesized that, regardless of interspecific differences in morphology and microhabitat use, diets would be more diverse during the warmer and drier period when prey are more available and frog metabolic rates are higher. Aside from adding to the knowledge about the trophic ecology of Neotropical frogs that are relatively understudied, our findings yielded insights about potential mechanisms that facilitate amphibian coexistence.

MATERIALS AND METHODS

Study Area

The study was conducted in a wetland (31.0651°S, 50.5121°W; datum = WGS84) in the northern portion of the Lagoa do Peixe National Park, an area of approximately 1.63 ha (Fig. 1). The wetland contains permanent and intermittent water bodies with a maximum depth of 50 cm. Predominant terrestrial vegetation was grasses of Family Poaceae, diverse shrubs, and herbaceous plants (*Eryngium* spp.) that commonly contain phytotelmata. The dominant aquatic macrophytes were *Salvinia herzogii*, *Azolla filiculoides*, *Eichornia crassipes* and *Cabomba* sp.

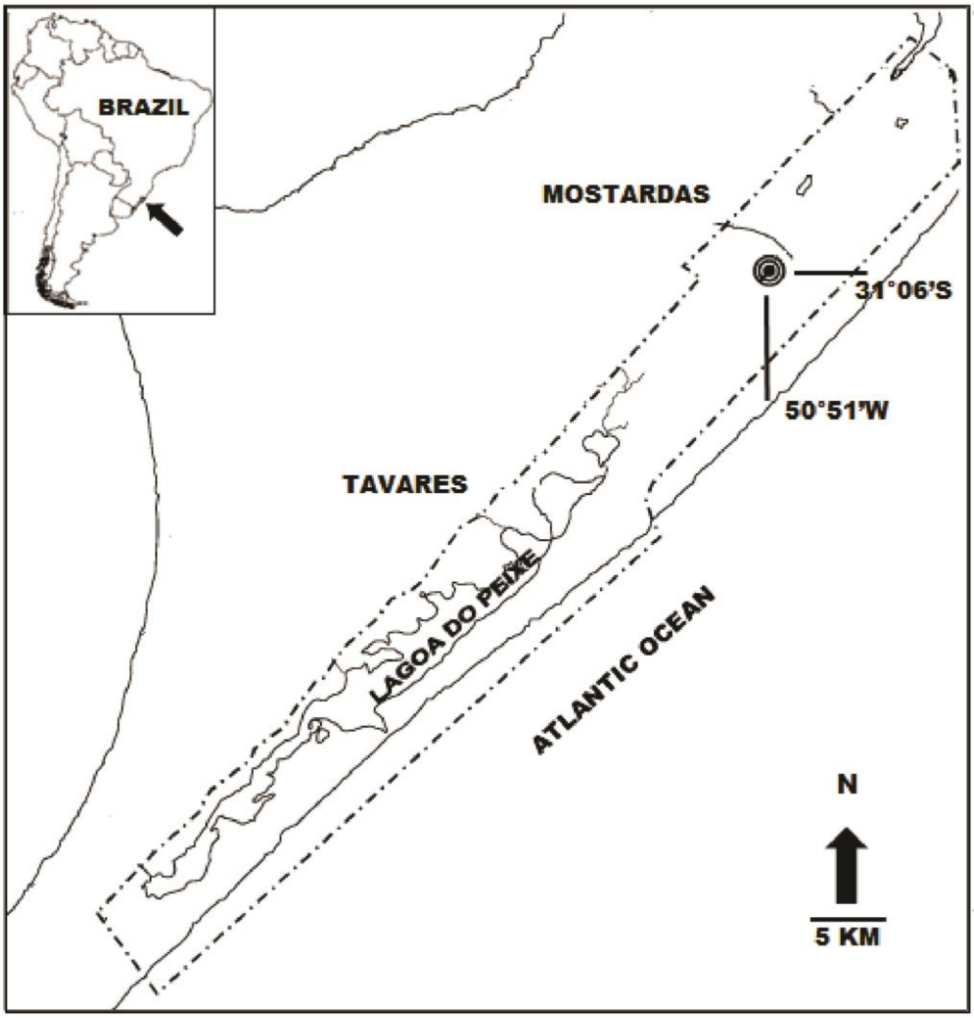

FIG. 1.—The location of the studied wetland (closed circle) in Lagoa do Peixe National Park (area enclosed by dotted line) within the extent of the coastal plain habitat of southern Brazil (indicated by black arrow within inset).

The regional climate is classified as subtropical humid. Precipitation, evapotranspiration and temperature data were obtained from Brazil's National Institute of Meteorology (Inmet 2010). We defined a cold/wet and a warm/dry period based on the mean monthly temperature, rainfall, evapotranspiration and water surplus (net balance rainfall and evapotranspiration; Fig. 2). The cold/wet period during our study period occurred from April–

September, when water temperature ranged from 14.1–19.8°C. The warm/dry period occurred from October–March, when air temperature ranged from 18.3–23.7°C.

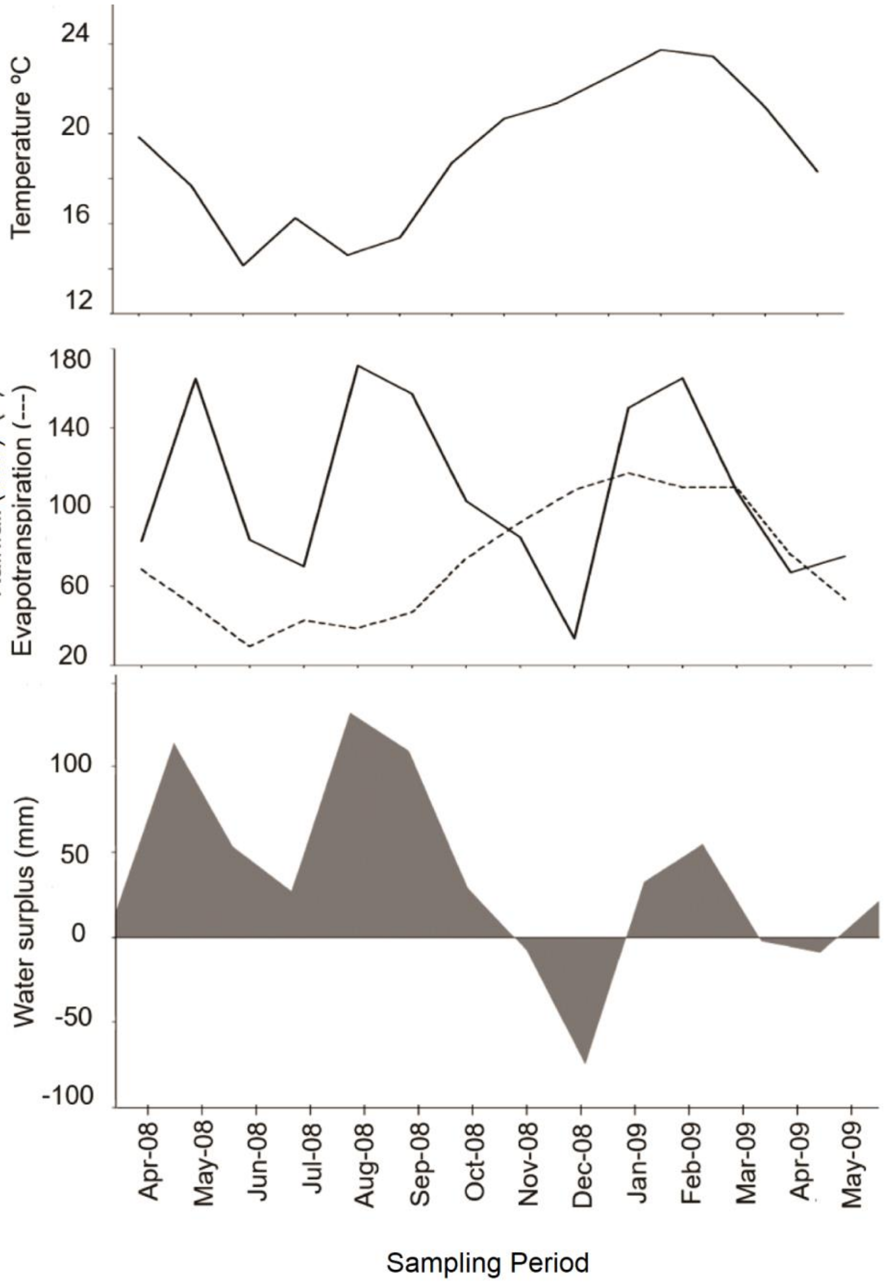

FIG. 2.—Monthly variation of temperature (°C), rainfall (mm^3) and water surplus (mm) during the study period (April 2008–May 2009) at Lagoa do Peixe National Park, Brazil.

Field Work and Data Collection

Field work was conducted monthly (one day per month) between April 2008 and May 2009 to sample frogs (*Pseudis minuta* and *Scinax squalirostris*) and representative species of invertebrates and primary producers. Specimens of *P. minuta* and *S. squalirostris* were collected by hand and euthanized in an ice bath before being transported to the laboratory for examination and processing. Frogs were collected at dusk, when these anurans were most active, and each monthly survey involved 2.5 hr of searching. Aquatic macroinvertebrates and macrophytes (*Salvinia herzogii* and *Eichornia crassipes*) were sampled using a drop sampler, which was a bottomless plastic bucket covering an area of 0.045 m^2. Each month, 18 samples were collected (three samples in three stands per macrophyte species). After collection, aquatic vegetation samples were washed in tap water over a 500-mm mesh sieve that retained associated macroinvertebrates. In addition to aquatic macrophytes, samples of periphyton, particulate organic matter (POM), and leaves from terrestrial plants were collected for estimation of isotopic composition of basal resources. Suspended samples of POM were obtained by filtering water through a pre-combusted (450°C, 4 h) Whatman glass fiber filter (porosity = 1.2 μm) with the aid of a manual vacuum pump. Periphyton was collected by carefully scraping a thin upper layer of flocculent or consolidated sediment from substrates. Samples of terrestrial vegetation (*Kyllinga vaginata* and *Sporobolus virginicus*) were collected by clipping with scissors, and invertebrates associated with terrestrial vegetation (ants, hemipterans, spiders) were sampled using pitfall traps consisting of 500 mL cans buried in the soil and containing 100 mL water (*n* = 10/month). A light trap also was used to collect winged insects (e.g., beetles, mosquitoes, moths). The pitfall traps and light traps were haphazardly distributed in the grassland near the edge of the main water body. All samples (primary producers, invertebrates and anurans) were stored on ice until transported to the lab where they were kept frozen until processing for SIA) and SCA.

Snout–vent length (SVL, ± 1 mm) and mandibular width (MW, ± 0.1 mm) were measured for each frog specimen, and stomachs were removed through an incision in the abdomen. Food items recovered from stomachs were preserved in 70% ethanol and later identified at the lowest feasible taxonomic level given available identification keys and degree of digestion. Partially digested prey (e.g., fragments of appendages, exoskeleton or muscle tissue) were classified as animal remains. Following Huckembeck et al. (2014), we estimated the numerical abundance of each prey category in each stomach and its area when spread in a Petri dish (with thickness ~1 mm and eliminating empty spaces) with the bottom marked in a grid with mm^2 squares.

Each frog specimen was dissected to obtain samples of muscle tissue (< 5 g) from the posterior limb. Because of their small size (< 10 mm), invertebrates could not be dissected to obtain sufficient samples of pure muscle tissue; therefore, invertebrates were processed whole for SIA. Macrophyte leaf samples were rinsed with distilled water and placed in a sterile Petri dish, then dried in an oven at 60°C (for 48 h) before being ground into a fine powder with a mortar and pestle, and placed in Eppendorf tubes for storage. Powdered material was weighed (1 to 3 mg), pressed into ultrapure tin capsules (Costech Analytical Technologies) and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen stable isotope ratios. We compared our samples against the carbon and nitrogen standards of marine sedimentary limestone and atmospheric nitrogen, respectively (Peterson and Fry 1987). On the basis of the SD of internal standard replicate samples, analytical precision was 0.14% and 0.13% for carbon and nitrogen stable isotope ratios, respectively.

Analysis of Microhabitat Use and Morphology

To evaluate habitat use by the two frog species, we followed the characterization of microhabitats performed by Huckembeck et al. (2012), that included the following parameters: vegetation spatial coverage (% grasses, shrubs, phytotelmata plants and aquatic plants); average height of the vegetation (cm); average water depth (cm); substrate type (dry, wet, flooded or underwater); and average distance from a water body (cm). Relative frequencies of occurrence of both species in each microhabitat were evaluated with Chi-square tests (Zar 1994). Differences in SVL and MW between species, and warmer versus colder periods, were assessed by the Mann–Whitney (U) test. Data were examined for normality (Kolmogorov–Smirnov tests), homoscedasticity (*F*-tests) and independence (autocorrelations of residuals; Hammer 2017).

Analysis of Diet and Prey Availability

Food items encountered during SCA were quantified by the prey-specific index of relative importance (%PSIRI) adapted from Brown et al. (2012), according to the formula:

$$\%\text{PSIRI} = \%\ \text{FO}\ (\%\ \text{NP}_i + \%\ \text{AP}_i)\ /\ 2,$$

where % FO is the relative frequency of occurrence of item *i* based on all stomachs; % NP_i is the relative abundance of prey *i* based on its numerical abundance; and % AP_i is the relative abundance of prey *i* based on the its estimated area. Relative abundance values were calculated as the number or area of item *i* divided by the number of stomachs containing item *i*.

Frog diet diversity was calculated by Shannon's Index, $H = -\sum p_i \times (\log p_i)$, where p_i is the proportion (by area) of each prey item found in the diet. Potential differences in H values within species, between seasons, and between species were evaluated by the diversity *t*-test (Hammer 2017). We also calculated interspecific dietary overlap based on Pianka's index:

$$O_{jk} = O_{kj} = [\sum (p_{ij} \times p_{ik})]\ /\ \sqrt{}(\sum (p_{ij}^2 \times p_{ik}^2),$$

where p_{ij} and p_{ik} are the proportions (by area) of food item *i* consumed by species *j* and *k*, respectively. Differences in prey availability (based on prey abundance and diversity) were evaluated by the student's *t*-test and diversity *t*-test (Hammer 2017).

Analysis of Stable Isotope Ratios

In order to verify the assumption that the isotopic composition of consumer muscle tissue was derived from food assimilated during periods when consumers and resources were collected together (Phillips et al. 2014), we only analyzed isotopic composition of frogs sampled during the final three months of both cold/wet and warm/dry periods. To evaluate patterns of isotopic variation within and between species and periods, we constructed biplots of $\delta^{13}C$ vs. $\delta^{15}N$ for frogs, representative prey, and dominant aquatic and terrestrial basal sources. The statistical significance of differences between average values of $\delta^{13}C$ and $\delta^{15}N$ was evaluated with the Mann–Whitney (*U*) test. The relative importance of material assimilated by frogs from various prey and basal food sources and relative vertical trophic positions of the two frog species in the food web were indicated by $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Following Phillips et al. (2014), food sources with similar isotopic values were pooled together to achieve better resolution in the isotopic mixing models used to estimate assimilation of material from sources. Therefore, when the variability (SD) around the average $\delta^{13}C$ values of a prey group (e.g., Aranea) was high (SD > 2.85), the group was divided using a non-hierarchical cluster analysis K-means (Hammer 2017). We attributed this variability to the diversity of species that compose each group. This procedure resulted in most prey taxa being divided into two groups, one relatively depleted (D) and one enriched (E) in ^{13}C (e.g., Araneae-D vs. Araneae-E; Table 1). We used Mann–Whitney (*U*) tests to assess the statistical significance of differences in prey isotopic values between periods. Based on isotopic similarity, basal production sources aggregated into two groups: aquatic producers (aquatic and emergent macrophytes, POM and periphyton) and terrestrial producers (plants with C_4 photosynthesis). Fully terrestrial C_3 plants were uncommon in the riparian zone of the wetland and, therefore, were not included in the sampling.

