PRIMARY RESEARCH PAPER

The contrasting feeding ecology of great egrets and roseate spoonbills in limnetic and estuarine colonies

Vanessa Oliveira Britto · Leandro Bugoni

Abstract Multispecies colonies of wading birds frequently occur in both freshwater and estuarine environments, in locations with potentially safe places for nesting where the nearby shallow waters provide food for rearing chicks. In 2011/2012, we investigated the feeding ecology of two large-sized waterbirds, the great egret, *Ardea alba*, and the roseate spoonbill, *Platalea ajaja*, which breed sympatrically in limnetic and estuarine colonies 65 km apart in southern Brazil. Whole blood from chicks was sampled for $\delta^{13}C$ and $\delta^{15}N$ stable isotopes, and their diets were assessed using direct (conventional) methods. The diet of spoonbills consisted of fish, insects, crustaceans, mollusks, and seeds, whereas great egrets fed mainly on fish, insects, and crustaceans. Bayesian stable isotope mixing models indicated that spoonbills fed exclusively in limnetic habitats, regardless of where they bred, whereas egrets breeding in the estuarine colony fed on both estuarine and limnetic prey, expanding their isotopic niche considerably. Dietary data confirmed this result, with the diets of egrets in the freshwater colony showing high similarity to the diets of spoonbills in both freshwater and estuarine colonies. The isotopic niche overlap was the lowest between species in the estuarine colony, suggesting that the feeding plasticity of egrets reduces interspecific competition during breeding.

Keywords Diet · Pellets · Stable isotopes · Mixing models · *Platalea ajaja* · *Ardea alba*

Introduction

Coexistence of sympatric species with similar morphological characteristics and similar ecological roles implies there are some differences in the ways they explore the environment. This was formalized by Hutchinson (1957) in terms of how species occupied *n*-dimensional hypervolumes of the ecological niches. In waterbirds, differences in foraging patterns, prey preferences, and physiological limitations result in trophic segregation during the breeding period (Croxall et al., 1999; Post, 2008). It has been proposed that colonies are located in places where adults can obtain enough food for chicks and themselves in the surrounding areas, because these birds are central-place foragers during breeding (Brzorad et al., 2004), while minimizing trophic niche overlap.

V. O. Britto (✉) · L. Bugoni
Waterbirds and Sea Turtles Laboratory, Institute of Biological Sciences, Universidade Federal do Rio Grande (FURG), CP 474, Rio Grande, RS CEP 96203-900, Brazil
e-mail: vanessa.obritto@gmail.com

V. O. Britto · L. Bugoni
Graduate Program in Biology of Freshwater Ecosystems, Institute of Biological Sciences, Universidade Federal do Rio Grande (FURG), CP 474, Rio Grande, RS CEP 96203-900, Brazil

Pelecaniformes (herons, egrets, ibises, spoonbills, and allies) are strongly associated with coastal or inland shallow waters and depend on these habitats for foraging and breeding (Frederick, 2002; Lorenz, 2014). The great egret, *Ardea alba* Linnaeus, 1753, hereafter egret, is widespread in Americas and the Old World, using a range of brackish and freshwater habitats for feeding (McCrimmon et al., 2001; Kushlan & Hancock, 2005). Egrets usually capture prey such as fish, crustaceans, amphibians, reptiles, and insects by detecting them visually and capturing a single prey with a rapid downward or lateral stroke (Beltzer & Quiroga, 2007; Maccarone & Brzorad, 2007; Post, 2008). In estuarine areas of North Carolina (USA), egrets forage and respond opportunistically to local prey availability (Post, 2008), foraging mainly during the daytime (Rojas et al., 1999).

The roseate spoonbill, *Platalea ajaja* Linnaeus, 1758, hereafter spoonbill, occurs from the southeastern USA to Argentina (Matheu & del Hoyo, 1992). Spoonbills have been reported to forage from hypersaline ponds and marine areas to freshwater lakes (Dumas, 2000). Spoonbills prefer freshwater in some areas, such as in Florida (USA) (Lorenz et al., 2009), which may be related to a limited ability to deal with hyperosmotic prey, as demonstrated for white ibis *Eudocimus albus* (Linnaeus, 1758) (Johnston & Bildstein, 1990). The Eurasian spoonbill *Platalea leucorodia* Linnaeus, 1758 relies entirely on marine resources during breeding in Mauritania (El-Hacen et al., 2013), but may switch from limnetic to marine prey during the breeding period in The Netherlands (El-Hacen et al., 2014). Roseate spoonbills are not only tactile foragers (Dumas, 2000), but also have visual capabilities, enabling them to forage at dawn, dusk, and night (Rojas et al., 1999). They are mainly ichthyophagous, but significant proportions of crustaceans, insects, and other aquatic invertebrates also occur in the diet (Dumas, 2000; Teixeira & Nacinovic, 2003).

There are many methods of assessing the diet of aquatic birds, from observations of food ingested to chemical analysis of the consumer's tissues, with advantages and limitations inherent to each procedure (Barrett et al., 2007). A combination of different methods has been demonstrated to provide a realistic picture of the trophic relationships in aquatic food webs. Thus, a combined approach of conventional dietary analysis and stable isotope analysis (SIA) for studies of feeding ecology is increasingly common in the dietary studies of waterbirds (Bearhop et al., 2001; Weiser & Powell, 2011). For instance, stable isotopes (SI) and their resulting isotopic mixing models provide key insights into opportunistic foraging patterns and have shown that many waterbirds are able to switch quickly from one food source to another (Ramos et al., 2009), which seems to be the case of egrets and spoonbills (Martínez-Vilalta & Motis, 1992; Matheu & del Hoyo, 1992; El-Hacen et al., 2014). Furthermore, dietary analysis provides key clues about important food sources to be included in isotopic mixing models, besides allowing identification of food items to species level. Combined techniques also provide information on different time scales: SIs from whole blood of birds reveal the diet that has been ingested and assimilated during the last 3–4 weeks before sampling, whereas the regurgitates and stomach contents are a snapshot of the last meal (Auman et al., 2011).

In addition to providing different pictures of the foraging ecology, analysis of data from traditional dietary methods and SIA have advanced and now allow comparisons of the results of the two methods in relation to the importance of different food sources (e.g., prey-specific index of relative importance—PSIRI% vs. isotopic mixing models in SIAR), inferred the niche breadth (e.g., Levin's niche breadth vs. isotopic niche width in SIBER), and dietary overlap (Morisita–Horn index vs. isotopic niche overlap in SIBER).

The study reported in this paper investigated the diet of spoonbill and egret chicks by examining the spontaneous regurgitates, pellets, gastrointestinal contents of dead chicks, stomach flushing contents, and SIA of whole blood. The study aims to understand the extent and mechanisms of dietary segregation of these two large wading birds (spoonbills vs. egrets), which have distinct foraging techniques (tactile vs. visual predator), distinct foraging periods (crepuscular and nocturnal in spoonbills vs. diurnal in egrets), and feed in two distinct environments (estuarine vs. limnetic). We hypothesize that egrets and spoonbills target different species of prey because these birds feed in different habitats; their prey differ in size, with spoonbills ingesting smaller prey due to tactile foraging; and their prey differ in trophic level, with egrets placed higher on the food chain through consuming larger prey. Furthermore, we expect that spoonbills are connected to benthic prey/food sources, and egrets are essentially piscivorous.

Materials and methods

Study area

This study was carried out in two waterbird colonies in southern Brazil, one near a federal protected area (Taim Ecological Reserve) in a limnetic marsh (Aguirre/Taim Marsh), and another at Marinheiros Island in the Patos Lagoon estuary (Fig. 1), both study areas close to marshes, swamps, lakes, channels, and rice fields, which comprise typical wetlands in southern Brazilian coastal plains. The climate in the region is warm-temperate, with an annual mean air temperature of approximately 18°C and annual rainfall 1,200–1,500 mm (Klein, 1998).

Marinheiros Is. (32°04′S, 052°09′W) is the largest island in the Patos Lagoon (~40 km²). The heronry there is a mixed colony where roseate spoonbill, great egrets, cattle egrets *Bubulcus ibis* (Linnaeus, 1758), snowy egrets *Egretta thula* (Molina, 1782), cocoi herons
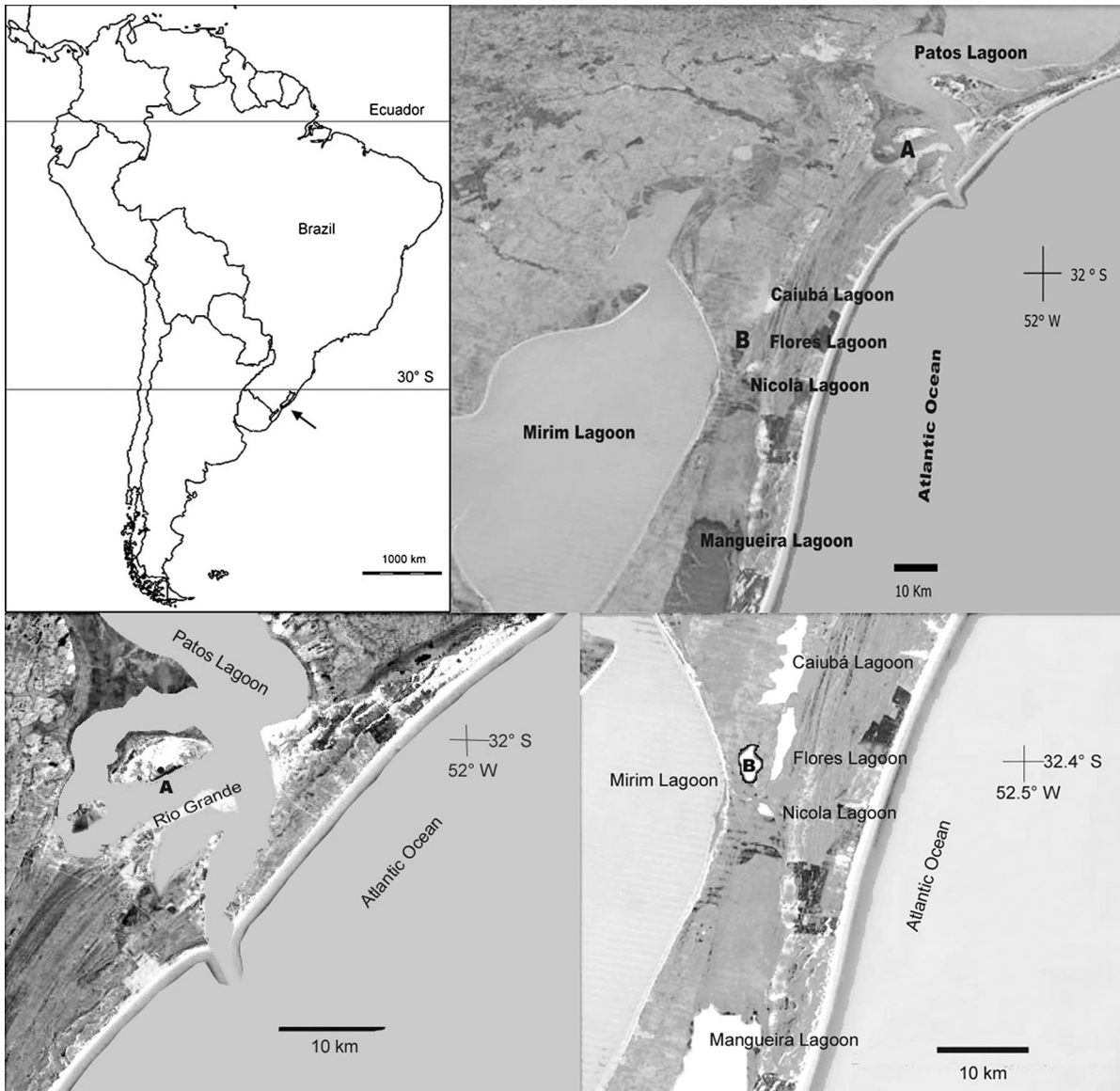
Fig. 1 The location of waterbird colonies in southern Brazil (*arrow*). This shows (*A*) Marinheiros Is., in the Patos Lagoon estuary, approximately 65 km from the freshwater colony (*B*) at Aguirre/Taim Marsh. Lagoons, the Atlantic Ocean, and other landmarks within the foraging range of studied birds

