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

Dieta e repartição de recursos entre garças reproduzindo em simpatria no Sul do Brasil

Amanda Oliveira Travessas

Orientador: Leandro Bugoni

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Aluno: Amanda Oliveira Travessas
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Dissertação apresentada ao Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais como requisito parcial para a obtenção do título de Mestra em Biologia de Ambientes Aquáticos Continentais.

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Às 14:00 hs (quatorze horas) do dia 10 (dez) do mês de agosto de 2021 (dois mil e vinte e um), via Webconferência (https://conferenciaweb.rnp.br/events/defesa-de-mestrado-amanda-travessas) reuniram-se docentes, discentes e comunidade em geral, para a Defesa Pública de Dissertação de Mestrado da acadêmica Amanda Oliveira Travessas. A dissertação intitulada **"Dieta e repartição de recursos entre garças reproduzindo em simpatria no Sul do Brasil"** foi avaliada pela Banca Examinadora composta pelo Profº Dr. Leandro Bugoni (Orientador), Prof. Dr. Alexandre Miranda Garcia (FURG) e Dr. Dimas Gianuca Neto (BirdLife International). Após a defesa e arguição pública, a Banca Examinadora reuniu-se, para deliberação final, e considerou a acadêmica **APROVADA**. Desta forma, a acadêmica concluiu mais uma das etapas necessárias para a obtenção do grau de **MESTRA EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS**. Nada mais havendo a tratar, às 16:20h **(dezesseis horas e vinte minutos)** foi lavrada a presente ata, que lida e aprovada, foi assinada pelos membros da Banca Examinadora, pela Acadêmica e pelo Coordenador do Curso.

Profº Dr. Leandro Bugoni

Prof. Dr. Alexandre Miranda Garcia

Profº Dr. Dimas Gianuca Neto

Amanda Oliveira Travessas

Profº Dr. Rogério Tubino Vianna

Coordenador do Curso

Dedico esta dissertação aos meus pais e ao meu irmão que sempre estiveram ao meu lado me incentivando. Sem o apoio e o incentivo deles, nada seria possível.

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RESUMO

Estudos de ecologia trófica comparativa informam sobre o uso de recursos alimentares, permitindo estimar a sobreposição e amplitude de nicho entre diferentes espécies em um determinado ambiente. Diversas espécies de aves aquáticas predadoras, como as garças, reproduzem-se em colônias mistas, podendo compartilhar os mesmos recursos obtidos em áreas adjacentes. No estuário da Lagoa dos Patos, área com abundância de recursos e de importância para aves aquáticas, há uma grande colônia de Pelecaniformes, localizada na Ilha dos Marinheiros, Rio Grande do Sul, Brasil. Dentre as sete espécies de Ardeidae presentes na colônia, três das mais abundantes (*Bubulcus ibis*, *Egretta thula* e *Nycticorax nycticorax*), de tamanhos semelhantes e com potencial sobreposição de nicho, ainda não foram estudadas nesse aspecto. Neste sentido, o presente estudo teve como objetivo investigar a dieta e repartição de recursos entre estas espécies, combinando métodos convencionais com análise de isótopos estáveis. Para isso, foram coletados regurgitados e pellets, para análise da dieta, e sangue de ninhegos, para a análise das razões isotópicas do carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$). As presas mais representativas na dieta de *B. ibis* foram insetos e anuros. *Egretta thula* alimentou-se principalmente de peixes e insetos, enquanto o inverso, insetos seguidos por peixes, caracterizou a dieta de *N. nycticorax*. A maior sobreposição de nicho isotópico foi de *N. nycticorax* sobre os nichos de *E. thula* e de *B. ibis*. Diferente do predito nas hipóteses do estudo, o nicho trófico e isotópico de *N. nycticorax* não diferiu das demais garças, possivelmente devido ao hábito de forrageio noturno. Porém, houve elevada sobreposição e esta espécie teve os nichos trófico e isotópico mais amplos. *Bubulcus ibis*, parece ter forrageado principalmente em ambientes terrestres e *E. thula* principalmente nos ambientes límnicos. As três espécies diferiram na dieta, quanto aos principais habitats de forrageio, mas ambas compartilharam recursos alimentares entre si, destacando a importância da abundância de recursos alimentares no entorno de colônias situadas em ambiente estuarino. Ademais, evidenciou-se que pelas espécies possuírem papéis ecológicos com algumas diferenças, podem coexistir na mesma colônia estuarina, compartilhando recursos alimentares. Conclui-se que os estudos sobre a dieta e repartição de recursos de garças, em colônias situadas não apenas na região do estudo, mas também em diversos ambientes aquáticos continentais são fundamentais para o entendimento das interações ecológicas entre consumidores e suas presas.

Palavras-chave: Ardeidae, estuários, colônias de aves aquáticas, espécies simpátricas, isótopos estáveis, Lagoa dos Patos.

ABSTRACT

Studies on the comparative trophic ecology provide information on the use of food resources, allowing us to estimate the overlap and niche width between different species in a given environment. Several species of predatory waterbirds, such as herons, breed in mixed colonies, and may share the same resources obtained in nearby areas. In the Patos Lagoon estuary, an area of abundant resources and importance for waterbirds, there is a large colony of Pelecaniformes, located on Marinheiros Island, Rio Grande do Sul state, Brazil. Among the seven species of Ardeidae present in the colony, three (*Bubulcus ibis*, *Egretta thula* and *Nycticorax nycticorax*), of similar size and with potential niche overlap, have not yet been studied. In this way, the present study aimed to investigate diet and resource partitioning with conventional methods and with stable isotope analysis, as complementary methods. Regurgitates and pellets were collected for diet analysis, and blood from nestlings, for carbon (δ^{13}C) and nitrogen (δ^{15}N) analysis. The most representative prey items in the diet of *B. ibis* were insects and anurans. *Egretta thula* fed mainly on fish and insects, while the reverse, insects followed by fish, characterized the diet of *N. nycticorax*. The greatest isotopic niche overlap was of *N. nycticorax* over the niches of *E. thula* and *B. ibis*. Unlike expected, in the study hypotheses, the trophic and isotopic niche of *N. nycticorax* did not differ from the other herons, possibly due to the nocturnal foraging habit. However, there was high overlap and this species had the broadest trophic and isotopic niches. *Bubulcus ibis*, had the narrowest niche and seems to have foraged mainly in terrestrial environments and *E. thula* primarily in limnic environments. The three species differed in diet, regarding the main foraging habitats, but both shared food resources between themselves, highlighting the importance of the abundance of food resources around colonies located in estuarine environments. Furthermore, it was evidenced that because the species have ecological roles with some differences, they can coexist in the same estuarine colony, sharing food resources. We conclude that studies on the diet and resources shared by herons in colonies located not only in the study region, but also in a range of continental aquatic environments, are fundamental to the understanding of ecological interactions between consumers and their prey.

Key-words: Ardeidae, estuaries, waterbird colonies, sympatric species, stable isotope, Patos Lagoon.

APRESENTAÇÃO

A Dissertação apresenta-se com Resumo, Abstract, Sumário, Lista de Figuras, Lista de Tabelas e pela Introdução Geral, junto às suas seções e Referências Bibliográficas. A seguir, o Capítulo 1 (manuscrito) apresenta-se de acordo com as normas do periódico *Estuaries and Coasts*. Após, encontram-se as figuras e as tabelas referentes ao manuscrito. Por fim, a estrutura da Dissertação conta com Considerações Finais e Perspectivas, finalizando com a seção de Anexos.

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INTRODUÇÃO GERAL

Nicho ecológico e partição de recursos

O nicho ecológico de uma espécie é definido como o hipervolume *n*-dimensional, onde as dimensões são os recursos utilizados e as condições ambientais encontradas em uma comunidade (Hutchinson 1957). Enquanto o nicho trófico define o uso do recurso alimentar e as posições tróficas das espécies através da análise de dieta (Bearhop *et al.* 2004). Estudos sobre ecologia trófica auxiliam no entendimento das interações ecológicas, pois estes informam sobre o uso dos recursos alimentares (Begon *et al.* 2007), além de possibilitarem uma compreensão mais robusta sobre as interações tróficas, como por exemplo, as relações entre predador-presa e como estas relações podem influenciar na estrutura e biologia evolutiva das comunidades (Kojadinovic *et al.* 2008). Com isso, a sobreposição e amplitude de nicho trófico são indicadores bastante utilizados e considerados imprescindíveis para o adequado conhecimento sobre as relações tróficas das espécies (Moser *et al.* 2018).

Recursos podem ser definidos como tudo o que é utilizado pelos organismos (Tilman 1982) e dependendo da quantidade consumida podem ser reduzidos ou não estarem mais disponíveis para outros consumidores (Begon *et al.* 2007). Desta forma, a ocupação de nicho é a utilização de recursos pelas espécies (Bearhop *et al.* 2004). Espécies que apresentam características de vida similares têm maior tendência de ocupar nichos semelhantes, com compartilhamento e potencial competição pelo mesmo recurso (Pianka 2000). A competição ocorre dependendo da disponibilidade do recurso compartilhado pelos organismos, ou seja, se o recurso for muito abundante, mesmo havendo uma grande sobreposição de nicho trófico não haverá competição entre as espécies (Pianka 1981). Outros fatores que influenciam na ocorrência da competição ou na partição de recursos alimentares são a plasticidade trófica, o oportunismo alimentar e as variações espaciais e temporais que irão depender conforme a espécie (Hancock e Kushlan 2010). Portanto, pesquisas sobre a partição de recursos são importantes para que seja possível compreender quais os mecanismos que influenciam na estrutura das comunidades (Faria *et al.* 2018).

Aves aquáticas predadoras

As aves predadoras possuem impacto considerável sobre as presas de ambientes aquáticos, por serem agentes importantes na dinâmica das teias alimentares nesses ambientes e por isso é relevante estudá-las, para compreender, por exemplo, a estrutura das assembleias e fluxo de energia e nutrientes em ecossistemas aquáticos (Steinmetz *et al*. 2003). Além disso, as aves aquáticas são importantes devido às suas interações com outros táxons, beneficiando a biodiversidade no geral, como, por exemplo, no controle populacional de peixes e auxiliando no ciclo de nutrientes (Green e Elmberg 2014). Entre as aves predadoras há a ordem Pelecaniformes que está representada pelas garças, socós, colhereiros e outras aves que estão associadas a ambientes aquáticos rasos continentais ou costeiros (Frederick 2002). A primeira colônia de Pelecaniformes do estuário da Lagoa dos Patos, localizada na Ilha dos Marinheiros, foi descrita por Gianuca (2010), com sete espécies de garças da família Ardeidae (*Ardea alba*, *Ardea cocoi*, *Egretta thula*, *Egretta caerulea*, *Bulbucus ibis*, *Nycticorax nycticorax* e *Nyctanassa violacea*), além do colhereiro (*Platalea ajaja*, Threskiornithidae).

As garças ocorrem em todos os continentes, exceto a Antártica (Martínez-Vilalta e Motis 1992), com um total de 60 espécies da família (Hancock e Kushlan 2010), a maioria dependente de áreas úmidas para se alimentar (Kushlan e Hancock 2005) e reproduzir (Frederick 2002). As garças são predadoras generalistas, com dieta composta por uma quantidade grande e variável de táxons em relação a sua disponibilidade em diferentes habitats e regiões (Fasola 1994). Devido às colônias de garças serem formadas em áreas onde há abundância de alimento próximo, os indíviduos da colônia podem frequentemente explorar os mesmos recursos alimentares (Bildstein *et al*. 1990).