Relative contributions of food sources to the biomass of the two anuran species, expressed as 95% credibility intervals, were estimated using a Bayesian isotopic mixing model as implemented in the SIAR program (Stable Isotope Analysis in R; Parnell et al. 2010). Based on the $\delta^{13}C$-$\delta^{15}N$ biplots in which we visualized the spatial trends in the isotopic composition of frogs and their stomach contents, we considered the following resources for the mixing model: Araneae depleted, Odonata, Coleoptera depleted, and Coleoptera enriched for *Pseudis minuta*; Araneae depleted, Araneae enriched, and Coleoptera enriched for *Scinax squalirostris*. Mixing models were fit using the Markov Chain Monte Carlo (MCMC) method, which generates simulations of the resources contribution to the anurans. These simulations were generated using a Dirichlet prior distribution (Parnell et al. 2010). Each model was run based on 500,000 iterations, discarding the first 50,000 because they are considered non-informative for guiding simulations. We used the mean values (±1 SD) for trophic discrimination factor (TDF) as determined for post-metamorphic anurans ($\delta^{13}C$: 1.13 ± 0.50 ‰; $\delta^{15}N$: 2.56 ± 0.50 ‰; Cloyed et al. 2015; Appendix).

The isotopic space (i.e., areas occupied in C-N isotopic space; Newsome et al. 2007) of each frog species was plotted as a standardized ellipse area corrected for small samples (SEAc) using the program SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al. 2011). SEAc is unaffected by bias associated with sample size, allowing comparison among groups with distinct sample sizes (Jackson et al. 2011). Overlap between isotopic spaces was calculated between periods and species and reported as a percentage of each SEAc (asymmetrical overlap; Albernaz et al. 2016).

RESULTS

Morphological Traits and Microhabitat Use

Forty-three *P. minuta* specimens (20 from the warm/dry and 23 from the cold/wet period) and 21 *S. squalirostris* specimens (11 from the warm/dry and 10 from the cold/wet period) were collected in the wetland. The values for body size and mouth width were both greater in *P. minuta* (SVL = 29.6 ± 4.9 mm, MW = 10.35 ± 1.60 mm) than in *S. squalirostris* (SVL = 22.2 ± 2.1 mm, MW = 6.40 ± 0.90 mm; SVL: $U = 36$, $z = -5.33$, $P < 0.001$; MW: $U = 7.5$, $z = -3.79$, $P < 0.001$). Values for SVL and MW in *P. minuta* were similar between periods (SVL: $U = 177.50$, $z = -0.83$, $P > 0.40$; MW: $U = 42.50$, $z = -0.32$, $P > 0.74$). The mean SVL of *S. squalirostris* was greater during the warm/dry period (23.0 ± 0.7 mm) than the cold/wet period (20.2 ± 3.1 mm; $U = 72$, $z = -2.44$, $P < 0.01$).

We observed interspecific differences in microhabitat use. Most specimens of *S. squalirostris* were captured on or near plants containing phytotelmata (13 occurrences versus 3.5 expected at random; $\chi^2 = 25.78$, df = 4, $P < 0.00$), with only one specimen captured from aquatic macrophytes (1 occurrence vs. 3.5 expected; $\chi^2 = 1.78$, df = 4, $P < 0.00$). In contrast, all *P. minuta* were captured from aquatic microhabitats, including floating macrophytes and wetted margins of the main water body, and none were observed on plants bearing phytotelmata.

SCA and Prey Availability

Regardless of the study period, the diversity of diet differed between the two frog species (cold/wet: $t = 18.98$, df = 119.61, $P < 0.001$; warm/dry: $t = 13.05$, df = 58.03, $P < 0.001$; Table 1), with *P. minuta* having greater diet diversity (cold/wet: $H = 2.08$; warm/dry: $H = 1.89$) than *S. squalirostris* (cold/wet: $H = 0.26$; warm/dry: $H = 0.34$). Interspecific dietary overlap was low in both periods (cold/wet: $O_{ij} = 0.12$; warm/dry: $O_{ij} = 0.17$).

For both of the frog species, diet diversity did not differ between study periods (*P. minuta*: $t = -1.59$, df = 87.79, $P > 0.11$; *S. squalirostris*: $t = -0.31$, df = 21.19, $P > 0.75$). Proportional consumption of prey categories varied between periods for *P. minuta*: Araneae (%PSIRI = 14.96), Coleoptera (%PSIRI = 9.65) and Hymenoptera (%PSIRI = 8.14) were predominant in the diet during the cold/wet season, whereas Odonata (%PSIRI = 17.19), Hemiptera (%PSIRI = 16.20) and Coleoptera (%PSIRI = 10.88) were more important in the diet during the warm/dry period. In contrast, diet composition of *S. squalirostris* revealed relatively little temporal variation. Hemiptera was the more important prey in the diet of *S. squalirostris* during both seasons (%PSIRI, cold/wet = 22.36; warm/dry = 18.80; Table 1).

Prey abundance in the wetland was greater during the warm/dry period ($t = -1.92$, $P < 0.05$), but prey diversity was higher during the cold/wet period (cold/wet, $H = 2.79$; warm/dry, $H = 2.59$; $t = 4.87$, $P < 0.00$; Fig. 3).

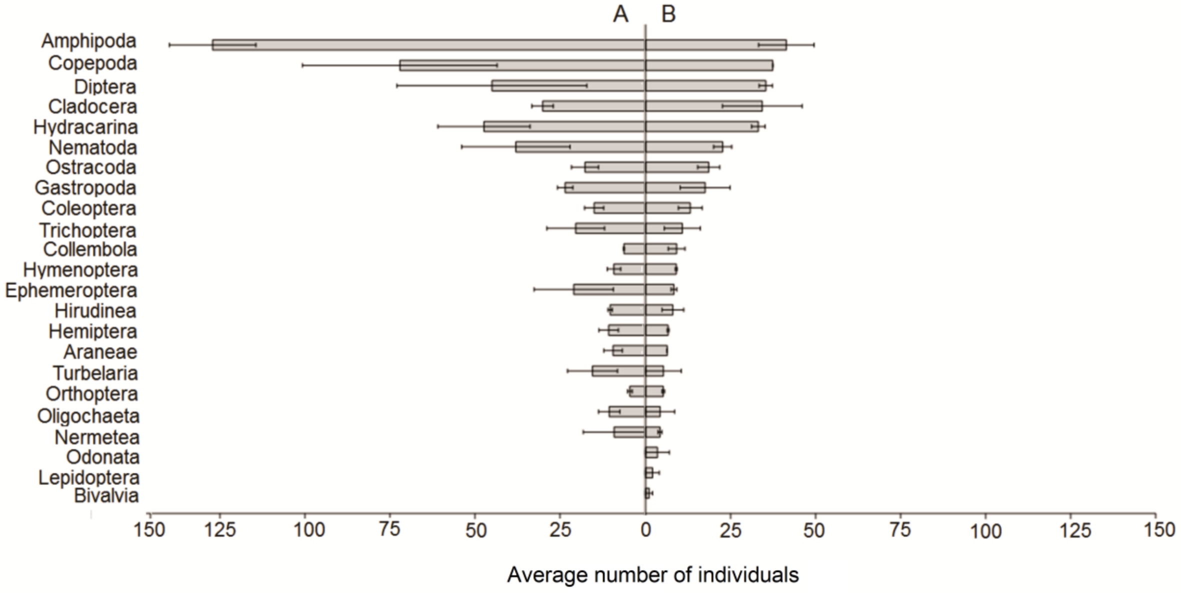

FIG. 3.—Mean (± 1 SD) number of individual invertebrate taxa obtained from surveys conducted during warm/dry (A) and cold/wet (B) periods at the wetland in Lagoa do Peixe National Park, Brazil. Data were square-root transformed.

TABLE 1.—Relative importance of food items, as measured by frequency of occurrence (FO), numerical percentage (%NP) and area percentage (%AP), and prey-specific index of relative importance (%PSIRI) in diets of *Pseudis minuta* and *Scinax squalirostris* during cold and warm periods at the wetland of Lagoa do Peixe National Park. Bold font indicates highest %PSIRI values for each period.

	Pseudis minuta							
	Cold/wet (*n* = 23)				Warm/dry (*n* = 20)			
Food items	FO	%NP	%AP	%PSIRI	FO	%NP	%AP	%PSIRI
Araneae	0.30	8.38	8.24	**14.96**	0.15	6.94	6.21	5.40
Cladocera					0.05	6.94	0.53	1.02
Coleoptera	0.22	6.84	8.16	**9.65**	0.20	8.67	11.17	**10.88**
Diptera	0.09	12.21	6.69	4.86	0.10	10.40	4.70	4.14
Gastropoda	0.04	4.89	0.69	0.72				
Hemiptera	0.13	4.89	3.07	3.07	0.45	10.79	2.34	**16.20**
Hydracarina	0.09	4.89	0.17	1.30	0.10	6.94	0.18	1.95
Hymenoptera	0.17	12.21	3.60	**8.14**	0.20	6.94	2.44	5.14
Isopoda	0.04	4.89	0.52	0.70	0.10	13.87	4.70	5.09
Lepidoptera	0.04	4.89	0.35	0.67				

Odonata	0.04	4.89	4.34	1.19	0.20	6.94	24.43	**17.19**
Orthoptera	0.04	4.89	26.06	3.98	0.05	6.94	26.60	4.60
Ostracoda	0.13	4.89	0.17	1.95				
Trichoptera	0.04	4.89	8.69	1.75				
Others (unidentified)								
Animal remains	0.74	6.61	11.69	40.02	0.45	7.71	4.89	15.53
Plant remains	0.13	4.89	8.86	5.30	0.25	6.94	11.81	12.85
Eggs	0.04	4.89	8.69	1.75				
	Scinax squalirostris							
	Cold/wet ($n = 10$)				Warm/dry ($n = 11$)			
Food items	FO	%NP	%AP	%PSIRI	FO	%NP	%AP	%PSIRI
Diptera	0.25	8.21	0.48	2.54				
Hemiptera	0.17	24.63	71.93	**18.80**	0.20	19.05	71.93	**22.36**
Hydracarina	0.17	12.31	0.72	2.54	0.20	19.05	2.88	5.39
Hymenoptera	0.17	12.31	0.72	2.54	0.40	28.57	2.16	**15.10**

Isopoda	0.92	2.24	0.39	**2.82**				
Others (unidentified)								
Animal remains	0.92	40.30	25.76	70.76	0.80	33.33	24.81	57.16

Isotopic Variability and Food Assimilation

Relative positions of the two frog species and their prey in the $\delta^{13}C$-$\delta^{15}N$ biplot indicated interspecific differences in assimilation of carbon and nitrogen from prey and basal sources (Fig. 4). Mean $\delta^{13}C$ values of *P. minuta* ranged from –26.64 to –22.28 ‰ during the cold/wet period, and from –25.08 to –21.79 ‰ during the warm/dry period (Fig. 4A). These values were similar between periods ($U = 66$, $z = –0.68$, $P > 0.49$; Table 2), and largely reflected isotopic values of important prey that varied between seasons (Fig. 4A). Mean $\delta^{15}N$ values of *P. minuta* differed between study periods ($U = 36$, $z = –2.21$, $P < 0.02$; Fig. 4A), and ranged from 5.66–7.48 ‰ during the cold/wet period and 4.25–7.13 ‰ during the warm/dry period.

Mean $\delta^{13}C$ values of *S. squalirostris* differed between periods ($U = 9$, $z = –2.32$, $P <$ 0.01; Table 2), with values ranging from –23.53 to –19.35 ‰ during the cold/wet period and from –20.84 to –17.28 ‰ during the warm/dry period (Fig. 4B). Mean $\delta^{15}N$ values of *S. squalirostris* differed between periods ($U = 13$, $z = –2.09$, $P < 0.03$; Table 2), with values ranging from 6.16–7.51 ‰ during the cold/wet period and from 3.08–7.24 ‰ during the warm/dry period (Fig. 4B).

Carbon isotope ratios of aquatic producers during both periods (cold/wet = –27.26 ± 2.49 ‰; warm/dry = –29.68 ± 1.91 ‰) were lower than those of terrestrial producers (cold/wet = –13.91 ± 2.40 ‰; warm/dry = –10.52 ± 2.40 ‰; Fig. 4). Average $\delta^{13}C$ and $\delta^{15}N$ values were similar between periods for any insect prey group (Table 2).

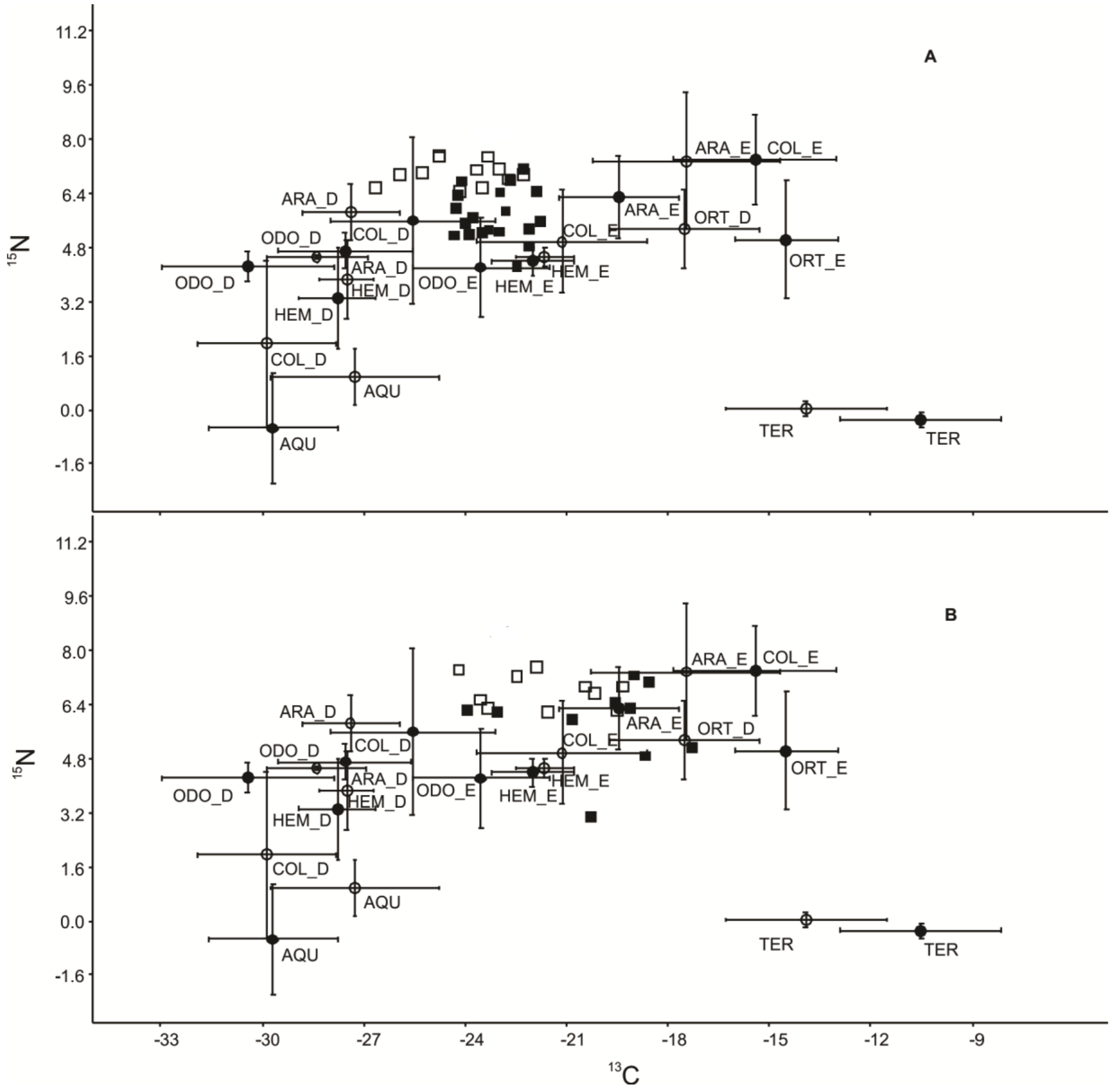

FIG. 4.—Average (± 1 SD) of δ13C and δ15N values of *Pseudis minuta* (PSE), (A) and *Scinax squalirostris* (SCI), (B) during the cold period (gray symbols) and the warm period (black symbols) at the wetland in Lagoa do Peixe National Park, Brazil. Acronyms: ARA-D = Araneae-depleted, ARA-E = Araneae-enriched, COL-D = Coleoptera-depleted, COL-E = Coleoptera enriched, HEM-D = Hemiptera-depleted, HEM-E = Hemiptera-enriched, ODO-D = Odonata-depleted, ODO-E = Odonata-enriched, ORT-D = Orthoptera-depleted, ORT-E = Orthoptera-enriched, AQU = aquatic producers and TER = terrestrial producers.