Table 1 The diet of great egret, *Ardea alba*, and roseate spoonbill, *Platalea ajaja*, chicks in estuarine (Marinheiros Is., Patos Lagoon), and limnetic (Aguirre/Taim Marsh) environments in southern Brazil during the spring/summer of 2011/2012

Food items	Estuary (Marinheiros Is., Patos Lagoon)								Freshwater (Aguirre/Taim Marsh)							
	Great egret (Regurgitates $n = 20$; Stomach contents $= 2$)				Roseate spoonbill (Regurgitates $n = 8$; Stomach contents $n = 2$; Pellets $n = 2$)				Great egret (Stomach contents $n = 12$; Stomach flushing $n = 1$)				Roseate spoonbill (Stomach contents $n = 7$; Pellets $n = 1$; Regurgitates $n = 1$; Stomach flushing $n = 5$)			
	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%
Fish total	**90.9**	**62.2** (56.7)	**75.1** (60.4)	**53.3**	**100.0**	**18.1** (17.5)	**9.6** (7.8)	**12.6**	**53.9**	**34.4** (28.4)	**75.7** (75.2)	**27.9**	**64.3**	**49.7** (35.9)	**29.4** (25.7)	**19.8**
Characidae total	4.5	0.9 (17.6)	1.1 (19.9)	0.9	**20.0**	2.1 (10.7)	1.0 (2.5)	1.3	–	–	–	–	**14.3**	19.9 (31.1)	11.8 (28.3)	4.2
Astyanax sp.	4.5	0.3 (5.9)	0.4 (6.6)	0.3	**6.7**	0.1 (0.3)	0.1 (0.3)	<0.1	–	–	–	–	–	–	–	–
Oligosarcus robustus	–	–	–	–	**6.7**	0.4 (16.7)	0.2 (2.4)	0.6	–	–	–	–	–	–	–	–
Unidentified Characidae	–	–	–	–	**20.0**	1.6 (5.0)	0.8 (1.6)	0.7					**14.3**	19.9 (31.1)	11.8 (28.3)	4.2
Curimatidae																
Cyphocharax voga	–	–	–	–	–	–	–	–	–	–	–	–	**7.1**	1.2 (3.5)	0.7 (2.9)	0.2
Atherinopsidae																
Odontesthes argentinensis	**22.7**	2.8 (52.7)	4.2 (45.4)	**11.1**	–	–	–	–	–	–	–	–	–	–	–	–
Pimelodidae																
Rhamdia quelen	**9.1**	0.9 (26.8)	1.1 (49.5)	3.5	–	–	–	–	–	–	–	–	–	–	–	–
Anablepidae																
Jenynsia multidentata	4.5	0.9 (42.9)	<0.1 (0.5)	1.0	**13.3**	1.6 (4.8)	1.2 (4.0)	0.6	–	–	–	–	**7.1**	0.3 (1.0)	0.2 (0.8)	0.1
Mugilidae																
Mugil sp.	**9.1**	1.3 (19.9)	0.4 (6.6)	1.2	**13.3**	1.0 (2.4)	0.4 (2.8)	0.3	–	–	–	–	–	–	–	–

Table 1 continued

Food items	Estuary (Marinheiros Is., Patos Lagoon)								Freshwater (Aguirre/Taim Marsh)							
	Great egret (Regurgitates n = 20; Stomach contents = 2)				Roseate spoonbill (Regurgitates n = 8; Stomach contents = 2; Pellets n = 2)				Great egret (Stomach contents n = 12; Stomach flushing n = 1)				Roseate spoonbill (Stomach contents n = 7; Pellets n = 1; Regurgitates n = 1; Stomach flushing n = 5)			
	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%
Sciaenidae																
Micropogonias furnieri	9.1	0.6 (15.8)	0.2 (8.3)	1.1	6.7	0.1 (5.9)	0.2 (4.6)	0.3	–	–	–	–	–	–	–	–
Pomatomidae																
Pomatomus saltatrix	4.5	0.3 (6.3)	4.8 (53.7)	1.4	–	–	–	–	–	–	–	–	–	–	–	–
Unidentified fish	63.6	54.1 (48.1)	62.9 (55.2)	32.9	100.0	13.4 (14.0)	6.8 (6.1)	10.0	53.9	34.4 (28.4)	75.7 (75.2)	27.9	21.4	28.3 (28.5)	16.7 (19.0)	15.3
Crustaceans total	68.2	6.6 (27.5)	23.4 (62.8)	30.8	66.7	2.6 (9.4)	59.1 (81.7)	30.4	30.8	2.6 (6.6)	20.2 (48.7)	8.5	21.4	0.5 (12.4)	35.8 (90.0)	11.0
Decapoda																
Penaeidae (shrimp)	9.1	1.6 (14.4)	2.6 (49.9)	2.9	20.0	1.5 (15.4)	20.4 (65.8)	8.1	–	–	–	–	–	–	–	–
Brachyura (crabs)	22.7	1.9 (27.5)	7.8 (53.9)	9.3	53.3	1.0 (5.5)	34.4 (73.1)	21.0	30.8	2.6 (6.6)	20.2 (48.7)	8.5	21.4	0.5 (12.4)	35.8 (90.0)	11.0
Callinectes sp.	31.8	2.5 (20.7)	10.4 (59.5)	12.8	–	–	–	–	–	–	–	–	–	–	–	–
Callinectes sapidus	9.1	0.6 (50.0)	2.6 (77.9)	5.8	–	–	–	–	–	–	–	–	–	–	–	–
Trychodactylidae	–	–	–	–	6.7	0.1 (3.0)	4.3 (35.2)	1.3	–	–	–	–	–	–	–	–
Insects total	77.3	27.4 (34)	1.0 (2.1)	13.9	100.0	58.2 (64.5)	17.4 (29.8)	47.2	100.0	61.1 (77.5)	4.0 (40.39)	58.9	100.0	44.1 (66.2)	26.1 (53.6)	59.9
Hemiptera	–	–	–	–	6.7	0.1 (3.0)	<0.1 (0.3)	0.1	–	–	–	–	–	–	–	–
Belostomatidae (water bugs)	27.3	3.4 (5.8)	0.1 (0.2)	0.8	80.0	10.8 (10.7)	3.2 (4.2)	6.0	53.9	12.3 (17.5)	0.8 (6.9)	6.6	64.3	6.6 (12.9)	3.9 (9.4)	7.2

Table 1 continued

| Food items | Estuary (Marinheiros Is., Patos Lagoon) | | | | | | | | Freshwater (Aguirre/Taim Marsh) | | | | | | | |
| | Great egret (Regurgitates n = 2; Stomach contents = 2) | | | | Roseate spoonbill (Regurgitates n = 8; Stomach contents n = 5; Pellets n = 2) | | | | Great egret (Stomach contents n = 12; Stomach flushing n = 1) | | | | Roseate spoonbill (Stomach contents n = 7; Pellets n = 1; Regurgitates n = 1; Stomach flushing n = 5) | | | |
	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%
Coleoptera (bugs)	50.0	10.0 (19.6)	0.4 (1.6)	5.3	100.0	18.1 (24.9)	5.4 (11.8)	18.4	84.6	26 (40.9)	1.7 (20.8)	26.1	92.9	27.6 (40.1)	16.3 (33.3)	34.1
Odonata (dragonflies)	40.9	7.5 (11.9)	0.3 (1.0)	2.7	86.7	27.5 (30.5)	8.2 (16.3)	20.3	76.9	17 (34.5)	1.1 (23.8)	22.4	71.4	6.3 (15.0)	3.7 (9.5)	8.8
Hydrophilidae	4.5	0.6 (3)	<0.1 (0.1)	0.1	13.3	0.5 (7.0)	0.1 (1.2)	0.5	38.5	5.2 (11.6)	0.3 (1.4)	2.5	35.7	3.2 (7.7)	1.9 (7.5)	2.7
Hymenoptera (ants, wasps)	4.5	0.3 (1.5)	<0.1 (0.1)	<0.1	–	–	–	–	–	–	–	–	7.1	0.2 (0.5)	0.1 (0.4)	<0.1
Formicidae (ants)	4.5	0.3 (2.9)	<0.1 (0.1)	0.1	–	–	–	–	–	–	–	–	–	–	–	–
Apoidae	–	–	–	–	6.7	0.1 (3.0)	<0.1 (0.3)	0.1	–	–	–	–	–	–	–	–
Diptera	–	–	–	–	6.7	0.4 (9.1)	0.1 (0.9)	0.3	–	–	–	–	–	–	–	–
Arachnida (spiders)	4.5	0.3 (33.3)	<0.1 (0.8)	0.8	13.3	0.4 (3.2)	0.1 (0.4)	0.2	–	–	–	–	–	–	–	–
Unidentified insects	36.4	5.0 (22.4)	0.2 (0.8)	4.2	6.7	0.4 (33.3)	0.1 (2.2)	1.2	7.7	0.6 (33.3)	<0.1 (2.8)	1.4	7.1	0.2 (100)	0.1 (100)	7.1
Mollusks total	13.6	1.6 (16.9)	0.4 (4.3)	1.4	26.7	4.5 (6.5)	8.7 (13.1)	2.6	–	–	–	–	14.3	1.7 (9.2)	7.0 (37.6)	3.3
Gastropoda	–	–	–	–	6.7	0.1 (1.1)	0.2 (3.0)	0.1	–	–	–	–	7.1	0.2 (7.1)	0.7 (26.9)	1.2
Planorbidae	–	–	–	–	6.7	3.9 (10.7)	7.5 (38.1)	1.6	–	–	–	–	7.1	0.3 (1.0)	1.3 (5.4)	0.2
Hydrobiidae	–	–	–	–	20.0	0.5 (4.8)	0.9 (3.7)	0.8	–	–	–	–	7.1	0.9 (2.5)	3.3 (13.4)	0.6

