Assessing the trophic niche of South American Terns integrating conventional and isotopic methods

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Abstract. Conventional dietary studies combined with stable isotope analysis allowed the characterisation of the trophic niche of South American Terns (*Sterna hirundinacea*) breeding in Argentina. Direct observation of prey deliveries during mate- and chick-provisioning showed that the diet of South American Terns comprised at least 18 prey items, mainly pelagic schooling fish with smaller proportions of marine invertebrates. Argentine Anchovy (*Engraulis anchoita*) was the main fish prey. Adults delivered a higher proportion of fish and smaller prey to their chicks than to their mates. Analysis of stable isotope ratios in blood samples, using Bayesian stable isotope mixing models, indicated that adult Terns used a higher proportion of marine invertebrates for self-feeding than for mate- or chick-provisioning. Analyses of stable isotopes ratios in blood samples from chicks were consistent with the determination of diet from direct observation, and indicated differences from adults in both the proportion of different prey in the diet and trophic niche. In addition, isotopic niche analysis showed that chicks had a significantly higher trophic position compared with self-feeding adults during both mate-provisioning and chick stages. The results highlight the importance of combining stable isotope and conventional methods to adequately assess the food requirements of a population during the breeding season.

Additional keywords: breeding diet, isotopic niche, Patagonia, prey provisioning, self-feeding, *Sterna hirundinacea*.

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Introduction

The niche concept has a central place in ecological theory. Ecologists have most often quantified ecological niche in terms of location in a multidimensional space, the axes of which represent relationships to environmental and biological factors influencing life history (Chase and Leibold 2003). The analysis of the ecological requirements of an organism, population or species needs the measurement and estimation of these key niche dimensions. However, these estimations are extremely challenging to obtain through conventional methods alone (Milesi and López de Casenave 2005; Newsome *et al*. 2007).

Dietary studies allow the characterisation of an important dimension of a population's ecological niche, contributing information not only on the diversity of resource categories used but also on niche overlap with other populations or species (Wiens 1989; Jaksic and Marone 2007). Many studies of seabirds have shown that the use of prey resources during breeding may vary geographically, between years, or seasonally in response to fluctuations in availability of prey and to energetic requirements or restrictions imposed by reproductive factors (Barrett *et al*. 1987; Pierotti and Annett 1991; Suryan *et al*. 2002; Wilson *et al*. 2004). The analysis of temporal patterns in dietary requirements during different life stages is, therefore,

fundamental to interpreting several aspects of the ecology of a population. In addition, dietary studies provide valuable information on predator–prey relationships and contribute to the identification of changes in marine ecosystems (Montevecchi 1993; Piatt *et al*. 2007).

The use of stable isotope analyses of animal tissues (mostly nitrogen and carbon) has become a valuable tool in the study of key aspects relating to the use of trophic resources by individuals or populations (Inger and Bearhop 2008), helping to overcome some of the difficulties associated with the use of conventional methods (Barrett *et al*. 2007; Newsome *et al*. 2007; Karnovsky *et al*. 2012). Stable isotope Bayesian mixing models allow the assessment of the relative proportion of different prey consumed by a predator based on the isotopic values of the predator's tissues and that of their prey (Moore and Semmens 2008; Parnell *et al*. 2010). Bearhop *et al*. (2004) and Newsome *et al*. (2007) suggested that stable isotope analysis could be a powerful tool to evaluate trophic position and trophic niche breadth. Layman *et al*. (2007) elaborated that proposal and developed a set of metrics on the isotopic data to infer characteristics of the niche width that were later reformulated in a Bayesian framework by Jackson *et al*. (2011), who also developed multivariate ellipse-based metrics for estimation and comparison of niche widths.

The combination of stable isotope and conventional methods may help provide a broader view of the use of resources by a species or population.

The South American Tern (*Sterna hirundinacea*) is endemic to South America, breeding from the coasts of southern Peru and central Brazil to Tierra del Fuego, Argentina, including the Malvinas (Falkland) Islands (Gochfeld and Burger 1996; Yorio 2005). Despite its wide distribution, little is known about its feeding ecology. The only quantitative information on diet is from studies in the breeding season in Argentina (Fernández Ajó *et al*. 2011) and southern Brazil (Fracasso *et al*. 2011) and from the non-breeding season in Argentina (Favero *et al*. 2000; Silva Rodríguez *et al*. 2005; Mariano-Jelicich *et al*. 2011) and Uruguay (Alfaro *et al*. 2011). This paper presents data on the trophic niche of South American Terns breeding in Patagonia, Argentina, gathered over two years. Like many other terns, South American Terns transport only one prey item in their bill when provisioning mates and offspring, and this is visible to an observer. This provides an excellent opportunity to evaluate how South American Terns use food resources during two different stages of the reproductive cycle (mate- and chick-provisioning). Our analysis of resource use by South American Terns includes the quantification of prey-types and prey-sizes, and an assessment of the amplitude of resource use based on conventional and isotopic niche approaches. In addition, stable isotope analysis allowed the evaluation of prey used by adults for self-feeding during the two stages of the breeding cycle and comparison with the prey delivered to mates and chicks.

Materials and methods

Study area and study species

The study was conducted in the Punta Loma Provincial Reserve (42°49′S, 64°53′W), Chubut Province, in Patagonian Argentina. The coast of this area is characterised by cliffs ~20 m high and bush steppe vegetation. The breeding colony of South American Terns was on the edge of the cliffs and comprised ~3200 pairs (A. Gatto and P. Yorio, unpubl. data). South American Terns arrive at Punta Loma in mid-October and settle at the colony site during the first and second week of November. First chicks hatch during the first and second week of December and start to fledge at about 4 weeks old (Scolaro *et al*. 1996; Villanueva-Gomila *et al*. 2009).