TABLE 2.—Number of samples (*n*), mean (± 1 SD) values for $\delta^{13}C$ and $\delta^{15}N$, and *P*-values from Mann–Whitney U-tests comparing mean isotopic values of frogs and their prey in the wetland of Lagoa do Peixe National Park.

	$\delta^{13}C$				
	Cold/wet		Warm/dry		
Group	*n*	Mean ± SD	*n*	Mean ± SD	*P*-values
Amphibians					
Pseudis minuta	8	–23.74 ± 1.31	20	–23.22 ± 0.96	0.49
Scinax squalirostris	10	–21.64 ± 1.74	7	–19.25 ± 1.15	0.02
Prey					
Araneae D	4	–27.39 ± 1.44	3	–27.55 ± 1.98	0.86
Araneae E	5	–17.46 ± 2.79	11	–19.46 ± 1.77	0.26
Coleoptera D	2	–29.87 ± 2.05	6	–25.55 ± 2.43	
Coleoptera E	3	–21.13 ± 2.54	3	–15.42 ± 2.42	0.15
Hemiptera D	4	–27.49 ± 0.8	2	–27.76 ± 1.14	
Hemiptera E	5	–21.64 ± 0.88	2	–22.00 ± 1.22	
Odonata D	3	–28.39 ± 1.49			
Odonata E			5	–23.54 ± 2.02	
Orthoptera D	2	–17.52 ± 2.21			
Orthoptera E	6	–14.50 ± 1.53			
Producers					
Aquatic	13	–27.26 ± 2.49	5	–29.68 ± 1.91	0.09
Terrestrial	2	–13.91 ± 2.4	2	–10.52 ± 2.4	

	$\delta^{15}N$				
	Cold/wet		Warm/dry		
Group	*n*	Mean ± SD	*n*	Mean ± SD	*P*-values
Amphibians					
Pseudis minuta	8	6.46 ± 0.72	20	5.67 ± 0.79	0.03
Scinax squalirostris	10	6.79 ± 0.49	7	5.58 ± 1.36	0.04
Prey					
Araneae D	4	5.83 ± 0.84	3	4.7 ± 0.53	0.15
Araneae E	5	7.36 ± 2.03	11	6.28 ± 1.2	0.28
Coleoptera D	2	1.95 ± 2.48	6	5.59 ± 2.48	
Coleoptera E	3	4.97 ± 1.53	3	7.40 ± 1.34	0.39
Hemiptera D	4	3.85 ± 1.16	2	3.30 ± 1.51	
Hemiptera E	5	4.61 ± 0.26	2	4.40 ± 0.42	
Odonata D	3	4.50 ± 0.05			
Odonata E			5	4.21 ± 1.46	
Orthoptera D	2	5.34 ± 1.15			
Orthoptera E	6	5.03 ± 1.75			
Producers					
Aquatic	13	0.97 ± 0.83	5	–0.55 ± 1.64	0.1
Terrestrial	2	0.03 ± 0.23	2	–0.30 ± 0.23	

Proportional assimilation of food resources by frogs was estimated using isotopic mixing models. *Pseudis minuta* had a similar pattern of prey assimilation during the two periods (Fig. 5). Odonata-E was estimated to be the prey assimilated in greatest proportions by *P. minuta* during the cold/wet period (95% Bayesian credibility interval = 12–61%), followed by Coleoptera-E (30–59%) and Araneae-D (0–42%). This species assimilated prey in similar proportions during the warm/dry period. In contrast, *S. squalirostris* assimilated prey in different proportions during the two periods. During the cold/wet period, Coleoptera-E was assimilated in greatest proportions (37–86%), followed by Araneae-D (11–47%) and Araneae-E (0–22%). Coleoptera-E, Araneae-D and Araneae-E made similar contributions to *S. squalirostris* biomass during the warm/dry period (9–58%, 10–56%, and 1–57%, respectively; Fig. 5).

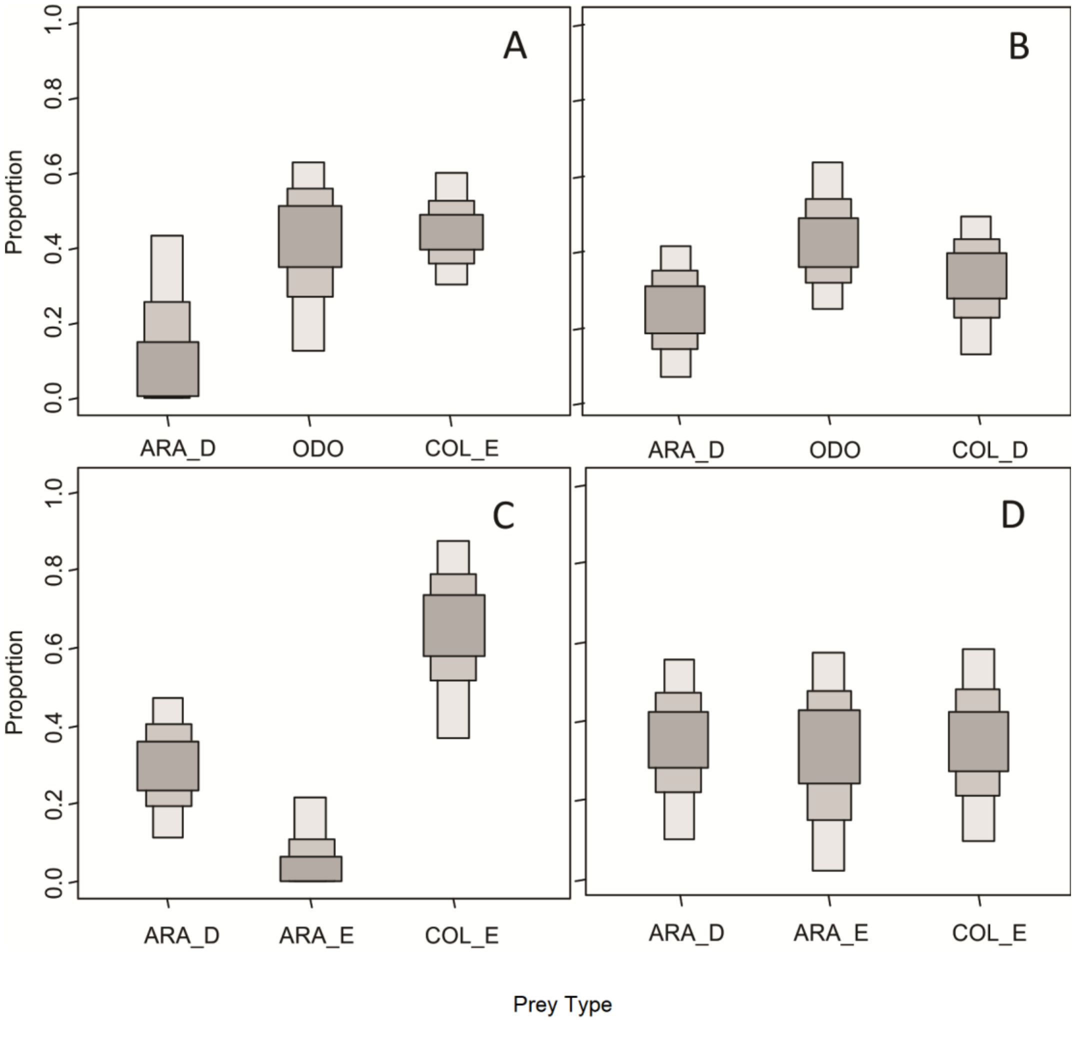

FIG. 5.—The relative contribution of potential prey to biomass of *Pseudis minuta* (A = cold/wet period; B = warm/dry period) and *Scinax squalirostris* (C = cold/wet period; D = warm/dry period) at the wetland in Lagoa do Peixe National Park, Brazil. Bayesian credible intervals of the feasible contributions of each prey category: 50% (dark gray), 75% (medium

gray) and 95% (pale gray). Acronyms: ARA-D = Araneae-depleted, ARA-E = Araneae-enriched, COL-D = Coleoptera-depleted, COL-E = Coleoptera enriched, ODO-D = Odonata-depleted.

Both frog species showed shifts in the relative position and size of their isotopic spaces between survey periods (Fig. 6). *Pseudis minuta* had a larger isotopic space during the cold/wet period (SEAc = 3.41 for cold/wet, and 2.45 for warm/dry), and ellipses of the two periods overlapped in isotopic space (Fig. 6). In contrast, the isotopic space occupied by *S. squalirostris* was greater during the warm/dry period (SEAc = 5.88 for warm/dry, and 2.45 for cold/wet). The two periods had little overlap within isotopic space, with separation mostly along the $\delta^{13}C$ axis, with the cold/wet-period having lower values (Fig. 6). Overlap between isotopic ellipses of the two periods was much lower for *S. squalirostris* (0.008) than for *P. minuta* (0.86). This finding indicated that the resources assimilated by *P. minuta* had similar isotopic composition during both periods, whereas the resources assimilated by *S. squalirostris* varied between periods (Fig. 6). There was interspecific overlap in isotopic spaces of the two anurans during only the cold/wet period (asymmetrical overlap = 17.64% for *P. minuta*, 20.68% for *S. squalirostris*).

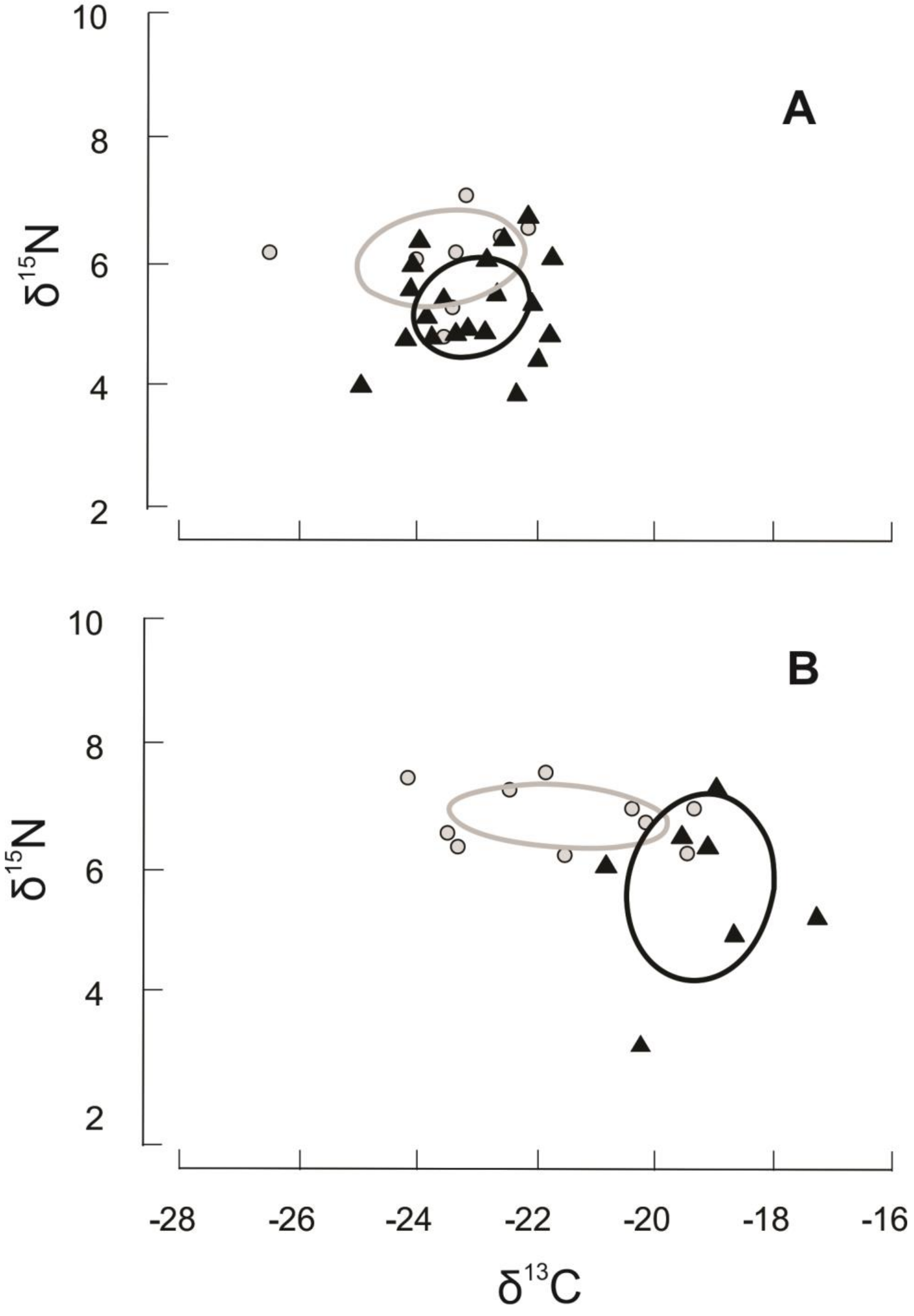

FIG. 6.—Isotopic niche space of *Pseudis minuta* (A) and *Scinax squalirostris* (B) during the cold/wet period (gray circles) and the warm/dry period (black triangles) at the wetland in Lagoa do Peixe National Park, Brazil.

DISCUSSION

Our findings support the hypothesis that among sympatric frog species, that having a larger body and mouth gape, and that uses a greater array of microhabitats, should have a broader trophic niche. Prior studies have suggested that anurans representing several families exhibit a correlation between body size and diet composition, with larger individuals consuming larger prey and having greater volumes of stomach contents (Kittel and Solé 2015). Our findings are consistent with patterns observed for hylid frogs inhabiting a permanent pond in Southern Brazil (Miranda et al. 2006). *Pseudis minuta* is larger, occurred in a wider range of microhabitats and, as predicted, had a more diverse diet than *S. squalirostris*. *Pseudis minuta* often was captured from aquatic microhabitats, including floating macrophytes, but sometimes can be found in terrestrial microhabitats of riparian areas (Huckembeck et al. 2012). In our study system, *P. minuta* was not associated with herbaceous plants bearing phytotelmata. *Scinax squalirostris* had a more restricted distribution and was usually captured from plants growing along the wetland margin, either within, or in close proximity to, phytotelmata. Similar to our findings, an investigation of hylids inhabiting wetlands in Colombia found interspecific differences in microhabitat use and diet that were associated with variation in body size (Muñoz-Guerrero et al. 2007). Other studies have concluded high niche overlap among frogs sharing the same habitat in tropical and subtropical forests (Toft 1980; Wu et al. 2005). In addition to being larger, *Pseudis* has traits that are adaptive in aquatic habitats, such as interdigital membranes on the hind feet and the absence of digital pads that are possessed by most hylids (Huckembeck et al. 2014). *Scinax* lacks interdigital membranes on the hindfeet and possesses digital pads typical of treefrogs.