Table 1 continued

| Food items | Estuary (Marinheiros Is., Patos Lagoon) | | | | | | | | Freshwater (Aguirre/Taim Marsh) | | | | | | | |
| | Great egret (Regurgitates n = 20; Stomach contents = 2) | | | | Roseate spoonbill (Regurgitates n = 8; Stomach contents n = 2; Pellets n = 2) | | | | Great egret (Stomach contents n = 12; Stomach flushing n = 1) | | | | Roseate spoonbill (Stomach contents n = 7; Pellets n = 1; Regurgitates n = 1; Stomach flushing n = 5) | | | |
	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%	FO%	N% (PN%)	M% (PM%)	PSIRI%
Ancylidae	–	–	–	–	–	–	–	–	–	–	–	–	**7.1**	0.2 (0.5)	0.7 (2.7)	0.1
Ampullariidae																
Pomacea sp.	4.5	0.3 (25)	0.1 (6.5)	0.7	–	–	–	–	–	–	–	–	**7.1**	0.2 (7.1)	0.7 (26.9)	1.2
Unidentified mollusks	**9.1**	1.3 (12.9)	0.3 (3.2)	0.7	–	–	–	–	–	–	–	–	–	–	–	–
Vegetation	22.7	2.2 (5.1)	0.1 (0.2)	0.6	**80.0**	**16.6** (12.5)	5.2 (5.5)	7.2	23.1	1.9 (22.5)	0.1 (17.9)	4.7	57.1	3.9 (11.9)	2.1 (9.1)	**6.0**
Oryza sativa (rice, seeds)	**9.1**	0.3 (4.1)	<0.1 (0.2)	0.2	–	–	–	–	7.7	0.6 (9.1)	<0.1 (< 0.1)	0.35	21.4	0.7 (3.5)	<0.1 (0.3)	0.4
Ludwigia sp. (seeds)	–	–	–	–	26.7	9.4 (11.7)	2.9 (3.8)	2.1	–	–	–	–	**7.1**	0.3 (28.6)	0.2 (29.6)	2.1
Unidentified seeds	**13.6**	1.9 (5.7)	0.1 (0.3)	0.4	**80.0**	7.3 (8.6)	2.3 (4.3)	5.2	15.4	1.3 (29.2)	0.1 (26.8)	4.3	**42.9**	2.9 (9.3)	1.8 (7.1)	3.5

The samples obtained from the different methods were pooled. The values of contribution that were higher than 5% are in bold, except for the prey-specific values (PN% and PM%)

FO% frequency of occurrence, N% contribution by number, PN% prey-specific contribution by number, M% contribution by mass, PM% prey-specific contribution by mass, PSIRI% prey-specific index of relative importance, – item not found

Ardea cocoi Linnaeus, 1766, black-crowned night herons *Nycticorax nycticorax* (Linnaeus, 1758), yellow-crowned night herons *Nyctanassa violacea* (Linnaeus, 1758), and little blue herons *Egretta caerulea* (Linnaeus, 1758) nest in spring/summer every year (Gianuca, 2010). The colony is located in a swamp with horticultural crops nearby and is 300 m from the estuarine waters of the Patos Lagoon (Fig. 1). The vegetation in the nesting areas is composed of trees, small bushes, and bambuses (Gianuca, 2010). The Patos Lagoon is composed mainly of open waters (80% of the area), with the margins containing fresh and salt water marshes, as well as sand dunes and sandy beaches (Asmus, 1998). Samples from this heronry have been termed estuarine, despite the fact that they could be from limnetic habitats on the island's inland areas or from the nearby mainland that is approximately 3 km away.

The Aguirre/Taim Marsh colony (32°30′S, 052°32′W) consists of roseate spoonbills, great, cattle and snowy egrets, cocoi herons, and plumbeous ibises *Theristicus caerulescens* (Vieillot, 1817). The colony is deep within a large marsh composed of *Juncus* sp. (Juncacea) and shrubs (*Cephalanthus glabratus* Martius, Rubiaceae). The area is on a private farm with other marshes, cattle ranching and rice paddies nearby. It is approximately 1.9 km from the Flores Lagoon, 4.3 km from the Nicola Lagoon, 5.2 km from the Mirim Lagoon, 9.8 km from the Caiubá Lagoon, 15 km from the Atlantic Ocean and 56 km from the Patos Lagoon estuary (Fig. 1). Samples from this colony are referred to as limnetic/freshwater samples. The estuarine and freshwater colonies are 65 km apart.

Dietary sampling

Blood and regurgitate sampling started during mornings, about 09:00 h, and lasted until about 15:00 h. In order to minimize disturbance, no sampling occurred during critical periods such as nest settlement early in the breeding season, when visits were limited to the border of the colony, to monitor the stage of the breeding cycle. We entered the colony twice during incubation period, for egg collection for another study on metals, or when chicks were at the age expected for blood sampling (i.e., ~3 weeks old). Four samplings at each colony occurred from 08 November to 05 December 2011. Food remains were gathered from inside the nests or from the ground below them, when we were sure about the origin of regurgitates or pellets.

Spontaneous regurgitates egested during chick handling (semi-digested material), pellets (undigested hard parts of prey egested regularly, involved in mucous), and the digestive tracts of dead chicks found in colonies, were collected. In addition, stomach flushing was attempted. Esophageal probes (6 mm diameter, 44 mm length) were inserted through the bill to the bottom of the proventriculus, and warm freshwater was injected with a syringe until the excess flowed out of the bill's edge (Gales, 1987). Then, the probe was gently removed and the bird was turned upside down (Gales, 1987). This method had very limited effectiveness (see Results) and was, therefore, discontinued after the first few trials. The stomach contents of each individual obtained using different methods were placed in plastic zip-loc bags and frozen until laboratory analysis. It was not possible to standardize the sample sizes among the species, colony, and sampling method, and the following samples sizes were obtained: the spontaneous regurgitates of egrets (estuary $n = 20$) and spoonbills (estuary $n = 8$, limnetic $n = 1$); the pellets from spoonbills (estuary $n = 2$, limnetic $n = 1$); the digestive tracts of egret chicks (estuary $n = 2$, limnetic $n = 12$) and spoonbills (estuary $n = 5$, limnetic $n = 7$); and the stomach flushings from egrets (limnetic $n = 1$) and spoonbills (limnetic $n = 5$). The samples were pooled for data analysis, with a total sample size for egrets of $n = 22$ (estuary) and $n = 14$ (limnetic), and for spoonbills $n = 15$ (estuary) and $n = 14$ (limnetic) (Table 1).

Samples were sorted under a dissecting microscope and the food taxa present were separated and identified at the lowest taxonomic level possible. The fish were identified by their otoliths using Naves (1999) and the reference collections at the Demersal Fish Resources and Cephalopods Lab and the Waterbirds and Sea Turtles Lab, both at the Universidad Federal do Rio Grande (FURG). Whole fish or their undigested remains were identified according to Fischer et al. (2011), crustaceans according to Buckup & Bond-Buckup (1999), insects and mollusks according to Borror & Long (1969) and Mugnai et al. (2010).

SIA samples

The chicks were captured by hand and approximately 0.5 ml of whole blood was obtained from the tarsal or the brachial vessels of egrets (estuary $n = 18$, limnetic

Table 2 The mean ± 1 standard deviation (SD) values of $\delta^{15}N$ and $\delta^{13}C$ in the tissues of waterbirds and their potential food sources that were used for the Bayesian stable isotope mixing model (SIAR)

	Birds/Food sources	Sampling method	Tissue	$\delta^{15}N$	$\delta^{13}C$	Location	n	Reference
Bird	*Ardea alba*	Manual	Whole blood	8.0 ± 0.9	−24.9 ± 2.8	Aguirre/Taim Marsh	16	This study
	Ardea alba	Manual	Whole blood	10.0 ± 1.7	−22.2 ± 3.4	Marinheiros Is.	18	This study
	Platalea ajaja	Manual	Whole blood	9.4 ± 1.3	−24.8 ± 1.4	Aguirre/Taim Marsh	16	This study
	Platalea ajaja	Manual	Whole blood	8.1 ± 0.4	−25.4 ± 1.5	Marinheiros Is.	14	This study
Fish	Characidae							
	Astyanax jacuhiensis	Chicks regurgitate (found on the ground in colony)	Muscle	8.0	−22.2	Marinheiros Is.	1	This study
	Astyanax jacuhiensis	Net	Muscle	9.2	−23.5	Taim Marsh	1	This study
	Astyanax eigenmanniorum	Net	Muscle	7.2 ± 0.9	−28.7 ± 0.8	Taim Marsh	3	This study
	Oligosarcus robustus	Fisherman	Muscle	12.6 ± 0.9	−22.7 ± 0.5	Mirim Lagoon	5	Silva-Costa & Bugoni (2013)
	Bryconamericus iheringii	Net	Muscle	8.7	−19.4	Taim Marsh	1	This study
	Hyphessobrycon bifasciatus	Net	Muscle	6.9	−30.5	Taim Marsh	1	This study
	Cichlidae							
	Geophagus brasiliensis	Net	Muscle	8.0	−28.8	Taim Marsh	1	This study
	Crenicichla lepidota	Net	Muscle	10.4	−24.9	Taim Marsh	1	This study
	Cichlasoma portalegrense	Net	Muscle	7.0	−31.2	Taim Marsh	1	This study
	Erythrinidae							
	Hoplias malabaricus	Net	Muscle	8.2	−29.1	Taim Marsh	1	This study
	Mugil sp.	Egret regurgitate	Muscle	10.0	−15.5	Marinheiros Is.	1	This study
	Mugil platanus	Net	Muscle	10.6	−19.6	Taim Marsh	1	This study
	Pimelodidae							
	Rhamdia aff. *quelen*	Net	Muscle	8.9 ± 0.7	−27.4 ± 3.5	Taim Marsh	3	This study
	Rhamdia aff. *quelen*	Egret regurgitate	Muscle	6.8 ± 0.2	−17.7 ± 0.4	Marinheiros Is.	2	This study

