

Combining a geographic information system, known dietary, foraging and habitat preferences, and stable isotope analysis to infer the diet of Magellanic Penguins in their austral distribution

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Abstract. The Magellanic Penguin (*Spheniscus magellanicus*) is the most abundant and widely distributed top marine predator of Atlantic Ocean waters off the Patagonian coast. During the breeding season, this species consumes thousands of tonnes of its prey and has a significant effect on populations of fish and squid near the Penguin's breeding colonies. We combined data on stomach contents, known habitat preferences of their prey, stable isotope analysis and a geographic information system to describe the diet of Magellanic Penguins in their austral distribution. On this basis, we present a comprehensive analysis of the trophic ecology of the species, the diet of which is poorly known (only a few analyses of stomach contents). Stable isotopes revealed a mean trophic level of 5.3 (range 4.5–5.8) and a diet in accordance with spatio-temporal overlap analysis. This study shows that Magellanic Penguins forage on few prey species not targeted by commercial fisheries in this region, mostly Patagonian Sprat and Longfin Squid. Moreover, we demonstrated that a combination of complementary tools can be used to describe the trophic relationships of wide-ranging marine predators, such as seabirds.

Additional keywords: prey distribution, *Spheniscus magellanicus*.

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Introduction

Trophic interactions are fundamental components of ecosystem organisation and dynamics (Walters *et al.* 1997) and accurate understanding and quantification of the strengths of trophic interactions remains lacking for most ecological systems (Paine 1988). A first logical step in determining trophic interactions is to provide a detailed description of the diet of organisms within the system. Seabirds are top-order predators with high energetic and food demands, and colonial breeding seabirds could impose significant predation pressure on prey populations in areas close to breeding colonies (Lewis *et al.* 2001). Understanding the trophic relationships between seabirds and their prey is necessary to incorporate seabird populations into regional models of marine ecosystems (e.g. Bergamino *et al.* 2012). Information on trophic interactions is also essential to assess seabird conservation and management issues, such as seabird–fisheries interactions (Montevecchi 2002; Berón *et al.* 2013), or for use of seabirds to monitor changes in marine ecosystems, as proposed by several authors (Cairns 1987; Piatt *et al.* 2007; Boersma 2008; Cury *et al.* 2011; Moreno *et al.* 2013).

Approximately 1 000 000 pairs of Magellanic Penguin (*Spheniscus magellanicus*) breed along the Patagonian coast of Argentina (Schiavini *et al.* 2005), making them a dominant marine predator in terms of prey consumption in this region (Brooke 2004). About half of the global population of the species (Schiavini *et al.* 2005) breeds in the Southern Patagonian Continental Shelf Ecosystem (SPSE). There have been few studies of trophic relationships of Magellanic Penguins in this ecosystem, and based on analysis of stomach contents only. Samples in these studies were collected during only a few days in the early chick-rearing period in the 1990s (Frere *et al.* 1996; Scolaro *et al.* 1999; Wilson *et al.* 2005).

Although analysis of stomach contents provides information on species composition of the diet, the sampling is restricted in time (Barrett *et al.* 2007). Stable isotope analysis (SIA) provides a reliable and integrative tool for trophic studies; depending on the tissue sampled, SIA can reveal the diet from the preceding few days to the preceding weeks, months or even years (Boecklen *et al.* 2011; Hobson 2011). Recently, Bayesian stable-isotope mixing models became available to estimate the proportion of

prey consumed by a predator (Moore and Semmens 2008; Parnell *et al.* 2010). When a Bayesian uninformative prior is used (no known information on diet) in a mixing model, all sources are assumed to contribute equally to a predator's diet *a priori* (Yeakel *et al.* 2011). This model will incorrectly assign a high proportion of the diet to an included potential prey not actually consumed by the predator but that satisfies stable isotope mixing conditions. In addition, estimates of dietary contributions may be inaccurate when multiple prey are isotopically similar. For these reasons input prey species included in any mixing model have to be chosen carefully. Taking this into consideration is particularly important for predators exploiting vast marine areas (Moreno *et al.* 2010), such as Magellanic Penguins, which could be consuming a diverse array of species with similar stable isotope signatures. In this study we developed a framework to infer the trophic ecology of a wide-range marine predator using Magellanic Penguins in the SPSE as a model species in two steps. First, we reviewed the published information on the trophic ecology of Magellanic Penguins and distribution and life histories of potential prey to identify the most likely prey. Second, we developed stable-isotope mixing models using the identified prey species to estimate their proportions in the diet at different localities.

Methods

Magellanic Penguins breed along the Patagonian coast of Argentina from 41°26'S to 54°54'S (Schiavini *et al.* 2005). Penguins in the north and the south of this distributional range feed in different marine ecosystems with different prey species present (Wilson *et al.* 2005). In this study, we focussed on the breeding colonies in the Southern Patagonian Continental Shelf Ecosystem (SPSE), which extends from 47°S to 55°S over the Patagonian Continental Shelf (see Ciancio *et al.* 2008 for a detailed description of this ecosystem, including the area and trophic relationships of main species). It includes 30 breeding colonies supporting ~436 517 pairs of Magellanic Penguins, more than a third of the world's population (see Fig. 1 for locations of largest colonies). We concentrated our studies on the five largest colonies (between 47°S and 52°S), which were arranged, for analysis, into northern, central and southern sectors of the study area: Puerto Deseado (northern), San Julián (central), Monte Entrance (central), Monte León (central) and Cabo Vírgenes (southern).