Contrary to our prediction, the isotopic space occupied by the smaller species, *S. squalirostris*, was larger than that occupied by the larger species. Large isotopic variation among prey inhabiting phytotelmata could explain this pattern. Phytotelmata in subtropical wetlands harbor diverse assemblages of terrestrial and semi-aquatic invertebrates (Campos 2010). Given that invertebrates can exhibit substantial variation in their isotopic values over small spatial scales in systems with high environmental heterogeneity (e.g., Willson et al. 2010), we suggest that large levels of isotopic variation might exist among invertebrates from phytotelmata.

Results from SCA did not provide strong evidence corroborating our hypothesis that diets of both frogs would be more diverse during the warm/dry period. We also disregard the influence of body size on the diet between the periods. *S. squalirostris* was greater in the warm/dry period, but we did not consider this minor difference (< 3 mm) biologically significant. Diets of both species were fairly consistent during the two survey periods. The relative importance of only a few prey categories varied seasonally within the diet of *P. minuta*, and Hemiptera were dominant in the diet of *S. squalirostris* during both periods. In contrast, the diet of a hylid (*Hypsiboas pulchellus*) inhabiting ponds in temperate southern Uruguay varied among microhabitats and seasons (Maneyro and Rosa 2004). Our dietary results should be interpreted with caution, because sample sizes for SCA were small, and some of the material recovered from stomachs was in an advanced state of digestion, especially during the cold/wet period.

Stable isotope analysis did not show differences in proportions of prey assimilated by *P. minuta* during the two periods. In contrast, *S. squalirostris* revealed between-period differences in the proportional assimilation of prey categories. The isotope space occupied by *S. squalirostris* during the warm/dry period was more than double the size of that occupied during the cold/wet period, with higher values of $\delta^{13}C$ during the warmer period. We speculate that this difference in carbon isotopic ratios might reflect assimilation of material from herbivorous insects that feed on plants using the C_4 photosynthetic pathway (e.g., terrestrial grasses) that typically are enriched in ^{13}C. During the warm/dry period, these insects probably become more available to frogs as wetted marginal areas shrink. In Panamanian highland streams, frogs showed a variation in their isotopic values, indicating that sources of prey vary in riparian areas (Whiles et al. 2006). In order to evaluate our hypothesis, we suggest future studies at this site should examine the isotopic variation of more invertebrate taxa, as well as C_4 grasses.

Our prey availability survey revealed higher prey densities during the warm/dry period. Seasonal variation in invertebrate communities has been reported in other studies of subtropical wetlands, with invertebrate densities increasing and species richness decreasing as water levels recede (Moraes et al. 2014). Both adult and larval communities of anurans in tropical latitudes have been found to respond to changes in resources availability (Toft 1980; Whiles et al. 2006; Altig et al. 2007). However, the two hylids in the present study did not exhibit seasonal dietary variation. Both hylids have been described as opportunistic, generalist predators (Huckembeck et al. 2014; Kittel and Solé 2015). Changes in diets of both frog species seemed to be driven largely by changes in the relative proportions of prey types in their respective microhabitats. Given small sample sizes, however, this inference is tentative.

Isotopic spaces occupied by *P. minuta* and *S. squalirostris* overlapped broadly during the cold/wet period when higher rainfall produced a hydrologic pulse that connected aquatic habitats in the wetland (Garcia et al. 2017). Hydrologic connectivity promotes entry of terrestrial invertebrates, vegetation and riparian detritus into aquatic habitats (Rezende and Mazzoni 2005). During floodplain inundation, new growth of floating aquatic vegetation and emergent riparian plants support spiders, ants and other terrestrial invertebrates (Campos 2010). Terrestrial arthropods are probably more vulnerable to predation by hylid frogs under these conditions, resulting in stronger linkages between terrestrial and aquatic food-web compartments. Moreover, higher dispersal of invertebrate prey between phytotelmata housed in emergent plants and floating aquatic vegetation (Zilli and Marchese 2011) could have contributed to higher overlap between isotopic spaces of the two frog species during the cold/wet period.

Aquatic ecosystems in subtropical and tropical latitudes are strongly influenced by unimodal or bimodal annual rainfall (Winemiller 1990; Bunn and Arthington 2002), and amphibian ecology should show corresponding temporal responses (Babbit 2005; Maltchik et al. 2008). Our study revealed an appreciable influence of seasonality on the trophic ecology in only one of the two hylid species. Both of these anurans are trophic generalists that feed opportunistically on insects, but only *P. minuta* underwent a major seasonal diet shift. Microhabitat use and morphological constraints appear to be important factors that influence the trophic ecology of these species. Nonetheless, both species link aquatic and terrestrial compartments of the wetland food web, and therefore can serve as sensitive indicators of environmental impacts to the system.

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APPENDIX

Percent contributions of prey taxa (Bayesian credible interval of 95%) to *Pseudis minuta* and *Scinax squalirostris* muscle tissue during cold and warm periods in the wetland of Lagoa do Peixe National Park based on calculations using different values for the trophic discrimination factor: (1) Cloyed et al. (2015), (2) Post (2002), and (3) Vanderklift and Ponsard (2003). Acronyms: ARA-D = Araneae depleted, ARA-E = Araneae enriched, COL-D = Coleoptera depleted, COL-E = Coleoptera enriched, ODO-D = Odonata depleted, ODO-E = Odonata enriched, PSE = *Pseudis minuta*, SCI = *Scinax squalirostris*.

	Cold/wet					
	PSE			SCI		
TDF	1	2	3	1	2	3
ARA-D	0–42	0–45	0–40	11–47	3–43	4–40
ARA-E				0–22	0–35	0–28
COL-E	30–59	35–65	35–67	37–86	26–94	37–92
ODO-D	12–61	3–53	7–56			
ODO-E						
	Warm/dry					
	PSE			SCI		
TDF	1	2	3	1	2	3
ARA-D	7–42	13–49	1–37	10–56	6–52	6–52
ARA-E				1–57	3–61	2–59
COL-D	13–49	4–38	13–51			
COL-E				9–58	9–58	12–62
ODO-E	25–63	30–64	28–68			

CAPÍTULO 2

Manuscrito em revisãona revista *Wetlands*

Trophic structure of anuran assemblages in two coastal habitats: comparison of patterns from dietary and stable isotope analyses

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Abstract

We evaluated the hypothesis that contrasting characteristics of wetland and sand dunes result in differences in food resource use and trophic structure of anuran assemblages. We used stomach content (SC) and stable isotopes (SI) to compare trophic parameters of both assemblages. The parameters were trophic niche size (Shannon's index), overlap (Pianka's index and SEA_c), and trophic diversity and redundancy (using isotopic metrics like variations of $\delta^{13}C$ (CR), and $\delta^{15}N$ (NR), mean distance to centroid (CD) and standard deviation of nearest neighbour distance (SDNND)). We also used Bayesian isotope mixing models to estimate the relative contribution of primary sources for both assemblages. Isotopic metrics providing insights on vertical trophic length (NR), diversification of basal sources (CR) and trophic diversity (CD) had higher values in the dunes (0.40, 0.27, 0.18) than in the wetland (0.20, 0.19, 0.09). SCA and SIA showed higher segregation of food niches in sand dunes compared with the wetland. Additionally, anurans assimilated a greater number of primary sources (particulate organic matter, C_3 plants, periphyton) in the wetland compared with the dunes (mainly C_3 plants). These findings corroborated the hypothesis that contrasting coastal environments are associated with differences in food resource use and trophic structure of anuran assemblages.

Keywords: amphibians, diet, isotope-based community metrics, niche overlap, Lagoa do Peixe National Park, Neotropics

Introduction

Studies on food web structure are crucial to understand how temporal and spatial environmental changes interfere with species interactions and ecosystem functioning (Winemiller and Polis 1996). Biotic (e.g. productivity, prey availability, interspecific interactions) and abiotic factors (e.g. flood, temperature, wind disturbance) may influence the food web structure, which change in response to new environmental conditions (Vander Zanden et al. 2000; Schriever and Willians 2013). For example, basal food sources can be more diverse in some coastal aquatic environments that are highly productive. In these habitats, diverse primary sources may promote low trophic redundancy among consumers,i.e. lower proportion of species with similar trophic functions (Catry et al. 2016). Ecosystem size and primary productivity may also influence vertical trophic structure (Power et al. 1996; Persson et al. 1996; Post 2002). For example, food chain length is expected to be limited due to the inefficient transfer of productivity between trophic levels. Hence, only large and/or highly productive ecosystems would have sufficient resources to sustain high biomass of consumers at high trophic levels (Takimoto and Post 2013; Sanders et al. 2016). Another important factor shaping trophic structure is prey availability, which play a crucial role on diet composition, food strategy and trophic niche breadth of consumers (e. g. Oliveira et al. 2018). Also, the interplay between low prey availability and high demand for food by consumers interferes in the competition for resources. In this case, the coexistence of competing species may be achieved through differentiation of the trophic niche, which reduces overlap between competing species (MacArthur 1958; Pianka 1974).

A part of the coast in the southern Neotropical region are characterized by extensive wetlands and coastal sand dunes harboring considerable biodiversity, including many endemic and endangered species (Seeliger and Kjerfve 2013). These coastal ecosystems are characterized by contrasting productivity levels and physiographic features (Ferreira et al.

2009; Portz et al. 2016). Wetlands are considered transition areas between aquatic and terrestrial environments (Junk 2002) dominated by diverse aquatic macrophytes (~176 species in subtropical coastal wetlands, Rolon et al. 2011) and characterized by high primary production (Junk et al. 1989; Whigham and Simpson 1992). For instance, the mean primary production of dominant marsh plants in wetlands in southern Brazil range from 2,438 to 3,599 g dry weight m^{-2} $year^{-1}$ (Ferreira et al. 2009). This primary production sustains a great diversity of invertebrates (e.g., 23 orders recorded by Huckembeck et al. 2014). In contrast, sand dunes are transitional areas between marine and terrestrial environments dominated by sparse and less diverse vegetation (~71 species, Seeliger et al. 1998). This habitat is usually characterized by limitations in water supply and nutrients, resulting in comparatively much lower levels of primary productivity (McLachlan 1991). For example, the mean plant productivity in sand dunes in southern Brazil ranges from 0.49 to 22.85 g dry weight m^{-2} $year^{-1}$ and are associated with lower diversity of invertebrates (e.g. 12 orders recorded by,Gianuca (1997) and Seeliger et al. (2000)).

In this coastal system, amphibians have a high ecological plasticity and the same species can occur both in wetlands and dunes (Loebmann and Vieira 2005; Oliveira et al. 2013). Anurans are considered good models for trophic ecology studies because they present diverse life histories and, in many cases, play a crucial role promoting the flow of energy between aquatic and terrestrial habitats (Kupfer et al. 2006). Although there are numerous studies on the trophic ecology of anurans (Brandão et al. 2003; Huckembeck et al. 2014), little information is currently available on the influence of contrasting environmental conditions on diet and food resources partitioning of anuran assemblages in the Neotropical region (e.g. Peltzer et al. 2010).

Studies on the trophic ecology of anurans have been traditionally performed using stomach content analysis (Leivas et al. 2018; Oliveira et al. 2018). However, this technique has some limitations, such as the difficult to correctly identity prey in advanced stages of digestion and the actual assimilation of ingested food (Jepsen and Winemiller 2002). Stable isotope analysis is another technique that had become one of the main tools to reconstruct consumer's diets and unravel trophic links in food webs (Fry 2006; Layman et al. 2012). The most commonly isotopes used in trophic ecology studies are carbon (^{12}C, ^{13}C, expressed as $\delta^{13}C$) and nitrogen (^{14}N, ^{15}N, expressed as $\delta^{15}N$), which can provide information on the primary food sources supporting food chains and consumers' trophic positions (Peterson and Fry 1987). Isotopic metrics based on distribution of isotope values in a two-dimensional $\delta^{13}C$-$\delta^{15}N$ space (Layman et al. 2007) have been used for characterizing food webs (Abrantes et al. 2013; Catry et al. 2016). More recently, isotopic metrics have been improved by Bayesian statistics that take into account uncertainty regarding isotopic composition of food sources and consumers and trophic fractionation to produce estimates with credibility intervals (Jackson et al. 2011; Parnell 2016).

We analyzed data for stomach contents and stable isotopes to compare the trophic structure of anuran assemblages between sand dunes and a wetland in coastal plain in southern Brazil. We hypothesized that contrasting characteristics of these habitats will result in differences in food resources use and trophic structure of anuran assemblages. More specifically, we predicted that (i) the more productive habitat (wetland) will have higher diversity of basal food sources and lower trophic redundancy among anurans, and (ii) lower food resources in the less productive habitat (sand dunes) will be associated with lower niche overlap among anurans.

Material and Methods

Study area and field work

The field work was carried out in the "Lagoa do Peixe" National Park located on state of Rio Grande do Sul, southern Brazil (31°S). Anurans and primary producers were collected monthly from October 2008 to May 2009 in wetland and sand dune habitats. These habitats were separated by a distance of 4.6 km (Fig. 1). The wetland (31°06'53.00" S, 50°51'40" W) was characterized by ephemeral and permanent water bodies with floating and rooted aquatic macrophytes. The terrestrial vegetation adjacent to the water bodies were dominated by grass fields and shrubs sparsely distributed. The sand dune (31°08'28.03" S, 50°49'30.10" W) is characterized by incipient and frontal dunes with height varying from few centimeters up to three meters. In some areas between dunes, there were ephemeral water bodies with maximum depth of 50cm. The terrestrial vegetation adjacent to the ephemeral water bodies was scarce and dominated by *Andropogon arenarius* Hackel., *Senecio crassiflorus* (Poir.) DC, *Polygala cyparissias* A. St.- Hill. Et. Moq., *Androtrichum trigynum* (Spreng.) and *Panicum racemosum* (P. Beauv.) Spreng.. The study area has a subtropical climate with an average annual temperature of 17.5 °C, rainfall ranging from 1.200 to 1.500 mm. year^{-1}, and warmer and colder periods from January to February and from June to July, respectively (Seeliger et al. 1998).