Table 2 continued

Birds/Food sources	Sampling method	Tissue	$\delta^{15}N$	$\delta^{13}C$	Location	n	Reference
Sciaenidae							
Micropogonias furnieri	Egret regurgitate/Fisherman	Muscle	15.1 ± 0.8	−14.7 ± 0.8	Patos Lagoon	3	This study
Poeciliidae							
Phalloceros caudimaculatus	Net	Muscle	9.1	−29.0	Taim Marsh	1	This study
Cnesterodon decemmaculatus	Net	Muscle	9.8 ± 0.7	−27.9 ± 0.9	Taim Marsh	3	This study
Anablepidae							
Jenynsia multidentata	Net	Muscle	9.5 ± 0.4	−25.4 ± 0.2	Taim Marsh	2	This study
Jenynsia multidentata	Egret regurgitate	Muscle	13.5 ± 3.9	−10.9 ± 4.8	Marinheiros Is.	2	This study
Atherinopsidae							
Odonthestes argentinensis	Egret regurgitate	Muscle	13.6 ± 0.5	−15.1 ± 0.2	Marinheiros Is.	4	This study
Callichthyidae							
Corydoras paleotus	Net	Muscle	9.8 ± 0.6	−24.0 ± 1.1	Taim Marsh	3	This study
Heptapteridae							
Pimelodella australis	Net	Muscle	9.1 ± 0.6	−27.9 ± 0.9	Taim Marsh	3	This study
Clupeidae							
Platanichthys platana	Fisherman	Muscle	10.7	−17.2	Patos Lagoon	1	This study
Brevoortia pectinata	Fisherman	Muscle	10.0 ± 1.7	−14.8 ± 0.4	Patos Lagoon	3	This study
Crustaceans Penaeidae (shrimps)							
Farfantepenaeus paulensis	Fisherman	Muscle	6.5 ± 0.4	−8.8 ± 0.2	Patos Lagoon	4	This study
Palaemonetes argentinus	Dipnet	Muscle	9.2	−30.4	Taim Marsh	1	This study
Palaemonetes argentinus		Muscle	7.3 ± 0.3	−24.4 ± 0.2	Mangueira Lagoon	3	Rodrigues et al. (2014)
Brachyura (crabs)							
Callinectes sp.	Egrets regurgitate	Muscle	10.8 ± 1.2	−16.6 ± 0.5	Marinheiros Is.	2	This study
Callinectes sapidus	Fisherman	Muscle	6.8 ± 0.1	−11.3 ± 0.6	Patos Lagoon	2	This study
Amphibians Amphibians (frogs) Hylidae							
Pseudis minuta	Dipnet	Muscle	6.2	−23.8	Taim Marsh	1	This study
Mollusks Ampullariidae							
Pomacea canaliculata	Dipnet	Muscle	8.7 ± 1.2	−22.5 ± 0.8	Taim Marsh	4	This study

Table 2 continued

	Birds/Food sources	Sampling method	Tissue	δ^{15}N	δ^{13}C	Location	n	Reference
Insects	Belostomatidae (water bugs)	Dipnet	Muscle	5.7	−28.3	Taim Marsh	1	This study
Vegetation	Ludwigia sp.	Spoonbill stomach content	Seed	5.9	−26.1	Taim Marsh	1	This study
	Oryza sativa (rice)	Rice field	Seed	9.3	−27.5	Taim Marsh	1	This study
	Periphyton			5.2 ± 0.1	−24.7 ± 0.2	Mangueira Lagoon	3	Rodrigues et al. (2014)
	POM (particulate organic matter)			6.1 ± 0.7	−19.9 ± 1.0	Patos Lagoon	3	Claudino et al. (2013)

The sampling method, tissue analyzed, sampling location, sample size (n), and data reference source are indicated. The samples collected for the current study were collected from September 2011 to March 2012

$n = 16$) and spoonbills (estuary $n = 14$, limnetic $n = 16$). Drops of blood were placed on glass slides, dried in the sun, scraped, and stored in 1.5 ml plastic vials. Drying blood does not alter values of SI in birds (Bugoni et al., 2008). The tarsus and culmen lengths of the chicks were measured with a caliper as a proxy of age, and the chicks were banded with metals rings to avoid resampling the same individual. Sampling chicks from the same nest was also avoided. The chicks sampled were older than 3 weeks to ensure that the isotopic values of the blood were changed toward the diet provided by the adult birds and not by the egg nutrients, as blood half-life turnover in adult birds occurs at approximately 3–4 weeks (Bearhop et al., 2002; Sears et al., 2009).

Samples of the prey taxa used for the Bayesian isotopic mixing models were obtained from the prey remains in the birds' diets or by other methods (Table 2) in areas near freshwater and estuarine colonies within the potential foraging range of adult birds. Small muscle fragments or whole organisms had lipids extracted in a Soxhlet apparatus over 6 h with a petroleum ether solvent. The samples of potential food sources and chick blood were lyophilized, ground, homogenized, and weighed (approximately 1 mg) in tin capsules (5×9 mm, Costech®) for analysis in a mass spectrometer coupled with an elemental analyzer (Thermo Finnigan Delta Plus XP) at the University of Georgia (USA). The results from the SIA were expressed based on a delta notation (δ), using Eq. 1 (Bond & Hobson, 2012):

$$\delta^{13}\text{C or }\delta^{15}\text{N‰} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1, \qquad (1)$$

where the R_{sample} is the ratio between the heavy and the light isotope in the sample, and R_{standard} is the ratio between the heavy and the light isotope in either Pee Dee belemnite limestone, the international standard for C, or atmospheric N$_2$, the international standard for N. Internal laboratory standards, calibrated according to international standards, were run after every 12 samples to correct for drift. The standard deviation of the internal standards indicated the precision of the analysis, which was ±0.10 for δ^{15}N and ±0.11 for δ^{13}C.

Data analysis

The importance of each food item in the diet for the species and location of the colony was determined

from samples obtained by the four direct methods that were pooled. Calculated parameters were the relative frequency of occurrence (*FO%*), the relative contribution by number (*N%*), and the relative contribution by mass (*M%*) of each food item in the diet, based on the reconstructed mass (as in Bugoni & Vooren, 2004 and detailed below). Following Brown et al. (2012), the prey-specific contribution per bird species and location were also calculated, i.e., the mean contribution as a percentage by number (PN%) or mass (PM%), taking into account only the samples in which a given prey item occurred (Eq. 2):

$$\text{PN\% or PM\%} = \frac{\sum_{j=1}^{n} N\%(\text{or } M\%)_{ij}}{n_i}, \qquad (2)$$

where n_i = the number of samples in which the food item i was found; j = sample j; i = prey i.

With the PN% and PM% values, as well as *FO%*, a Prey-specific Index of Relative Importance (PSIRI%) was calculated, as proposed by Brown et al. (2012) (Eq. 3):

$$\text{PSIRI}_i\% = \frac{FO\%(\text{PN}_i\% + \text{PM}_i\%)}{2}. \qquad (3)$$

For reconstruction of the ingested mass, when the prey were digested or semi-digested, a corresponding allometric regression was used if possible to estimate the original body mass of each individual prey item based on the length of a rigid structure, e.g., otoliths for fish. Fish otoliths were measured and used for diet reconstruction only if they had an index of digestion (ID) of '0' or '1', i.e., with no or slight digestion, as in Bugoni & Vooren (2004). For otoliths with an ID of '2' or '3', or species without available allometric equations, the mean mass of the lowest corresponding taxonomic category was applied. For food items without allometric equations, the mean body mass was calculated based on the prey/food items weighted for the region: brachyuran crustaceans = 23.7 g (Oliveira et al., 2006); Penaeidae crustaceans = 9.37 g (D'Incao & Calazans, 1978); and Coleoptera insects = 0.2 g (Bugoni & Vooren, 2004). The body mass of Belostomatidae insects was calculated based on the mean body mass measured from four undigested specimens found in the samples (0.2 g). For all of the other insects, an arbitrary value of 0.2 g was used (Bugoni & Vooren, 2004). The mass of mollusks was based on the mean mass of seven *Pomacea canaliculata* (Lamarck, 1822), with sizes similar to

those found in the diet (1.3 g). For seeds, a mean mass (0.02 g) of six intact rice grains (*Oryza sativa* L.) and a mean mass (0.21 g) of four *Ludwigia* sp. found in the diet were used.

The niche trophic overlap was calculated using the Morisita index, modified by Horn, based on the contribution of the number (*N%*) of each taxon (Magurran, 2011). The values higher than 0.6 were regarded as an indication of high overlap in the diet (Diamond, 1983). In addition, the standardized Levin's niche breadth (B_S) was calculated based on contribution by mass (as in Barquete et al., 2008). For comparison, the isotopic niche width and isotopic niche overlap were calculated from $\delta^{13}C$ and $\delta^{15}N$ values, using SIBER—Stable Isotope Bayesian Ellipses in R software (Jackson et al., 2011).

To determine the contribution of each food group to the diet of spoonbills and egrets, Bayesian stable isotope mixing models were built using R software (SIAR) (Parnell et al., 2010); the birds were separated into four groups according to location and species (2 species × 2 colonies). The sources used in the models were determined from the diet data or were inferred from the differences in foraging techniques, i.e., spoonbills are tactile foragers and egrets are visual predators (Table 2). Prey with similar isotopic values or representative of similar guilds were grouped, as recommended by Phillips & Gregg (2003). Sources with limited contribution or wide credibility intervals (CI) were successively removed from models. Periphyton from the Mangueira Lagoon and POM from the Patos Lagoon are important carbon sources that sustain the trophic webs in the region (Rodrigues et al., 2014) and are potentially ingested by spoonbills due to their tactile foraging technique. Similarly, the amphibian *Pseudis minuta* Günther, 1858 was included because it is an abundant aquatic frog (Huckembeck et al., 2012) and, therefore, serves as potential prey for egrets, given their foraging tactics.

The predator–prey diet–blood discrimination factors that were used in the model were $\Delta^{13}C = 0.46 \pm 0$ and $\Delta^{15}N = 1.86 \pm 0$ (Cherel et al., 2005). Some of the models were tested with other discrimination factors, as reviewed by Hahn et al. (2012). Silva-Costa & Bugoni (2013) used these models to evaluate the diet of kelp gulls, *Larus dominicanus* Lichtenstein, 1823, which are generalist omnivorous waterbirds in the region with a similar diet to spoonbills and egrets. Nevertheless, the models with

the best fits, i.e., with the lowest CIs, were those with isotopic discrimination factors that were measured for the rockhopper penguin, *Eudyptes chrysocome* (Forster, 1781) (Cherel et al., 2005); therefore, these values were used in our models and presented in this study.