Dietary and niche evaluation through feeding observations

Direct observations of prey items delivered to mates and chicks were conducted to estimate trophic niche (prey-type and prey-length) and trophic niche width (prey richness, prey diversity and diversity of fish lengths). Although this method is direct, it does not allow the estimation of the trophic niche of self-feeding adults. We considered 'mate provisioning' as the delivery of prey to a mate during both the egg-laying and incubation periods. Observations were conducted using binoculars (Audubon 10 × 42 Equinox) throughout daylight hours and different weather conditions and stages of the tidal cycle over 12 days in 2004 and 13 days in 2005, evenly distributed between mate- and chick-provisioning stages. Observations were carried out from a blind located at the periphery of the Tern colony. After the researcher entered the blind in each sampling period, Terns re-settled rapidly on their nests or within their territories and did not show further obvious signs of disturbance. The study area was scanned continuously for adults attempting to land. Once an adult was selected, it was observed with binoculars until the prey could be identified, often while the adult was attempting to feed its mate or chick. Terns were not individually marked, and repeated sampling of the same individuals was kept to a minimum by selecting different groups of nests in each observation period. As adults may land and take off again with their prey, possible pseudoreplication was kept to a minimum by noting the nest location and the position of prey in the bill (orientation of tail and abdomen of prey item). Each item was identified to the lowest taxonomic level possible using morphological characters, and length noted relative to the bill-length of the adult. Size of prey was categorised as tiny (<0.5 × bill-length), small (0.5–1.0 × bill-length), medium (1–1.5 × bill-length) and large (>1.5 × bill-length) (Shealer 1998*a*; Gatto and Yorio 2009). Mean bill-length of adult South American Terns is 41.2 mm (± 2.4 mm s.d.) (Lisnizer *et al*. 2014). To reduce observer bias in determination of prey, all feeding observations and estimations of length of prey were made by only one observer (A. Gatto) in both years. Observations from the blind positioned very close to the birds allowed the detection of even small differences among prey species, including different silversides, marine invertebrates and terrestrial insects.

Generalised linear models (GLM) with binomial error structure and logit link function (Faraway 2006) were used to test the effects of breeding stage and season on the proportions of fish vs marine invertebrates. The effects of main type of fish prey, breeding stage and season on lengths of fish were evaluated using ordered logistic regressions (OLR) (Agresti 2002). GLM and OLR were fitted using R software version 3.1.1 (R Development Core Team 2014). Models with all possible combinations of predictor variables were considered, and best-fitting models were selected using Akaike's Information Criterion (AIC) (Burnham and Anderson 2002). Prey richness was defined as the observed number of prey species, and prey- and fish-length diversity was calculated using the Shannon Diversity Index (Krebs 1999). Unidentified fish prey ($n = 82$, 4.5%) were not included in the former analyses. To allow for the comparison between breeding stages, the variables prey richness and prey- and fish-length diversity were rarefied (Sanders 1968; Krebs 1999), scaling the larger sample to the size of the smaller one using EcoSim software (Gotelli and Entsminger 2010). Differences in richness and diversity were tested using 95% confidence intervals given by this software. Means are presented ± 1 standard deviation (s.d.).

Dietary and niche evaluation through stable isotope analysis

To complement the information obtained through direct observations of prey delivered to mates or chicks, stable isotopes analysis was performed. Stable isotopes analysis allowed the estimation of the trophic niche of adults self-feeding and feeding chicks (prey consumed and isotopic niche) and trophic niche width (through isotopic niche width). Whole blood samples were collected during 2005 to assess the carbon (C) and nitrogen (N)

isotopic composition that reflected the diet during the two breeding stages studied. Stable isotope analysis is based on the evaluation of the proportion of specific stable isotopes in a tissue sample from an organism (Fry 2004). The relationship between C and N stable isotopes in animal tissues reflects the isotopic relationships of their prey when the tissue was formed: the ratio $^{15}N : {^{14}N}$ is incremented along the trophic web and the ratio $^{13}C : {^{12}C}$ reveals trophic spatial information, such as a coastal or pelagic diet in marine environments (Newsome *et al.* 2007; Inger and Bearhop 2008).

Whole blood samples integrate the isotopic composition of the prey ingested by an individual during a period of *c.* 1 month before the sample was collected (Hobson and Clark 1992). Thus, blood samples used to assess adult diet during the mate-provisioning stage were collected when chicks were hatching, and those used to assess adult diet during the chick-provisioning stage and to assess chick diet were collected 25–30 days after the mean hatching date. Samples were obtained from a total of 33 adult South American Terns captured at night using mist-nets at the colony in both breeding stages, and from 17 chicks captured at their nests. Captured individuals were ringed with numbered metal rings provided by Centro Nacional de Pesquisa e Conservação de Aves Silvestres (CEMAVE), Brazil, and handmade colour-coded Darvic rings. Most of the ringed adults were later seen at their nests, indicating that the sample was predominantly or entirely of breeding adults. Whole blood samples (0.5–1 mL) were extracted from the brachial vein of each individual and conserved in 70% ethanol. When freezing is not available, blood preservation in 70% ethanol has been recommended as it has no significant effects on the isotopic values of the blood (Hobson *et al.* 1997; Halley *et al.* 2008), although this may not be the case in all situations (Bugoni *et al.* 2008). Fasting and lipid content can also affect isotopic values in plasma (Cherel *et al.* 2005). However, stable isotope ratios measured in whole blood are usually similar to those in cells, which are the main component by mass (Cherel *et al.* 2005). Blood samples were dried at 60°C over 24 h and then ground in a micro-mortar (Hobson *et al.* 1997). A subsample of 1 ± 0.2 mg was set in a tin capsule for stable isotope analysis. Sample analyses were performed by the Stable Isotope Facility of the University of California at Davis (California, USA). Stable isotope abundance is expressed using standard δ notation relative to Vienna Pee Dee Belemnite and atmospheric N. The internal laboratory standard used was a mixture of sucrose and ammonium sulfate ($\delta^{13}C = -24.44‰$, $\delta^{15}N = 1.33‰$) calibrated with the mentioned international standards. Observed measurements were $-24.44 \pm 0.05‰$ and $1.32 \pm 0.1‰$, for $\delta^{13}C$ and $\delta^{15}N$ laboratory standards respectively.