A dieta de três espécies de garças foi estudada previamente nesta colônia na Ilha dos Marinheiros. No estudo sobre a dieta da garça-moura *A. cocoi* confirmou-se que a mesma é generalista, e tem os peixes grandes como os itens alimentares predominantes tanto em ambientes estuarinos como em uma colônia límnica no Taim (Faria *et al*. 2016). No estudo da dieta da garça-branca-grande *A. alba*, constatou-se que a dieta é semelhante à de *A. cocoi*, ou seja, predominantemente piscívora, mas insetos e crustáceos também fazem parte de sua dieta (Britto e Bugoni 2015). A dieta da garça-azul *E. caerulea* é representada principalmente por crustáceos predados em águas rasas do estuário da Lagoa dos Patos, mas também alimenta-se de peixes (Gianuca *et al*. 2012) e em outro estudo, os crustáceos também foram os itens alimentares mais encontrados na dieta de *E. caerulea* (Gianuca, Klein & Vooren 2010).

Estuários e área de estudo

Estuários são zonas de transição entre as zonas límnica e marinha, com alta produtividade, resultando na abundância de nutrientes e de oportunidades para a alimentação (Day-Jr. *et al*. 2013). Essa alta produtividade em estuários também é a responsável pela grande variedade de habitats, utilizados para a alimentação e nidificação pelas aves (Weller 1999; Takekawa *et al*. 2001). O estuário da Lagoa dos Patos abriga uma diversidade considerável de espécies de aves e trata-se de uma área relevante para a conservação dos Pelecaniformes na América do Sul (Dias *et al*. 2017).

A Ilha dos Marinheiros está localizada na região central do estuário da Lagoa dos Patos (32°00'S, 52°09'W; Figura 1) e possui uma área de 40 km^2 (Gianuca *et al*. 2012). Há várias propriedades rurais que realizam a prática de horticultura na zona periférica da ilha, que abriga terras férteis, matas paludosas e matas de restinga. Paisagens arenosas com vegetação esparsa estão presentes de maneira predominante na porção central da ilha, com algumas zonas úmidas, uma lagoa de aproximadamente 7 km e plantações de *Pinus elliottii* (Gianuca 2010). A colônia das aves aquáticas está situada na zona periférica da ilha, voltada para a cidade de Rio Grande (Figura 2), em mata paludosa com predomínio de espécies arbóreas *Sebastiana brasiliensis*, *Sapium glandulosum*, *Erythrina crista-galli*, *Schinus terebinthifolius*, *Salix humboldtiana*, *Ficus cestrifolia* e algumas moitas de bambu do gênero *Bambusia* (Gianuca 2010). A colônia está localizada próxima a hortas, além de estar a 300 m da margem da Lagoa dos Patos (Britto e Bugoni 2015).

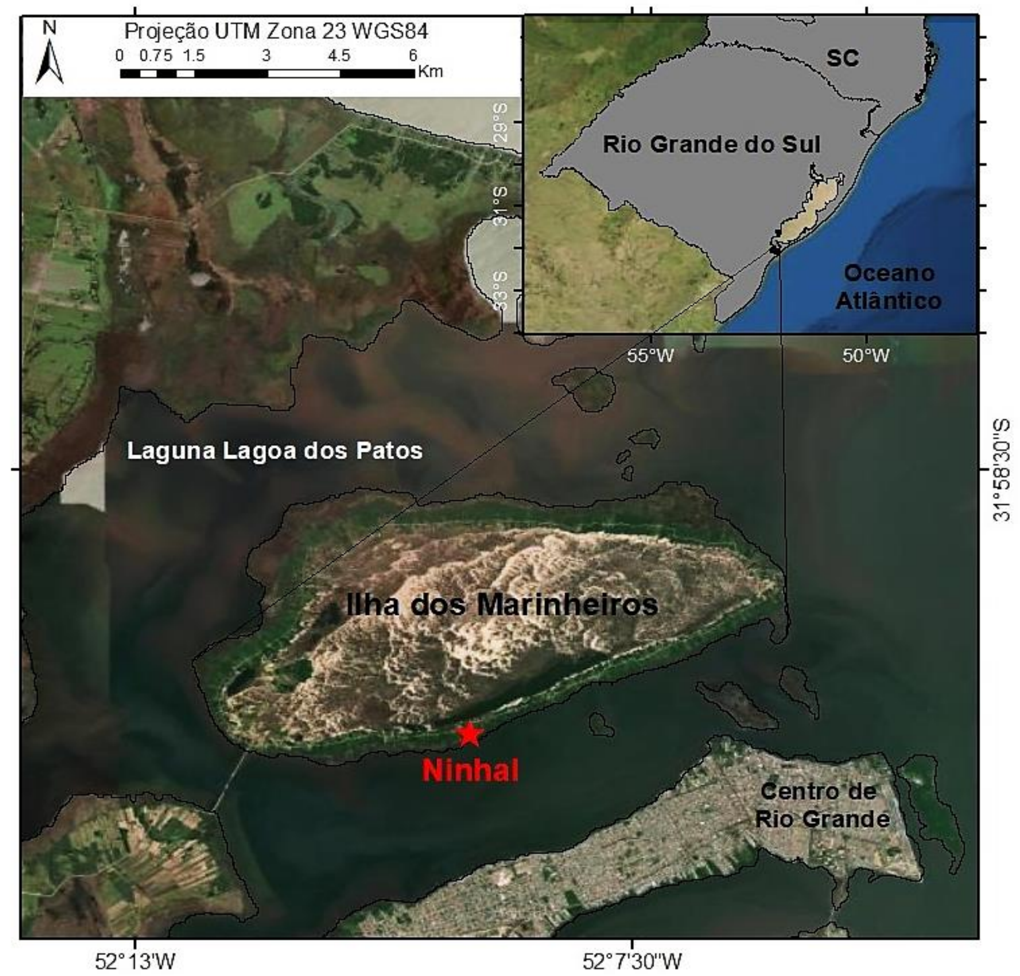

Figura 1. Mapa da Ilha dos Marinheiros, no estuário da Lagos dos Patos, no município de Rio Grande, Rio Grande do Sul, Brasil.

Figura 2. Vista da colônia de Pelecaniformes na Ilha dos Marinheiros, voltada para a Lagoa dos Patos e para a cidade de Rio Grande, RS – Brasil (Foto: A. O. Travessas).

Espécies de aves do estudo

Entre as sete espécies de garças encontradas na colônia na Ilha dos Marinheiros (*A. alba*, *A. cocoi*, *B. ibis*, *E. caerulea*, *E. thula*, *N. violacea* e *N. nycticorax*), três espécies foram selecionadas para o estudo (*B. ibis*, *E. thula* e *N. nycticorax*) por possuírem tamanhos semelhantes e ainda não haver estudos de dieta comparativa na região. A garça-vaqueira *B. ibis* (Figura 3), é uma espécie gregária (de la Peña 1992) e exótica oriunda do continente africano (Ducommun *et al*. 2008) que possui grande capacidade de invadir e ocupar novas áreas em vários lugares do mundo (Nunes *et al*. 2010) e por isso, recentemente, colonizou todos os continentes com exceção do Antártico (Martínez-Vilalta e Motis 1992). A espécie habita pradarias (Bó e Darrieu 1993) alimentando-se principalmente de insetos, além de outras presas que são espantadas com o movimento do gado (Kaufman 1996). Porém, também utiliza o ambiente aquático para a alimentação (Bó e Darrieu 1993) e nidificação em colônias (de la Peña 1992).

Figura 3. Indivíduo adulto de garça-vaqueira (*B. ibis*) com plumagem reprodutiva (Foto: A. O. Travessas).

A garça-branca-pequena *E. thula* (Figura 4), possui ampla distribuição em ambientes aquáticos (Brzorad e Maccarone 2014), ocorrendo em todo o continente americano (Martínez-Vilalta e Motis 1992). Estudos indicam que a espécie se estabelece em áreas com maiores densidades de presas (Master *et al*. 2005), sendo por isso considerada oportunista (Brzorad e Maccarone 2014). Consequentemente, possuiu uma dieta diversificada, alimentando-se principalmente de peixes, porém crustáceos, anfíbios, répteis, insetos e outros animais também são predados (Smith 1997). Possui dieta muito semelhante à dieta da garça-branca-grande *A. alba* (Miranda e Collazo 2006) e, consequentemente, costumam ocupar nichos semelhantes.

Figura 4. Indivíduo adulto de garça-branca-pequena (*E. thula*) alimentando-se (Foto: A. O. Travessas).

O socó-dorminhoco *N. nycticorax* (Figura 5), tem ocorrência na América Central, América do Norte, América do Sul, Europa, África e Ásia (Hancock e Kushlan e 2010) e é uma espécie considerada oportunista que forrageia durante o período noturno ou crepuscular (Maccarone *et al*. 2015) geralmente de forma solitária (Kushlan e Hancock 2005). *Nycticorax nycticorax* também possui uma dieta variada (Kazantzidis e Goutner 2005) ingerindo itens como peixes, anfíbios, répteis, pequenos roedores, morcegos, insetos, aranhas, crustáceos, moluscos, ovos de outras espécies, entre outras presas. Possui uma ampla distribuição, utilizando inclusive os habitats modificados pela ação humana, porém ocorre com maior frequência nas margens com vegetação, em ambientes de água rasa doce ou rios de água salgada, riachos, lagos, lagoas, pântanos e manguezais (Kushlan e Hancock 2005).

Figura 5. Indivíduo adulto de socó-dorminhoco (*N. nycticorax*) durante forrageio (Foto: A. O. Travessas).

Métodos convencionais para estudo de dieta e análise de isótopos estáveis

Para compreender as teias alimentares no ecossistema é necessário ter conhecimento sobre os recursos alimentares utilizados (Twining *et al*. 2020) e para isso utilizam-se métodos convencionais para a análise de dieta de consumidores. Entre os métodos convencionais de estudo de dieta de aves, há a coleta de regurgitados e pellets e ambas representam alimentos ingeridos recentemente. Os regurgitados representam presas parcialmente digeridas (Britto e Bugoni 2015, Figura 6A) os quais permitem a identificação da espécie e medição do tamanho das presas, para após quantificar a contribuição dos diferentes itens alimentares na dieta. Os regurgitados espontâneos são o alimento semidigerido expelido pelos adultos para alimentação dos filhotes, ou em situações de estresse como estratégia de defesa contra predadores. Os pellets, diferente de regurgitados, são restos rígidos do alimento envolto em muco, que não são digeridos e são expelidos regularmente (Britto e Bugoni 2015, Figura 6B).