Literature review, analysis of spatio-temporal overlap and prey selection to include in models

To analyse the overlap of Magellanic Penguins and their potential prey, we reviewed: (1) the available information on the main prey species consumed by Magellanic Penguins, as well as food of other top-order predators (fishes, other seabirds, mammals) in the SPSE; (2) available information on the foraging areas and diving habits of Magellanic Penguins; and (3) the stable isotope values, temporal distribution, abundance and habitat preferences of potential prey species. We reviewed the scientific literature for these three aspects. We also interviewed researchers currently working on the abundance and distribution of potential marine prey of Magellanic Penguins and other species; most research on pelagic, benthopelagic, and

demersal marine species in the SPSE is conducted by the federal Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), the main fisheries research agency in Argentina, located in Mar del Plata.

To evaluate the spatio-temporal overlap between potential prey and Magellanic Penguins we used unpublished INIDEP databases, and published maps of distribution and abundance for individual species of potential prey (see Table S1 of the Supplementary Material, available online only). We georeferenced the foraging areas of Magellanic Penguins and distribution of potential prey from published maps and created layers in a geographic information system (GIS) using the georeferencer tool from the open-source software QGIS (Quantum GIS Development Team 2014). Once an image was converted into a GIS layer, the position of fishery research hauls or the polygon of Magellanic Penguin foraging areas were manually digitised. In identifying the foraging areas of colonies of Magellanic Penguins, we took a conservative approach and chose the largest foraging area described in the literature from the incubation or early chick periods (Wilson *et al.* 2005; Boersma *et al.* 2009). GIS polygons were created that included all positions reported in either kernel or simple point distributions and we used these areas as the maximum potential foraging area for a particular colony throughout the breeding season. To identify the distributions of potential prey species, in addition to the GIS layers created from published maps, we also included georeferenced data from acoustic and bottom-trawl research surveys conducted by INIDEP (e.g. Brunetti *et al.* 1997; Pineda *et al.* 1998; Sánchez *et al.* 1995; Madirolas 1997; INIDEP unpubl. data for research survey trawl OB0397; sources listed in Table S1). These data consist of acoustic estimation of the abundance of pelagic species (e.g. Sánchez *et al.* 1995; Madirolas 1997) or biomass of fish from bottom trawl-surveys (INIDEP unpubl. data for research survey OB0397). We evaluated the temporal overlap between foraging areas of Magellanic Penguins and the distribution of potential prey by grouping available information for spring or summer (Fig. 1; Sánchez *et al.* 1995; Brunetti *et al.* 1997; Madirolas 1997; Whöler *et al.* 1997; Pineda *et al.* 1998).

We created maps combining the foraging areas of the four colonies of Magellanic Penguin and the abundance and distribution of potential prey species, and then estimated the percentage overlap of the Penguin foraging area with the distribution of the potential prey species. We classed the overlap as: (1) high, when the Penguin foraging areas overlapped fully with the distribution prey species (e.g. Patagonian Sprat (*Sprattus fuegensis*) occur in all Penguin foraging areas); (2) medium overlap, when the overlap of Penguin foraging areas and prey distributions was >10% of the foraging area of the Penguins but <100% (e.g. Hake (*Merluccius hubbsi*)); and (3) low overlap, with overlap of Penguin and potential prey distributions was <10% of the foraging area of the Penguins (e.g. Argentine Squid (*Illex argentinus*)). We also classified potential prey species in terms of their vertical distribution in the marine environment: pelagic, demersal or benthic.

In the SPSE, Magellanic Penguins forage in coastal and inshore waters between the 100 m isobath and the coast (see Fig. 1 for foraging ranges for colonies). Although foraging dives up to 110 m deep have been recorded, most do not exceed 50 m