Finally, the $\delta^{13}C$ and $\delta^{15}N$ values from the whole blood of birds were analyzed using a generalized linear model, GLM (McCullogh & Searle, 2001), with a Gaussian distribution and residual values of the dependent variable that were normally distributed and homoscedastic. The factor 'species' (2 levels—egret and spoonbill), 'colony' (2 levels—estuary and freshwater), and the continuous covariate 'culmen length' or 'tarsus length' were used as an indication of the size/age of the chicks. The interaction between the independent variables was also tested. The non-significant variables were progressively removed, and the model with the lowest Akaike Information Criterion (AIC) was selected and presented in this study. To detail the GLM and test its significance, an ANOVA with residuals from the selected model was calculated, which provided a percentage of variation explained by the full model in addition to a significance level, as well as for each variable individually (Ye et al., 2001). All of the analyses were performed using R software (R Development Core Team, 2012).

Results

Diet

A total of 320 individual prey were found in the diet of great egrets from the colony sampled at the Patos Lagoon estuary (mean = 14.5 prey per sample). In the limnetic colony, 154 prey (mean = 11.8, Table 1) were detected. The total number of prey in the spoonbill's diet and the mean per sample were much higher than for egrets: 823 prey (mean = 54.9) in the estuary and 587 prey (mean = 41.9) in the limnetic colony (Table 1). The egret chicks' diets in the estuary were composed mostly of fish (199 individuals), insects (88) and crustaceans (21), whereas in the limnetic area insects (94) and fish (53) predominated. Mollusks and vegetation/seeds were also present in the egret chicks' diets, but with low abundance. Spoonbill chicks in the estuarine colony fed mostly on insects (479), fish (149), vegetation/seeds (137), with lower amount of mollusks (37) and crustaceans (21).

In the freshwater colony, the chicks also relied on fish (292) and insects (259), whereas there were lower numbers of crustaceans, mollusks and vegetation/ seeds, including rice grains (Table 1).
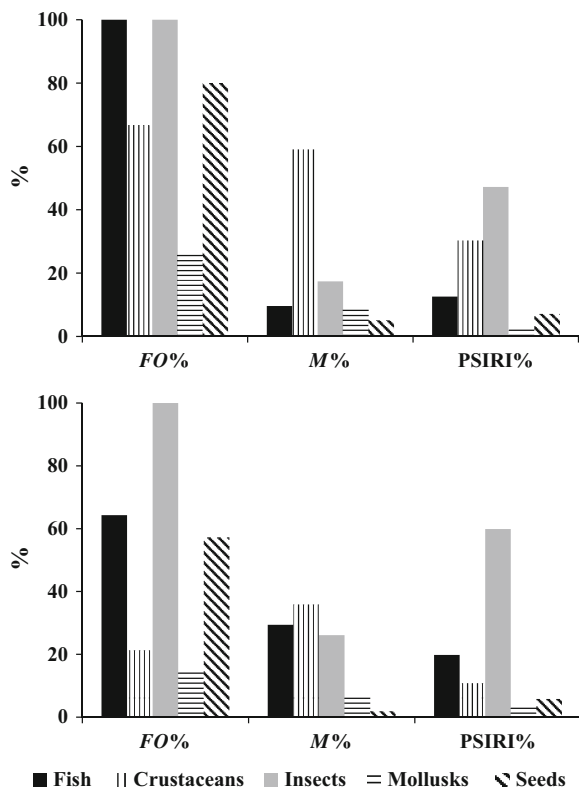
Considering all of the dietary parameters that were analyzed together and integrated by PSIRI% values, the diet of the estuarine egret chicks was mainly fish (PSIRI 53.3%), followed by crustaceans (30.8%) and insects (13.9%). Fish had the largest values of biomass $M\% = 75.1\%$, followed by crustaceans (23.4%) and insects (1.0%). All of the parameters that were pooled (Fig. 2) confirmed a predominantly piscivorous diet. However, the chick diet in the limnetic colony included a large proportion of insects (PSIRI = 58.9%), followed by fish (27.9%) and crustaceans (8.5%). Due to its larger size, fish contributed more in biomass ($M = 75.7\%$) than insects ($M = 4.0\%$), with crustaceans accounting for 20.2% of the $M\%$. Therefore, in terms of the energy obtained, represented by
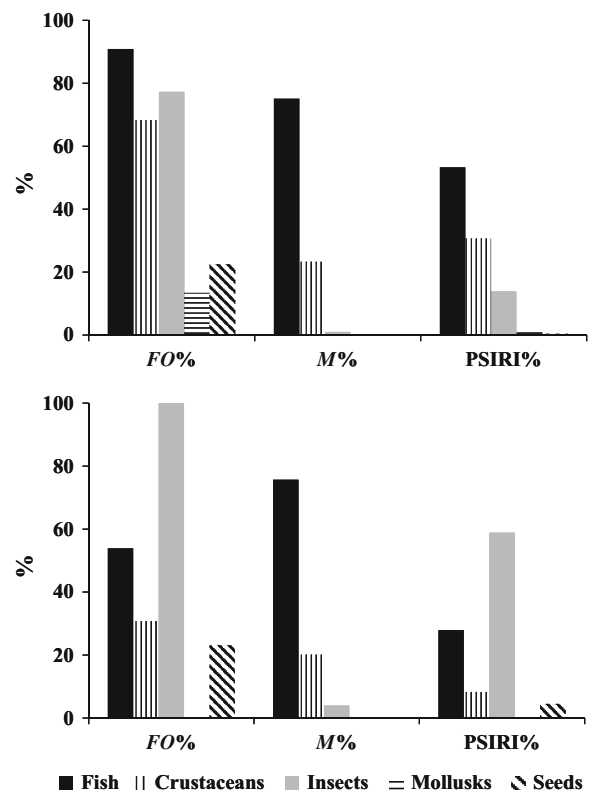
Fig. 2 The diet composition of great egrets, *Ardea alba*, chicks. *Above* the estuarine environment (Marinheiros Is.); *below* the freshwater environment (Aguirre/Taim Marsh). *FO%* Frequency of occurrence; *M%* contribution in reconstructed mass; *PSIRI%* Prey-specific Index of Relative Importance

ingested biomass, the limnetic diet is also piscivorous; nevertheless, other food items, such as insects and crustaceans, were also important in the diet as a whole (PSIRI%, Fig. 2).

The spoonbill chicks' diets in the estuary had an insect PSIRI% of 47.2%, followed by crustaceans (30.4%) and fish (12.6%). Fish and insects were present in all of the samples (FO% = 100%). Crustaceans predominated in biomass (59.1%), followed by insects (17.4%) and fish (9.6%). The seeds had a PSIRI% = 7.2% and FO% = 80.0% (Fig. 3). Therefore, in the estuary, the spoonbill chicks fed on a range of prey taxon, but the energetically important (M%) foods were crustaceans and insects. The insects were common and frequent, but the biomass contribution was small. Despite also relying on the same varied group of food items (Fig. 3) in the freshwater colony, the spoonbills fed their chicks mainly insects

(PSIRI% = 59.9%, FO = 100% and M = 26.1%) and fish (PSIRI% = 19.8%, M = 29.4%), although again crustaceans contributed more in biomass (M% = 35.8%), despite their low PSIRI% (11.0%). Vegetation (seeds) was frequently consumed (FO = 57.1%), but had a low PSIRI% (6.0%).

Trophic niche overlap and niche breadth

The diet overlap measured by the Morisita–Horn index at each location/species varied from 14 to 81% (Table 3). Remarkably, the largest diet overlap was between the estuarine spoonbills and limnetic egrets (81%), and the lowest (14%) occurred between the limnetic spoonbills and estuarine egrets. Large overlaps were also found between the estuarine and limnetic egrets (65%), indicating that freshwater prey were also captured by egrets that were breeding in the estuary. In addition, a large diet overlap (77%) was found between species of the freshwater colony (Table 3).

Standardized Levin's niche breadth (B_S) was lower for egrets (0.06 in the estuary and 0.08 in limnetic area), and higher for spoonbills (0.18 in the estuary and 0.22 in the limnetic area).

The GLM of SI values

The mean $\delta^{13}C$ values of the blood of estuarine egret chicks were significantly higher than those of freshwater egrets and spoonbills in both environments (Fig. 4). The $\delta^{15}N$ values also formed two groups but were unrelated to the species or sampling colony, instead reflecting the trophic level. $\delta^{15}N$ values from

Fig. 3 The diet composition of roseate spoonbills, *Platalea ajaja*, chicks. *Above* estuarine environment (Marinheiros Is.); *below* freshwater environment (Aguirre/Taim Marsh). *FO%* Frequency of occurrence; *M%* contribution in reconstructed mass; *PSIRI%* Prey-specific Index of Relative Importance

Table 3 The Morisita–Horn similarity index for the diet of great egret, *Ardea alba*, and roseate spoonbill, *Platalea ajaja*, chicks in estuarine (Patos Lagoon), and limnetic (Aguirre/Taim Marsh) environments in southern Brazil

	Estuary		Freshwater
	Great egret	Roseate spoonbill	Great egret
Roseate spoonbill (estuary)	0.55		
Great egret (freshwater)	**0.65**	**0.81**	
Roseate spoonbill (freshwater)	0.14	0.56	**0.77**

The index is calculated from the number of prey in the diet. Values >0.6 indicate high diet overlap and are in bold

the two habitats could not be directly compared because the birds from limnetic and estuarine colonies are part of distinct food webs (Fig. 4).
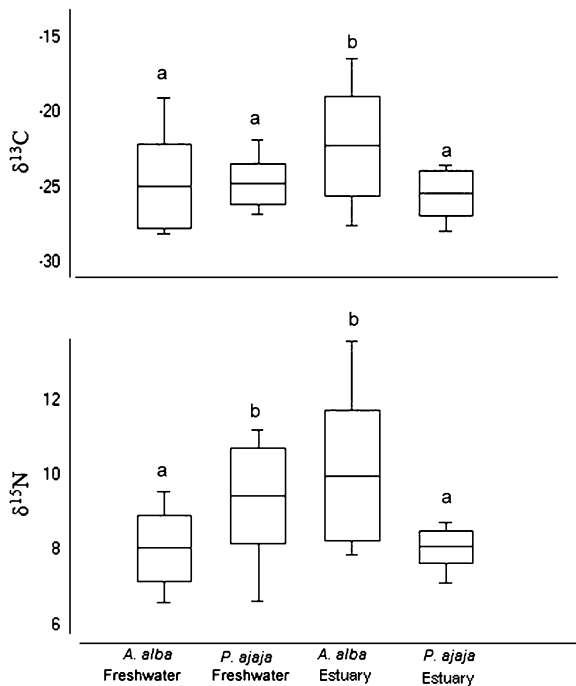
The GLM model with the best fit for the δ^{13}C values selected only two variables, the 'species' and 'colony', and the interaction among them (Table 4), as in Eq. 4:

$$\delta^{13}C \sim \text{Species} + \text{Colony} + \text{Species} * \text{Colony}. \quad (4)$$