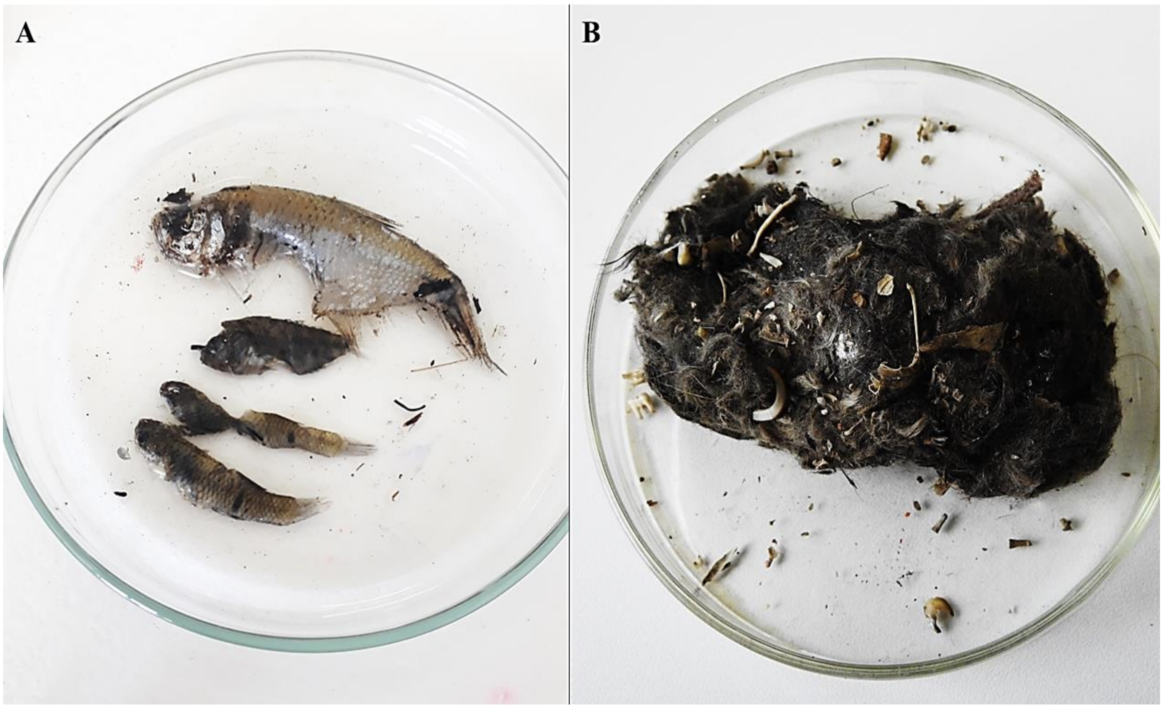

Figura 6. Distinção entre regurgitados (A) e pellets (B) em placas de Petri (Fotos: A. O. Travessas).

Aves aquáticas são utilizadas para responder várias questões ecológicas, entre eles, a partição de recursos tróficos, devido à facilidade de coletar os alimentos regurgitados espontaneamente por elas (Kushlan 1992) ou encontrados próximos aos seus ninhos. Após a coleta é possível identificar os alimentos, determinar a abundância e compreender quais variáveis podem influenciar nos nichos dessas aves (Kushlan *et al*. 1985). Além disso, o conhecimento preciso sobre a dieta dessas aves contribui para a identificação das interações com as atividades humanas em estuários (Ruiz-Guerra e Echeverry-Galvis 2019).

Estudos sobre relações tróficas das aves aquáticas utilizam como metodologia complementar a análise de isótopos estáveis (Britto e Bugoni 2015; Faria *et al*. 2016; Faria *et al*. 2018), pois esta técnica, além de fornecer informações quantitativas sobre a largura (ou amplitude) do nicho trófico dos organismos (Newsome *et al*. 2007), pode confirmar a exploração em comum de recursos (Layman *et al*. 2012). Assim, esta técnica complementa informações de estudos de dieta tradicionais (Karnovsky *et al*. 2012) como, por exemplo, a análise de regurgitados e pellets. Resultados da análise convencional da dieta fornecem ainda informações de base para aprimorar as análises isotópicas como, por exemplo, subsidiar a escolha das fontes alimentares (presas) nos modelos de misturas isotópicas usados para estimar a assimilação das presas nos tecidos dos consumidores (Parnell *et al*. 2013; Britto e Bugoni 2015). Portanto, a combinação de métodos convencionais e análises isotópicas resultam em inferências mais precisas e conclusões mais robustas.

Por estas técnicas convencionais representarem alimentos ingeridos recentemente, a técnica de análise de isótopos estáveis auxilia, ao fornecer dados sobre as proporções de alimentos que foram assimilados em diferentes escalas temporais, porque cada tecido possui diferentes taxas de renovação (Bearhop *et al*. 2002). O sangue total das aves, por exemplo, possui taxa de renovação isotópica relativamente lenta, representando a dieta ingerida e assimilada, considerando semanas anteriores, em um período de 1 a 5 semanas (Auman *et al*. 2011). Diferente do sangue total, o plasma é um tecido que possui a taxa de renovação isotópica mais rápida e informa a dieta ingerida e assimilada, considerando apenas na escala de dias anteriores (Phillips *et al*. 2014).

Os isótopos estáveis mais utilizados para identificar as presas assimilada nos tecidos dos consumidores são os isótopos estáveis de carbono (^{13}C e ^{12}C; expressos pela notação $\delta^{13}C$) e de nitrogênio (^{15}N e ^{14}N; $\delta^{15}N$). Os valores de $\delta^{13}C$ costumam ser utilizados para diferenciar as origens dos recursos alimentares (Barrett *et al*. 2007) uma vez que são influenciados pelo ciclo fotossintético dos produtores na base de cada cadeia trófica e o tipo de dieta (Martinelli *et al*. 2009), enquanto os valores de $\delta^{15}N$ são mais utilizados para descrever o nível trófico dos organismos nos ecossistemas (Martinelli *et al.* 2009), porque possuem maior variação a cada nível trófico (Hussey *et al*. 2014).

O nicho isotópico é estimado a partir da composição isotópica dos consumidores e pode ser comparado com o nicho trófico *n*-dimensional (Newsome *et al*. 2007; Layman *et al*. 2012; Hette-Tronquart 2019). Em outras palavras, o nicho isotópico é utilizado como um indicador para mensurar através da técnica de isótopos estáveis o nicho trófico dos consumidores em estudos de dieta (Layman *et al.* 2012). Entretanto, vale ressaltar as limitações envolvidas na técnica de isótopos estáveis, para que se possa utilizar a técnica idealmente, pois por essa ser uma técnica complementar, a amplitude de nicho isotópico obtida como um parâmetro, pode demonstrar tamanho hiperbólico em comparação ao da amplitude de nicho trófico. Além disso, fontes de táxons distintos podem através dessa técnica, apresentar assinaturas isotópicas idênticas ou semelhantes (Newsome *et al.* 2007), dificultando na interpretação dos modelos de mistura isotópicos. Por isso o conhecimento a respeito das limitações da técnica de isótopos estáveis auxilia para utilizarmos da melhor forma possível essa técnica de forma complementar em estudos de ecologia.

Portanto, a utilização conjunta de métodos como o de análises convencionais de dieta e a análise de isótopos estáveis fornecem informações mais completas sobre a dieta assimilada e a partição de presas entre as espécies. Isso porque pode-se através dos valores de $\delta^{13}C$ e $\delta^{15}N$ compreender a partição de alimento (Power *et al*. 2002) e uso de habitats distintos pelas espécies (Bearhop *et al*. 2004). A união desses métodos pode até mesmo acrescentar informações relevantes, tais como as diferenças intraespecíficas em áreas de alimentação e a especialização da dieta (Newsome *et al*. 2007).

Objetivos

1) Objetivo geral

O objetivo geral do estudo é compreender a ecologia trófica, a repartição de recursos e a sobreposição de nichos tróficos de três espécies de garças (Ardeidae): *B. ibis*, *E. thula* e *N. nycticorax*) no Sul do Brasil.

2) Objetivos específicos:

a) Comparar a dieta das espécies de garças através de dois métodos: a análise de regurgitados e/ou pellets e os modelos de mistura isotópicos;
b) Analisar a posição trófica das espécies através da análise de isótopos estáveis ($\delta^{13}C$ e $\delta^{15}N$);
c) Identificar as amplitudes de nicho trófico das espécies, com base na análise de pellets e regurgitados, e do nicho isotópico, através de isótopos estáveis no sangue;
d) Quantificar a sobreposição de nicho trófico entre as espécies de garças.

Hipóteses

H1. *Bubulcus ibis* terá um nicho distinto por forragear principalmente em ambientes terrestres;

H2. *Egretta thula* será a espécie com o nicho isotópico mais estreito por alimentar-se preferencialmente em ambientes costeiros nas áreas límnicas e estuarinas.

H3. O nicho mais distinto será o de *N. nycticorax* devido ao seu hábito de forrageio noturno, que possibilita uma dieta com presas distintas, comparada às outras espécies com hábito de forrageio diurno.

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CAPÍTULO 1

Manuscrito a ser submetido para a revista *Estuaries and Coasts*.

CAPÍTULO 1

Diet and resource partitioning between egrets and herons in an estuarine colony in southern Brazil

Amanda Oliveira Travessas, Cindy Tavares Barreto, Daiane Carrasco Chaves e Leandro Bugoni

Manuscrito formato de acordo com as normas para a submissão no periódico *Estuaries and Coasts*

Diet and resource partitioning between egrets and herons in an estuarine colony in southern Brazil

Amanda Oliveira Travessas[1,2,*], Cindy Tavares Barreto[1,4], Daiane Carrasco Chaves[3], Leandro Bugoni[1,2]

[1] Waterbirds and Sea Turtles Lab, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande - FURG, Campus Carreiros, 96203-900, Rio Grande, RS, Brazil

[2] The Graduate Program in Biology of Continental Aquatic Environments, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande - FURG, Campus Carreiros, 96203-900, Rio Grande, RS, Brazil.

[3] Laboratory of Genetics, Institute of Biological Sciences, Federal University of Rio Grande - FURG, Campus Carreiros, 96203-900, Rio Grande, Rio Grande do Sul, Brazil.

[4] University of Connecticut, Department of Ecology and Evolutionary Biology, Storrs, Connecticut, USA

[*] Corresponding author: amandatravessas@gmail.com

Abstract Studies on the comparative trophic ecology provide information on the use of food resources, allowing us to estimate the overlap and niche width between sympatric species. Several species of predatory waterbirds, such as herons, breed in mixed colonies, and may share the same resources obtained in nearby areas. Here we studied the feeding ecology of *Bubulcus ibis*, *Egretta thula* and *Nycticorax nycticorax*, which are similar in size and thus with potential niche overlap, nesting together with other five species at Patos Lagoon estuary, southern Brazil. Regurgitates and pellets were collected for diet analysis, and blood from nestlings, for stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) analyses. The most representative prey items in the diet of *B. ibis* were insects and anurans, while *Egretta thula* fed mainly on fish and insects, and insects followed by fish, characterized the diet of *N. nycticorax*. The greatest isotopic niche overlap was of *N. nycticorax* over the niches of *E. thula* and *B. ibis*. Both the trophic and the isotopic niches of *N. nycticorax* did not differ from egrets, possibly due to the nocturnal foraging habit. However, it showed a high overlap with the other species, in addition to the broadest niche. Just as expected about *B. ibis* having a distinct niche by foraging primarily in terrestrial environments, the species showed the narrowest niche and appeared to have used primarily terrestrial environments. The three species had different diets in terms of foraging habitat use, although they all shared some food resources, which can be explained by the abundance of food resources around colonies located in estuaries. Furthermore, it was evidenced that because species of the study have ecological roles with some differences, they can breed sympatrically, sharing food resources obtained nearby. We conclude that studies of diet and resource partitioning among herons in colonies located not only in the study region, but in numerous locations around the world, are critical if we are to add information regarding ecological interactions between consumers and their prey and what factors may influence these interactions. As future perspectives, we suggest considering long-term temporal and spatial variations and investigating which aspects help in the occurrence or not of competition between species in these colonies. In particular for species that breed in estuarine colonies like the one in this study, because these colonies are more stable in terms of availability of food resources compared to those in limnic environments.