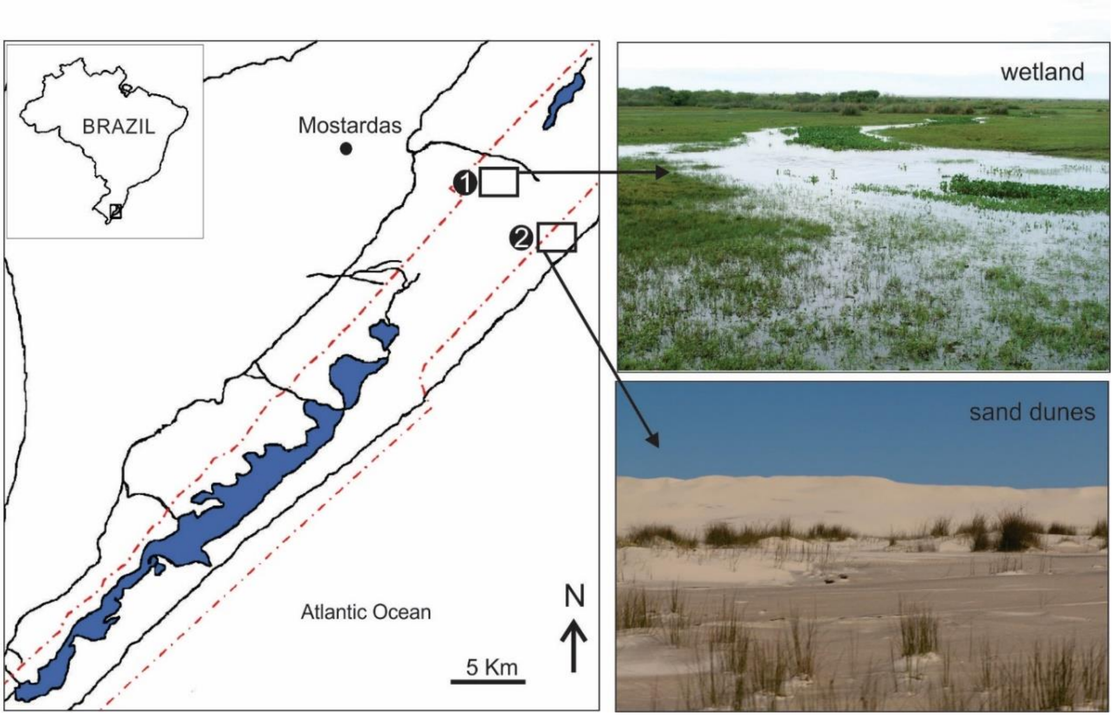

Fig.1 Southern Brazil and the location of the Lagoa do Peixe National Park (LPNP) (red dotted line) in the coastal plain with the location of the wetland (1) and dunes (2) study areas

Anurans were collected manually, and the sampling started at the dusk simultaneously at the wetland and the sand dune and lasted ca. for 2.5 hours in each habitat. The following primary producers were collected in each habitat: periphyton (PERI), suspended particulate organic matter (POM) and C_3 and C_4 plants. The leaves from C_3 and C_4 plants were collected manually; PERI samples were obtained by scraping the substrate (submerged stems and leaves); and POM samples were obtained by filtering water with a manual pump and using a pre-combusted (450°C, 4 h) glass-fiber filter (porosity: 1.2 µm) (Huckembeck et al. 2014). All samples were kept frozen until they were processed in the laboratory. Collection permits were issued by ICMBio-SISBIO (license number 14523-3). In the wetland, *Bacopa monnieri* (L.) Wettstein, *Desmodium* cf. *adscendens* (Sw.) DC and *Enydra sessilifolia* (Ruiz & Pav.) Cabrera were considered as C_3 plants, whereas *Sporobolus virginicus* (L.) Kunth and *Kyllinga vaginata* Lam. were classified as C_4 plants. In the dune, *Achyrocline atureiodes* (Lam.) DC, *Baccharis trimera* (Less.) DC, *Senecio crassiflorus* (Poir.) DC*, Vernonia* cf. *brevifolia* Less., *D. cf. adscendens*, *Drosera* sp.L. and *P. cyparissius* were considered as C_3 plants, whereas grass species (Poaceae not identified) were classified as C_4 plants.

Dietary analysis

We measured the snout–vent length (SVL ± 1 mm) for each anuran. We applied the Kruskal-Wallis test to analyze the differences in SVL between species in each habitat. When the result was significant, we applied the Mann-Whitney test to compare the SVL among species (Hammer 2017). The anurans were dissected through an incision in the ventral region to remove the stomach and the food items were removed and stored in 70% alcohol. Invertebrates (mostly insects) found in the stomach content were identified up to the order level. Food items digested as pieces of exoskeleton and locomotor appendages were classified as animal remains and fragments of vegetation were classified as plant remains.

For each food item identified, we estimated the following parameters: Frequency of occurrence (%FO), which corresponds to the frequency in percentage that a food item occurred in the analyzed stomachs, and Percentage of Area (%A), which corresponds to the percentage of the total area occupied (in mm^2) by a food item in relation to the total area occupied by the item in all the stomachs with food. To measure this area, we spread the food item until its height was no greater than 1 mm on a Petri dish with the bottom marked in a grid with mm^2 squares (Huckembeck et al. 2014).

We calculated the trophic niche breadth of the anurans for each habitat by Shannon's Index, $H = –\Sigma\, p_i \times (\log p_i)$, where p_i is the proportion of each prey item found in the diet. Differences in the niche breadth between habitats were evaluated by the diversity t-test (Hammer 2017). We used the Pianka's index to calculate the interspecific dietary overlap: $O_{jk} = O_{kj} = [\Sigma\, (p_{ij} \times p_{ik})] / \sqrt{}\, (\Sigma\, (p_{ij}^2 \times p_{ik}^2)$, where p_{ij} and p_{ik} are the proportions of food item *i* consumed by species *j* and *k*. The significance of Pianka's index was evaluated through a null model (Winemiller and Pianka 1990). We calculated this null model through 10,000 randomized simulations of the observed percentage of area (%A) of each items. The Pianka's index was calculated for each randomization and, from this process, the p-value was estimated. The p-value is based on the proportion of the randomized index that was greater than the observed value. These analyses were calculated using EcoSimR 1.0 (Gotelli and Ellison 2013).

Stable isotope analysis

After thawing the samples, we dissected the anurans and collected muscle samples from the posterior limb (< 5 g). Muscle and primary food sources (PERI, POM, C_3 and C_4 plants) samples were rinsed with distilled water and placed in sterile Petri dishes, then dried in an oven at 60°C (for 48 h). Dried samples were ground to a fine powder with a mortar and pestle and stored in clean Eppendorf tubes. We weighed sub-samples (1 - 3 mg) into tin capsules, and sent to the Analytical Chemistry Laboratory, Institute of Ecology at the University of Georgia for measurement of stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). Differences between samples and standards (carbon: ‘‘marine limestone fossil’’; nitrogen: atmospheric air) are reported as parts per thousand (‰) (Peterson and Fry 1987):

$$\delta^{13}C\ (‰) = [(^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard}) - 1] \times 1000$$

$$\delta^{15}N\ (‰) = [(^{15}N/^{14}N_{sample})/(^{15}N/\ ^{14}N_{standard}) - 1] \times 1000$$

Standard deviations of $\delta^{13}C$ and $\delta^{15}N$ replicate analyses were 0.13 and 0.14%, respectively.

$\delta^{13}C$-$\delta^{15}N$ biplots for anurans and primary food sources were used to evaluate patterns of isotopic variation between habitats. Differences between average $\delta^{13}C$ and $\delta^{15}N$ values of anuran muscle and primary sources samples were evaluated with the Mann-Whitney (U) test.

We used four quantitative community metrics based on the position of individuals in the $\delta^{13}C$-$\delta^{15}N$ space to characterize the trophic structure in both habitats (Layman et al. 2007). We used the following metrics: $\delta^{13}C$ range (CR), distance between the two individuals with the most enriched and most $\delta^{13}C$-depleted values, which provides information on the niche diversification of basal resources; $\delta^{15}N$ range (NR), distance between the two individuals with the most enriched and most $\delta^{15}N$-depleted values, which provides information on the vertical trophic structure; and the mean distance to centroid (CD), measured by mean Euclidean distance of each individual's to the $\delta^{15}N$ – $\delta^{13}C$ centroid, which gives an estimative of the average degree of trophic diversity. In relation to trophic redundancy, we used the standard deviation of nearest neighbor distance (SDNND), calculated as the standard deviation of Euclidean distances of each individual to its nearest neighbor; which provides a measure of evenness of community members packing, with low values suggesting a more uniform distribution of trophic niches (Layman et al. 2007). These metrics and their respective Bayesian credibility intervals were calculated using the Stable Isotope Analysis package in R (SIAR; Parnell et al. 2008; Jackson et al. 2011).

The isotopic space based on $\delta^{13}C$ and $\delta^{15}N$ values of each anuran species was calculated using a Bayesian standardized ellipses area corrected for small samples (SEAc). SEAc is an isotopic metric unaffected by differences in sample sizes among groups and can be obtained using the SIBER (*Stable Isotope Bayesian Ellipses* in R) (Jackson et al. 2011). An isotopic baseline correction is required to compare the trophic structure of communities between habitats exhibiting substantial variation in isotopic composition of primary food sources (Newsome et al. 2007, Hoeinghaus et al. 2008). To meet this assumption, we applied the standardization method suggested by Catry et al. (2016), which consist in subtracting the mean value of site-specific major primary sources from each consumer and dividing by the range value of the same sources. We calculated the overlap between isotopic spaces among anurans for each habitat based on asymmetrical overlap (Albernaz et al. 2016). We applied a *t-test* to compare the total overlap between anurans in each habitat. We previously tested the data for normality (Kolmogorov-Smirnov), homoscedasticity (*F-test*) and independence (autocorrelation test of the residuals) (Hammer 2017). Average overlap per anuran assemblage was estimated, which correspond to the average of all overlaps involving the anuran species in both habitats.

Before estimating the relative contribution of primary food sources, we tested if the isotopic variability of the anurans was contained within the isotopic mixing polygon formed by primary sources (Phillips et al. 2014). We carried this test using isotope mixing polygon simulations, which determine the limits of source values in the $\delta^{13}C$-$\delta^{15}N$ biplot that may contribute to consumers (Smith et al. 2013). In order to use isotope mixing polygon simulations, previously, we determined the trophic position (TP) to each anuran species in both habitats using *tRophicPosition* package in R (Quezada-Romegialli et al., 2018) (Table 1). After, based on TP values, we corrected the effect of fractionation adding TDF (trophic discrimination factor) to $\delta^{15}N$ values of primary producers (Phillips et al. 2014). We used average TDF values (± 0.1SD) of 1.3 ± 0.30 for δ13C and 2.9 ± 0.32 for δ15N (McCutchan et al. 2013). The samples located outside the 95% mixing polygon region were excluded from the mixing models, because they may not be explained by the primary sources (Smith et al. 2013). These analyses were computed using the *sp* (*Classes and Methods for Spacial Data* in R, Pebesma and Bivand 2005) and *splancs* packages (Bivand et al. 2017).

Table 1. Average in estimated TP (±SD) of anuran in the wetland and in the dunes and the credible interval (95%). Anurans: BOAPUL (*Boana pulchella*), LEPLAT (*Leptodactylus latrans*), PHYBIL (*Physalaemus biligonigerus*), PHYGRA (*Physalaemus gracilis*), PSEMIN (*Pseudis minuta*)

	Wetland			Dunes		
Species	average TP	SD	95%	average TP	SD	95%
BOAPUL	2.73	0.11	(2.49 - 2.97)	2.75	0.44	(1.86 - 3.66)
LEPLAT	2.68	0.13	(2.43 - 2.96)	3.37	0.23	(2.92 - 3.86)
PSEMIN	2.59	0.14	(2.31 - 2.88)	3.59	0.19	(3.21 - 3.99)
PHYBIL				2.48	0.17	(2.15 - 2.84)
PHYGRA	2.54	0.11	(2.33 - 2.88)			

We applied Bayesian isotopic mixing models to estimate the relative contributions of primary food sources for anurans in both habitats using the SIMMR (*Stable Isotope Mixing Models* in R) package (Parnell 2016). These simulations were generated using a Dirichlet prior distribution (Parnell et al. 2010). We fitted these models using the Markov Chain Monte Carlo (MCMC) method, which generates simulations of the resources contribution to the anurans. We run 500,000 iterations for each model and discarded the first 50,000 because they are non-informative for guiding simulations. We used the same method described above to correct TDF to estimate the relative contributions of primary producers through Bayesian isotopic mixing models (Phillips et al. 2014).

In this study, primary producers in the wetland classified as C_4 plants correspond to plants of Poaceae and Cyperaceae family. Despite this, some samples of C_4 plants showed lower values of $\delta^{13}C$ (e.g., $\delta^{13}C$ *K. vaginata*: -20.37), which are characteristic of plants with C_3 metabolism. Probably the plants used in this study, may change their carbon fixation mechanism in response to environmental changes such as floods and variations in rainfall and temperature. According to Bruhl and Wilson (2007), most Cyperaceae presents C_4 metabolism, but there are species of this family with intermediary metabolism and others with the ability to change their carbon fixation mechanism (e.g., the genus *Eleocharis*). Species belonging to Poaceae family also have a wide variation in carbon fixation mechanism, including plants with intermediary metabolism (C_3 - C_4) (Christin et al. 2009). Furthermore, variation in the metabolism of plants can be observed along an environmental gradient. C_4 plants are less dominant in environments exhibiting flooded conditions, where is more common the predominance of C_3 plants (Wantzen et al. 2002). In order to obtain a food source group with more homogeneous isotopic values, the less dominant C_4 plants with lowest $\delta^{13}C$ values in the wetland were excluded from the analysis (total n excluded= 2; total n used in the study: 2) (Table 1).

Results

Dietary patterns

A total of 200 individuals were collected, 97 in the wetland and 103 in the sand dunes (Online Resource 1). *Leptodactylus latrans* was the largest species in both habitats (wetland: 37.60 ± 13.90 mm, n = 24; dunes: 46.50 ± 7.77mm, n = 11). *Physaleamus gracilis* (25.71 ± 2.61mm, n = 25) and *B. pulchella* (23.88 ± 2.84 mm, n = 12) were the smallest species in the wetland and in the dunes, respectively (Online Resource 2). *Boana puchella* (28.97 ± 2.84 mm, n = 23) and *P. minuta* (wetland: 37.27 ± 2.91 mm, n = 25; dunes: 23.88 ± 2.84 mm, n = 58) were larger in the wetland compared to individuals in the sand dunes (Online Resource 2). *Physalaemus biligonigerus* (n = 22 was similar in size to *P. gracilis*, with average SVL equal 26.59 ± 2.72 mm (Online Resource 2).

A total of 16 and 18 items were identified in the diet of anurans in the wetland and in the dunes (Online Resource 1). The diet comparison of those species co-occurring in both habitats (*B. pulchella*, *L. latrans* and *P. minuta*) revealed changes in the average relative abundance (%A) of the dominant preys (Fig. 2). Hemiptera (%A = 21.3), Coleoptera (%A =

19.7) and Lepidoptera (%A = 15.3) showed greater contribution in the anurans' diets in the wetland, whereas Coleoptera (%A = 35.3), Odonata (%A = 28.0), Hymenoptera (%A = 10.6) were the most abundant food items in the dunes (Fig. 2).