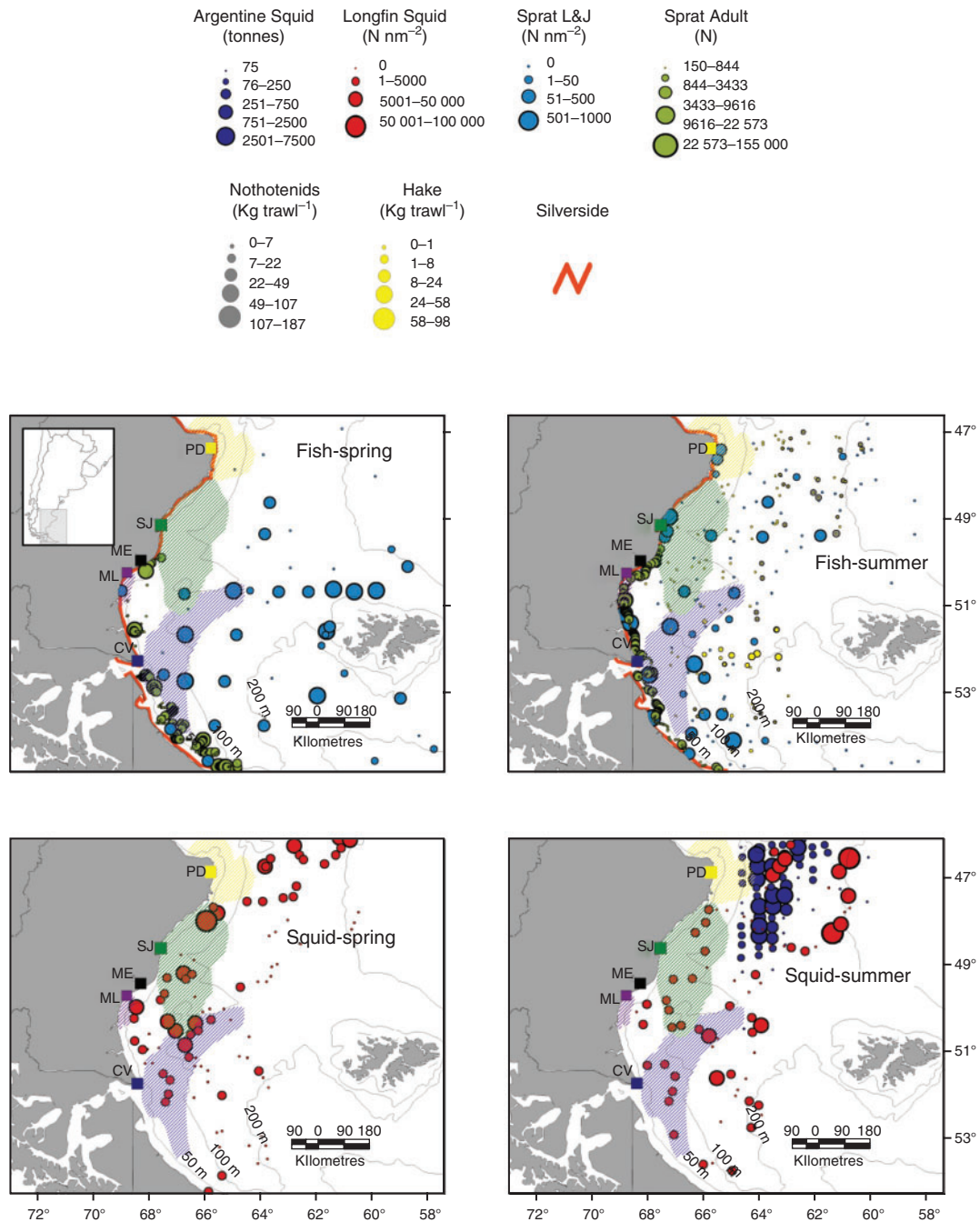


Fig. 1. Examples of the spatio-temporal overlap analysis of Magellanic Penguin foraging areas (coloured hatching – for colour, see online version of this paper available at <http://www.publish.csiro.au/nid/96.htm>) and the distribution of potential prey: Argentine Squid (from Brunetti *et al.* 1997); Longfin Squid (Pineda *et al.* 1998); larval and juvenile (L&J) and adult Patagonian Sprat (Sánchez *et al.* 1995; Madirolas 1997); Hake and notothenid fish (research trawls; OB0397 Instituto Nacional de Investigaciones Pesqueras INIDEP; and Silverside). Breeding colonies: PD, Puerto Deseado; SJ, San Julián; ME, Monte Entrance; ML, Monte León; CV, Cabo Vírgenes. Foraging areas for each colony shown by hatching of matching colour; foraging areas for PD and ML from Boersma *et al.* (2009); for SJ and CV from Wilson *et al.* (2005); foraging range of ME colony has not been studied. Spring, September–December; summer, January–March. N, number of individuals. $N\ nm^{-2}$, number of individuals per square nautical mile.

(Wilson *et al.* 2005). We thus considered pelagic and demersal prey as likely to be consumed by Penguins and removed benthic prey from analysis. Potential prey were discarded from further

consideration using the following criteria. (1) Potential prey that do not coexist with Magellanic Penguins, such as benthic prey or prey that showed low overlap with Magellanic Penguin

foraging areas were discarded from further analysis (see Table S1). (2) For prey that did coexist with Magellanic Penguins (medium or high spatial overlap), prey were discarded from analysis if: (2a) they were either not recorded or formed only a small proportion of the diet (<5% mass) stomach content analyses of Magellanic Penguins (see Table S2), or (2b) they had very different stable isotope values compared to those for Magellanic Penguins (after discrimination factor corrections; see below, Fig. 2).

Stable isotope analysis for determination of diet

We collected whole blood from Magellanic Penguins from the five large colonies in the SPSE – Puerto Deseado, San Julián, Monte Entrance, Monte León and Cabo Vírgenes – during spring (early December, small chicks) and summer (early February, large chicks) in the breeding season of 2012–13. However, we were not able to sample the Monte León colony during spring owing to heavy rain. We collected 0.5 mL of blood from the metatarsal vein of 15 individuals (5 females, 5 males, 5 chicks) from each colony and season (overall $N=135$). We determined the sex of adults following Gandini *et al.* (1992). Blood was preserved in 70% ethanol before processing in the laboratory. Previous studies have shown little effect of alcohol preservation on blood of birds and other organism (Hobson *et al.* 1997; Sarakinos *et al.* 2002).