Keywords Ardeidae, pellets, regurgitations, stable isotopes, trophic niche

Introduction

Studies diet provide information on species requirements, trophic webs structure and food resource partitioning between species with similar ecological requirements (Britto et al. 2018). The trophic niche is defined by the resources used by a particular species (Bearhop et al. 2004), which can be shared in a community depending on consumer, and their availability in the environment (Begon et al. 2007). By obtaining the trophic niche, parameters such as overlap and niche breadth are used to investigate the common use of resources between different species (Moser et al. 2018).

Predatory birds have a direct relationship with prey in aquatic environments and investigating trophic levels, energy and nutrient fluxes, are relevant (Steinmetz et al. 2003) to understand interactions with other taxa and the role of waterbirds in their habitats (Green and Elmberg 2014). Among common predatory birds in aquatic shallow environments are herons and egrets, generalist birds that capture a wide diversity of prey, such as fish, crustaceans, amphibians, mollusks, small mammals, young birds, reptiles, and various aquatic invertebrates and larvae of various taxa. The feeding habits of these birds could be related to the availability of food in the environment, in turn influencing its breeding success, because during the nesting period the herons depend on superabundant concentrations of available food (Hancock and Kushlan 2010). For these reasons, heronries, i.e. colony of herons, egrets and other large-sized waterbirds, are constantly formed in areas where there is abundant food nearby and, consequently, allowing for the exploitation of food resources within a suitable distances from nests (Bildstein et al. 1990).

Estuaries, as transitional areas between continental and marine environments, have abundant nutrient and food resources (Day-Jr. et al. 2013) and are, therefore, commonly used by birds for feeding, breeding, and roosting (Weller 1999). The Patos Lagoon Estuary in southern Brazil holds the Marinheiros Island, where a colony of Pelecaniformes has been described, with eight species, of which seven are herons - Ardeidae (great egret *Ardea alba*, cocoi heron *Ardea cocoi*, cattle egret *Buculcus ibis*, little blue egret *Egretta caerulea*, snowy egret *Egretta thula*, yellow-crowned night-heron *Nyctanassa violacea* and black-crowned night-heron *Nycticorax nycticorax*; Gianuca 2010). Heron nestlings >3-weeks old have been used as biological models in trophic ecology studies at this site (Gianuca et al. 2012, Britto and Bugoni 2015; Faria et al. 2016). The little blue beron feeds mainly on crustaceans from coastal areas of the estuary and may also feed on fish (Gianuca et al. 2012). The great egret feeds mainly on fish, followed by insects and crustaceans, exploring both the region around the estuarine colony and nearby limnetic regions (Britto and Bugoni 2015). Finally, the cocoi heron has shown a feeding preference for large fish, exploring both the region surrounding the same colony and limnetic areas (Faria et al. 2016). However, there are other heron species that have not yet been studied in the estuary, such as the cattle and snowy egrets and the black-crowned night heron.

The cattle egret is a species native to the Africa that usually feeds in both grassland areas and wetlands (estuaries, mangroves and marshes). It is a cosmopolitan species, except Antarctica and mountainous areas with low temperatures (Hancock and Kushlan 2010). It has a commensalistic relationship with livestock, feeding on insects that are dispersed by the movement of these large mammals. In addition to insects, it may feed on spiders, mollusks, crustaceans, amphibians, reptiles, fish, and small rodents (Kushlan and Hancock 2005; Hancock and Kushlan 2010). The snowy egret is a species present only in the Americas (Martínez-Vilalta and Motis 1992) and widely distributed in aquatic environments. It is considered opportunistic (Brzorad and Maccarone 2014), as it generally inhabits areas with greater prey availability (Master et al. 2005). It has a diverse diet and feeds primarily on fish, but crustaceans, amphibians, reptiles, and insects can also be preyed upon (Smith 1997). The black-crowned night heron is a bird with nocturnal and crepuscular habits (Maccarone et al. 2015) that usually has a diverse diet, consisting of fish, amphibians, small mammals, birds, crustaceans, insects, among other taxa (Kushlan and Hancock 2005). It has a wide distribution, except Oceania and Antarctica (Beletsky et al. 2006). Due to its wide distribution, it is common to find this species in human-altered habitats, including cities, but the species is most often found in vegetated sites, in shallow areas of freshwater environments, such as rivers, estuaries, lakes, streams, ponds, swamps and mangroves (Kushlan and Hancock 2005).

Regurgitates represent partially digested prey, while pellets, unlike regurgitates, are hard remnants of the food wrapped in mucus, which are not digested and are regularly expelled (Britto and Bugoni 2015). Conventional methods for studying diet, such as regurgitates and pellets analyses, used simultaneously with stable isotope analysis, provide more complete information on which prey are important in the consumer's diet and how resource partitioning occurs among different consumers (Layman et al. 2012). As well, the results of regurgitated and pellet analysis can provide background information to improve isotopic analyses, such as in the choice of food sources (prey) in isotopic mixture models (Parnell et al. 2013; Britto and Bugoni 2015), because they allow the identification of the species and measurement of the size of prey or food items, and subsequently allow calculating the contribution of different food items in the diet of each consumer. The prey effectively assimilated by consumers could be inferred by isotopic data, as well as provide information on isotopic niche overlap and breadth, which can be compared to the various trophic niche dimensions (Newsome et al. 2007; Layman et al. 2012; Hette-Tronquart 2019). In this study the diet was studied using complementary methods, i.e., regurgitation and pellet analysis and isotopic mixing models with δ^{13}C and δ^{15}N, which allowed the determination of trophic and isotopic niche breadth and quantification of niche overlap.

Cattle, snowy egrets and black-crowned night heron were chosen for the study because they present similar body sizes and there are no studies on their diet in the region, besides their unknown ecological roles. They are potentially interesting models for investigating niche overlap and food resource partitioning in morphologically similar and phylogenetically close vertebrates. Thus, the objectives of the study were to understand the trophic ecology, resource partitioning, and overlapping of trophic and isotopic niches of three species of small estuarine herons. Based on the ecology of the species it is expected that i) cattle egret will have a distinct niche, for foraging mainly in terrestrial environments compared to other species; ii) snowy egret will have a restricted niche, by feeding preferentially in coastal environments in estuarine and limnetic areas; iii) due to the nocturnal and crepuscular foraging habit, black-crowned night heron will have a distinct diet, with low trophic and isotopic niche overlap.

Methods

Study area

Marinheiros Island (32°00'S, 52°09'W; Fig. 1), is located in the Patos Lagoon Estuary, municipality of Rio Grande, state of Rio Grande do Sul, Brazil. It has an area of 40 km^2, with fertile land used for horticulture, areas of swamp forests and ‘restinga’ forests in the peripheral zone of the island, and dunes in the center of the island, as well as a lagoon and several wetland environments (Gianuca 2010). A colony of Pelecaniformes is located in the peripheral zone of the island, a few meters from the shore of Patos Lagoon (Britto and Bugoni 2015).

Diet sampling

Regurgitates and pellets from nestlings of three ardeids species were collected in or near nests between September 2019 and January 2020 (*B. ibis* n = 6; *N. nycticorax* n = 23; *E. thula* n =

3). Pellets and regurgitates collections were made according to the knowledge of the nestlings' nests or direct observation of the nestlings expelling regurgitates or pellets. Samples found on the ground were very difficult to identify according to the species that expelled them, and therefore they were not collected without knowing for sure which species expelled them. Samples that were possible to identify the species that expelled the regurgitate or pellet were collected and deposited in plastic bags and frozen until analysis. Subsequently, each sample was washed under running water in a 0.075 mm opening metal sieve. Afterwards, prey and food items were separated, quantified, measured, weighed, and identified under a stereoscopic microscope using reference collections and identification guides for comparison (Fisher et al. 2004; Malabarba et al. 2013; Conversani et al. 2017; Hamada et al. 2018).

Sampling for Stable Isotope Analysis (SIA)

In the same period as the regurgitated and pellet collection, nestlings at least 3-weeks old, when blood is expected to represent food delivered by parents rather than egg nutrients (Barreto 2013), were captured, weighed, measured, and individually identified with metal rings provided by CEMAVE/ICMBio to avoid resampling. A total of 0.1 mL of blood was collected (cattle egret n = 15, black-crowned night heron n = 20, and snowy egret n = 8), stored in 1.5 mL vials, and frozen. In the laboratory, the blood samples were freeze-dried, homogenized, weighed, placed in tin capsules, and analyzed in an isotope ratio mass spectrometer, coupled to elemental analyzer, at the Integrated Analysis Center (CIA) of the Federal University of Rio Grande (FURG), Rio Grande city, Brazil, using the equation in delta notation (δ) of Bond and Hobson (2014):

$$\delta^{13}\text{C ou } \delta^{15}\text{N (‰)} = \left(\frac{R\text{sample}}{R\text{standard}}\right)\text{-1} \qquad \text{eq. 1}$$

where R_{sample} is the ratio between the heavy and light isotopes of the sample and R_{standard} is the ratio between the heavy and light isotopes of the standard used at CIA-FURG, which are glutamic acid and caffeine, for both carbon and nitrogen.

In addition to blood samples, muscle samples from potential prey found in regurgitates and pellets or in environments near the colony were also collected. Insects of the orders Orthoptera and Coleoptera and spiders were collected with tweezers, whole fish or fish with limited digestion from regurgitates were collected manually. From these samples, muscle fragments or whole organisms were prepared and placed in the Soxhlet apparatus for about 6 h with petroleum ether for lipid removal (Post et al. 2007; Elliott, Roth and Crook 2017; Cloyed et al. 2020). Subsequently, the samples were lyophilized, macerated, homogenized, and weighed in tin capsules and analyzed in the same way as the blood. For taxonomic groups not found in regurgitated samples or not possible to extract the muscle due to advanced digestion, isotopic data found in the bibliography (Santi-Júnior 2013) and unpublished data from the Waterbirds and Sea Turtles Laboratory (LAATM-FURG) were used.

Diet data analysis

For the diet analysis we calculated for cattle egret and black-crowned night heron the frequency of occurrence (FO%), which is the presence of the taxon in relation to the total number of samples analyzed of each species, and the average percentage contribution in number of each food item (PN%), considering only the samples in which the item was found, according to the equation in Brown et al. (2012):

$$PN\% = \frac{\sum_{j=1}^{n} N\%_{ij}}{n_i} \qquad \text{eq. 2}$$

Where:

$N\%$ = contribution in number of each taxon;

n_i = number of samples in which the food item was found;

j = sample;

i = prey-specific.

The percentage contribution in reconstituted mass of each food item (PM%) was also calculated, considering only the samples where the item was found. Prey mass was reconstructed using allometric equations based on measurements of fish otoliths, or from mean values of similar sized prey (as in Bugoni and Vooren 2004). The equation for PM% is:

$$PM\% = \frac{\sum_{j=1}^{n} M\%_{ij}}{n_i} \qquad \text{eq. 3}$$

Where:

$M\%$ = mass contribution of each taxon;

n_i = number of samples in which the food item was found;

j = sample;

i = prey-specific.

Then, after obtaining the values of PN%, PM% and FO%, the prey-specific relative importance index (PSIRI%) was calculated, according to Brown et al. (2012):

$$PSIRI\% = \frac{FO\% \, (PN_i\% + PM_i\%)}{2} \qquad \text{eq. 4}$$

Where:

$N_i\%$ = contribution in number of each taxon;

M_i % = mass contribution of each taxon;

i = prey-specific.