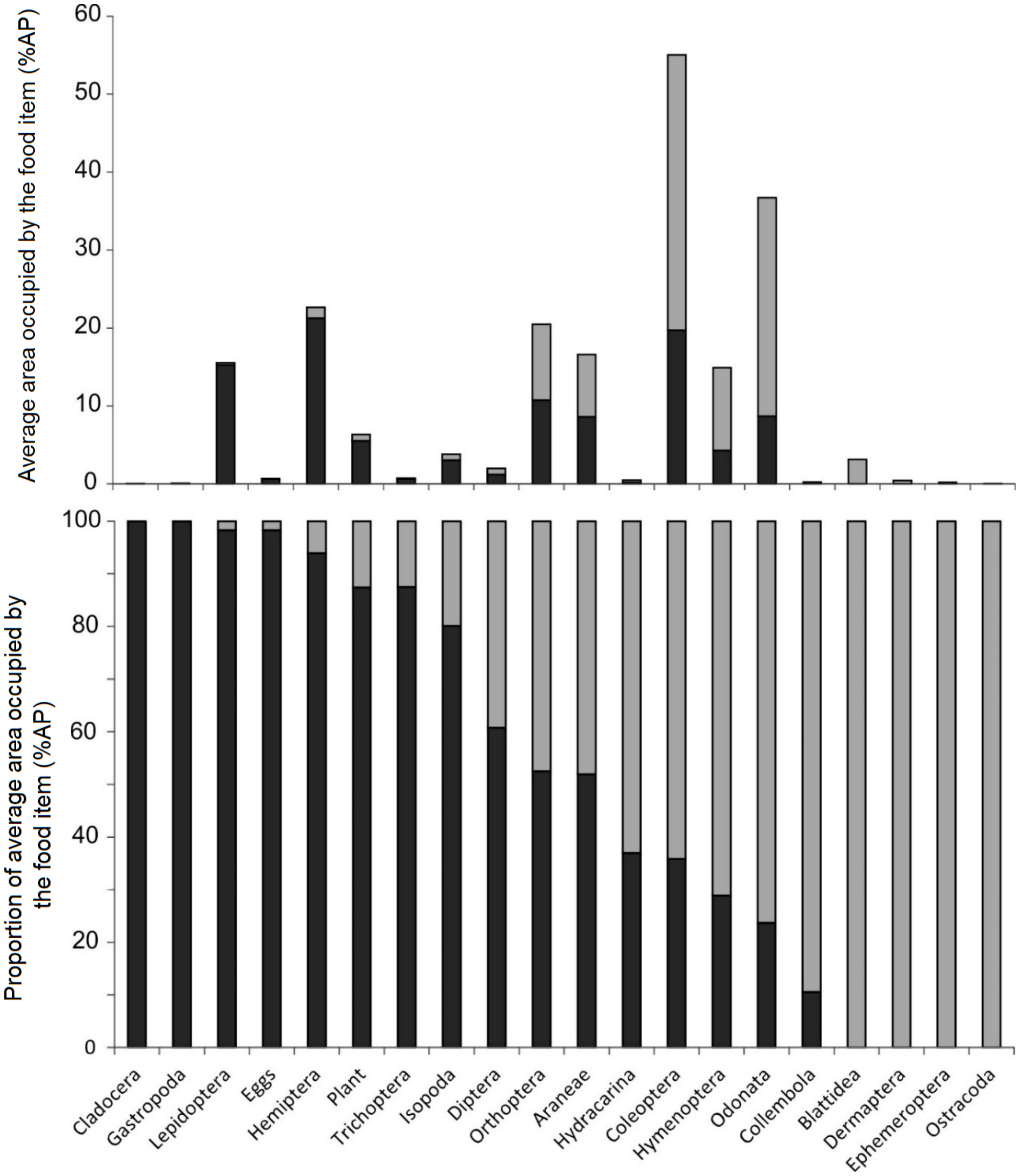

Fig.2 Area percentage (%AP) of the stomach content of the anurans in the wetland (black bars) and dunes (gray bars) (upper graphic) and its proportion AP% (bottom graphic)

The anurans had larger trophic niche breadths in the wetland than in the dunes (Wetland, H'= 1.62; Dunes, H' = 1.16; t = 3.44, df = 177.6, $p < 0.05$). The trophic niche overlap observed was greater than expected by chance for anurans in the wetland (observed: 0.50; expected: 0.29; $p < 0.01$) and dunes (observed: 0.32; expected: 0.12; $p < 0.02$). This result suggests a dietary overlap in both habitats, with lesser dietary overlap in sand dunes than wetland (t = -47.65; $p < 0.05$).

Stable isotope patterns

We analyzed isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of 77 anurans and 16 primary food sources in wetland and 76 anurans and 18 primary sources in dunes (Table 2). The range in average $\delta^{13}C$ values of primary sources was wider in the wetland (-38.7‰ to -10.5‰) compared to the dunes (-31.6‰ to -12.2‰) (Fig. 3), but mean $\delta^{13}C$ for frog assemblages of the two habitats were not significantly different (U = 114; $p > 0.45$). In contrast, average $\delta^{15}N$ values of primary sources showed higher amplitude in the dunes (-7.4‰ to 1.8‰) than in the wetland (-2.6‰ to 1.8‰), and the overall $\delta^{15}N$ average value of primary sources was lower in dunes (U = 37; $p < 0.001$). Between-habitat comparisons of average $\delta^{13}C$ values for each primary food source revealed statistically significant differences for POM ($p < 0.01$) and PERI ($p < 0.01$), with both primary sources showing lower average values in the wetland (Table 1). In contrast, average $\delta^{15}N$ values showed statistically significant differences only for POM ($P < 0.04$), which had lower average value in the dunes (Table 2).

Table 2. Number of samples (n) and mean values (±SD) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios (in permil) of anurans and primary food sources collected in the wetland and dunes. Anurans: BOAPUL (*Boana pulchella*), LEPLAT (*Leptodactylus latrans*), PHYBIL (*Physalaemus biligonigerus*), PHYGRA (*Physalaemus gracilis*), PSEMIN (*Pseudis minuta*). Sources: POM (particulate organic matter), PERI (periphyton), C_3 (plant C_3), C_4 (plant C_4), BACMON (*Bacopa monnieri),* DESADS (*Desmodium cf. adscendens*), ENYSES (*Enydra sessilifolia*), ACHSAT (*Achyrocline satureiodes*), BACTRI (*Bacharis trimera*), DROSER (*Drosera* sp.), POLCYP (*Polygala cyparissius*), MARPIN (*Margynicarpus pinnatus*),

SENCRA (*Senecio crassiflorus*), VERBRE (*Vernonia cf. brevifolia*), SPOVIR (*Sporobolus virginicus*), KYLVAG (*Kyllinga vaginata*). C_3 plant* and C_4 plant* correspond to means of all plants C_3 and C_4

			$\delta^{13}C$ (‰)					$\delta^{15}N$ (‰)				
	Wetland	Dunes	Wetland		Dunes			Wetland		Dunes		
Anuran	n	n	Mean	SD(±)	Mean	SD(±)	p-value	Mean	SD(±)	Mean	SD(±)	p-value
BOAPUL	15	4	-22.8	2.4	-20.3	1.3	0.06	6.2	0.5	3.7	1.5	0.01
LEPLAT	17	6	-21.6	2.7	-20.6	1.0	0.24	5.6	0.8	6.0	0.9	0.42
PHYBIL		22			-17.4	1.5				2.9	0.8	
PHYGRA	27		-17.9	2.6				5.5	0.4			
PSEMIN	18	44	-23.3	0.9	-22.6	2.0	0.16	5.4	0.8	6.6	0.9	<0.00
Sources												
POM	7	2	-26.0	1.9	-22.7	3.5	0.01	1.0	0.6	-0.5	0.6	0.04
PERI	4	2	-36.9	1.5	-17.0	3.7	0.01	0.7	0.8	1.0	1.1	0.24
C_3 plants*	3	12	-29.3	1.5	-29.1	1.4	0.95	-1.7	0.8	-2.7	1.4	0.09
BACMON	1		-27.8					-1.4				
DESADS	1	2	-30.7		-29.0			-2.6		-2.8		
ENYSES	1		-29.5					-1.0				
ACHSAT		1			-27.9					-3.2		
BACTRI		1			-31.2					-5.5		
DROSER.		1			-27.0					-1.5		
POLCYP		1			-28.5					-2.2		
MARPIN		1			-30.1					-1.2		
SENCRA		3			-29.4					-2.4		
VERBRE		2			-29.1					-3.3		
C_4 palnts*	2	2	-12.2	2.4	-12.3	0.1	0.69	-0.3	0.2	-5.3	3.0	0.24
SPOVIR	1	1	-13.9	0.03								
KYLVAG	1	1	-10.5	-0.30								
Poaceae		2			-12.3	0.1				-5.3	3.0	

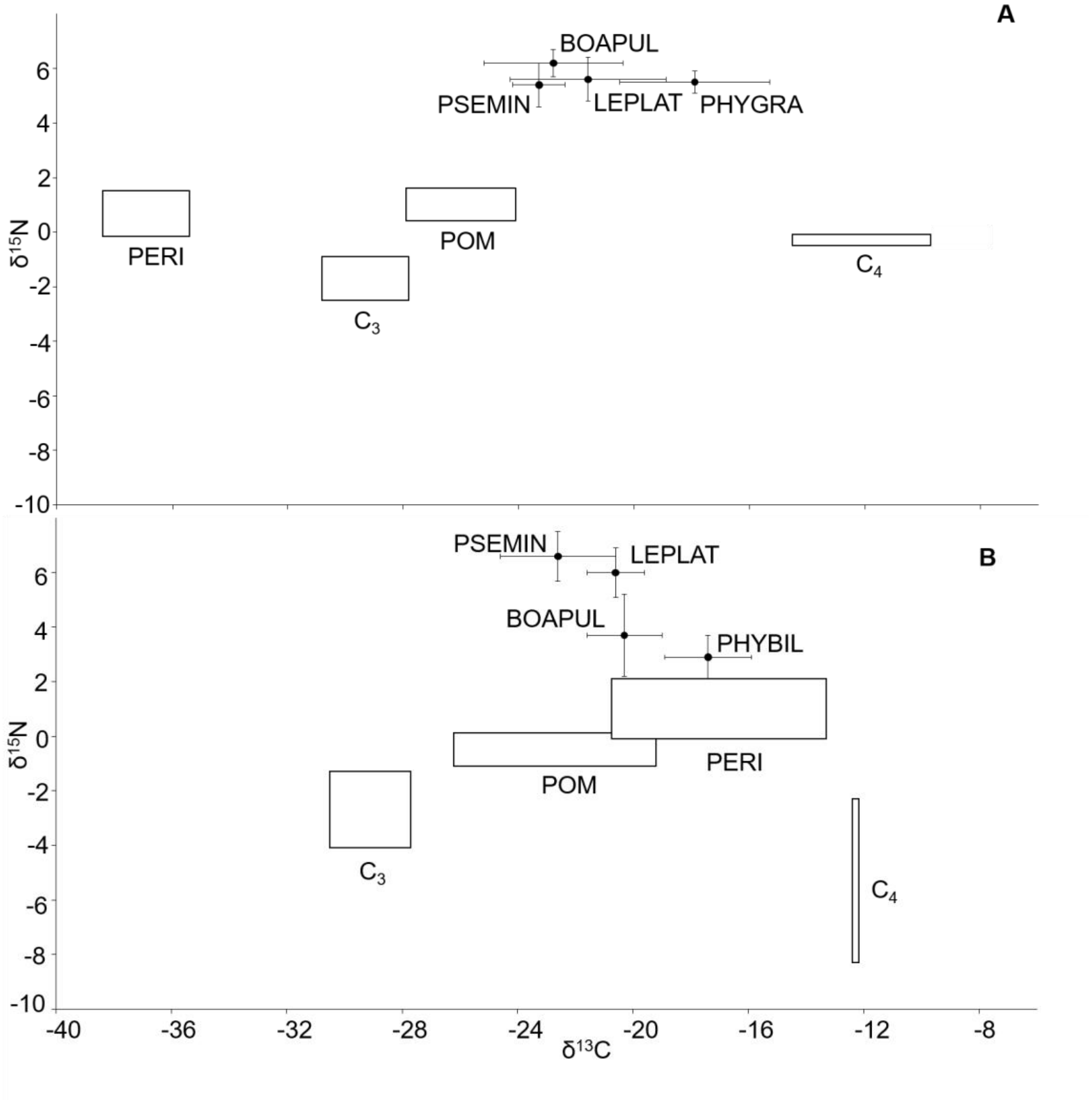

Fig.3 Average (±SD) values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of anurans and primary sources (open boxes) in the wetland and dunes. Anurans: BOAPUL (*Boana pulchella*), LEPLAT (*Leptodactylus latrans*), PHYBIL (*Physalaemus biligonigerus*), PHYGRA (*Physalaemus gracilis*), PSEMIN (*Pseudis minuta*). Sources: POM (particulate organic matter), PERI (periphyton), C_3 (plant C_3), C_4 (plant C_4)

Anuran assemblages in both habitats had similar amplitude of variation in their $\delta^{13}C$ values, with values ranging from 25.68‰ to -13.76‰ in the wetland and from -26.50‰ to -14.79‰ in the dunes (Fig. 3). In contrast, there was higher variation in $\delta^{15}N$ values in the dunes (1.64‰ to 8.13‰) than in the wetland (3.42‰ to 7.26‰) (Fig. 3). Regarding those anurans co-occurring in both habitats, differences were significant in average values only in $\delta^{15}N$ for *B. pulchella* (p < 0.01) and *P. minuta* (p <0.00). They had contrasting patterns, with the former showing higher average value in the wetland (6.2‰) than in the dunes (3.7‰) and the latter the opposite pattern (5.4‰ in the wetland and 6.6‰ in the dunes) (Table 2).

The isotopic metrics providing information on vertical trophic length (NR) and niche diversification of food basal sources (CR) had higher values in the sand dunes (0.40 and 0.27) than in the wetland (0.20 and 0.19, respectively). Similarly, the metric reflecting trophic diversity (CD) was higher in the sand dunes (0.18) than in the wetland (0.09). In contrast, there was no between-habitat difference in the metric reflecting trophic redundancy (SDNDD) (0.03 in both habitats). Despite the tendency of higher values of NR, CR, and CD in the dunes than in the wetland, the overlapping of credible intervals created a considerable degree of uncertainty in the estimation of these metrics, especially, due the great variability in the dunes (Fig. 4).

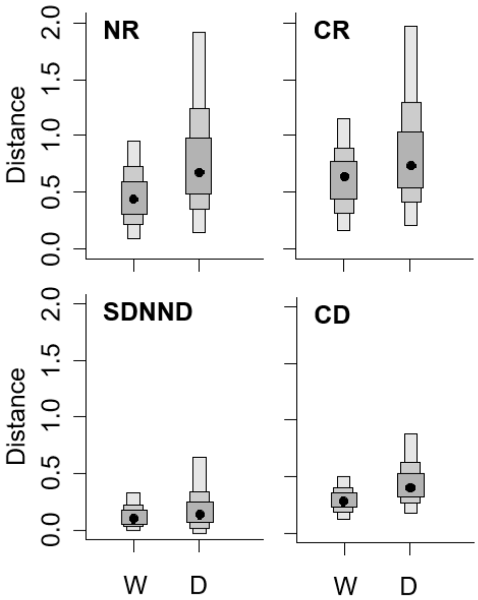

Fig.4 Layman's metrics for the communities of anurans in the wetland (W) and dunes (D) Black dots represent the mode, and boxes present the 50%, 75% and 95% credible

intervals. NR = δ^{15}N range, CR = δ^{13}C range, CD = mean distance to centroid, SDNND = standard deviation of mean nearest neighbour distance

In general, the contribution of primary sources along the food chain in the wetland was more diversified (i.e. higher number of sources showing substantial contribution to consumers). In contrast, there was a dominant contribution of carbon coming from C_3 plants for most species in the dunes (Fig. 5). In the wetland, through the prey consumed by anurans, the primary food sources showing the highest contribution (95% credibility intervals) for *B. pulchella*, *P. minuta* and *P. gracilis* were POM (10 - 78%), C_3 (21 - 53%) and C_4 (40 - 58%), respectively. *Leptodactylus latrans* showed similar levels of assimilation of carbon coming from the four primary sources (Fig. 5). In contrast, carbon coming from the C_3 plants had the highest contribution for all species (*B. pulchella*: 9 - 63%, *L. latrans*: 44 - 72%, *P. minuta*: 60 - 78%), except for *P. biligonigerus* who assimilated more carbon from C_4 plants (30 - 42%) (Fig. 5). Only 11 individuals (five *L. latrans* and two *P. minuta* in the wetland and four *P. minuta* in the dunes) were excluded from the mixing model analyses because their isotopic variability was not within the isotopic mixing polygon formed by the primary food sources (Online Resource 3).