At the same time as collecting blood, we also collected sampled Penguin stomach contents using the methods described by Wilson (1984). We flushed stomachs of 4–7 Penguins from one colony in each of the northern (Puerto Deseado), central (San Julián) and southern sectors (Cabo Vírgenes) of the study area. The least damaged prey were washed with distilled water and frozen until processing in the laboratory; the rest of the stomach contents was discarded.

The whole blood and muscle samples taken from the prey items in the stomach contents were dried at 60°C for 48 h and ground to a fine powder. All samples were analysed for C and N content and for stable isotopic values at the Stable Isotope Facility, University of California, Davis, California. Stable isotope ratios are expressed as δ values (‰):

$$\delta X = (R_{\text{sample}} \div R_{\text{standard}}) - 1$$

where X is either ^{13}C or ^{15}N , and R is the corresponding ratio $^{13}\text{C} : ^{12}\text{C}$ or $^{15}\text{N} : ^{14}\text{N}$. Standards used were Vienna Pee Dee belemnite for $\delta^{13}\text{C}$ and atmospheric N_2 (air) for $\delta^{15}\text{N}$. Secondary isotopic reference materials used were nylon (standard deviation 0.056 for $\delta^{13}\text{C}$ and 0.05 for $\delta^{15}\text{N}$), bovine liver (s.d. 0.007 for $\delta^{13}\text{C}$, 0.07 for $\delta^{15}\text{N}$), USGS-41 glutamic acid (s.d. 0.17 for $\delta^{13}\text{C}$, 0.16 for $\delta^{15}\text{N}$), and glutamic acid (s.d. 0.06 for $\delta^{13}\text{C}$, 0.26 for $\delta^{15}\text{N}$). Lipids from prey species samples were extracted using chloroform–methanol (1 : 1) (Post *et al.* 2007).

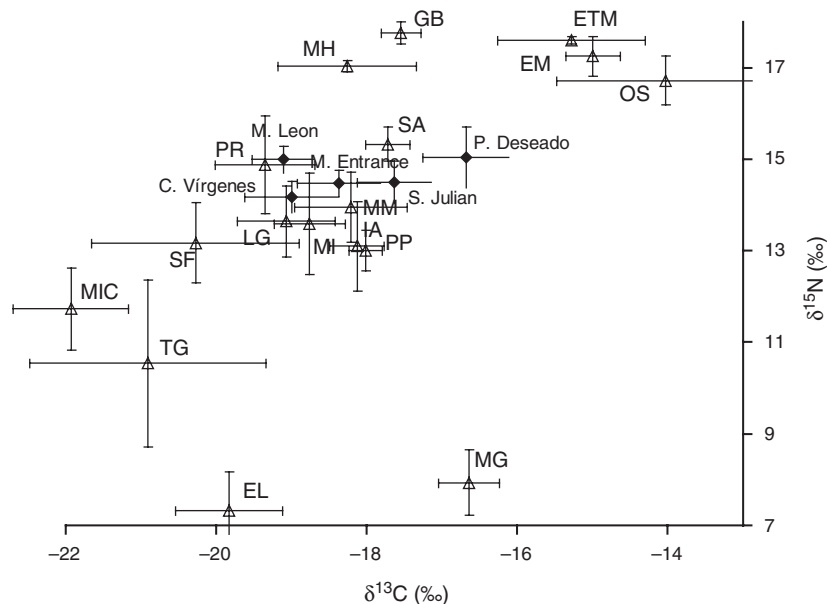


Fig. 2. Stable isotope niche space for potential prey items (open triangles) and Magellanic Penguins from the five breeding colonies studied (solid diamonds). Values are means \pm s.d. Stable isotope values for prey are from Ciancio *et al.* (2008) and estimates from the present study (see Methods). Stable isotope values for the colonies were corrected by subtracting the discrimination factors (2.29 for $\delta^{15}\text{N}$, 0.24 ‰ for $\delta^{13}\text{C}$; from Cherel *et al.* 2005). Prey items: EL, *Euphausia lucens*; EM, *Eleginops maclovinus* (Haddock); ETM, *Enteroctopus megalocyathus* (octopus); GB, *Genypterus blacodes* (Kingklip); IA, *Illex argentinus* (Argentinean Squid); LG, *Loligo gahi* (Longfin Squid); MH, *Merluccius hubbsi* (Hake); MI, *Moroteuthis ingens*; MIC, mictophids (lanternfish); MM, *Macroronus magellanicus* (Hoki); MS, *Myxinus* spp. (hagfish); OS, *Odontesthes* spp. (silverside); PP, *Peisos petrunkevitchi*; PR, *Patagonotothen ramsayi*; SA, *Salilota australis* (Tadpole Codling); SF, *Sprattus fuegensis* (Patagonian Sprat); TG, *Themisto gaudichaudii*.