For calculating the trophic niche overlap, the Morisita-Horn similarity index proposed by Horn (1966) was used, which is based on the contribution in number of each taxon (N%), with the following equation:

$$CH = \frac{2\, \Sigma n_i \, p_{ji} \, pik}{\Sigma n_i p^2_{ij} + \Sigma n_i p^2_{ik}}$$

eq. 5

Where:

CH = Morisita-Horn similarity index;

p_{ij} = proportion of resource i on the total resources used by species j;

p_{ik} = proportion of resource i on the total resources used by species k;

n = total number of resources used.

The Morisita-Horn similarity index ranges from 0 to 1, and the closer to 1, the greater the overlap.

The trophic niche range was also calculated based on the contribution in number of each taxon using Levin's standardized Index (Magurran 2004), according to the following equation by Hurlbert (1978):

$$Bs = \frac{(\Sigma p_i^2)^{-1} - 1}{(n - 1)} \quad \text{eq 6}$$

Where:

Bs = Levin's standardized Index;

p_i^2 = proportion of the resource belonging to resource category i;

n = total number of food items consumed.

The standardized Levin Index also ranges from 0 to 1, and the closer to 1, the greater the range of trophic niche.

Isotopic data analysis

Bayesian isotopic mixing models were generated using the 'simmr' package (Parnell et al. 2010) in the R environment, with the isotopic values of δ^{13}C and δ^{15}N from the blood of the consumer species and the most common potential prey in the diet (Table 1). The trophic discrimination factors (TDF) of the consumers used were -0.3 ± 0.5‰ and 2.61 ± 0.5‰ for δ^{13}C and δ^{15}N, respectively. These TDFs are values derived from experiments with penguins, predominantly piscivorous birds, fed whole fish (*Aptenodytes patagonicus*: δ^{13}C = -0.81‰ and δ^{15}N = 2.07‰; and *Eudyptes chrysocome*: δ^{13}C = 0.20‰ and δ^{15}N = 2.72‰; in Cherel et al. 2005) and with puffins (*Fratercula cirrhata*: δ^{13}C = -0.30 and δ^{15}N = 3.05‰; Williams et al. 2007). These TDFs have been used in previous studies with isotopic modeling of the diet of cocoi heron (*A. cocoi*) in the region (Faria et al. 2016) and the diet of kelp gull (*Larus dominicanus*; Silva-Costa and Bugoni 2013) and were the values that best fit the mixing model developed in the present study. Therefore, the TDFs selected for the studies were the same as those used by Faria et al. (2016) and Silva-Costa and Bugoni (2013). In addition to resulting in more realistic models, the species studied in both, are closer taxonomically, with similar body size, diet also based on a range of vertebrate and invertebrate prey, thus similar to the species in the present study.

Additionally, isotopic niche breadth and isotopic niche overlap considering absolute values (‰) and later calculated the relative area (%) among heron species were calculated using SIBER (Stable Isotope Bayesian Ellipses in R) package in R environment (Jackson et al. 2011). Standard ellipse areas adjusted for small sample sizes (SEAc) were used to determine the breadth and overlap of isotopic niches of consumers.

Results

Diet and trophic niche based on conventional methods

The cattle egret diet was composed mainly of insects, spiders, and anurans, but fish, crustaceans, and ticks were also found among the food items. The diet of black-crowned night heron was more varied, consisting of insects, crustaceans, fish, spiders, tadpoles, and heron chicks ($n = 2$), including cannibalism. Only three regurgitations of snowy egret were found, with 5 fish specimens: *Phalloceros caudimaculatus* ($n = 1$), *Cnesterodon decemmaculatus* ($n = 1$), unidentified fish (3) and 3 insects (Belostomatidae = 2 and Odonata = 1). The diet of this species was not analyzed in detail due to limited sampling.

The most frequently found prey items in the diet of cattle egret were insects (FO% = 100), anurans (83.3%) and arachnids (83.3%). The largest percentage contribution in reconstituted mass (PM%) in the diet of cattle egret were anurans (52.1%), followed by insects (27.4%). Black-crowned night heron had insects (FO% = 78.2) and fish (FO% = 73.9) as the most frequent prey in the diet and the highest contribution in reconstituted mass were bird chicks (PM% = 44.1) and fish (PM% = 29.1). According to the prey-specific index of relative importance (PSIRI%), the diet of cattle egret was composed mainly of insects (43.2%), anurans (26.6%) and arachnids (13%), although it also fed on fish and crustaceans. This index indicated insects (24.3%) and fish (15.2%) as the main components in the diet of black-crowned night herons, although it has a very generalist diet, also feeding on anurans, birds, spiders and crustaceans (Table 2). The trophic niche ranges confirm this result, as indicated by Levin's standardized Index, in which Bs = 0.12 was calculated for cattle egret and 0.63 for black-crowned night heron. The overlap between these two species, using the Morisita-Horn similarity index, was CH = 0.67.

Prey assimilation and isotopic niche based on SIA

According to the stable isotope analysis, mean $\delta^{13}C$ values were -22.7‰ in the blood of cattle egret chicks, -22.9‰ in blood of black-crowned night heron, and with the lowest value of -23.5‰ in chick blood of the snowy egret. For $\delta^{15}N$, the highest values were from the blood of black-crowned night heron chicks (9.9‰) and the lowest from cattle egret (7.2‰). As for the mean variation in isotopic values of consumer blood, the highest variation of $\delta^{13}C$ and $\delta^{15}N$ occurred in the blood of black-crowned night heron, and the lowest in snowy egret chicks (Table 1).

Mixing models indicated assimilation of anurans as the main source (credibility interval CI 95% = 0.82), in the blood of cattle egret chicks, followed by insects (95% CI = 0.58) (Fig. 2, 3a). For black-crowned night heron, *Callinectes* crabs (95% CI = 0.49) and estuarine fish, with values similar to *Atherinella brasiliensis*, were the most assimilated source in blood (95% CI = 0.40; Fig. 3b). Finally, insects (95% CI = 0.53) and freshwater fish (95% CI = 0.51) were the most assimilated prey sources by snowy egret (Fig. 3c).

The largest isotopic niche breadth was found for black-crowned night heron (SEAc = 93.4) followed by far by cattle (29.6) and snowy (7.8; Table 3; Fig. 4) egrets. Cattle egret had 73.8% of its area overlapped by black-crowned night heron and 17% by snowy egret. Black-crowned night heron overlapped 100% with the niche of snowy egret, but snowy egret overlapped only 8.3% with the niche of black-crowned night heron (Table 3; Fig. 4).

Discussion

The three ardeids studied in southern Brazil had substantial overlap in their diet and trophic niches. Insects were the most important food items in the diet of cattle egret and black-crowned night heron according to pellet and regurgitation analysis, while anurans and fish were the most assimilated sources according to SIA for all three species. Black-crowned night heron was the species with the most generalist feeding habit and most diverse diet, and also with larger trophic niche overlap with cattle egret, and isotope niche overlap with cattle and snowy egrets. The abundance of food resources in the Patos Lagoon Estuary seems to allow birds sharing resources and foraging habitats (Dias et al. 2017), as demonstrated by niche overlap, while breed in sympatry with low competition for food resources. Just as the coexistence and reproductive success of herons in the Marinheiros Island colony may be influenced by variations in prey in nearby areas. Because these species have been shown to utilize and share food resources available throughout the heterogeneous landscape surrounding the colony. Possibly because the diet of birds can fluctuate annually as influenced by variations in the effects of environmental conditions (Dias et al. 2017).

Diet of heron nestlings inferred by pellet and regurgitation analysis

Pellets and regurgitate analysis provide detailed information about the species and eventually the size of prey consumed (Britto and Bugoni 2015), which allowed us to determine that species have differences in their use of food resources. Cattle egret had a diet composed especially of terrestrial or semi-aquatic prey, such as insects and anurans. Other studies, such as in *La Albufera* Nature Park, Spain (Antón-Tello et al. 2021) and in a wetland in northeastern Iran (Ashoori et al. 2017), also demonstrated predominantly insectivorous diets, but with a high diversity of taxa. In addition to insects (Diptera, Orthoptera, and Hemiptera), other prey items were also frequent, such as small anurans and spiders, indicating using mainly terrestrial environments near colonies. However, aquatic prey, such as killifishes, and semiaquatic prey, such as giant waterbugs, although less consumed, confirm the generalist feeding habit of cattle egret, demonstrating that the species can explore other environments, in addition to terrestrial ones.

Black-crowned night heron had a generalist feeding habit, with insects (Coleoptera, Hemiptera and Hymenoptera) and fish of various sizes, such as Brazilian silverside (*Atherinella brasiliensis*) as the main prey, feeding on prey from terrestrial, limnetic, and estuarine environments. Insects are among the most consumed prey items by the species elsewhere, for example in the Axios Delta in Greece (Kazantzidis and Goutner 2005). Fish were the most important prey in a wetland area in northeastern Iran (Ashoori et al. 2017) and amphibians in rice plantation in northeastern Italy (Cardarelli et al. 2017). In other words, the species usually feeds on abundant and easily captured prey (Davis 1993). Fish of varying sizes can be found in both estuarine and limnetic areas on or near our study site (Quintela et al. 2018), also exploited by other herons, such as great egret (Britto and Bugoni 2015) and de cocoi heron (Faria et al. 2016). Heron chicks, tadpoles, crustaceans, and spiders were also among the items consumed by black-crowned night heron in our study. Cannibalism of adults on nestlings and of juveniles preying on nestlings, reported in other studies (e.g. Brussee et al. 2017) demonstrates the opportunistic feeding behavior of nigh herons. Juveniles feeding on nestlings of their own species have been reported in California, United States (Brussee et al. 2017), and nestlings ingested by juveniles as they fell from nests in a mixed colony of snowy egrets and black-crowned night heron in Louisiana, United States (Riehl 2006). Among the taxa found from the diet of the species in the present study, some had already been reported in South America, such as in the *Paraná* River, Argentina (Quiroga et al. 2013). Other studies have shown diet with high prey diversity, but with distinct main prey, such as adult anurans and tadpoles in a rice field in Italy (Cardarelli et al. 2017). The more diverse taxa in the diet of black-crowned night heron compared to the other species in the present study can be explained due to its opportunistic feeding behavior (Kazantzidis and Goutner 2005), showing a tendency to feed in varied environments.

For snowy egret, freshwater killifishes (*Phalloceros caudimaculatus* and *Cnesterodon decemmaculatus*), Odonata and Belostomatidae, both aquatic insects, were the main consumed prey. Even with the limited sampling, results were similar to previous studies in coastal limnetic environments, such as in the Colombian Caribbean, and on Cajual Island, northern Brazil (Martínez 2010; Ruiz-Guerra and Echeverry-Galvis 2019). Another study demonstrated similar results as snowy egret tended to use more limnetic environments compared to the other heron and egret species. But when it comes to the most important prey in the diet, the results were different from the few samples found in our study, with fishes being the most important prey in the diet of snowy egret, followed by crustaceans (Gianuca, Klein and Vooren 2010).

This study had been demonstrated that insects are important food items for all three heron species. Therefore, the current decline in the abundance and diversity of these prey items (Wagner et al. 2021), may play a role in ecosystem functioning. In addition to the concern about possible future impacts on insect abundance and diversity at the global scale reported in Wagner et al. 2021, due to the numerical importance of these prey items to the diet of the heron and egret species in the study, we are concerned about the decline in abundance of *Atherinella brasiliensis* in the Patos Lagoon estuary (Silva et al. 2021). This is because, *A. brasiliensis* represents one of the food items with the highest contribution in reconstituted mass to black-crowned night heron in the present study.

