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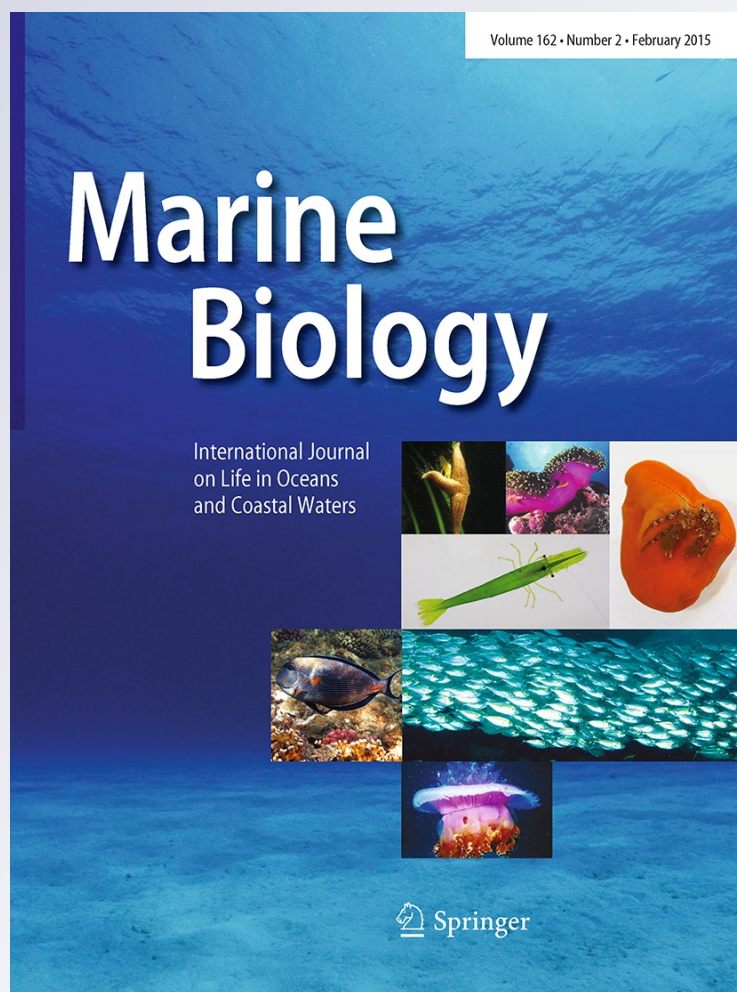
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Dietary consistency of male South American sea lions (*Otaria flavescens*) in southern Brazil during three decades inferred from stable isotope analysis

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Abstract Marine predators may undergo remarkable dietary changes through time as a result of both anthropogenic and natural changes in the environment, but this variability is often difficult to tackle and seldom incorporated into ecosystem models. This paper uses the stable isotope ratios of carbon and nitrogen in skeletal material of South American sea lions from Brazilian scientific collections to investigate whether these animals modified their diet from 1986 to 2009, as reported for other marine predators in the region. Stable isotope ratios indicated that demersal potential prey were always enriched in ^{13}C as compared with pelagic prey.

Accordingly, the absence of any statistically significant correlation between stranding year and the $\delta^{13}\text{C}$ values of adult males indicated no major increase in the consumption of pelagic prey from 1986 to 2009. Likewise, the results of the mixing model SIAR revealed a mixed diet including pelagic and demersal prey, with a central role for demersal fishes throughout the whole period. Furthermore, SIAR suggested no major changes in the proportion of pelagic and demersal prey in the diet of adult male South American sea lions during the past three decades. Demersal fishes were also relevant prey for juvenile South American sea lions during the whole period, but they always consumed a larger proportion of pelagic prey than the adults did. These results suggest no major changes in the diet of male South American sea lions during the past three decades in southern Brazil, contrary to what has been reported for other predators in the regions and for the species in northern Patagonia.