The relative contribution of the different prey to the isotope mixture was analysed using Bayesian mixing models within the R package SIAR (Parnell and Jackson 2013) following the guidelines suggested by Phillips *et al.* (2014). Before running the isotopic mixing models, the sensitive analysis proposed by Smith *et al.* (2013) was conducted in order to evaluate the feasibility of the isotopic mixing polygon. Neither element concentration dependence nor prior information on the proportion of prey on the diet of Terns was included in the

isotopic mixing models. As no specific diet-tissue discrimination factors (DTDFs) between food and whole blood were available for any tern species, we used DTDFs of Great Skua (*Catharacta skua*) from Bearhop *et al.* (2002) (1.1‰ for $\delta^{13}C$ and 2.8‰ for $\delta^{15}N$). Although published information on DTDFs of piscivorous seabirds is scant, it is likely they are qualitatively similar between species (Bodey *et al.* 2014). A standard deviation of $\pm 0.3‰$ was added to both values to account for potential differences in discrimination factors between actual values for South American Terns and those used in this study. This value was estimated as the mean dispersion of DTDFs of Charadriiformes in Bond and Diamond (2011). Unfortunately, isotopic information of prey from our study area is lacking, so prey isotopic values were carefully selected from published studies conducted in nearby areas and during, or as close as possible, to the breeding season. In order to keep to a minimum the parameters to be estimated (see Phillips *et al.* 2014), we included only the main prey in the mixing models. In addition, we pooled *a priori* prey species that were ecologically similar (different silverside and marine invertebrate species). We also conservatively managed uncertainty of isotopic prey values so as to represent as best as possible the isotopic scenarios of the known prey spatiotemporal dispersion. Information on the isotopic composition of Argentine Anchovy (*Engraulis anchoita*) corresponded to prey obtained at the nearby Golfo San José (J. Ciancio, unpubl. data; $\delta^{13}C = -18.7‰$, $\delta^{15}N = 15.9‰$, s.d. for both $= 0.5‰$). The isotopic composition of silversides (*Odontesthes* spp.) ($\delta^{13}C = -15.7‰$, $\delta^{15}N = 17.3‰$, estimated s.d. for both $= 0.3‰$) and Silver Warehou (*Seriorella porosa*) ($\delta^{13}C = -17.8‰$, $\delta^{15}N = 18.3‰$, added s.d. for both $= 0.1‰$) were obtained from Forero *et al.* (2004) (value for the former corresponds to the average between the two reported silverside species). The isotopic composition of marine invertebrates ($\delta^{13}C = -18.1‰$, $\delta^{15}N = 14.3‰$, added s.d. for both $= 1$ ‰) was calculated using the average composition of Patagonian Longfin Squid (*Loligo gahi*) (Ciancio *et al.* 2008) and the polychaete *Eunice argentinensis* (Galván *et al.* 2009), as these are the most likely candidate species for the unidentified squid and polychaetes in the diet of the South American Terns.

After normality and homoscedasticity were checked, mean $\delta^{13}C$ and $\delta^{15}N$ isotope values were compared using analysis of variance (ANOVA) and Tukey's test *a posteriori* comparisons (Crawley 2013). Isotopic niches were compared using the hypothesis-testing framework proposed by Turner *et al.* (2010) and the approach based on multivariate ellipse metrics (Jackson *et al.* 2011). Differences in centroid location, which provide information on isotopic position, and eccentricity, which provides insight into differences in the underlying distribution of $\delta^{13}C$ and $\delta^{15}N$ data, were tested using nested linear models and residual permutation procedures (see Turner *et al.* 2010 for statistical details). Niche width was estimated for each group using multivariate ellipse-based metrics (Jackson *et al.* 2011). The analysis generates standard ellipse areas (SEA) which are bivariate equivalents to standard deviations in univariate analyses. We used SEA values corrected for small sample size (SEA_C) to calculate niche overlap, and generated Bayesian estimates of SEA (SEA_B) to test differences in isotopic niche by comparing their 95% credible intervals (CI) (for examples see Jackson *et al.* 2012; Thomson *et al.* 2012; Zabala *et al.* 2013).

Results

Dietary and niche evaluation through feeding observations

A total of 1840 prey items were delivered by adult Terns in the two study years: 598 during mate-provisioning and 1242 during chick-provisioning. The breeding diet of South American Terns comprised at least 18 prey items: seven fish, six marine invertebrates and five terrestrial insects (Table 1). Fish were the main prey item carried to the colony in both study years (2004: 76%, $n = 657$; 2005: 87%, $n = 1183$). The main fish prey was the Argentine Anchovy, which constituted >60% of deliveries except during chick-feeding in 2004. Secondary prey included the silverside *Odontesthes nigricans* and unidentified prawns, and varied between breeding stages and years (Table 1). Prey richness and prey diversity were significantly higher in 2004 than in 2005 (both $P < 0.05$, Table 1). In both years, prey richness was significantly higher during chick-provisioning than when feeding mates (both $P < 0.05$, Table 1). Prey diversity was significantly higher when feeding chicks than during mate-provisioning in 2004 ($P < 0.05$), but the reverse pattern was observed in 2005 ($P < 0.05$) (Table 1).