Diet inferred by SIA

The sources most assimilated by cattle egret in the isotope mixing model were anurans, followed by insects, contrary to a recent isotopic study in Spain (Antón-Tello et al. 2021), where insects predominated, followed by anurans. Through the most assimilated sources in our model, cattle egret was shown to capture mainly prey from terrestrial environments, similar to the results found in the analysis of pellets and regurgitates.

Unlike cattle egret, black-crowned night heron assimilated more sources from estuarine environments, such as fish and crustaceans, in addition to other sources from terrestrial and limnetic environments. In the present study the species seems to use isotopically varied habitats, diverging from the species in the United States, where a narrower $\delta^{13}C$ and $\delta^{15}N$ isotopic niche in feathers were found (Craig et al. 2015).

Snowy egrets had greater assimilation of prey from limnetic environments, such as fish, showing little variation in $\delta^{13}C$ and $\delta^{15}N$ values in blood. The results suggest that individuals foraged at locations close to the colony, as was shown in egg isotope analysis in the Colorado River, in Mexico (Herzka et al. 2013).

Niche breadth and overlap

The overlap of the trophic niches of cattle egret and black-crowned night heron was due to the consumption of common prey in terrestrial environments, such as insects and spiders. As for the isotopic niche, black-crowned night heron showed a high overlap with the snowy egret niche (100%), followed by the overlap with the cattle egret niche (73.8%). This could had occurred because the species assimilated varied sources, compared to other ardeids, resulting in a broad niche, and thus, overlapping with other species studied.

SIA, coupled to diet analysis, confirmed that black-crowned night heron had the widest range of trophic and isotopic niches, and the greatest variety of taxa preyed upon. This refutes our prediction that the niche of black-crowned night heron would be the most distinct among the species, since it feeds in nocturnal and crepuscular periods, providing opportunities to capture different prey (Quiroga et al. 2013), which would lead to low trophic and isotopic niche overlap. Cattle egret showed narrow trophic and isotopic niches due to the exploitation of predominantly terrestrial prey, supporting our hypothesis that the species would have a distinct niche by foraging primarily in terrestrial environments. Finally, the narrow isotopic niche was that of snowy egret, as expected, due to a preference for prey from limnetic environments, which demonstrated a considerable distinction in sources assimilated, compared to other species in the study.

Conclusion

It was noticeable from the information in this study, based on conventional diet analysis and SIA, that the cattle egret, snowy egret, and black-crowned night heron share a range of upper-level taxa, but showed differences in prey species ingested, and sources assimilated. In addition, the species used distinct foraging environments, eventually sharing prey, resulting in the trophic and isotopic niche overlaps. The coexistence of these species may have occurred in the study colony because these species are morphologically, ecologically, and phylogenetically close. Consequently, species of herons and egrets that breed in sympatry may distinguish in at least one of the niche dimensions to allow coexistence between them, when considering the temporal, spatial dimensions, and their trophic niches, during the reproductive period (Ye et al. 2021). This confirms that the small egrets and herons coexisting in the colony shared food resources and/or assimilated common sources during the studied breeding period. But it is worth remembering that SI technique doesn't distinguish species or prey sizes, but provide information on sources assimilated by the consumers, complementary dietary methods is key for a better understanding of food web relationships (Layman et al. 2012; Karnovsky et al. 2012). In other words, there may be prey with the same isotopic values, but which are taxonomically distinct. Or prey common in regurgitates and pellets due to hard body pieces, but with low contribution for nutrients effectively assimilated.

The present study was the first to compare the diet and investigate resource partitioning among the three ardeid species (cattle egret, snowy egret, and black-crowned night heron) with conventional methods and SIA. Therefore, the importance of progressively obtaining information about the trophic ecology of predatory waterbirds in estuarine and freshwater colonies had been demonstrated. Further understanding on the consumer-prey relationships, movements between environments and the role of waterbirds for the energy and nutrients flow in food webs are needed to advance the current scientific knowledge to guide conservation. Finally, it is worth noting how important information from long-term studies on the diet, abundance, and reproductive parameters of herons and egrets are for us to understand how these species may respond to inter-annual variations in food availability related to environmental conditions.

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Declarations

Ethical and sampling approval This study had been approved by ICMBio/SISBIO (sampling permit No. 72947-1) and Ethical Comittee on Animal Use, issued by CEUA-FURG (Certificate No.P028/2020).

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Table 1 Values of $\delta^{13}C$ and $\delta^{15}N$ and the standard deviation in the blood of consumers: cattle egret (*Bubulcus ibis*), black-crowned night heron (*Nycticorax nycticorax*) and snowy egret (*Egretta thula*) and potential prey used in the Bayesian mixing models

Birds and potential sources	Species/taxon	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	N	Reference
Chick blood	*Bubulcus ibis*	-22.47 ± 2.53	7.17 ± 0.78	15	This study
	Egretta thula	-23.77 ± 2.26	8.25 ± 0.35	8	This study
	Nycticorax nycticorax	-22.87 ± 2.64	9.91 ± 1.91	20	This study
Anurans		-22.94 ± 1.15	4.22 ± 0.82	5	This study
	Pseudis minuta	-24.52	5.18	1	This study
	Scinax squalirostris	-23.51	4.32	1	This study
	Physalaemus gracilis	-22.22 ± 0.71	3.87 ± 0.83	3	This study
Estuarine fishes	*Atherinella brasiliensis*	-21.14 ± 0.38	9.74 ± 2.99	3	This study
Freshwater fishes		-27.32 ± 0.03	6.36 ± 0.33	2	This study
	Cichlasoma portalegrense	-27.29	6.60	1	This study
	Synbranchus marmoratus	-27.35	6.13	1	This study
Crustaceans	*Callinectes* sp.	-19.00 ± 0.72	8.36 ± 1.63	2	This study
Spiders	Theridiidae	-16.53	3.77	1	Santi-Júnior (2013)
Insects		-25.84 ± 0.59	4.57 ± 0.71	3	This study
	Coleoptera	-26.26	5.08	1	This study
	Odonata	-25.34	5.09	1	LAATM[1] Database
	Orthoptera	-25.42	4.07	1	LAATM[1] Database

[1]Waterbirds and Sea Turtles Lab

Table 2 Diet composition of nestlings of the cattle egret (*Bubulcus ibis*) and the black-crowned night heron (*Nycticorax nycticorax*) at the colony of Marinheiros Island, in the city of Rio Grande, Rio Grande do Sul, Brazil, between September 2019 and January 2020. FO% = Frequency of occurrence; PN% = Contribution in prey-specific numbers; PM% = Contribution in prey-specific reconstituted mass; PSIRI% = Prey-specific relative importance index

Food items	*Bubulcus ibis*				*Nycticorax nycticorax*			
	FO%	PN%	PM%	PSIRI%	FO%	PN%	PM%	PSIRI%
Anurans	**83.3**	**12.4**	**52.1**	**26.6**	**4.3**	**5.8**	**0.06**	**0.1**
Hylidae	66.6	9.7	41.7	17.0	-	-	-	-
Hypsiboas pulchellus	16.6	0.3	0.3	< 0.1	-	-	-	-
Pseudis minuta	33.3	5.8	14.3	3.3	-	-	-	-
Scinax squalirostris	33.3	3.7	27.1	5.0	-	-	-	-
Leiuperidae	50.0	2.1	1.6	0.9	-	-	-	-
Physalaemus gracilis	33.3	1.8	1.4	0.5	-	-	-	-
Pseudopaludicola falcipes	16.6	0.3	0.2	< 0.1	-	-	-	-
Leptodactylidae	33.3	0.5	8.8	1.5	-	-	-	-
Leptodactylus gracilis	16.6	0.3	0.7	< 0.1	-	-	-	-
Leptodactylus latrans	16.6	0.3	8.0	0.7	-	-	-	-
Unidentified	-	-	-	-	4.3	5.8	0.06	0.1
Fishes	**16.6**	**5.3**	**4.8**	**0.8**	**73.9**	**12.1**	**29.1**	**15.2**
Atherinopsidae	-	-	-	-	30.4	3.9	23.1	4.1
Atherinella brasiliensis	-	-	-	-	30.4	3.9	23.1	4.1
Characidae	-	-	-	-	8.7	0.8	0.2	< 0.1
Astyanax sp.	-	-	-	-	4.3	0.4	0.2	< 0.1
Pseudocorynopoma doriae	-	-	-	-	4.3	0.4	0.04	< 0.1
Poeciliidae	16.6	5.3	4.8	0.8	8.7	0.8	0.1	< 0.1
Phalloceros caudimaculatus	16.6	5.3	4.8	0.8	8.7	0.8	0.1	< 0.1
Cichlidae	-	-	-	-	4.3	0.4	0.09	< 0.1
Sciaenidae	-	-	-	-	4.3	0.4	0.3	< 0.1
Stellifer sp.	-	-	-	-	4.3	0.4	0.3	< 0.1
Ariidae	-	-	-	-	4.3	0.8	0.8	< 0.1

Genidens barbus	-	-	-	-	4.3	0.8	0.8	< 0.1
Loricariidae	-	-	-	-	8.7	0.8	0.8	< 0.1
Loricariichthys anus	-	-	-	-	8.7	0.8	0.8	< 0.1
Synbranchidae	-	-	-	-	8.7	0.8	1.0	< 0.1
Synbranchus marmoratus	-	-	-	-	8.7	0.8	1.0	< 0.1
Unidentified	-	-	-	-	17.4	2.3	2.8	0.4
Birds	-	-	-	-	**8.7**	**0.8**	**44.1**	**1.9**
Ardeidae	-	-	-	-	8.7	0.8	44.1	1.9
Bubulcus ibis (chicks)	-	-	-	-	4.3	0.4	20.7	0.4
Nycticorax nycticorax (chicks)	-	-	-	-	4.3	0.4	23.4	0.5
Insects	**100.0**	**58.9**	**27.4**	**43.2**	**78.3**	**57.0**	**5.2**	**24.3**
Blattaria	50.0	2.9	1.4	2.6	4.3	0.4	0.04	< 0.1
Blattidae	16.6	1.3	0.2	0.1	-	-	-	-
Coleoptera	66.6	13.9	2.1	5.3	56.5	41.0	3.8	12.6
Coccinelidae	16.6	0.3	0.2	< 0.1	-	-	-	-
Eriopis connexa	16.6	0.3	0.2	< 0.1	-	-	-	-
Hydrophilidae	-	-	-	-	4.3	0.4	0.04	< 0.1
Scarabaeidae	-	-	-	-	4.3	0.4	0.04	< 0.1
Diptera	66.6	15.3	9.3	8.1	-	-	-	-
Brachycera	66.6	7.9	5.1	4.3	-	-	-	-
Dolichopodidae	16.6	0.5	0.3	< 0.1	-	-	-	-
Muscidae	16.6	0.5	0.3	< 0.1	-	-	-	-
Sarcophagidae	33.3	5.0	3.0	1.3	-	-	-	-
Tabanidae	16.6	0.8	0.5	0.1	-	-	-	-
Nematocera	16.6	0.5	0.3	< 0.1	-	-	-	-
Tipulidae	16.6	0.5	0.3	< 0.1	-	-	-	-
Hemiptera	50.0	2.6	1.4	1.0	39.1	6.2	0.6	2.6
Auchenorrhyncha	-	-	-	-	21.7	2.7	0.2	0.3
Cercopidae	-	-	-	-	8.7	1.6	0.1	< 0.1
Heteroptera	33.3	1.0	0.6	0.3	17.3	1.9	0.2	0.18
Belostomatidae	33.3	0.5	0.3	0.1	13.0	1.6	0.1	0.1
Belostoma sp.	16.6	0.3	0.2	< 0.1	4.3	0.8	0.07	< 0.1