This model had limited performance, explaining only 22% of the variance in δ^{13}C values (Table 5). The ANOVA with residuals of the model indicated that the 'species' variable and the interaction of 'species:colony' were significant. 'Colony' alone was not significant, demonstrating that, according to the species and the place where the colony is located, the species could either change their chicks' diet (i.e., egret) or keep the same diet in both freshwater and estuarine colonies (i.e., spoonbills). The best GLM, with a lower AIC for δ^{15}N values, is represented by Eq. 5:

$$\delta^{15}N \sim \text{Species} + \text{Colony} + \text{Species} * \text{Colony} + \text{Tarsus\_Length} * \text{Colony}. \quad (5)$$

This model explained 44% of the variance in δ^{15}N values (Table 5) and indicated that species differ in their δ^{15}N, but with an interaction between 'species' and 'colony', as well as between 'tarsus length' and 'colony' (Eq. 5, Table 5). The lack of significance of 'colony', but the significance of the interaction of 'colony':'species' and colony:'tarsus length' suggests that there are differences in the δ^{15}N values between the colonies, depending on the species under scrutiny, as well as in the δ^{15}N values of chick blood, which differ according to chick size. The ANOVA of the model indicated a high explicability of the interaction of 'species:colony' (26.0%), the interaction of 'colony:tarsus length' (6.4%), and the variable 'tarsus length' alone (9.2%). This latter result shows that as chicks mature, they rely more on prey with higher δ^{15}N values; these are most likely larger prey that are higher in the trophic chain.

Bayesian SI mixing models

For the Bayesian SI mixing model, the spoonbills' tactile foraging technique and the prey identified by direct methods were taken into account. The following sources were incorporated into the model after testing several models with different combinations of sources. The sources were: two small limnetic fish [*Jenynsia multidentata* (Jenyns, 1842) and *Astyanax eigenmanniorum* (Cope, 1894)], the estuarine crustacean *Callinectes* sp., the limnetic shrimp *Palaemonetes argentinus* Nobili, 1901, and autotroph organisms at the base of the food web (periphyton and POM) (Fig. 5).

The limnetic sources were demonstrated to be important food sources for spoonbill chicks in the freshwater colony, with limnetic fish having credibility intervals (CI 95%) of 10.1–47.3% (*A. eigenmanniorum*) and CI of 3.1–41.2% for *J. multidentata* (Fig. 6); primary consumers, such as *P. argentinus* had CIs from 1.1 to 41.4%; and sources at the base of the food web, such as periphyton and POM, had CIs from 1.1 to 34.8% and 0.4 to 22.8%, respectively. *Callinectes* sp., the only typically estuarine prey, had the lowest CI (0.3–15.9%). However, estuarine spoonbills had CIs for fish of 0.1–12.7% for *A. eigenmanniorum* and 1.2–45.7% for *J. multidentata*. The

Fig. 4 Stable isotope values of δ^{13}C (above) and δ^{15}N (below) in the whole blood of roseate spoonbill, *Platalea ajaja*, and great egret, *Ardea alba*, chicks in limnetic and estuarine colonies in southern Brazil, from September 2011 to January 2012. The values indicate the mean (*central bars*) and standard deviation (*rectangles*), with minimum and maximum values (*external lines*). The *different letters* indicate statistically significant differences

Table 4 The generalized linear model (GLM) coefficients of the selected model

Term	Estimative	Standard error (SE)	t	P
δ^{15}N (AIC = 207.2; df = 58)				
Intercept	8.093	0.675	11.996	<0.001
Main effects				
Roseate spoonbill	−1.764	0.412	−4.281	<0.001
Freshwater	1.026	1.261	0.813	0.419
Tarsus length	0.030	0.010	3.064	0.003
Interactions				
Platalea ajaja:Freshwater	3.320	0.598	5.550	<0.001
Freshwater:Tarsus length	−0.045	0.017	−2.572	0.013
δ^{13}C (AIC = 302.3; df = 60)				
Intercept	−22.224	0.578	−38.447	<0.001
Main effects				
Roseate spoonbill	−3.146	0.874	−3.600	<0.001
Freshwater	−2.688	0.843	−3.190	0.002
Interactions				
Roseate spoonbill:Freshwater	3.293	1.231	2.675	<0.01

The intercept represents values of δ^{15}N or δ^{13}C for 'great egret' and colony 'estuary,' in relation to all of the other levels are compared. For example, an estimate of roseate spoonbills was $e^{-1.764}$, indicating that δ^{15}N values were 176.4% (statistically significant) lower than the species defined for comparison, i.e., the great egret; or the value for 'freshwater' ($e^{1.026}$) indicates a non-significant increase of 102.6%, in comparison with the 'estuary' value

df degrees of freedom for the residuals of each model

Table 5 A summary of ANOVA results with δ^{15}N and δ^{13}C values in the generalized linear model (GLM)

Source of variance	df	Deviance	% explained	df of residuals	Deviance residuals	F	P
δ^{15}N						0	
Null model				63	137.297		
Main effects							
Species	1	1.095	0.80	62	136.201	0.828	0.367
Colony	1	2.465	1.80	61	133.736	1.864	0.177
Tarsus length	1	12.645	9.21	60	121.091	9.562	0.003
Interactions							
Species:Colony	1	35.642	25.96	59	85.449	26.952	<0.001
Size:Colony	1	8.749	6.37	58	76.700	6.616	0.013
Total explained	5	60.596	44.14				
δ^{13}C							
Null model				63	463.52		
Main effects							
Species	1	38.711	8.35	62	424.80	6.436	0.014
Colony	1	20.902	4.51	61	403.90	3.475	0.067
Interactions							
Species:Colony	1	43.033	9.28	60	360.87	7.155	<0.01
Total explained	3	102.646	22.14				

The % explained is calculated as the ratio between the deviance residuals/deviance residuals of the null model*100, as in Ye et al. (2001)

df degrees of freedom
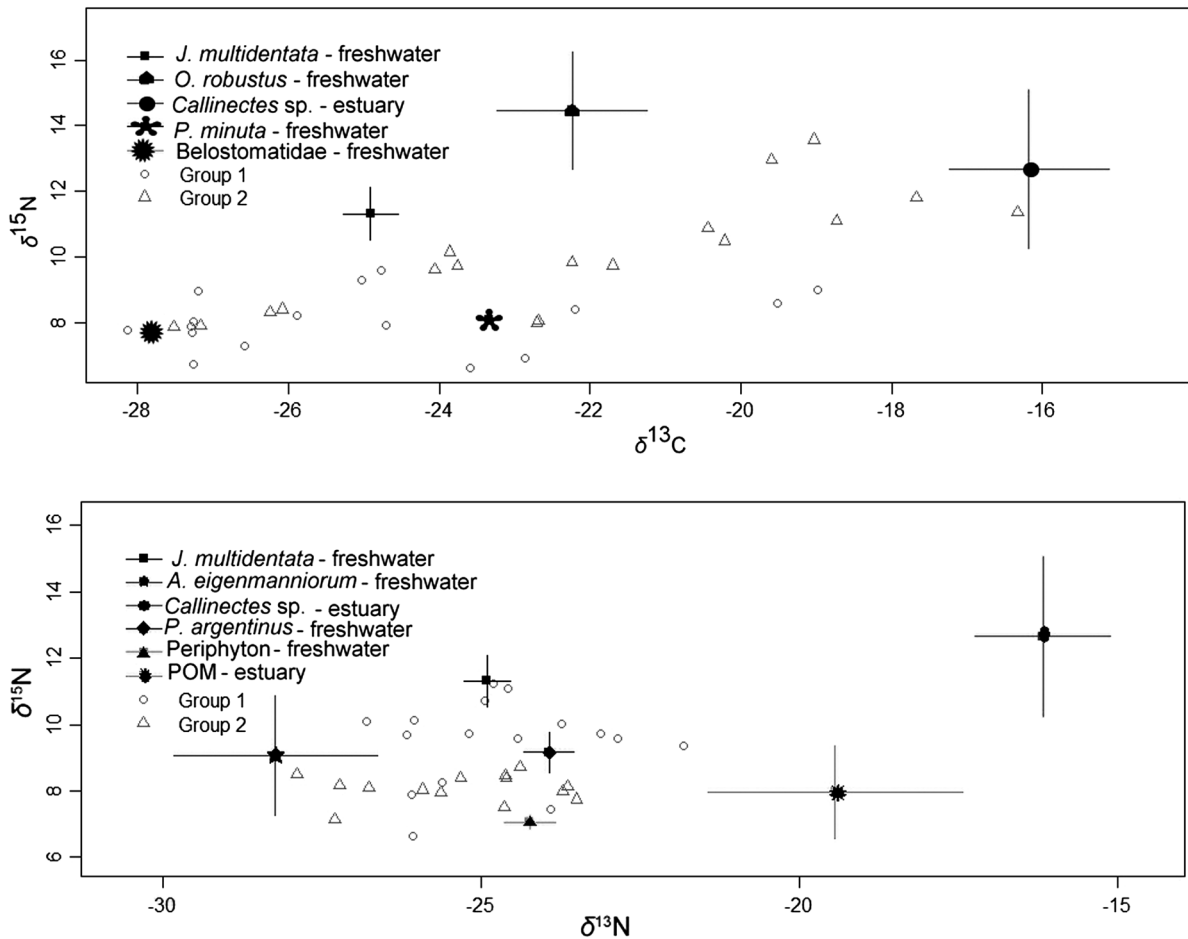
Fig. 5 The distribution of individual $\delta^{15}N$ and $\delta^{13}C$ values from the whole blood of great egret, *Ardea alba*, chicks (*above*) and roseate spoonbill, *Platalea ajaja*, chicks (*below*) that were sampled from a limnetic colony (Group 1, *circles*) and an estuarine colony (Group 2, *triangles*) in southern Brazil. The values of potential food sources that were used in mixing models, SIAR (*solid symbols*, followed by standard deviation), are shown

limnetic shrimp, *P. argentinus* had CIs from 0.3 to 25.1%, whereas limnetic periphyton and estuarine POM had CIs between 29.7–62.9% and 0.3–21.4%, respectively. *Callinectes* sp., the only estuarine prey species in the model, again had the lowest CIs (0.07–5.8%). Overall, it is clear that despite breeding in the estuary, spoonbills rely heavily on limnetic prey (Fig. 6) as shown by direct diet assessments and the GLM analyses above.