Estimation of trophic levels and dietary components

The Bayesian stable isotope mixing model SIAR v4.0 (Stable Isotope Analysis in R; Parnell *et al.* 2010) was used to evaluate the relative contribution of the different prey sources, implemented in R version 3.0.0 for Windows (R Core Team 2014). We ran the models with colonies grouped by coastal sector (northern, central, southern) to account for spatial variation in stable isotope values of prey species (Moreno *et al.* 2011). We fitted the models with prey isotope values (from our stomach samples) for the northern sector (model for Puerto Deseado), central sector (model for San Julián, Monte Entrance and Monte León), and south sector (model for Cabo Vírgenes) (Table 1). The three models for each sector were run for spring and summer (six models in total). We ran the models for 500 000 iterations, with 50 000 initial discards. The remaining iterations were thinned by a factor of 15 to estimate the proportional distribution of the prey. We checked the convergence of Markov chains by visually inspecting the trace plots. We used a surrogate mean discrimination factor (a key parameter for stable isotope mixing models; Bond and Diamond 2011) from blood of Rockhopper Penguins (*Eudyptes chrysocome*), which had been fed with whole Capelin

Table 1. Stable isotope values of (a) Magellanic Penguins and (b) prey species

The prey items shown were selected based on estimates of dietary proportions using stable isotope mixing models. Values are expressed as means and standard deviations. Sectors refer to the northern, central and southern sectors of the study area; the same stable isotope values for Patagonian Sprats were used for the three sector models

		<i>N</i>	$\delta^{15}\text{N}$ (s.d.)	$\delta^{13}\text{C}$ (s.d.)
(a) Magellanic Penguins				
Colony				
Northern sector				
Puerto Deseado	Season			
	Spring	15	17.03 (0.59)	-17.30 (0.68)
	Summer	15	17.19 (0.74)	-16.86 (0.34)
Central sector				
San Julián	Spring	15	16.66 (0.30)	-18.05 (0.46)
	Summer	15	16.46 (0.58)	-18.00 (0.54)
Monte Entrance	Spring	15	16.50 (0.27)	-18.48 (0.46)
	Summer	15	16.58 (0.28)	-19.04 (0.51)
Monte León	Spring	15	17.06 (0.29)	-19.50 (0.41)
	Summer	15	17.06 (0.29)	-19.50 (0.41)
Southern sector				
Cabo Vírgenes	Spring	15	16.23 (0.32)	-18.98 (0.48)
	Summer	15	16.25 (0.36)	-19.80 (0.47)
(b) Prey				
Northern sector				
Longfin Squid		7	14.70 (1.32)	-17.69 (1.30)
		5	17.59 (0.27)	-16.31 (0.32)
Central sector				
Longfin Squid		7	15.47 (0.18)	-18.49 (0.16)
		5	16.72 (0.27)	-14.02 (1.14)
Southern sector				
Longfin Squid		7	15.15 (0.10)	-19.58 (0.27)
		5	16.72 (0.27)	-14.02 (1.14)
Sectors combined				
Patagonian Sprat – larvae and juveniles		15	12.53 (0.87)	-20.16 (1.30)
Patagonian Sprat – adults		10	13.82 (1.14)	-19.64 (1.38)

(*Mallotus villosus*) or Capelin muscle, as described by Cherez *et al.* (2005). The values of the mean discrimination factor were $2.29\text{‰} \pm 0.46$ for $\delta^{15}\text{N}$ and $0.24\text{‰} \pm 0.59$ for $\delta^{13}\text{C}$. The Patagonian Sprat is the most abundant pelagic fish in the region and the main prey item in stomach contents of Magellanic Penguins in the SPSE (see Table S2). Size-classes of Patagonian Sprat are geographically segregated (see Fig. 1) and have distinct stable isotope values. We separated Patagonian Sprat into two size-classes: larvae and juveniles (<90 mm fork-length) and adults (>90 mm fork-length).

We estimated the trophic level of the Magellanic Penguin using the method described in Ciancio *et al.* (2008):

$$\text{TL}_{\text{penguin}} = ((\delta^{15}\text{N}_{\text{penguin}} - \delta^{15}\text{N}_{\text{euphausiid}})\text{DF}) + 2$$

where DF is the discrimination factor, and $\delta^{15}\text{N}_{\text{euphausiid}} = 7.33$. Residuals were checked for conformity to a normal distribution using visual Q–Q plots. We used a two-way analysis of variance (ANOVA) (Crawley 2007) with colony and season as fixed effects to account for colony and season effect on blood stable isotope values of Penguins, and Tukey's Honest Significant Difference was used for posterior comparisons. We used a multivariate analysis of variance (MANOVA) with prey species as the fixed effect to test if stable isotope values differed among prey species. We performed all statistical analyses in R (R Core Team 2014).