Pentatomidae	16.6	0.5	0.3	< 0.1	-	-	-	-
Naucoridae	-	-	-	-	4.3	0.4	0.04	< 0.1
Hymenoptera	50.0	1.0	0.8	0.5	30.4	7.0	0.6	1.1
Formicidae	16.6	0.3	0.2	< 0.1	8.7	0.8	0.07	< 0.1
Camponotus sp.	-	-	-	-	4.3	0.4	0.04	< 0.1
Solenopsis invicta	16.6	0.3	0.3	< 0.1	-	-	-	-
Neuroptera	33.3	0.8	0.6	0.2	-	-	-	-
Odonata	33.3	2.4	1.4	0.6	4.3	1.6	0.1	< 0.1
Coenagrionidae	16.6	0.5	0.3	< 0.1	-	-	-	-
Libellulidae	33.3	1.8	1.3	0.5	4.3	1.6	0.1	< 0.1
Orthoptera	66.6	20.0	12.2	10.6	4.3	1.2	0.1	< 0.1
Acrididae	50.0	3.9	2.4	1.6	-	-	-	-
Aleuas sp.	16.6	0.3	0.2	< 0.1	-	-	-	-
Gryllidae	50.0	7.4	4.5	3.0	-	-	-	-
Tettigonidae	33.3	5.3	1.6	1.1	-	-	-	-
Romaleidae	16.6	0.5	0.3	< 0.1	4.3	1.2	0.1	< 0.1
Alcamenes clarazianus	16.6	0.5	0.3	< 0.1	4.3	1.2	0.1	< 0.1
Arachnids	**83.3**	**16.6**	**14.7**	**13.0**	**17.3**	**6.6**	**0.9**	**0.6**
Araneae	83.3	15.5	14.3	12.4	17.3	6.6	0.9	0.6
Ixodidae	33.3	1.0	0.4	0.2	-	-	-	-
Rhipicephallus sanguineus	16.6	0.8	0.3	0.1	-	-	-	-
Crustaceans	**33.3**	**1.6**	**1.0**	**0.4**	**21.7**	**17.6**	**20.6**	**4.1**
Decapoda	-	-	-	-	13.0	4.3	18.1	1.4
Grapsidae	-	-	-	-	4.3	0.4	0.4	< 0.1
Portunidae	-	-	-	-	8.7	1.6	17.8	0.8
Callinectes sp.	-	-	-	-	8.7	1.6	17.8	0.8
Isopoda	33.3	1.6	1.0	0.4	8.7	13.3	1.2	0.6
Ligiidae	33.3	1.6	1.0	0.4	8.7	13.3	1.2	0.6
Ligia exotica	33.3	1.6	1.0	0.4	8.7	13.3	1.2	0.6
	n = 6	N = 380			*n* = 23	N = 256		

Table 3 Bayesian ellipse area for small samples (SEAc) and isotopic niche overlap and percentage range obtained with the $\delta^{13}C$ and $\delta^{15}N$ values of each ardeid species on Marinheiros Island, southern Brazil, from September 2019 to January 2020

Species (Species number)	SEAc	Species 1	Species 2	Overlap area (‰^{2})	% of Sp. 1 area	% of Sp. 2 area
B. ibis (1)	29.57	1	2	21.83	73.84	23.37
N. nycticorax (2)	93.42	1	3	5.03	17.02	64.80
E. thula (3)	7.77	2	3	7.77	8.31	100.00

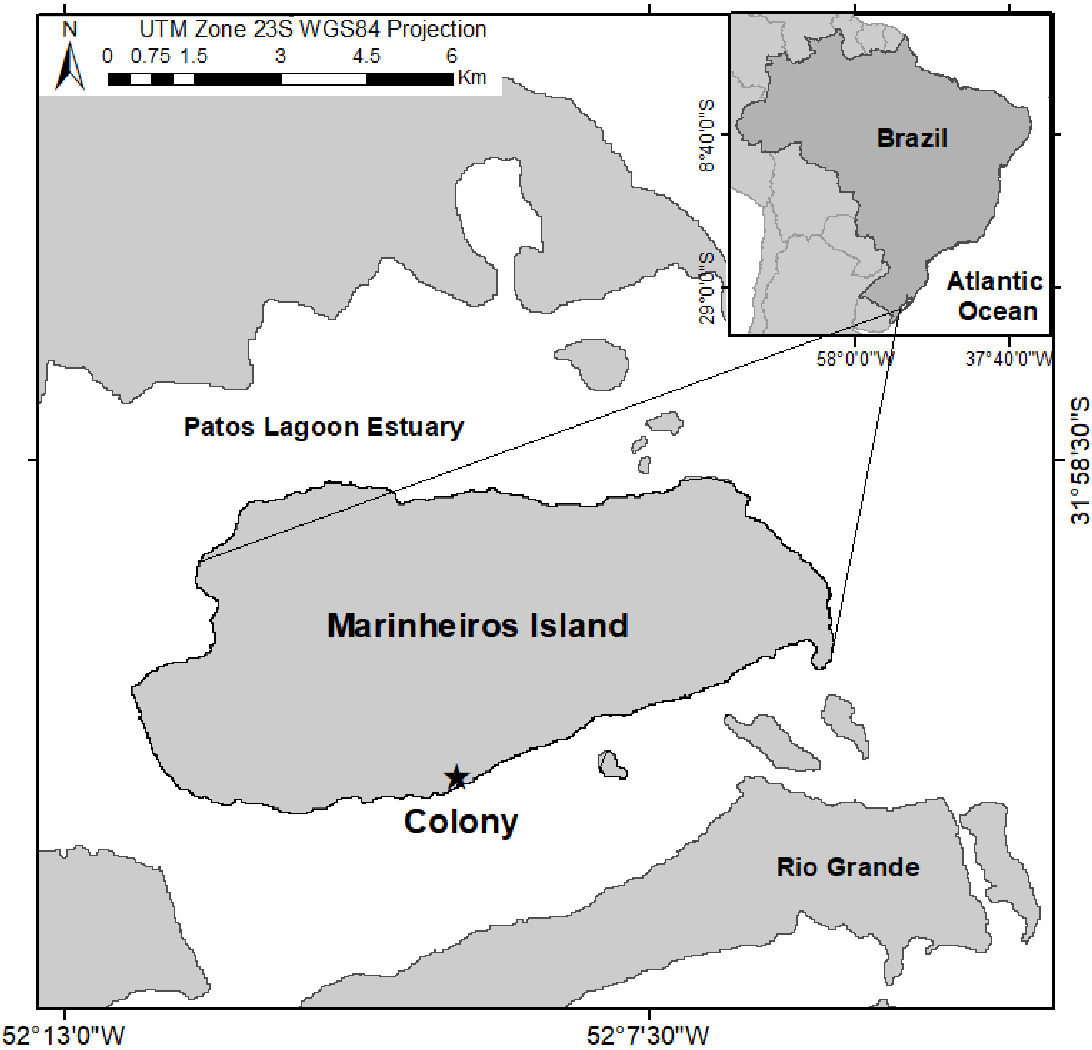

Fig. 1 Marinheiros Island, Patos Lagoon Estuary, city of Rio Grande, Rio Grande do Sul, Brazil. The star indicates the collection point, located in the Pelecaniformes colony

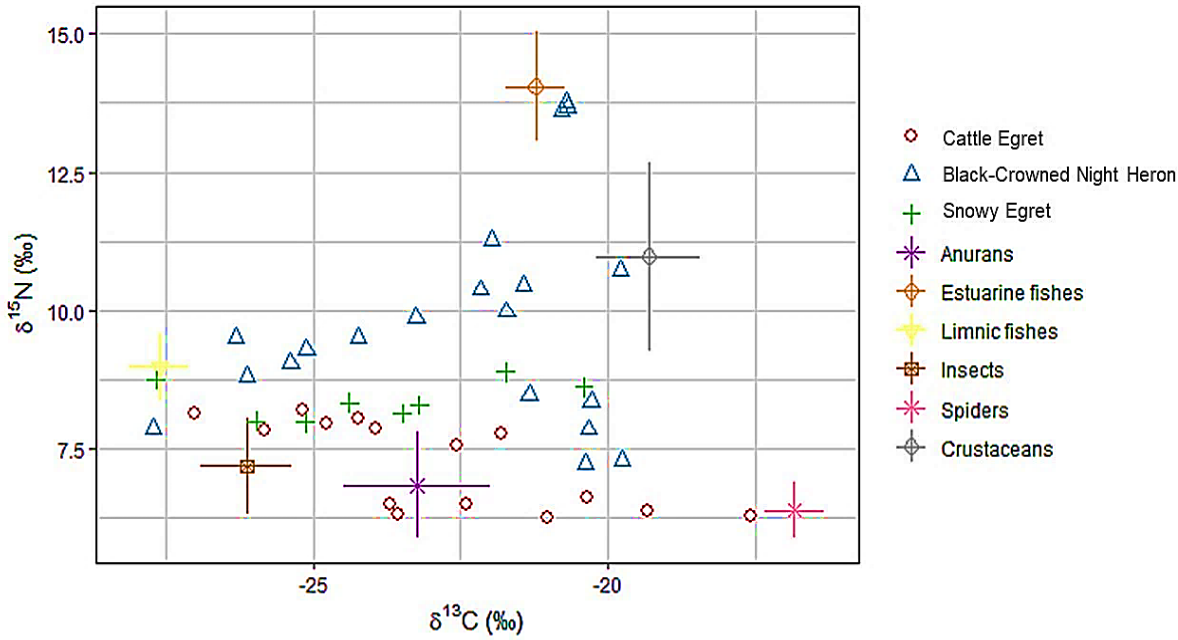

Fig. 2 Biplot output by simmr package for δ^{13}C and δ^{15}N with the sources from estuarine environment: **Estuarine fishes** (*Atherinella brasiliensis*) and **Crustaceans** (*Callinectes* sp.); of limnic environment: **Freshwater fishes** (*Cichlasoma portalegrense* and *Synbranchus marmoratus*); and terrestrial environment: **Anurans** (*Pseudis minuta*, *Physalaemus gracilis* and *Scinax squalirostris*), **Insects** and **Spiders**