SIAR models for egrets included a freshwater insect, the water bug Belostomatidae, the estuarine crab *Callinectes* sp., the limnetic amphibian *P. minuta*, and two limnetic fish representing distinct functional groups (*J. multidentata* and *O. robustus*) (Fig. 5). The model for egret chicks from the freshwater colony had

CI ranges for *J. multidentata* of 0.2–17.4% and for *O. robustus* of 0.1–8.9%. *Callinectes* sp. had CIs of 0.1–12.7%, and *P. minuta* had CIs of 1.0–71.1%, whereas values for Belostomatidae were 1.9–73.3% (Fig. 7). Egret chicks in the estuarine colony had CIs from contributing sources as follow: *J. multidentata* had range 0.8–36.9%, and *O. robustus* was 0.5–28.8%. *Callinectes* sp., or an estuarine prey with similar SI values, was an important source with CIs 6.0–40.0%; *P. minuta* was 4.0–50.5%, and Belostomatidae was 2.9–41.1%. Overall, the egret chicks in the freshwater colony seemed to rely more on prey with isotopic signatures that were similar to insects and amphibians despite the limnetic fish sources (*J. multidentata* or others with a similar SI signature) and saltwater
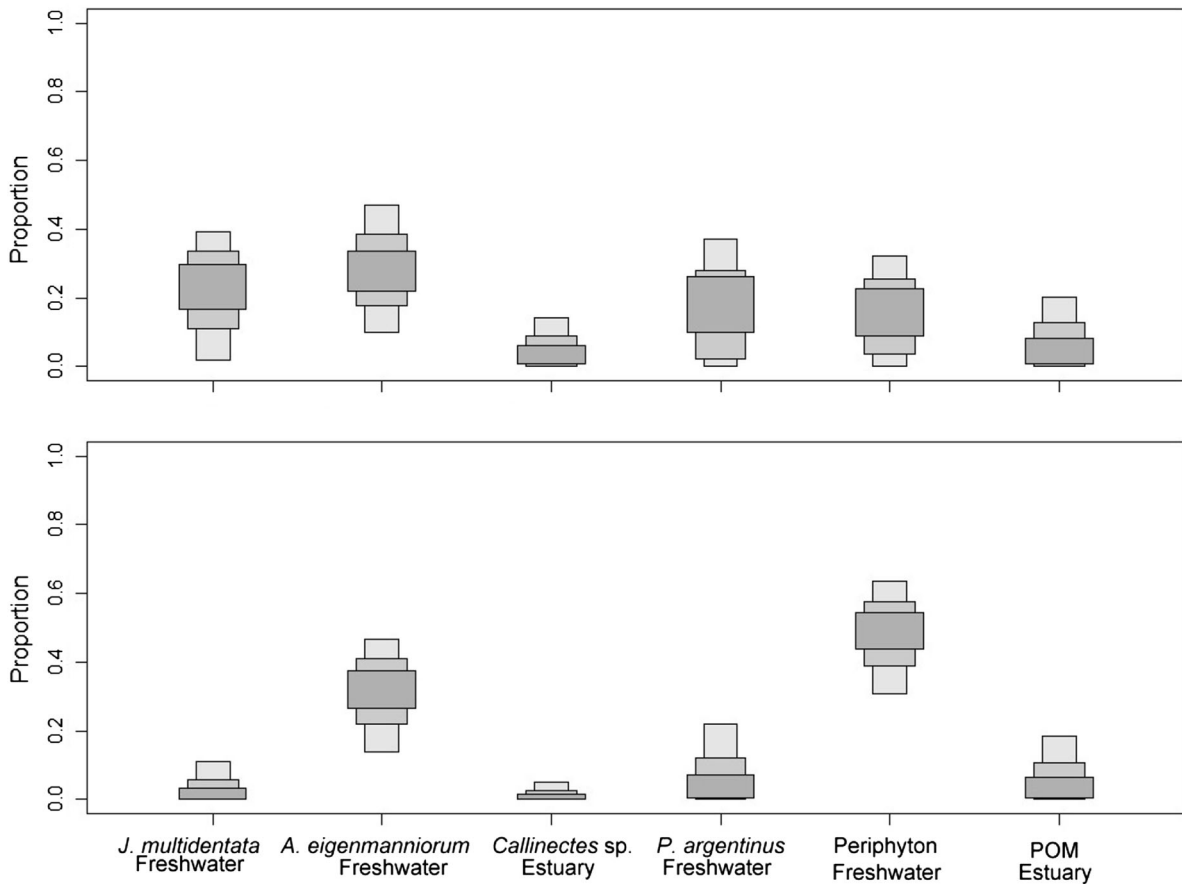
Fig. 6 The contribution of different food sources to the stable isotope values in the whole blood of roseate spoonbill, *Platalea ajaja*, chicks in the limnetic (*above*) and estuarine (*below*)

environments were obtained using the Bayesian stable isotope mixing model (SIAR). Credibility intervals are shown (95%, *dark gray*, 75% *medium gray*, 25% *light gray*)

sources (similar to *Callinectes* sp.), which also contributed consistently to the model. However, egrets that bred in the estuary had more varied sources, with similar proportions of both estuarine and freshwater sources (Fig. 7).

Isotopic niche

The isotopic niche width was greater for egrets than spoonbills in both sampling colonies (Fig. 8; Table 6), whereas isotopic niche overlap was similar to overlaps described in the diet by the Morisita–Horn index (Table 3). The highest isotopic niche overlap was again found between the limnetic egrets and estuarine spoonbills (20%), and the lowest was found between both species in the estuary (2%). Low overlaps were also found between the estuarine and freshwater egrets (8%) and between the estuarine and freshwater spoonbills (6%) (Table 6).

Discussion

Differences in the food type, prey size and habitats selected for foraging by great egrets and roseate spoonbills were strongly influenced by differences in foraging techniques, mediated by bill morphology and physiological constraints of chicks to salt. Despite being waterbirds of similar size and body mass, the spoonbills capture 4–6 times more prey per sample than egrets. The prey captured by spoonbills are smaller and have a predominance of insects. In addition, spoonbills feed lower in the trophic web; sources at the base of the food chain, such as the periphyton and/or POM, were detected by the SIA. Spoonbills are tactile foragers in shallow waters (Matheu & del Hoyo, 1992; Dumas, 2000; Teixeira & Nacinovic, 2003), inserting their bill in the water near the bottom and moving their partially opened bill
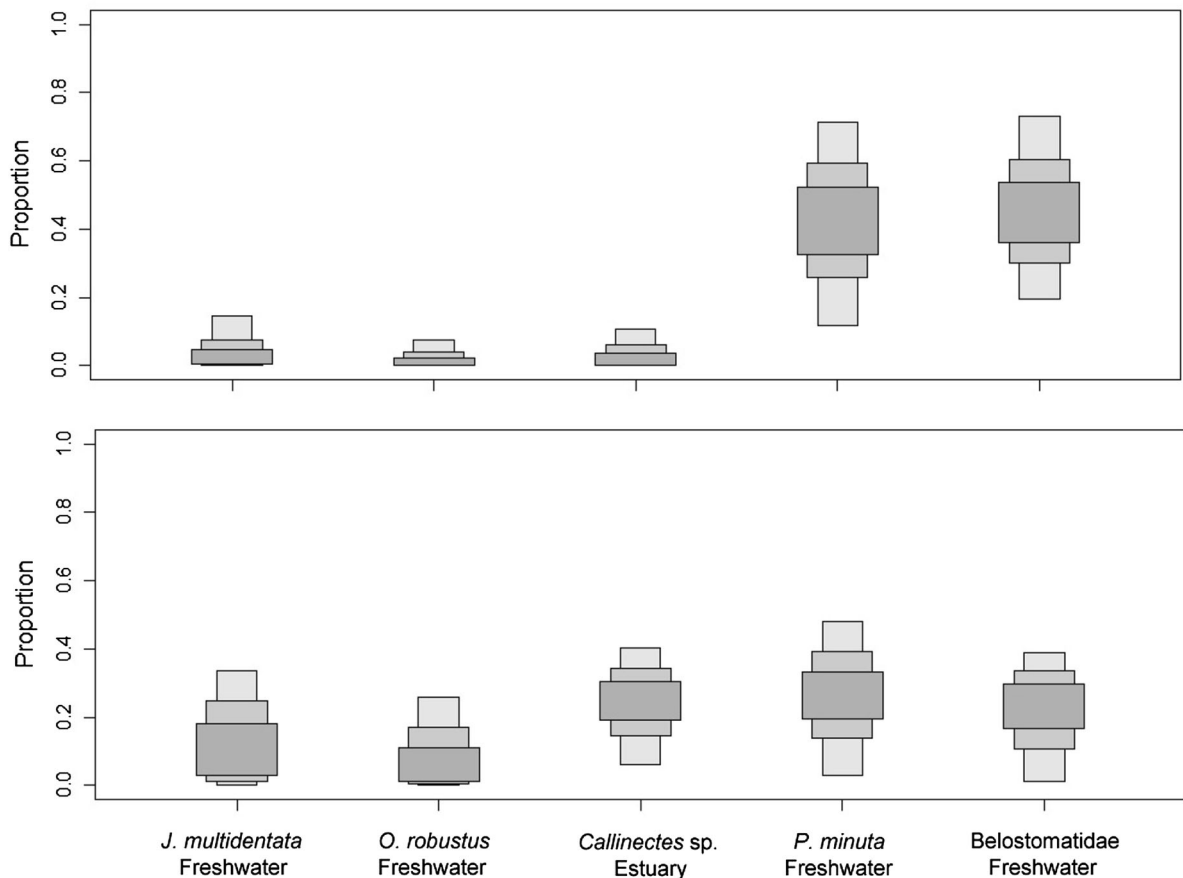
Fig. 7 The contribution of different food sources to the stable isotope values in whole blood of great egret, *Ardea alba*, chicks in the limnetic (*above*) and estuarine (*below*) environments were obtained using the Bayesian stable isotope mixing model (SIAR). Credibility intervals are shown (95%, *dark gray*, 75% *medium gray*, 25% *light gray*)

from side to side while walking, and closing their bill to capture prey once detected (Dumas, 2000; Teixeira & Nacinovic, 2003). The bill morphology and feeding techniques of spoonbills suggest that food sources at the base of the food chain could be important, but conventional dietary methods were unable to detect them. Using isotopes we could not exclude the possibility that periphyton, phytoplankton or C_3 macrophyte consumers, or consumers of vegetable organic matter, such as the small fish *A. eigenmanniorum* and *J. multidentata*, which include vegetation in their diets (Corrêa et al., 2010; Fischer et al., 2011), are food sources.