Results

Analysis of spatio-temporal overlap and prey selection

The literature review of the diet of Magellanic Penguins, and of other seabirds and marine mammals, combined with the spatio-temporal overlap analysis of prey distributions identified 18 potential prey species in the SPSE (see Table S1). Hyperiid amphipods (*Themisto gaudichaudii*) and euphausiids (*Euphausia lucens*) are the main prey of fish and squid species in the SPSE (see Table S1). Longfin Squid (*Loligo gahi*) is the most abundant prey species in the SPSE and the main squid species eaten by other top-order predators, whereas Patagonian Sprat and notothenid fish are the main prey for piscivorous predators (see Table S1). We found only three studies describing diet of Magellanic Penguins in the SPSE, all of them analyses of stomach contents done during the small-chick stage of breeding (October–January) in 1985–88 and 1996–97 (see Table S2). Only one study reported diet as the proportion of mass (Frere *et al.* 1996). The dietary studies recorded eight species of squid and fish in the diet of Magellanic Penguins, only three of which – Patagonian Sprat, silverside (*Odontesthes* spp.), Longfin Squid – in frequencies >5% frequency or mass and which accounted for the bulk of the Penguin's diet. Patagonian Sprat were the main item, comprising 15–85% mass, with silversides comprising 9–40% and Longfin Squid 6–30% (see Table S2).

The foraging ranges of Penguins from four of the five studied colonies did not overlap (Fig. 1; there were no data for the Monte Entrance colony), as was found for Magellanic Penguins on the Falkland Islands (Islas Malvinas) (Masello *et al.* 2010). The results of the prey-selection analysis are summarised in Table S1. This includes the summary of overlap of Magellanic Penguin foraging ranges and distribution of prey

species (column labelled ‘Spatial overlap with MP’), prey habitat-class (benthic, demersal or pelagic; column labelled ‘Habitat’; all benthic species were discarded), and summary of reasons prey were rejected. The stable isotope values of the 18 potential prey species are shown in Fig. 2. Based on the above analysis, three main prey species were selected for estimation of diet using the stable isotope mixing models: Patagonian Sprat, Longfin Squid and silversides.

Stable isotope analysis and diet predicted by the mixing models

Stable isotope values of Magellanic Penguin whole blood ranged between -20.96‰ and -15.98‰ for $\delta^{13}\text{C}$, and between 15.56 and 17.97‰ for $\delta^{15}\text{N}$ (Fig. 2, Table 1). Visual inspection of Q–Q plots showed normality for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Penguin blood and for prey species. ANOVA showed a significant effect of colony on stable isotope values for $\delta^{15}\text{N}$ ($F_{4,129}=18.2$, $P<0.001$; all *post hoc* pairwise comparisons $P<0.05$, except for Puerto Deseado–Monte León and San Julián–Monte Entrance comparisons $P>0.05$) but no effect of season ($F_{1,129}=0.04$, $P=0.8$). ANOVA also showed a significant interaction between season and colony for $\delta^{13}\text{C}$ ($F_{3,126}=10.2$, $P<0.001$). MANOVA showed a significant effect of prey species on isotope values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) included in the mixing model ($F_{30,2}=11.3$, $P<0.001$). The mixing models predicted Patagonian Sprat and Longfin Squid as the main prey in the study area (Fig. 3). Patagonian Sprat (larvae–juveniles and adults) were the main prey in all but the Puerto Deseado colony for both seasons, and ranged between 21 and 59% of the diet (median of proportions). The proportion of Patagonian Sprat in the diet increased from north to south (Puerto Deseado to Cabo Vírgenes). Longfin Squid were the second-most important item (19–57%), and were the main prey of the northernmost colony, Puerto Deseado colony (37% for spring, 47% for summer). Longfin Squid was also a large proportion of the diet at the San Julián and Monte Entrance colonies during summer. In contrast to the diet reported from published analyses of stomach contents (see above), silversides comprised only a small proportion of the diet at all colonies (<20% of prey consumed), with the exception of the Puerto Deseado colony. Overall, the diet during spring and summer was similar across the studied geographical range. The mixing models showed a decrease in consumption of silversides in the central sector (San Julián, Monte Entrance and Monte León colonies) during the summer, with an increase in consumption of Longfin Squid. Overall, the average trophic level of the Magellanic Penguin was 5.3 (range 4.5–5.8).

Discussion

Our study shows that Magellanic Penguins in the Southern Patagonian Shelf Ecosystem (SPSE) predominately forage on only a few prey species during the breeding season: Patagonian Sprat, Longfin Squid and silverside. The estimated proportions of prey eaten were based on stable isotope mixing models, which are very sensitive to the sources included (in this instance, prey) and have low power to distinguish between sources with similar stable isotope values (Yeakel *et al.* 2011). Previous studies using stable isotopes for estimating diets of