The proportion of fish in relation to marine invertebrates delivered by Terns was >80%, except during mate provisioning in 2004 (65%). The best model describing the variation in the proportion of fish and marine invertebrates included only the effect of the breeding stage (Table 2). There were significant independent effects of the breeding stage ($\chi^2_1 = 54.7$, $P < 0.0001$) on the proportion of fish and marine invertebrates. Model parameters indicated that South American Terns delivered a greater proportion of fish to their chicks than to their mates, independently of the year (0.99; $Z = 7.42$, d.f. = 1, $P < 0.0001$) (Table 1).

The sizes of fish were estimated in 99% of observations of adults carrying fish ($n = 1516$). South American Terns mainly carried medium and large fish (40–60+ mm, 64% in total, $n = 1511$). The best model describing the variation in fish-size included the effect of the main prey type and the breeding stage (Table 2). There were significant independent effects of main prey type ($\chi^2_1 = 246.7$, $P < 0.0001$) and breeding stage ($\chi^2_1 = 24.8$, $P < 0.0001$) on the size of fish. South American Terns delivered larger anchovies than silversides (2.35, $t = 15.0$, d.f. = 1, $P < 0.05$) and larger fish to their mates than to their chicks, although the value of this last parameter was not significant (0.58, $t = 5.0$, d.f. = 1, $P > 0.05$) (Fig. 1). There was no significant effect of year on fish-size ($\chi^2_1 = 0.51$, $P = 0.47$; Fig. 1).

In 2004, South American Terns carried a higher diversity of fish-lengths than in 2005 ($P < 0.05$), and in both years the diversity of fish-lengths was higher when feeding chicks than during mate-provisioning ($P < 0.05$ for both years) (Table 3). The diversity of Argentine Anchovy and silverside lengths was higher in 2005 than in 2004 (all <0.05). In both years, the diversity of Argentine Anchovy lengths was higher during

Table 1. Number (percentage in parentheses) of prey items and values for richness and diversity of prey delivered to mates and chicks by South American Terns during the 2004 and 2005 breeding seasons at Punta Loma, Argentina

Prey	2004		2005	
	Mate-provisioning ($n = 244$)	Chick-provisioning ($n = 413$)	Mate-provisioning ($n = 354$)	Chick-provisioning ($n = 829$)
Fish				
Argentine Anchovy (*Engraulis anchoita*)	147 (61)	103 (26)	220 (62)	643 (77)
Silverside (*Odontesthes nigricans*)	6 (2)	124 (30)	3 (<1)	13 (2)
Silver Warehou (*Seriolella porosa*)		21 (5)	49 (14)	38 (5)
Silverside (*Odontesthes argentinensis*)	1 (<1)	25 (6)	5 (1)	20 (2)
Silverside (*Odontesthes incisa*)		15 (4)		1 (<1)
Butterfish (*Stromateus brasiliensis*)		6 (1)		
Signathid		1 (<1)		
Unidentified fish	3 (1)	45 (11)	2 (1)	32 (4)
Marine invertebrates				
Polychete A	10 (4)	56 (14)	23 (6)	33 (4)
Prawn	75 (31)			2 (<1)
Squid A		1 (<1)	41 (12)	12 (1)
Polychete B		5 (1)		21 (2)
Tunicate		1 (<1)		3 (<1)
Squid B	2 (1)			
Terrestrial insects				
Grasshopper A		1 (<1)	10 (3)	7 (1)
Cricket		2 (<1)		
Grasshopper B		1 (<1)		1 (<1)
Bumblebee				1 (<1)
Moth			1 (<1)	
Richness	6.00	12.40[A]	8.00	10.85[A]
		16.00		11.80[A]
Diversity	0.95	1.73[A]	1.22	0.86[A]
		1.72		1.02[A]

[A]Larger sample scaled (rarefied scaling) to the size of the smaller.

chick-provisioning than during mate-provisioning (both $P < 0.05$), while the diversity of silverside lengths was similar between breeding stages (both $P > 0.05$) (Table 3).

Table 2. Summary of model-selection results for models explaining the variation of the proportion of fish vs marine invertebrates of prey carried to the colony by South American Terns in relation to the breeding stage (mate-provisioning and chick-provisioning) and year (2004 and 2005), and the variation of size of fish in relation to same variables plus prey-type (anchovies and silversides)

Models are listed in decreasing order of importance

Candidate model	No. of parameters	AICc	ΔAICc	Weight
Fish vs marine invertebrates				
Breeding stage	2	73.5	0.0	1.00
Year	2	94.8	21.4	0.00
Null model	1	122.9	49.4	0.00
Breeding stage + year	3	122.9	49.4	0.00
Fish-size				
Prey + breeding stage	5	2978.0	0.0	0.68
Prey + breeding stage + year	6	2979.5	1.5	0.32
Prey	4	3000.8	22.7	0.00
Prey + year	5	3002.4	24.3	0.00
Breeding stage + year	5	3161.1	183.1	0.00
Breeding stage	4	3222.7	244.7	0.00
Year	4	3243.6	265.6	0.00
Null model	3	3282.9	304.8	0.00