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Introduction

Human activities have impacted most of the marine ecosystems around the world (Halpern 2008), and only retrospective studies can give us a full account of the magnitude of the change (Jackson et al. 2001). This approach has revealed that some marine predators have undergone remarkable dietary changes through time as a result of natural changes in food web structure (e.g., Trites et al. 2007; Páez-Rosas et al. 2012) and interaction with fisheries (e.g., Drago et al. 2009a; Hanson et al. 2009; Gómez-Campos et al. 2011). Ecosystem models need to account for those changes to produce realistic reconstructions of historical changes in ecosystem dynamics, but this is often impossible due to the absence of retrospective studies on the diet of marine predators.

Otariids inhabiting the southeastern coast of South America were heavily exploited since the arrival of western settlers and exploitation lasted till the second half of the twentieth century (Pérez Fontana 1943; Godoy 1963; Rodríguez and Bastida 1998; Ponce de León 2000). The northernmost rookeries of the South American sea lion (*Otaria flavescens*) are found in Uruguay, where <15,000 South American sea lions were estimated to survive in 1995 and the production of sea lion pups would be descending at a rate of 4.5 % per year (Páez 2006). Conversely, the numbers of South American fur seals (*Arctocephalus australis*) breeding in the same colonies increased since the end of commercial sealing (Vaz-Ferreira 1982; Lima and Páez 1997; Franco-Trecu et al. 2012).

The reason for the differences in the post-harvest dynamics of these two species is unknown, although Costa et al. (2004, 2006) have argued that pelagic foragers recover faster than demersal ones after exploitation because pelagic resources are usually less exploited by humans than demersal ones. South American sea lions breeding in Uruguay forage over a large area spanning from southern Brazil to northern Argentina (Rodríguez et al. 2013), with high levels of individual variability in the foraging grounds used (Zenteno et al. 2013). The same region supports important demersal fisheries, but landing biomass, catch per unit effort and mean trophic level of landings declined in the mid-1990s in some areas and currently many stocks are fully exploited or overexploited (Haimovici 1998; Vasconcellos and Gasalla 2001; Jaureguizar and Milessi 2008; Milessi and Jaureguizar 2013). As a response to the above reported changes, franciscana dolphins (*Pontoporia blainvillei*) decreased the consumption of some demersal sciaenid fishes (Pinedo 1994; Secchi et al. 2003; Crespo and Hall 2002) and the overall contribution of demersal fishes to the diet of marine birds declined over the past 30 years (Bugoni 2008).

Available dietary information for the South American sea lion in the region is based on scats and stomach contents analysis and revealed no evident temporal changes off southern Brazil (Rosas, 1989; Oliveira et al. 2008; Machado, 2013) and Uruguay [Riet-Sapriza et al. (2012), but see Naya et al. (2000); Szteren et al. (2004)]. However, most of the information has been collected only recently, and scats and stomach contents are not appropriate to test long-term variation in food resources, since these methods provide only a single “snapshot” of the diet of each individual just before sampling (Iverson et al. 2004). Furthermore, repeated sampling of large animals for stomach content analysis is extremely difficult and assigning scats to particular individuals is highly unlikely in crowded rookeries (Drago et al. 2010a).

Stable isotope analysis offers an alternative method to reconstruct dietary changes in marine predators over long

periods of time (e.g., Drago et al. 2009a; Hanson et al. 2009; Newsome et al. 2010a). The method is based on the assumption that the stable isotope ratio in the consumer's tissues integrates the stable isotope ratio of its prey items in a predictable manner over a long period of time, although stable isotope ratios experience a stepwise enrichment in the heavier isotope relative to prey (DeNiro and Epstein 1978; Kelly 2000). This increase is more pronounced in $\delta^{15}\text{N}$ values (3–5 ‰), which consequently are used to assess trophic level (DeNiro and Epstein 1978; Minagawa and Wada 1984). Trophic enrichment in ^{13}C is smaller (0.5–1.1 ‰) (Fry and Sherr 1984; Wada et al. 1991), and as a consequence, animal $\delta^{13}\text{C}$ values are useful to identify consumption of prey with different $\delta^{13}\text{C}$ values at a local scale, as well as foraging areas at larger geographic scales (Rau et al. 1982; Hobson et al. 1997).