penguins in the South Atlantic Ocean have not taken into consideration the abundance, distribution or availability of prey in the foraging area of the colony (e.g. Forero *et al.* 2002). In this study, we performed a comprehensive analysis of the distribution (in a three-dimensional space) and life histories of prey to determine the potential prey items of Magellanic Penguins to be used in the stable isotope mixing models. We explored how the abundance of prey varied in time and space, changing the availability for Penguins, and which allowed us to determine more accurately the most likely prey eaten. Even though this spatio-temporal overlap analysis may be considered simplistic, it is based on a very strong assumption: if two species overlap in space and time it does not imply that trophic interactions occur, but if they do not overlap, no trophic interaction is possible. This was a key assumption in discarding some potential prey species from analysis, such as the most abundant squid species on the Patagonian Continental Shelf and the main target of one of the largest squid fisheries worldwide, the Argentine Squid (Brunetti *et al.* 1998), which has similar stable isotope signature to the Longfin Squid (Fig. 1). Argentine Squid occur at depths >80 m and migrates to the SPSE during summer–autumn, and high densities of this prey are within the foraging range of Magellanic Penguins breeding at the Puerto Deseado colony only, and at the end of the breeding season (Brunetti and Ivanovic 1992; Crespi-Abril and Barón 2012). In contrast, Longfin Squid are available for individuals breeding at all colonies throughout the breeding season (Fig. 1). The high consumption of Longfin Squid at the Puerto Deseado colony, which is regarded as low quality food for penguin growth (Heath and Randall 1985; Heezik and Davis 1990), may be a response to high local abundances of these squid compared to other higher quality prey items, such as Patagonian Sprat, rather than a result of prey preference. Previously, high consumption of squid by Magellanic Penguins has been reported only at New Island, in the Falkland Islands (Islas Malvinas) (Pütz *et al.* 2001), where high densities of squid also occur (Brickle *et al.* 2006).

Overall, our SIA mixing model simulations estimated proportions of chosen prey according to the spatio-temporal overlap analysis. For example, during the spring, adult Patagonian Sprat congregate in spawning areas in the southern SPSE (Sánchez *et al.* 1995; Fig. 1). As larvae develop, they drift north and west, becoming available as prey for Penguins in the northern SPSE during summer. The stable isotope mixing models showed that Patagonian Sprat are important food for the central sector (San Julián, Monte Entrance, Monte León) and southern sector (Cabo Vírgenes), located closer to these spawning congregations (Fig. 1). The mismatch between the proportions estimated by the stable isotope mixing models and stomach contents could be explained by inter-annual variation in diet or the different timing of the stomach content analysis and SIA. The published analyses of diet we reviewed had been conducted almost 20 years before our analyses, and sampling in these studies was performed over only a few days during the early chick-rearing period (e.g. Wilson *et al.* 2005), whereas the SIA method used in the present study provides estimates of diet composition over several weeks (Boecklen *et al.* 2011).

Our results also showed that the average foraging trophic level (TL) of Magellanic Penguins breeding in the SPSE