Dietary and niche evaluation through stable isotope analysis

In 2005, δ^{13}C values of adults and chicks varied between $-18.4‰$ and $-16.3‰$, whereas δ^{15}N values varied between $17.2‰$ and $19.0‰$ (Fig. 2a). Mixing polygon sensitivity analysis (using 1500 iterations) showed that isotopic values of all individual samples, given the DTDFs and prey isotopic values used, were included in more than 95% of the simulated mixing polygons, validating the proposed mixing model (Fig. 2b). Based on the isotopic values corresponding to adults during the mate provisioning stage (δ^{13}C $= -17.2 \pm 0.4‰$, δ^{15}N $= 18.1 \pm 0.4‰$, $n = 16$; Fig. 2a), the Bayesian mixing model output showed an important use of Argentine Anchovy ($50 \pm 9\%$) and marine invertebrates ($39 \pm 7\%$) for self-feeding (Fig. 3a). This isotope model output also indicated that higher proportions of marine invertebrates were found in self-feeding adults than that observed when adults were provisioning their mates (Fig. 3a, 3b). During the chick-stage, the mixing model output using the isotopic values of adults (δ^{13}C $= -17.7 \pm 0.4‰$, δ^{15}N $= 17.7 \pm 0.4‰$, $n = 17$) showed an important use of marine invertebrates ($49 \pm 7\%$) and Argentine Anchovy ($48 \pm 9\%$) for self-feeding (Fig. 3c). The model output using chick isotopic values (δ^{13}C $= -17.4 \pm 0.2‰$, δ^{15}N $= 18.8 \pm 0.1‰$, $n = 17$) showed that adults provisioned chicks with a significantly higher proportion of Argentine Anchovy ($60 \pm 9\%$) than marine invertebrates ($19 \pm 7\%$) (Fig. 3d). These results were comparable to those obtained from direct observations of adults feeding chicks,
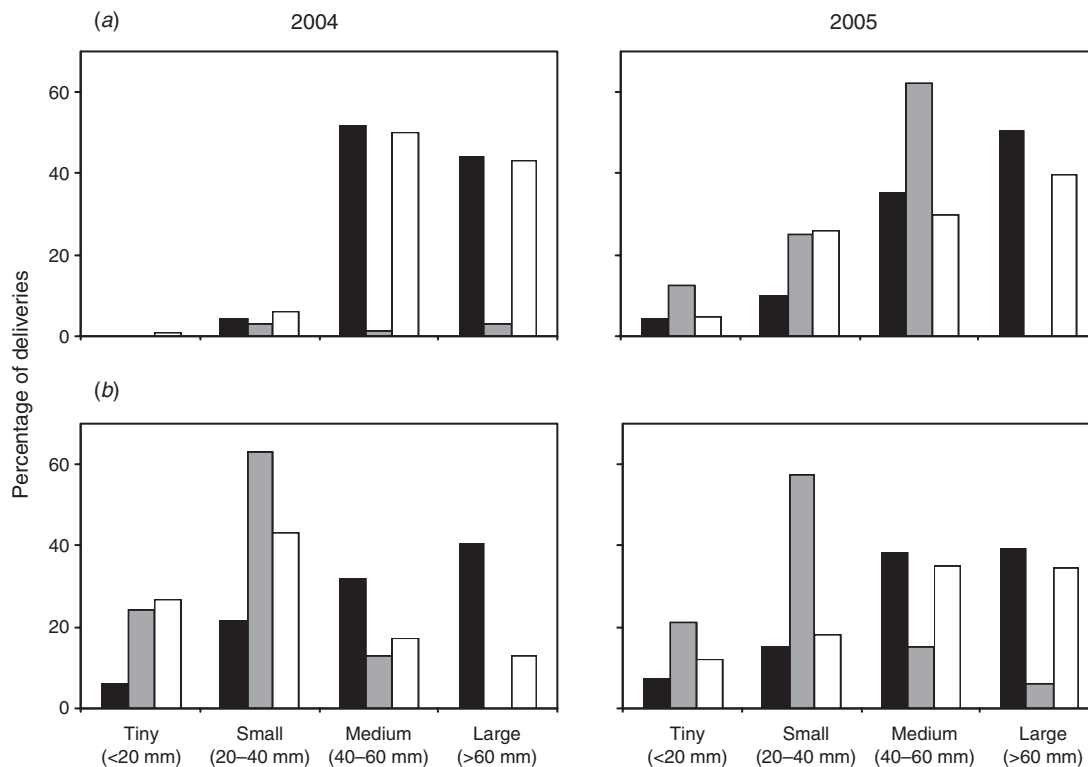

Fig. 1. Percentage occurrence of lengths of fish prey of South American Terns at Punta Loma, Patagonian Argentina, during: (a) mate-provisioning and (b) chick-provisioning in the 2004 and 2005 breeding seasons. Black bars: Argentine Anchovy; grey bars: silversides; white bars: all fish (including preceding).

Table 3.   Diversity of fish-lengths delivered to mates and chicks by South American Terns during the 2004 and 2005 breeding seasons at Punta Loma, Argentina

Species	2004			2005		
	Mates	Chicks	Season	Mates	Chicks	Season
Argentine Anchovy	0.83[A] ($n = 145$)	1.23 ($n = 103$)	1.06 ($n = 248$)	1.07 ($n = 220$)	1.21[A] ($n = 632$)	1.18[A] ($n = 852$)
Silversides	1.00 ($n = 7$)	0.71[A] ($n = 149$)	0.94[A] ($n = 156$)	0.90 ($n = 8$)	0.94[A] ($n = 33$)	1.15 ($n = 41$)
All fish	0.91 ($n = 155$)	1.28[A] ($n = 341$)	1.37 ($n = 496$)	1.22 ($n = 279$)	1.29[A] ($n = 736$)	1.28[A] ($n = 1015$)