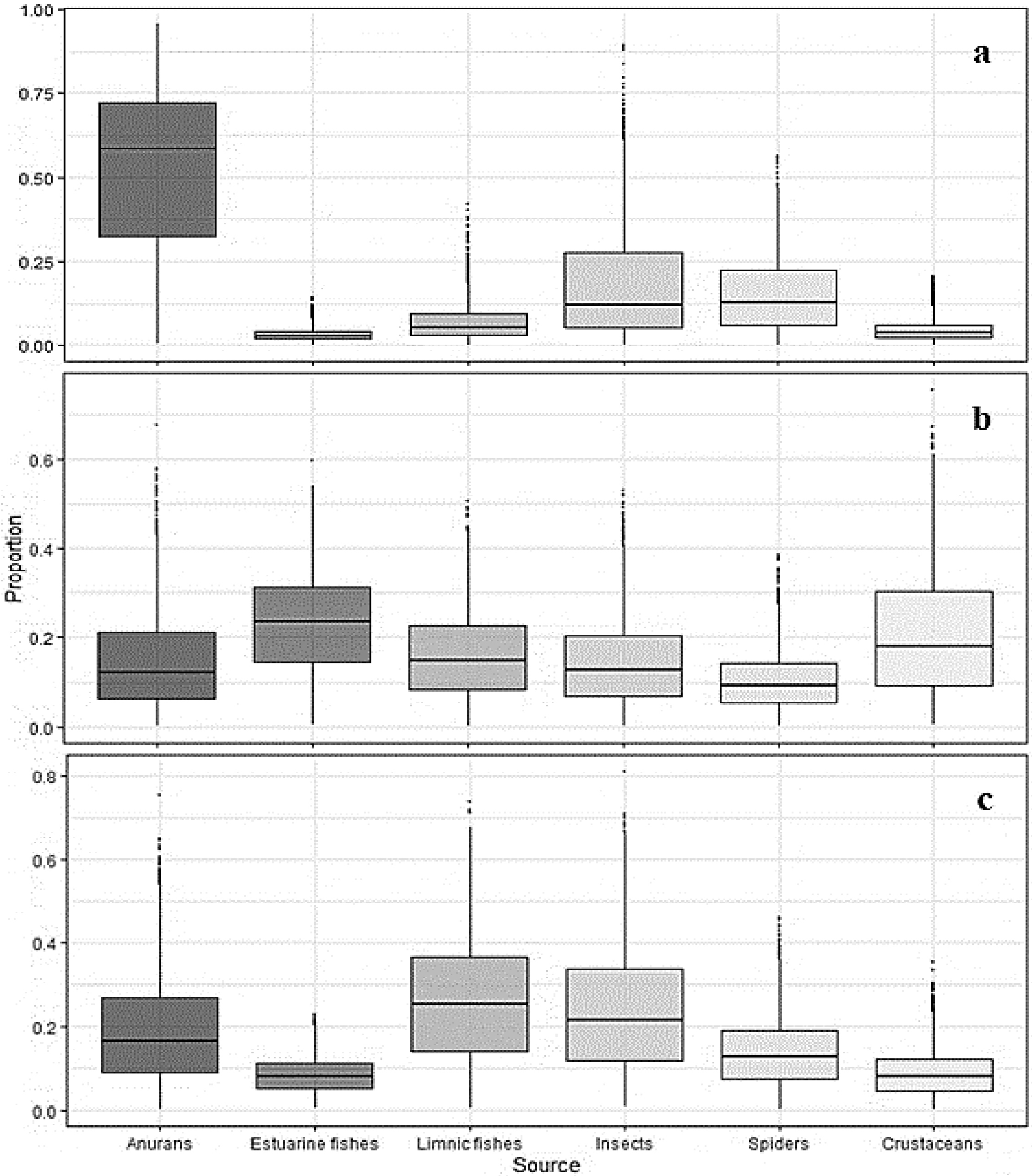

Fig. 3 Contribution of potential sources (**Anurans**, **Estuarine fishes**, **Freshwater fishes**, **Insects**, **Spiders** and **Crustaceans**) in the blood of consumers, obtained from mixing models with isotopic values of the blood of nestlings of the three herons: cattle egret (**a**), black-crowned night heron (**b**) and snowy egret (**c**) in the Pelecaniformes colony of Marinheiros Island, Southern Brazil. The horizontal line in the center of the rectangle represents the mean and the rectangle indicates the standard deviation. The vertical lines outside the rectangle indicate the minimum and maximum contribution values

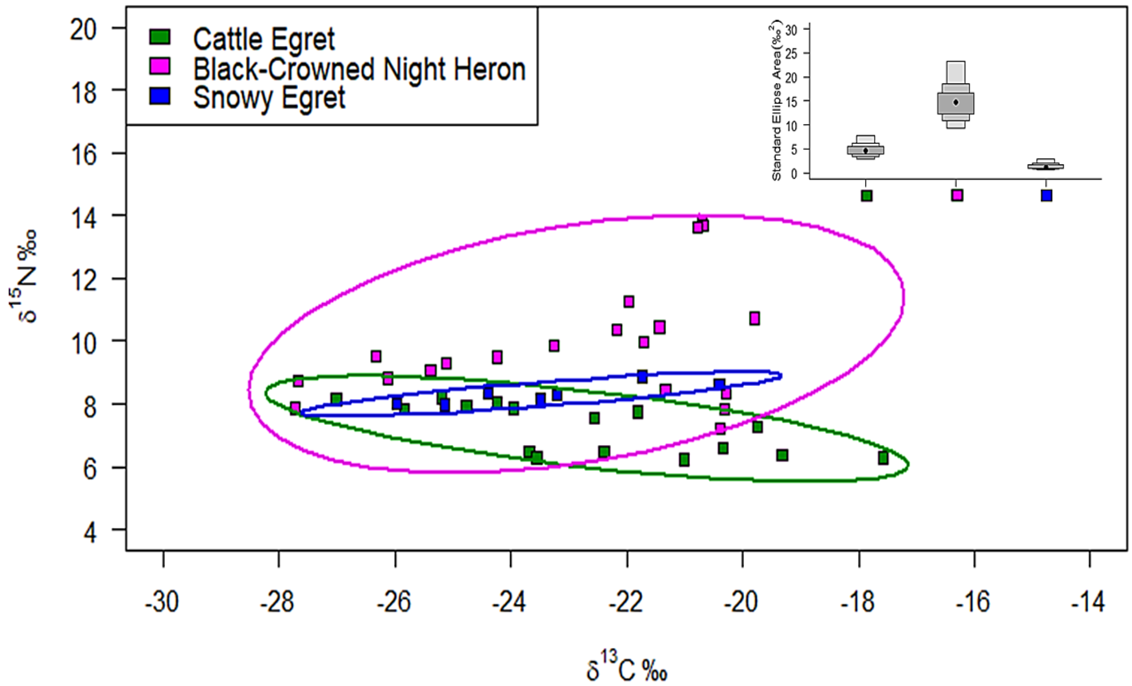

Fig. 4 Isotopic niche of cattle egret, black-crowned night heron and snowy egret in delta space (δ), obtained through standard corrected ellipse areas (SEAc) and comparison between standard ellipse areas at ‰^{2} (SEAc) based on isotopic values in whole blood, at the Marinheiros Island, municipality of Rio Grande, state of Rio Grande do Sul, Brazil

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Este estudo utilizou como método, as análises convencionais de dieta, ou seja a análise de regurgitados e pellets, em conjunto com a análise de marcadores intrínsecos, os isótopos estáveis (valores de δ^{13}C e δ^{15}N) no sangue de ninhegos, para investigar a dieta e repartição de recursos tróficos entre três espécies de pequenas garças - *Bubulcus ibis*, *Egretta thula* e *Nycticorax nycticorax* que reproduzem em simpatria em uma colônia no Estuário da Lagoa dos Patos, RS. Além desse, estudos prévios no mesmo local utilizaram estas técnicas para investigar a dieta de *Ardea cocoi* (Faria *et al*. 2016) e de *Ardea alba* (Britto e Bugoni 2015). No entanto, este foi o primeiro trabalho a investigar a repartição de recursos e a dieta de forma comparativa entre as três espécies de pequenas garças no local. Diante disso, e sabendo da importância de estudar a dieta e repartição de recursos com métodos em conjunto, recomendamos mais estudos da mesma natureza, não apenas na região como também em outros locais.

Os resultados obtidos neste estudo não acrescentaram apenas informações a respeito dos nichos trófico e isotópico das espécies e da sobreposição entre os nichos, mas também, acerca do papel ecológico dessas aves predadoras na manutenção e fluxo de energia nos ambientes aquáticos (Steinmetz *et al*. 2003). A espécie que apresentou a dieta mais generalista entre as três, *N. nycticorax*, utilizou ambientes estuarinos, límnicos e terrestres para a sua alimentação e apresentou alta sobreposição de nicho trófico com *B. ibis*. No entanto, *B. ibis*, diferente de *N. nycticorax*, utilizou principalmente ambientes terrestres para a sua alimentação. Entre os táxons terrestres compartilhados pelas duas espécies estão os insetos, que foram as presas mais representativas na dieta das duas garças. Destacamos o quão diversos foram os táxons de insetos presentes na dieta de *B. ibis*, com oito ordens identificadas. Sabendo-se do atual declínio da abundância e diversidade de insetos no mundo (Wagner *et al.* 2021), os resultados desse estudo nos fazem refletir sobre quais as futuras possíveis consequências e o que pode modificar na ecologia trófica de *B. ibis* e *N. nycticorax*. Com o atual declínio das presas mais importantes para ambas, além dos demais táxons que vivem no entorno dessa colônia estuarina.

Com o nicho isotópico de *N. nycticorax* apresentando alta sobreposição sobre os de *B. ibis* e *E. thula*, confirma-se que as três espécies de pequenas garças assimilaram fontes em comum ou fontes com valores de δ^{13}C e δ^{15}N bastante semelhantes. Em outras palavras, as garças coexistem e reproduzem na mesma colônia estuarina partilhando fontes e/ou recursos alimentares eventualmente. Recomendamos, como perspectivas futuras, investigar quais os fatores bióticos e abióticos que permitem a coexistência dessas três espécies na colônia, e estudos tratando da existência ou não de competição pelos recursos pelas garças.

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ANEXOS

COMISSÃO DE ÉTICA EM USO ANIMAL
Universidade Federal do Rio Grande
Pró-Reitoria de Pesquisa e Pós-Graduação - PROPESP
ceua@furg.br http://www.propesp.furg.br

CERTIFICADO Nº P028/2020

Certificamos que o projeto intitulado "Dieta e repartição entre garças reproduzindo em simpatria no Sul do Brasil", protocolo nº 23116.008789/2019-63, sob a responsabilidade de Leandro Bugoni - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi APROVADO pela COMISSAO DE ETICA EM USO ANIMAL DA UNIVERSIDADE FEDERAL DO RIO GRANDE (CEUA-FURG), em reunião de 27 de maio de 2020 (Ata 006/2020).
A CEUA lembra aos pesquisadores que qualquer alteração no protocolo experimental ou na equipe deve ser encaminhada à comissão para avaliação e aprovação. Um relatório final deve ser enviado à CEUA no término da vigência do seu projeto.

CEUA Nº	Pq021/2019
COLABORADORES AUTORIZADOS A MANIPULAR OS ANIMAIS	Amanda Oliveira Travessas
VIGENCIA DO PROJETO	01/03/2021
ESPECIE / GRUPOS TAXONOMICOS	*Bubulcus ibis*, *Egretta caerulea*, *Egretta thula* e *Nycticorax nycticorax*
NUMERO DE ANIMAIS	100 (25 de cada espécie)
Nº SOLICITAÇAO / AUTORIZAÇAO SISBIO	72947
ATIVIDADE(S)	(X) CAPTURA ( ) COLETA DE ESPÉCIMES (X) MARCAÇÃO (X) OUTRAS: coleta de sangue
LOCAL(is) REALIZAÇAO ATIVIDADES	Ilha dos Marinheiros, Rio Grande, RS
ENVIO DO RELATORIO PARCIAL	Janeiro de 2021
ENVIO DO RELATORIO FINAL	Abril de 2021

Rio Grande, 07 de agosto de 2020.

Alice Teixeira Meirelles Leite
Coordenadora da CEUA-FURG

Controle da CEUA - Parecer(es) prévio(s) relacionado(s) ao processo: P052/2019. Página 1/1