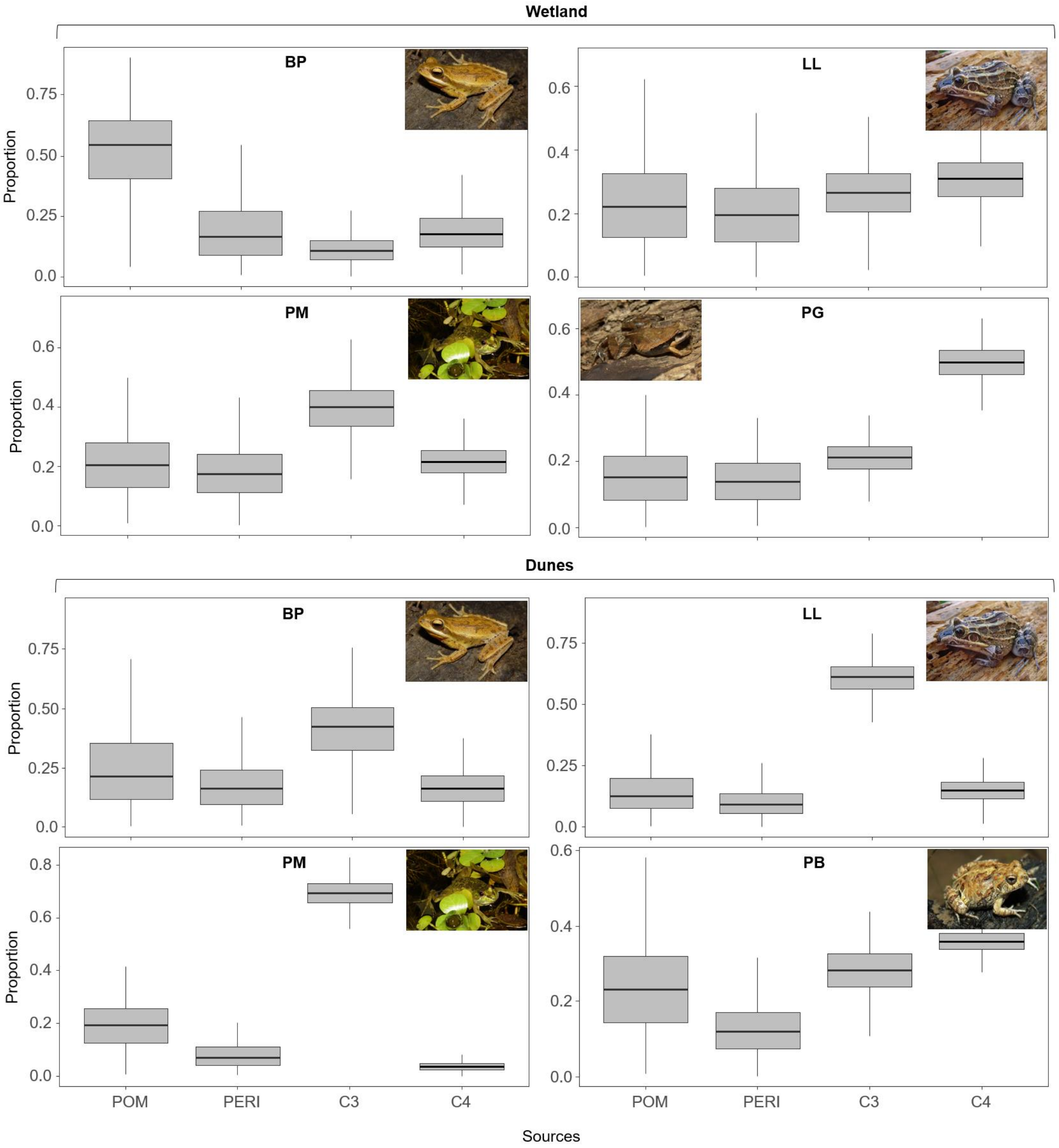

Fig.5 Results of the Bayesian isotopic mixing models (SIMMR) showing the proportion of the contributions (50%) of primary sources to anurans in the wetland and dunes. Anurans: BP (*Boana pulchella*), LL (*Leptodactylus latrans*), PB (*Physalaemus biligonigerus*), PG (*Physalaemus gracilis*), PM (*Pseudis minuta*). Photos: Daniel Loebmann. Sources: POM (particulate organic matter), PERI (periphyton), C_3 (plant C_3), C_4 (plant C_4)

Regarding isotopic niches, there were marked differences in the size and overlap of isotopic ellipses (SEAc) of anuran assemblages between habitats (Fig. 6). *Boana pulchella* and *L. latrans* had larger isotopic ellipses in the wetland (4.27‰ and 4.29‰, respectively) than in the dunes (2.56‰ and 2.86‰, respectively), whereas *P. minuta* showed an opposite pattern (2.43‰ vs 4.96‰, respectively) (Fig. 6). The mean overlap among isotopic ellipses (SEAc) was comparatively higher in the wetland (0.14‰) than in the dunes (0.04‰). In the wetland, all species had some degree of overlap among their isotopic ellipses, being the mean overlap higher for *P. minuta* (0.26 ± 0.27‰) and *L. latrans* (0.18 ± 0.12‰). In contrast, only two species (*L. latrans*: 0.12 ± 0.20‰; and *P. minuta*: 0.07 ± 0.12‰) had their isotopic ellipses overlapped in the anuran assemblage of the dunes (Fig. 6).

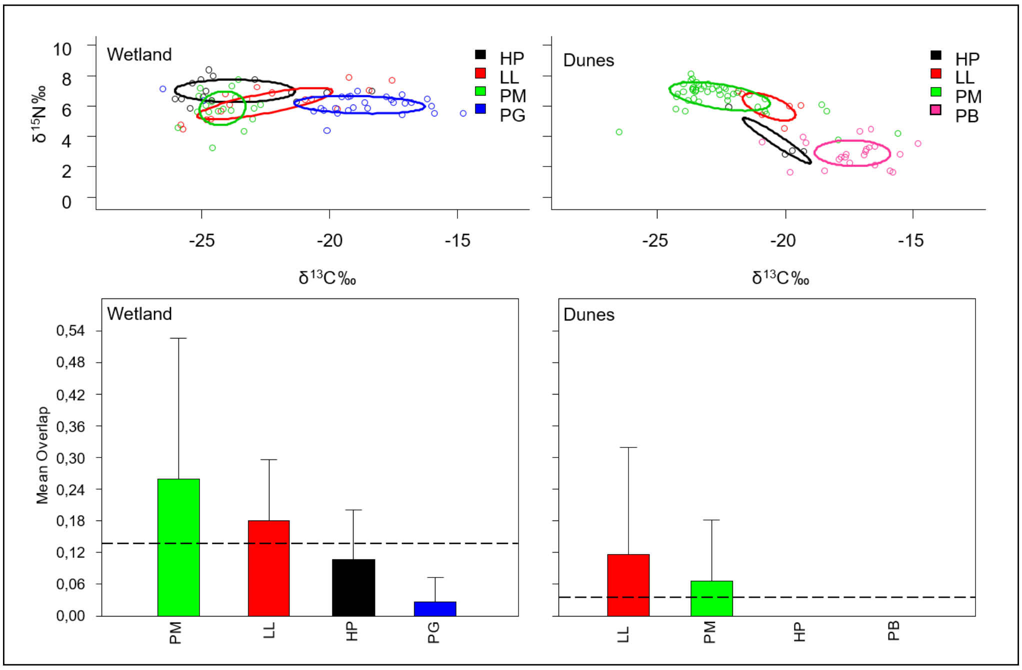

Fig.6 Isotopic niche space of anurans in the wetland and in the dunes in the Lagoa do Peixe National Park. The mean niche overlap for each species (±SD); the mean overlap for the assemblage is shown by a horizontal line. Anurans: BP (*Boana pulchella*), LL (*Leptodactylus latrans*), PB (*Physalaemus biligonigerus*), PG (*Physalaemus gracilis*), PM (*Pseudis minuta*)

Discussion

Our findings support the hypothesis that coastal systems with contrasting physiographic and productivity levels are associated with differences in trophic structure of anuran assemblages. Firstly, isotopic mixing models revealed that the anuran assemblage in the wetland is sustained by several basal sources, including POM, C_3 and C_4 plants. Wetlands generally have high primary production that sustains diverse aquatic consumers through complex trophic pathways (Brinson et al. 1981). Other studies have revealed multiple primary producers supporting food webs of coastal ecosystems (e.g. Catry et al. 2016). In contrast, the anuran assemblage in the sand dunes was sustained by a comparatively lower number of primary sources, with most of their biomass supported by food chains originating from C_3 plants. This pattern supports our prediction that the more productive habitat would be associated with more diverse basal resources and support a more diverse anuran trophic structure. Secondly, isotopically based community metrics (CD, CR, NR) were higher for frogs from the sand dunes. These estimates indicative of higher trophic diversity could reflect niche segregation among anuran species in response to competition for more limited invertebrate prey in sand dunes (Pianka 1974). Both stomach contents and stable isotope data revealed lower niche overlap among anuran species in the sand dunes. According to niche theory (Schoener 1974), species should reduce niche breadth under conditions of resource scarcity and competition (Pianka 1974; Oliveira et al. 2018). Accordingly, previous studies in subtropical regions suggested that the numerical abundance of invertebrate prey is approximately sixteen folds lower in the sand dunes compared with wetlands (Masson and McLachlan 1990; Huckembeck et al. 2018). These patterns corroborate our initial prediction that anurans would have lower overlap in trophic niches in the sand dunes.

However, in some instances, our findings did not corroborate our initial expectation. Regarding to the metric reflecting trophic redundancy (SDNND), there was not difference between habitats. Trophic redundancy corresponds to the trophic functional similarity between species (Catry 2015). Indeed, in both habitats the anurans are considered secondary and tertiary consumers, playing the same ecological function (diet composed mainly by insects) independent of the habitat. Furthermore, some community-based isotopic results (e.g. higher NR) suggested a more diverse vertical structure (i.e. larger food chains) in the sand dunes and not, as we initially suspected, in the more productive habitat (wetland). Although several studies have shown that environments with higher productivity are associated with greater diversity in their vertical trophic structure (Vander Zanden and Fetzer 2007; Takimoto and Post 2013), there are evidences corroborating the opposite (Post 2002). For example, consumers are known to change their feeding strategy to maximize nutritional gains relative to costs, especially in face of variations in quality and availability of resources (Schoener 1987; Gaeta et al. 2018). In some cases, omnivorous species may explore the high availability of primary resources (e.g. organic detritus) accumulated at the base of the food chain in productive habitats (Aresco 2010). A similar mechanism could lead to shorter vertical trophic structure in the studied wetland, which accumulated much more particulate organic matter (e.g. detritus) compared with the sand dunes (Seeliger et al. 2000; Ferreira et al. 2009).

Regardless between-habitat differences, we observed the highest overlap in isotopic spaces between *L. latrans* and *P. minuta* in both habitats. This is probably due to their wide foraging areas encompassing different microhabitats and the ability to ingest larger prey compared to the other studied anurans. For instance, *P. minuta* is a species associated with aquatic environments, but it also uses the adjacent habitats for its feeding activity, such as the margins of water bodies and grass fields (Huckembeck et al. 2012). Although feeding activity of *L. latrans* is more common in terrestrial habitats (Huckembeck, unpublished data), this species also feeds on invertebrates associated with the aquatic environment. In the wetland, *B. pulchella* showed low overlap with *P. minuta* and *L. latrans* and no overlap with anurans in the sand dunes. In the wetland, *B. pulchella* usually is associated with vegetated microhabitats (e.g. phytotelmata and aquatic macrophytes) and may use field areas for foraging (Huckembeck, unpublished data), whereas this species is associated only with terrestrial vegetation in the sand dunes. We expected higher overlap in isotopic spaces between *P. gracilis* and *L. latrans* because they use similar microhabitats (e.g. terrestrial fields and ephemeral water bodies) for foraging. We hypothesized that the lower overlap in their isotopic spaces in the wetland are associated with differences in their average body sizes (25.71 vs. 37.27 mm, respectively). A prior study has shown that smaller anurans that are less able to use different environments for foraging have a diet more restricted in this region (Huckembeck et al. 2018). Finally, *P. biligonigerus* did not show overlap in isotopic spaces with other species, probably because this species is more related to the terrestrial environment, being observed only in the sand substrate during the study. Such influence of microhabitats uses and morphology constraints on food resource partitioning has been reported among other species of anurans (Cloyed et al. 2017; Huckembeck et al. 2018).

It is worth noting that we observed some unexpected findings regarding consumers' assimilation of primary sources in the sand dunes, notably, the predominant assimilation of nutrients derived from C_3 plants. This pattern was somewhat surprising considering that the predominant vegetation in the studied sand dunes corresponds to plants with metabolism C_4 and CAM (Crassulacean Acid Metabolism) (Seeliger et al. 2000; Seeliger and Kjerfve 2013), which are characteristic of environments with water stress and high luminosity (Lane et al. 2008). Overall, C_4-CAM plants are more enriched in ^{13}C than C_3 plants (average $\delta^{13}C$: −14‰ and −27‰, respectively), mainly due to differences in the enzymes responsible for carbon fixation during photosynthesis (Marshall et al. 2007). Therefore, we expected that anurans in the sand dunes would have comparatively higher $\delta^{13}C$ values due to assimilation of the most abundant C_4-CAM plants. Rather, most anurans had comparatively lower carbon isotope ratios reflecting assimilation of C_3 plants. This finding led us to speculate if this substantial contribution of C_3 plants to anurans in the sand dunes was associated with spatial trophic subsidies deriving from the wetlands, which harbor high biomass of C_3 plants (Rolon et al. 2011). Spatial trophic subsidies have been recently recognized as one important mechanism promoting ecosystems connectivity (Polis et al. 1997). It is mainly observed between ecosystems with asymmetric productivity levels, with the energy flowing from the high-productivity (donor) to low-productivity (receiver) system (Koshino et al. 2013; Garcia et al. 2017). These contrasting ecosystem features and direction of flow are compatible with the conditions in our study; trophic subsidy flowing from the wetland (more productive) to the sand dunes (less productive). Among the potential biological vectors able to transport C_3 plants-derived nutrients from the wetland to the dunes are insects like Odonata and Diptera, which have a larval phase dependent on aquatic environments. After their aquatic development, these species migrate to adjacent areas, being considered an important energy pathway between aquatic and terrestrial environments (Gladyshev et al. 2011; Ciss et al. 2013). Although there are viable water bodies for the reproduction of Odonata in the sand dunes (Arthington and Watson 1982), dragonflies are abundant and conspicuously found in subtropical wetlands (Maltchik et al. 2010). They constitute an important prey for several groups of aquatic vertebrates (Huckembeck et al. 2014; Silva-Gonçalves et al. 2018), including the species of anurans we caught in the sand dunes. In fact, Odonata was the most consumed prey by *B. pulchella* (%A: 58.82) and *P. minuta* (%A: 52.97) in the dunes. Field studies on displacement patterns and trophic ecology of these potential biological vectors are needed to evaluate our hypothesis of lateral trophic subsidies between wetlands and sand dunes and their implications for anuran's population dynamics.