[A]Larger sample scaled (rarefied scaling) to size of the smaller.
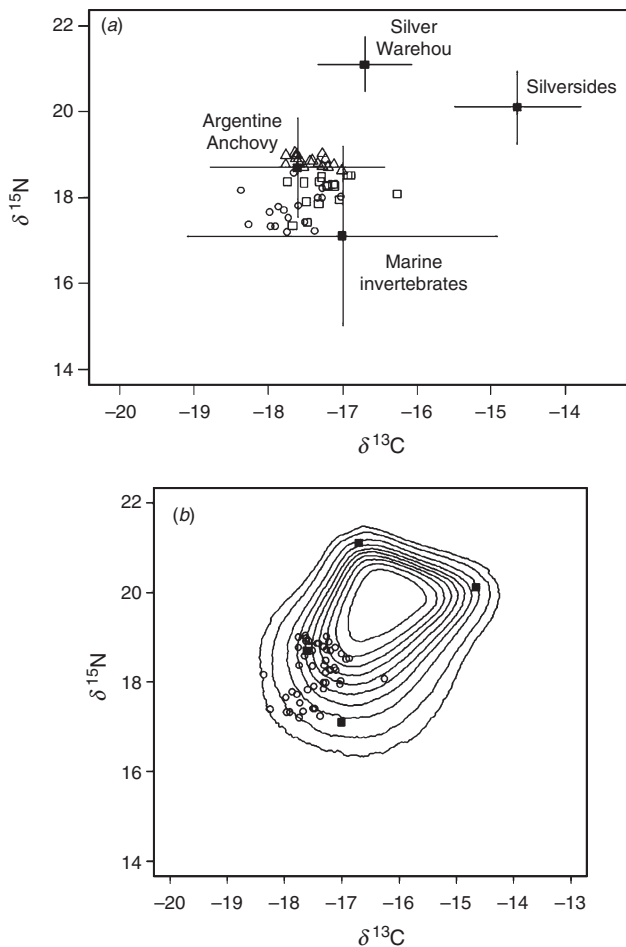
Fig. 2. Dual stable isotope plot of $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) showing the isotopic values of South American Tern whole blood during the 2005 breeding season at Punta Loma, Argentina, and their potential prey. (*a*) Isotopic mixing diagram. Open squares, adults during mate-provisioning; circles, adults during chick-provisioning; triangles, chicks. Potential prey values corrected for fractionation are represented by solid squares (values are means and error bars ± s.d.). (*b*) Simulated mixing region for the biplot in Fig. 2*a*. The positions of individual Terns (open circles) and the average source values (solid squares) are shown. Probability contours are at the 5% level (outermost contour) and at every 10% level.

Adults during both breeding stages and chicks showed differences in their isotopic positions, based on the observed differences in their centroid locations (adults in mate-provisioning vs adults in chick-provisioning, $P = 0.001$; adults in mate-provisioning vs chicks, $P = 0.001$; adults in chick-provisioning vs chicks, $P = 0.001$, using 1000 permutations; Fig. 4). These results were mainly due to differences in their $\delta^{15}N$ values (ANOVA: $F_{(2,47)} = 54.42$, $P < 0.0001$; Tukey HSD, all $P < 0.001$). In addition, differences in $\delta^{13}C$ were also found in adults between breeding stages (ANOVA: $F_{(2,47)} = 8.64$, $P < 0.001$; Tukey HSD, $P = 0.0004$). In accordance, the isotopic niches of adults estimated by SEA$_C$ (both in mate-provisioning and chick-provisioning) did not overlap with that of chicks (Fig. 4). During both breeding stages, adults showed a similar spread in their isotopic niche, as no differences were found neither in their SEA$_B$ (adults in mate provisioning: SEA$_B$ = 0.83, CI = 0.51–1.34; adults in chick feeding: SEA$_B$ = 0.84, CI = 0.53–1.34) nor eccentricity ($P = 0.85$) with an overlap in SEA$_C$ of 7%. Although the isotopic niche amplitude estimated by SEA$_C$ between chicks and adults in both mate-provisioning and chick-provisioning stages were different (adults in mate provisioning: SEA$_C$ = 0.41; adults in chick-provisioning: SEA$_C$ = 0.46; chicks: SEA$_C$ = 0.08), no significant differences were found using either Bayesian estimates of SEA (adult SEA$_B$ above; chicks: SEA$_B$ = 0.51, CI = 0.32–0.81) nor eccentricity (adults in mate provisioning vs chicks, $P = 0.17$; adults in chick feeding vs chicks, $P = 0.11$) (Fig. 4).

Discussion

This study is the first to describe differences in the prey of self-feeding South American Terns and the prey provided to chicks . At Punta Loma, Argentine Anchovies were the main prey provided by South American Terns to mates and chicks, although they also delivered other pelagic schooling fish, marine invertebrates and terrestrial insects. In general, significantly more Argentine Anchovies were provided than other prey species, reaching almost 80% of prey delivered during the chick-provisioning stage in 2005, possibly because of its relatively higher energetic value compared with other prey (Ciancio *et al*. 2007; García *et al*. 2010). The reason for the relatively lower contribution of this fish prey when feeding chicks in 2004 is not clear, as observations during mate-provisioning indicated that Anchovies were available earlier in the breeding season. The inclusion of marine invertebrates has also been recorded at the same South American Tern colony (Fernández Ajó *et al*. 2011), at a colony in southern Brazil (Fracasso *et al*. 2011) and at their wintering grounds (Favero *et. al.* 2000; Alfaro *et al*. 2011). Benthic prey, such as polychaetes, and which are generally