The great egret is a visual predator that uses the sit-and-wait technique (Kushlan & Hancock, 2005; Maccarone & Brzorad, 2007). Fish were three times more important than insects in the diet of egrets in the estuary, and insects were the predominant prey in freshwater. The use of small-sized prey is energetically plausible when they are abundant. Furthermore, during breeding, the egrets may select insects to deliver to chicks because they are unable to swallow large fish and other prey (Martínez-Vilalta & Motis, 1992). This may explain why the $\delta^{15}N$ values in blood of estuarine egret chicks were higher than those in freshwater egrets, as well as higher than in spoonbills from both environments, as demonstrated by the GLM analysis. The $\delta^{15}N$ values in freshwater fish tend to be higher than in estuarine fish (Garcia et al., 2007), which was detected in blood of spoonbills, but not in the egret blood, maybe due to predominance of insects in freshwater. Such differences in $\delta^{15}N$ values were further confounded by potential changes in prey size, as the chicks grew up.
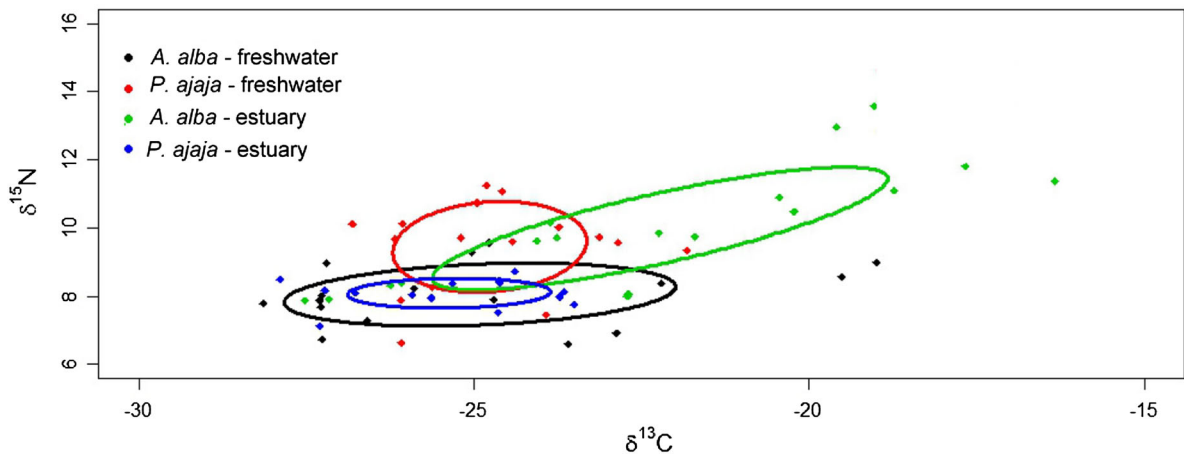
Fig. 8 The isotopic niche width and isotopic niche overlap between roseate spoonbill, *Platalea ajaja*, and great egret, *Ardea alba*, chicks in an estuarine and a freshwater colony in southern Brazil were estimated based on the $\delta^{13}C$ and $\delta^{15}N$ and were calculated using ellipse areas using the SIBER package in R software

Table 6 The isotopic niche overlap between great egret, *Ardea alba*, and roseate spoonbill, *Platalea ajaja*, chicks in estuarine (Patos Lagoon) and limnetic (Aguirre/Taim Marsh) environments in southern Brazil, based on $\delta^{13}C$ and $\delta^{15}N$ values and calculate using a Bayesian (SIBER) model

	Estuary	Freshwater		Niche width
	Roseate spoonbill	Great egret	Roseate spoonbill	
Great egret (estuary)	0.02	0.08	0.17	11.1
Roseate spoonbill (estuary)	0.20	0.06		2.1
Roseate spoonbill (freshwater)		0.10		6.0
Great egret (freshwater)				8.2

Methodological considerations

The number of dietary samples obtained per species, colony and method differed, but pooling all of the samples may have reduced some of the problems associated with different methodologies (e.g., Barrett et al., 2007; Cherel et al., 2007) and provided a moderately accurate estimate of diets (Harris & Wanless, 1993; Seefelt & Gillingham, 2006). For instance, pellets overemphasize prey with hard parts (e.g., insects) to the detriment of soft prey (e.g., small fish), which are only detectable in spontaneous regurgitates, stomach flushing or in the stomach contents of dead chicks. Contrary to expectations, spoonbills which ingest smaller prey than egrets, had lower numbers of unidentified prey. This is most likely due to the large number of fish in freshwater egrets' diets, as freshwater fish have smaller and more fragile otoliths than estuarine and marine fishes (Silva-Costa & Bugoni, 2013). Despite having being used for

penguins and other seabirds (Gales, 1987), stomach flushing was more invasive than the other methods used in the current study, and seems to be unsuitable for long-necked waterbirds.

The SIA provided information about the food sources effectively assimilated by consumers, complementing the dietary information provided by direct methods (Bearhop et al., 2001; Weiser & Powell, 2011; Silva-Costa & Bugoni, 2013). The relevance of such complementary approaches is clear in the current study because conventional dietary methods were unable to reveal the importance of primary producers in the diet of spoonbills, which was shown by the SIA. Furthermore, the congruence between the 'overlaps' and 'segregations' revealed by the Morisita–Horn index for the diet data, and the isotopic niche width revealed by the SIBER and SIAR for the stable isotope data, provide considerable strength to conclusions about dietary differences and overlaps. However, niche breadth calculated with dietary data (Levin's

niche breadth, B_S) and the isotopic niche from SIBER differed, with the B_S showing narrower niches for egrets than spoonbills and SIBER showing the opposite pattern. Dietary data indicated that spoonbills are less selective than egrets in both colonies, which might be expected of a tactile forager. The low values of B_S found for egrets are similar to the B_S 0.056 found for the estuarine piscivorous feeder Neotropic cormorant *Phalacrocorax brasilianus* (Gmelin, 1789) in the Lagoa dos Patos estuary (Barquete et al., 2008).

Foraging location inferred by diet and SIA

Spoonbills from the estuarine colony feed on freshwater prey, despite some fish prey tolerate a wide range of salinity (Vieira et al., 2010; Fischer et al., 2011). In the Florida Bay, spoonbills breeding on estuarine islands also move to inland foraging areas (Lorenz et al., 2009). The egrets seemed to forage closer to the colony because the estuary is <300 m from the colony, as indicated by SIA.

In the freshwater colony, the presence of rice grains in the spoonbills' diet indicates that they were foraging in irrigated rice fields, which are approximately 10 km from the colony; this is well inside the >30 km foraging radius of breeding roseate spoonbills feeding chicks in Florida (Lorenz et al., 2009).

Regarding egrets at the freshwater colony, SIA suggested that some adults forage in marine/estuarine waters. The nearest salt water is 15 km from this colony, and the estuary is approximately 55 km away, suggesting that some adults were able to travel to the marine beaches to forage. Tracking studies coupled with simultaneous use of dietary and SIA data (e.g., Caron-Beaudoin et al., 2013) will greatly expand our understanding of the foraging strategies of waterbirds. However, this result differs from niche breadth calculated using diet data, which suggest a more specialized niche by egrets and a lower specialization by spoonbills, consistent with foraging techniques. In summary, both wading species use limnetic prey when breeding in the estuary, but spoonbills rely on freshwater prey exclusively.

Feeding ecology and segregation

During breeding, spoonbills and egrets are central-place foragers, and their sympatry may have contributed to overlaps in the trophic niche. Similar isotopic values may be from distinct prey sources, despite undistinguishable. Dietary overlap was higher between freshwater egrets and estuarine spoonbills, suggesting that competition in freshwater is higher. When breeding in the estuary, there is a reduction in the potential competition, with spoonbills relying on prey similar to that of freshwater egrets and egrets switching to estuarine prey. Furthermore, the ability of spoonbills to feed during twilight periods and at night (Rojas et al., 1999) may reduce competition with diurnal egrets. Niche partitioning through spatial segregation or prey choice is a common feature of species breeding in sympatry (Connan et al., 2014).

The diet of both species was mainly composed of fish, insects, crustaceans, mollusks, and a minor proportion of seeds. Egrets most likely ingested the seeds of native species through secondary consumption (food ingested by their prey); however, spoonbills are known to eat aquatic plants, fruits, stems, and rhizomes, despite their predominantly carnivorous diet (Matheu & del Hoyo, 1992). The use of vegetation, periphyton, or POM at the base of the food chain was demonstrated with stable SIA, a technique not used before in this species.

Previous studies have indicated the occurrence of estuarine/coastal foraging by spoonbills (Dumas, 2000; Teixeira & Nacinovic, 2003; Lorenz et al., 2009). We were unable to detect foraging in salt water by spoonbills; possibly because the fish and crustaceans found in the diet were euryhaline species and the SIA did not indicate a marked estuarine signature in the blood. Bayesian mixing models with stable isotope data indicated the diet of spoonbills consisted predominantly of freshwater prey, with a small isotopic niche width.

The diets of great egrets described in previous studies (e.g., Figueroa & Stappung, 2003; Brzorad et al., 2004; Pretelli et al., 2012) are similar to this study and are predominantly piscivorous, despite insects and crustaceans making important contributions to the diet in both estuarine and freshwater environments. Other wading birds have similar diets: little blue heron that breed in sympatry at the Patos Lagoon estuary feed on estuarine fish and crustaceans (Gianuca et al., 2012), whereas adult cocoi herons in Argentina, ingest the same major taxa in addition to amphibians and small mammals (Ducommun & Beltzer, 2010). Dietary data and SIA also demonstrated the ability of egrets to exploit prey in both

estuarine and freshwater environments according to their breeding place.

Conclusion

When analyzing diet by both direct (pellets, spontaneous regurgitations, stomach flushing, stomach contents of dead chicks) and indirect (SI) methods, we found congruence in the vast majority of results. Our results suggest that during breeding great egrets exhibited no preference for either freshwater or brackish habitats for feeding and relied taking on prey larger than spoonbills. There is some overlap in the diet of egret and spoonbill chicks during the early chick rearing period, particularly in freshwater environments and when chicks are unable to ingest large prey.

The switch to estuarine prey by egrets that are breeding in the estuary seems to reduce potential competition with spoonbills, which feed exclusively on limnetic habitats, no matter where they breed. Spoonbills exhibit a predominantly carnivorous diet (fish, crustaceans, and insects) but, because they are tactile foragers, they also obtain a significant proportion of the diet from the base of the food chain, filtering, or ingesting incidentally plant matter, seeds, or periphyton. This later food source was detected by the SIA and mixing models, but was undetected by conventional diet analysis methods. Differences in the foraging techniques and trophic niche of these two large wading birds demonstrated mechanisms for segregation between the species and the reduction of potential competition between them.

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