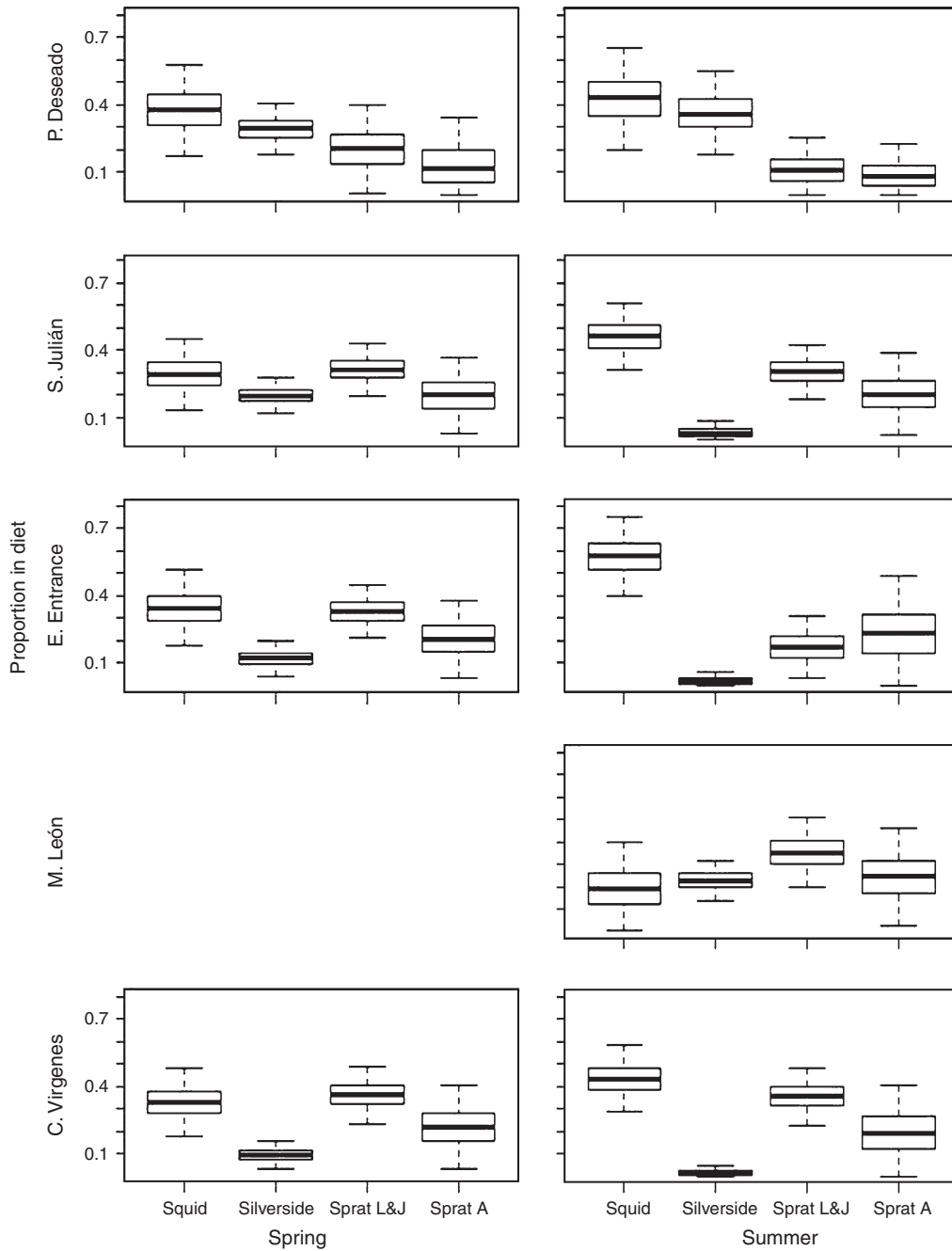


Fig. 3. Diet of Magellanic Penguins from five breeding colonies in spring and summer as identified by stable isotope mixing models including prey selected by the spatio-temporal overlap analyses. Boxplots (outliers not shown) show the median proportion of diet (dark horizontal line), the 25th and 75th percentiles (the bottom and top of the box), and whiskers at 1.5 times the interquartile range of the data. Prey selected: Squid: Argentine Squid, Sprat; L&J: larval and juvenile Patagonian Sprat; Sprat A: adult Patagonian Sprat.

was unusually high (5.3; between-colony range 4.5–5.8). The estimated TL of Magellanic Penguins in the north of their range was 4.14 (Forero *et al.* 2004), and on the Falkland Islands (Islas Malvinas) was 3.6 (Weiss *et al.* 2009). The TL values were also higher than those of other species of penguin from the Southern Ocean that feed mainly on crustaceans (3.6–4.1) and of the

piscivorous King Penguin (*Aptenodytes patagonicus*; Cherel *et al.* 2008; Cherel *et al.* 2010), for which the TL is 4.5. The occurrence of coastal species of prey may have affected $\delta^{15}\text{N}$ values in colonies within the SPSE in the present study (e.g. colonies with high consumption of silverside). In addition, high abundances of the main prey, Patagonian Sprat, are found

closer to shore compared to prey in other regions, such as anchovy (*Engraulis anchoita*) in the northern range of Magellanic Penguins (Hansen *et al.* 2001) or squid in the Falkland Islands (Islas Malvinas) (Weiss *et al.* 2009). Nearshore species in this marine ecosystem seem to be enriched in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values compared to offshore species (Ciancio *et al.* 2008).

Our results have implications for conservation of Magellanic Penguins and ecosystem level studies of them. Large decreases in numbers owing to competition for food with fisheries has been suggested for the closely related African Penguin (*Spheniscus demersus*) in the Benguela Current (Crawford *et al.* 2006; Crawford *et al.* 2011) and for all species of penguin nesting in the Falklands Islands (Islas Malvinas), including the Magellanic Penguin (Bingham 2002). In the north of the distribution of Magellanic Penguins, fisheries target Argentine Squid (Crespi-Abril and Barón 2012), one of its main prey there (but not in our study area, as above). These populations may also be threatened by increasing anchovy fishing (Skewgar *et al.* 2007). In this study we showed that Magellanic Penguins that breed in the SPSE forage on only a few prey species (Patagonian Sprat, Longfin Squid and silverside). Unlike the situation for other populations of *Spheniscus* penguins, none of these prey stocks are being targeted by any industrial fisheries, reducing the potential for direct interactions between Penguins and fisheries in this region (the Longfin Squid fishery in the nearby Falkland Islands (Islas Malvinas) targets a different stock; Pineda *et al.* 1998). The main conservation implication of our study is that the breeding colonies of Magellanic Penguin within the SPSE (about one-third of the global population) are not threatened by competition with commercial fisheries. This study also identifies the likely diet of what is the most abundant top-order predator in this ecosystem. Preliminary analyses, based on daily energy requirements, estimated that Magellanic Penguins in the SPSE may consume almost 30 000 tonnes during the breeding season (Ciancio *et al.* 2010). More recent studies using points of inflection in diving profiles (Sala *et al.* 2012) suggested these estimates could be even larger, highlighting the importance of Magellanic Penguins as predators and the need to understand their dietary and trophic relationships.

There is no gold-standard tool for studying the diet of organisms, including seabirds (Barrett *et al.* 2007). Analysis of stomach contents of seabirds at their breeding colonies has the disadvantages of being time-consuming, identifies the diet only during the immediate sampling period, usually only samples food carried to the nest, and may provide no information about prey eaten by adults for their own maintenance. In contrast, use of SIA and mixing models integrates longer periods of time and can provide a broader analysis of diet. However, uncertainty related to including or excluding particular prey and parameters, such as the discrimination factor, are important considerations to keep in mind when using these methods (Hobson 2011). Our study shows that combining tools that complement each other provide a more appropriate framework for developing an understanding of seabird trophic preferences in Patagonia. Furthermore, it provides vital data for the conservation of Magellanic Penguins in the region and the first step for ecological modelling aimed at understanding their full role in marine ecosystems.

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