validating the isotope mixing model outputs (Fig. 3*d*, 3*e*). More importantly, isotopic models revealed that during this stage adults consumed a higher proportion of marine invertebrates for self-feeding than that delivered to chicks and similar to that used for self-feeding when provisioning mates (Fig. 3).
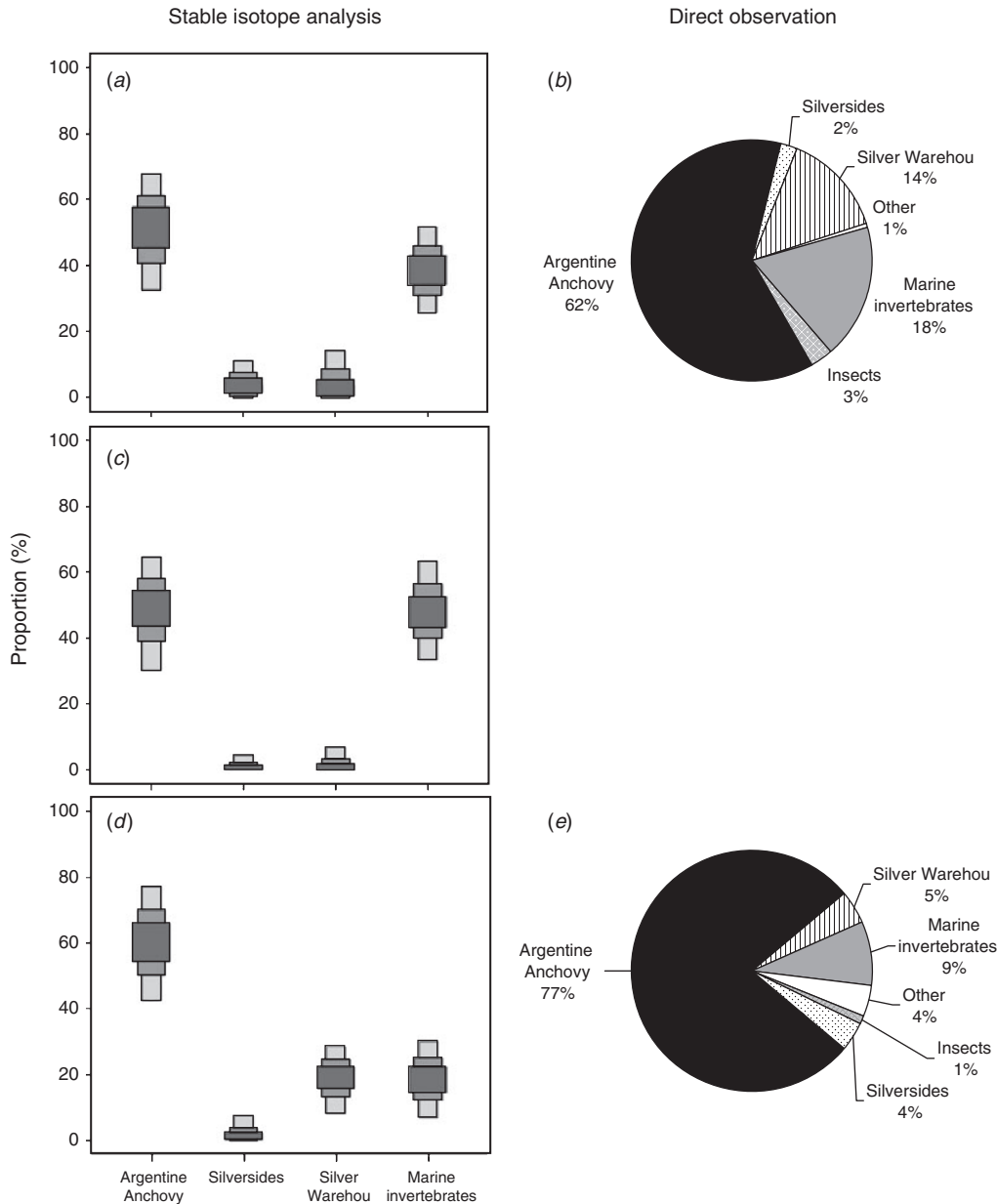
Fig. 3. Comparison among the trophic resource utilisation of South American Terns during the mate-provisioning and chick-provisioning stages in the 2005 breeding season at Punta Loma, Argentina, revealed by direct observations of prey deliveries and stable isotope analysis. Left panel: results of SIAR Bayesian isotope mixing models (50%, 75% and 95% credibility intervals) showing the estimated prey contributions to the self-feeding diets of adults during: (*a*) the mate-provisioning stage; (*c*) the chick-provisioning stage; and (*d*) chick diet. Right panel: percentage of prey types delivered to (*b*) mates and (*e*) chicks (see Table 1 for detailed information).

unavailable to foraging Terns, could be obtained from macroalgae stranded along beaches or from breaking waves along the shore during strong winds or large swells. Provisioning of benthic marine invertebrates was generally recorded after storms or periods of strong winds (A. Gatto, pers. obs.), suggesting that their use may often result from opportunistic feeding when faced with difficulties in obtaining their main fish prey. Several studies have shown that feeding ability of terns decreases during strong winds (Taylor 1983; Stienen *et al.* 2000).

The use of prey resources by South American Terns varied between years, with the inclusion of a higher proportion of Argentine Anchovies and a higher proportion of fish prey in relation to invertebrates during the 2005 breeding season. In addition, prey composition was in general less rich and diverse in 2005 than 2004. These differences between years may have been partly the result of changes in prey availability, but unfortunately we lack independent information on food resources in the study area. Interestingly, a similar pattern of changes in prey
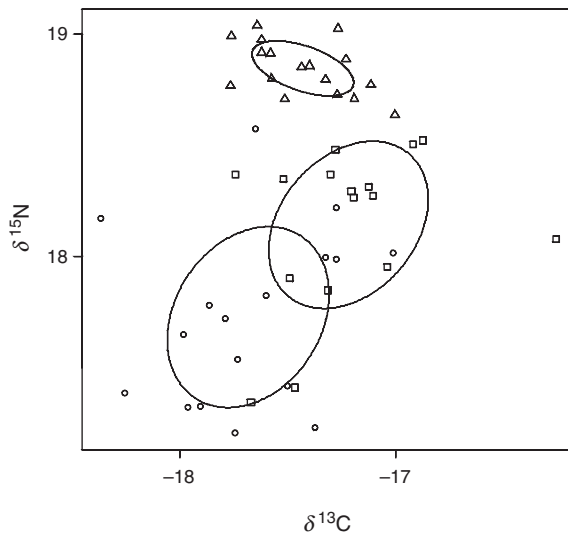
Fig. 4. δ^{15}N (‰) and δ^{13}C (‰) values for adult South American Tern during mate-provisioning (squares), adults during chick-provisioning (circles), and chicks (triangles). Isotopic niches are represented as the standard ellipses (solid lines) used to calculate SEA$_C$.

richness and diversity was recorded in the diets of Royal Terns (*Thalasseus maximus*) and Cayenne Tern (*Thalasseus sandvicensis eurygnatha*) at a nearby colony (Gatto and Yorio 2009) during the same years. Prey delivered by adults also differed between the two breeding stages, as they fed their chicks with a more diverse diet than that delivered to mates. A wider trophic spectrum during chick-provisioning has been already reported in other terns (Shealer 1998*a*; Ramos *et al*. 1998; Catry *et al*. 2006; Gatto and Yorio 2009), and it has been argued that the increase in prey diversity may benefit breeding performance (Shealer 1998*a*).