In conclusion, our work provides evidences corroborating the hypothesis that contrasting coastal environments (e.g. in terms of primary productivity) are associated with differences in food resources use and trophic structure of anuran assemblages. The more productive habitat (wetland) had a higher contribution of distinct primary sources (POM, C_3 and C_4 plants) sustaining the anuran assemblage. In contrast, anurans in the less productive habitat (sand dunes) were sustained by a comparatively lower number of primary sources (mainly C_3 plants). Additionally, community-based isotope metrics and comparison of species' isotopic niche areas revealed that the anuran assemblage had higher trophic diversity and food niche segregation in the sand dunes. These patterns could reflect anurans adaptations to avoid food niche overlap and to promote species coexistence to cope with the lower food availability in the arid conditions of the sand dunes.

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Online Resource 1. Area percentage (%AP) of the stomach content of the anurans in the wetland and dunes. Anurans: BOAPUL (*Boana pulchella*), LEPLAT (*Leptodactylus latrans*), PHYBIL (*Physalaemus biligonigerus*), PHYGRA (*Physalaemus gracilis*), PSEMIN (*Pseudis minuta*).

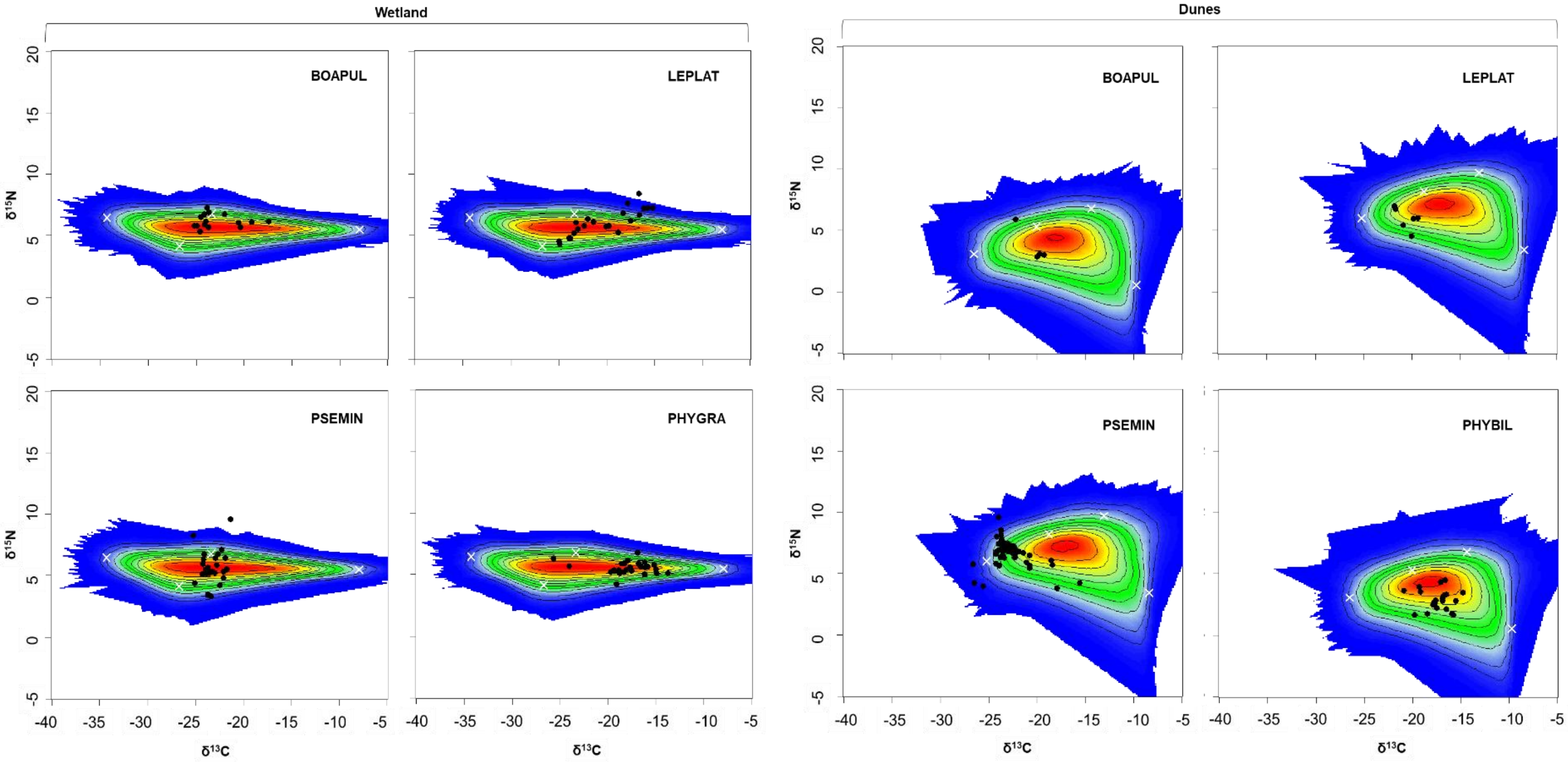
	Wetland					Dunes				
	BOAPUL (n=23)	LEPLAT (n=24)	PSEMIN (n=25)	PHYGRA (n=25)	Mean PA%	BOAPUL (n=12)	LEPLAT (n=11)	PSEMIN (n= 58)	PHYBIL (n=22)	Mean PA%
Araneae	6.59	9.90	5.70	12.26	8.61	4.12	7.27	4.93	15.6	7.98
Blattidea							12.53			3.13
Cladocera			0.15		0.04					
Coleoptera	33.33	11.63	12.94	20.94	19.71	2.35	69.42	24.61	44.9	35.32
Collembola		0.10			0.02			0.03	0.8	0.21
Dermaptera								1.47	0.3	0.44
Diptera		1.89	2.72	0.19	1.20	0.59	0.75	1.76		0.78
Ephemeroptera								0.78		0.19
Gastropoda		0.10	0.21		0.08					
Hemiptera	39.53	37.99	6.26	1.32	21.28			4.93	0.6	1.38
Hydracarina	0.40	0.10	0.21		0.17	1.18		0.01		0.30
Hymenoptera	0.40	1.89	3.03	11.89	4.30	1.18	7.77	4.75	28.7	10.60
Isopoda			2.72	9.43	3.04		1.50	1.51		0.75

	Wetland					Dunes				
	BOAPUL (n=23)	LEPLAT (n=24)	PSEMIN (n=25)	PHYGRA (n=25)	Mean PA%	BOAPUL (n=12)	LEPLAT (n=11)	PSEMIN (n= 58)	PHYBIL (n=22)	Mean PA%
Lepidoptera	6.59	19.96		34.53	15.27		0.75		0.3	0.26
Odonata		6.53	28.29		8.70	58.82		52.97	0.2	28.00
Orthoptera	13.18	5.00	15.40	9.43	10.75	29.41		0.99	8.6	9.75
Ostracoda								0.01		
Trichoptera			2.57		0.64			0.37		0.09
Others										
Eggs			2.57		0.64			0.04		0.01
Plant		4.90	17.25		5.54	2.35		0.84		0.80

Online Resource 2. Mean snout–vent length (±SD) and the Mann-Whitney p-values among anuran species. Anurans: BOAPUL (*Boana pulchella*), LEPLAT (*Leptodactylus latrans*), PHYBIL (*Physalaemus biligonigerus*), PHYGRA (*Physalaemus gracilis*), PSEMIN (*Pseudis minuta*).

		Wetland		
	mean±SD (mm)	LEPLAT	BOAPUL	PHYGRA
PSEMIN	37.27 ± 2.91	0.124	< 0.001	< 0.001
LEPLAT	37.60 ± 13.90		0.28	< 0.001
BOAPUL	28.97 ± 5.08			< 0.001
PHYGRA	25.71 ± 2.61			
		Dunes		
	mean±SD (mm)	LEPLAT	BOAPUL	PHYBIL
PSEMIN	23.88± 2.84	< 0.001	< 0.001	< 0.001
LEPLAT	46.50± 7.77		0.01	< 0.001
BOAPUL	31.30± 3.34			0.01
PHYBIL	28.59± 2.72			

Online Resource 3. Biplots of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios with simulated mixing polygons, where the positions of the anurans are represented by black dots and the average source signatures is represented by white crosses. Probability contours are at the 5%level (outermost contour) and at every 10% level. Anurans: BOAPUL (*Boana pulchella*), LEPLAT (*Leptodactylus latrans*), PHYBIL (*Physalaemus biligonigerus*), PHYGRA (*Physalaemus gracilis*), PSEMIN (*Pseudis minuta*). Sources: POM (particulate organic matter), PERI (periphyton), C_3 (plant C_3), C_4 (plant C_4). TDF values: 1.3 ± 0.30 for $\delta^{13}C$ and 2.9 ± 0.32 for $\delta^{15}N$ (McCutchan et al. 2013)



CONSIDERAÇÕES FINAIS E PERSPECTIVAS

No primeiro capítulo os resultados mostraram que a sazonalidade influenciou a dieta de *Pseudis minuta,* porém não foi observada uma mudança considerável na composição da dieta de *Scinax squalirostris.* Esse resultado pode estar relacionado com a grande quantidade de conteúdo digerido dessa espécie e o pequeno número de espécimes analisados. Similarmente, estudos prévios sobre o efeito da sazonalidade apontam uma mudança na composição da dieta de anuros entre as estações, tanto na região tropical quanto na subtropical, relacionado com a disponibilidade de presas (TOFT, 1980; BERAZATEGUI et al., 2007). As duas espécies são descritas como generalistas e oportunistas (HUCKEMBECK et al. 2014; KITTEL; SOLÉ, 2015), portanto é esperado que ocorra uma resposta a variação da disponibilidade de presas (HUCKEMBECK et al., 2014). Nesse ponto, esse estudo traz uma informação importante sobre a relação com a disponibilidade de presas, pois existem poucos trabalhos que combinam a análise da dieta com um esforço amostral sobre a variabilidade das presas na região subtropical (MANEYRO; ROSA, 2004; ATTADEMO et al., 2007; HUCKEMBECK et al., 2014). Outro resultado desse estudo mostrou que *P. minuta,* a qual comparada com *S. squalirostris* é uma espécie maior e que usa mais amplamente os micro-habitats, apresentou uma dieta mais diversificada. Além disso, as duas espécies de anuros apresentaram valores isotópicos distintos, indicando a utilização de diferentes recursos, o que provavelmente está relacionado com as restrições morfológicas e diferenças na utilização dos micro-habitats.

Esses resultados corroboram estudos prévios que indicaram mudanças na dieta de anuros que utilizam diferentes micro-habitats (MANEYRO; ROSA, 2004) e que apresentam correlação entre os seus tamanhos e o tamanho das presas ingeridas, mostrando que esses fatores são relevantes em estudos sobre a abrangência do nicho trófico de anuros (MUÑOZ-GUERRERO et al., 2007). Apesar disso, a utilização de micro-habitats pelos anuros não tem sido um assunto recorrente na literatura disponível nos ambientes costeiros (HUCKEMBECK et al., 2012; OLIVEIRA et al., 2015).

Além dos objetivos propostos nesse estudo terem sido atingidos, os resultados desse trabalho levantaram algumas questões, principalmente, com relação a influência do hidroperíodo. Nesse estudo, o espaço isotópico ocupado pelos dois hilídeos se sobrepõe durante o período de cheia do banhado, indicando uma possível conectividade entre os ambientes utilizados pelos anuros. Previamente, em áreas alagadas costeiras foi observada a conectividade entre os ambientes terrestre e aquático durante o pulso de inundação, ocorrendo uma maior contribuição de recursos terrestres para a assembleia de peixes (BASTOS, 2016). Portanto, estudos futuros devem ser realizados para a identificação de um padrão do efeito do hidroperíodo sobre a dieta de anuros nos ambientes costeiros da região subtropical.

No segundo capítulo a Tese forneceu evidências que corroboraram a hipótese de que os ambientes costeiros com níveis de produtividade primária e disponibilidade de recursos distintos estão associados a diferenças no uso de recursos alimentares e na estrutura trófica de assembleias de anuros. O habitat mais produtivo (banhado) teve uma contribuição de fontes primárias distintas (plantas POM, C_3 e C_4) sustentando as espécies de anuros. Em contraste, os anuros no habitat menos produtivo (dunas) foram sustentados, predominantemente, por carbono oriundo de plantas C_3. A vegetação das dunas apresenta, predominantemente, metabolismo C_4 (SEELIGER; KJERFVE, 2013). Portanto, essa alta contribuição de plantas C_3 para as espécies de anuros das dunas levou a hipótese de que ocorra um subsídio trófico dos ambientes adjacentes para as dunas, aonde seriam assimilados pelos anuros. Alguns trabalhos têm registrado essa relação de conectividade entre ambientes costeiros da planície costeira do RS (OLIVEIRA et al., 2014; GARCIA et al., 2017). Por exemplo, a conectividade hidrológica foi observada durante um ciclo de seca e cheia, no qual durante o período de inundação houve assimilação por organismos estuarinos de nutrientes oriundos do ambiente de água doce (banhado) (GARCIA et al., 2017). Outra possibilidade de via de fluxo de biomassa a ser considerado é por meio da dispersão de vetores biológicos como insetos alados (por exemplo, Odonata e Diptera) (GLADYSHEV et al. 2011). Assim, a execução de estudos sobre o fluxo de biomassa e energia é necessária para determinar quais os processos e vetores (fisícos e/ou biológicos) que poderiam promover subsídios tróficos entre o banhado e as dunas e suas implicações para a dinâmica populacional dos anuros.

Com relação as métricas isotópicas baseadas na comunidade e a comparação do espaço isotópico das espécies, os resultados revelaram que a assembleia de anuros tinha maior diversidade trófica e segregação de nicho trófico nas dunas. Esses padrões podem refletir as adaptações dos anuros para evitar a sobreposição de nicho trófico e promover a coexistência das espécies em um ambiente com menor disponibilidade de presas (PIANKA, 1974). Segundo OLIVEIRA; ROCHA (2015), embora haja inúmeros trabalhos sobre a dieta de anuros (e. g. OLIVEIRA et al., 2018), ainda existe uma lacuna no conhecimento sobre questões relacionadas ao nicho trófico dos anuros em ambientes costeiros. Portanto, o resultado sobre a partição de nicho trófico (inferido pela análise dos nichos isotópicos) obtido na presente Tese, somado ao conhecimento disponível sobre a ecologia trófica de anuros (e. g. MOLINA-BURGOS et al., 2018), pode contribuir para o entendimento sobre como as variações espaciais interferem nas interações interespecíficas desse importante grupo de consumidores em ecossistemas de planície costeira.

Do ponto de vista regional, a presente Tese gerou informações sobre a ecologia trófica de seis espécies de anuros em dois habitats (banhado e dunas costeiras) do Parque Nacional da Lagoa do Peixe, uma importante área de conservação da Biodiversidade no sul do Brasil. Além disso, com a combinação da análise do conteúdo estomacal e da análise dos isótopos estáveis, foi possível avançar no conhecimento sobre o funcionamento desses dos habitats importantes para os anuros, os quais se estendem ao longo da costa do extremo sul do Brasil e que vem sofrendo crescente degradação devido aos impactos das atividades humanas.

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ANEXOS

Anexo 1. *Boana pulchella.* Foto: Daniel Loebmann

Anexo 2. *Scinax squalirostris.* Foto: Daniel Loebmann

Anexo 3. *Pseudis minuta.* Foto: Daniel Loebmann

Anexo 4. *Leptodactylus latrans.* Foto: Daniel Loebmann

Anexo 5. *Physalaemus biligonigerus.* Foto: Daniel Loebmann

Anexo 6. *Physalaemus gracilis.* Foto: Daniel Loebmann