South American Terns delivered fish of similar sizes between years. Lengths of fish (40–60+ mm) were also within the range of sizes recorded at the same colony in the following season (Fernández Ajó *et al*. 2011), indicating that although prey composition may vary, Terns select similar sized prey among years. South American Terns provisioned their mates with larger prey than that delivered to their chicks. Studies in other species of tern have shown that during laying, males select larger prey to provision their mates (Taylor 1979; Shealer 1998*b*; Nisbet 2002) so as to fulfil their mate's nutritional and energetic requirements (Nisbet 1977). Smaller fish prey during chick-provisioning could also result from size-selective predation. Prey sizes recorded during mate-provisioning indicate that larger prey were available in the study area before chicks hatched, suggesting parents were selecting smaller prey when feeding their chicks. This prey selection should be particularly important during the early chick-stage, as smaller chicks have more difficulties manipulating and swallowing large prey (Shealer 1998*b*). In addition, the higher diversity of fish-lengths recorded when feeding chicks suggests changes in the prey requirements of chicks during their development. An increase in prey-size with age of chicks has been recorded for South American Terns breeding in the same study area (Fernández Ajó *et al.* 2011) and in several other tern species (Shealer 1998*b*;

Rossell *et al.* 2000; Stienen *et al.* 2000; Wambach and Emslie 2003; Ramos *et al.* 2004; McLeay *et al.* 2009; García *et al.* 2010), very likely as a result of growing energy demands and the capacity of chicks to handle larger prey as they grow.

Most studies using novel tools in stable isotope analysis report dietary reconstructions based solely on the results of isotopic mixing models, without analysis of complementary direct analyses (Boecklen *et al.* 2011) or without performing sensitive analyses despite the considerable influence of both diet-tissue discrimination factors (DTDFs) and prey inputs (Phillips *et al.* 2014). Although the stable isotope ratios for prey used in our models were not measurements from samples obtained in our study area, the sensitivity analysis showed that the mixing polygon for our consumer data was statistically feasible. In addition, stable isotope analyses of chick samples provided results that were consistent with those determined through direct observations of chick-provisioning, and indicated both a different trophic niche and a different contribution of prey to the diet between adults and chicks. Chicks had a significantly higher trophic position compared with adults during adult self-provisioning and mate-provisioning. This could be the result of differences between the DTDFs of adults and chicks. Sears *et al.* (2009) showed that growing Rhinoceros Auklet (*Cerorhinca monocerata*) chicks had depleted δ^{15}N values and no changes in δ^{13}C. Thus, assuming the same in South American Terns, chick δ^{15}N values would have been larger than those measured, and thus the difference in trophic level between adults and chicks would in fact be larger. Isotopic values could also reflect that adults or chicks were nutritionally stressed. However, Cherel *et al.* (2005) showed that in fasting King Penguins (*Aptenodytes patagonicus*) the effects on whole blood was only a small enrichment on δ^{15}N values. Adult diet included an important contribution of both anchovies and marine invertebrates while chicks were fed mostly with anchovies. Dietary differences between adults and chicks have been reported in several seabird studies (e.g. Ydenberg 1994; Barrett *et al.* 2007), including terns (Ramos *et al.* 1998; Shealer 1998*b*; Nisbet 2002; Catry *et al.* 2006). In central-place foragers that are single-prey loaders, it is expected that parents would provision their offspring with higher quality prey than those used for self-feeding (Bradstreet and Brown 1985; Davoren and Burger 1999; Wilson *et al.* 2004; Sonntag and Hüppop 2005). Adult Common Terns (*Sterna hirundo*), for example, feed their offspring mostly with fish, whereas they use also crustaceans, insects and other invertebrates for self-feeding (Nisbet 2002). Similarly, the diet of Little Tern (*Sterna albifrons*) chicks consists of a higher proportion of fish with a higher energetic value than the diet of adults (Catry *et al.* 2006).

Although breeding South American Terns at Punta Loma fed on a variety of fish and invertebrate prey, results clearly indicate the importance of the Argentine Anchovy as a prey resource. Similar importance of Argentine Anchovy has been reported for this Tern at its wintering grounds (Alfaro *et al.* 2011; Mariano-Jelicich *et al.* 2011). Although still not considerably exploited by commercial fisheries in our study area, Anchovies represent an attractive alternative target species to other partially over-exploited fish stocks (Skewgar *et al.* 2007). Several studies have indicated the negative effect of fisheries on seabirds (Schaffner 1986; Crawford 2004; Cury *et al.* 2011), highlighting the need

of further studies in order to understand the effects of anchovy fisheries on South American Tern populations.

In summary, South American Terns breeding at Punta Loma mainly provisioned their mates and chicks with fish, although the composition of delivered prey differed from that used for self-feeding. Resource use by terns is often based on observations of food delivered to mates and chicks, and may therefore result in a biased assessment of resource use and requirements. The use of stable isotope analysis to characterise the use of prey resources by breeding adults showed that although Argentine Anchovies may be a key prey resource, marine invertebrates also constituted an important component despite their lower nutritional quality. The results highlight the importance of combining stable isotope and conventional methods of dietary analysis to adequately assess the food requirements of a population during the breeding season.

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