Here, we use stable isotope ratios in skeletal material of South American sea lions available at scientific collections from Brazil to investigate whether major dietary shifts have occurred during the past three decades.

Materials and methods

Study site and sample collection

The scientific collection of Universidade Federal do Rio Grande (FURG) stores skeletal material from South American sea lions dead stranded in southern Brazil (29°S–32°S; Fig. 1) from 1986 to 1988, whereas the collection of Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul (GEMARS) stores skeletal material from animals dead stranded in the same area from 1994 to 2009.

Although the South American sea lion is one of the most frequently pinniped species observed off Brazil, there are no breeding colonies of the species in the area (Pinedo 1990; Simões-Lopes et al. 1995), and South American sea lions are thought to come from the breeding colonies in Uruguay, 300 km south of Rio Grande do Sul (Pinedo 1990; Rosas et al. 1994). Satellite telemetry has revealed that during the breeding season South American sea lions forage in a wide area ranging from southern Brazil to northern Argentina, but stable isotopes of oxygen have revealed limited exchange of adult male South American sea lions with other regions in the southwestern Atlantic Ocean (Zenteno et al. 2013).

Additional South American sea lion samples (bone and vibrissae) were collected from the scientific collection of Centro Nacional Patagónico (Puerto Madryn, Argentina) to calculate diet-to-predator discrimination factors (see below).

Maxillo-turbinal bones were initially selected for the present study, as sampling them preserved the collected

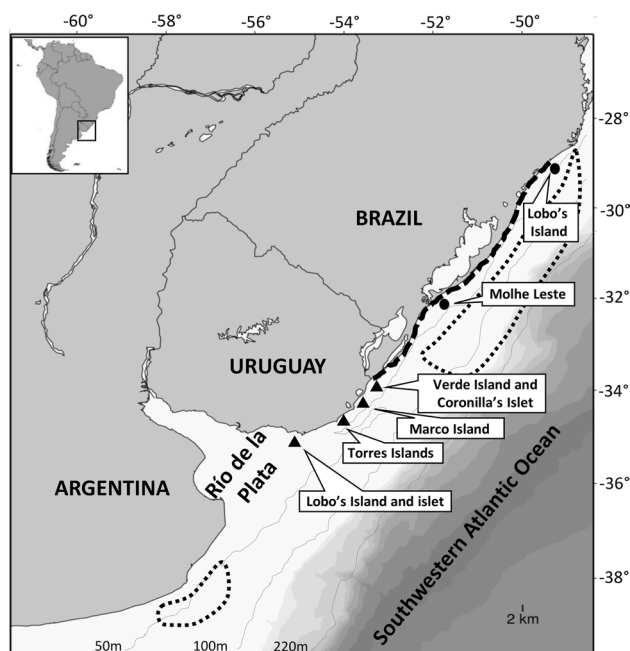


Fig. 1 Study area. South American sea lion samples were collected along the *dashed line*. Potential prey were collected along northern Argentina and southern Brazil. The *triangles* show the main breeding rookeries of South American sea lions in Uruguay, whereas the *circles* show the main haul-outs sites occupied by South American sea lions in southern Brazil. Potential prey were collected within the *dotted polygons* (Source: www.seaturtle.org)

skulls for further study. However, only the canine teeth of the earlier specimens had been preserved in the collection. Since stable isotope ratios of carbon and nitrogen may vary between tissues [Koch (2007), but see Riofrío-Lazo and Aurióles-Gamboa (2013)], differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in paired samples of bone and dentine (all the layers after the second annuli) from 12 individuals were tested. Additional paired samples of vibrissae and bone from eight adult individuals were also analyzed to calculate a diet-to-bone and diet-to-dentine discrimination factors (see below).

South American sea lions may forage over a wide area including southern Brazil, Uruguay and northern Argentina (Rodríguez et al. 2013). The stable isotope ratios of some South American sea lion prey from southern Brazil, Uruguay and northern Argentina have been reported by Abreu et al. (2006), Bugoni et al. (2010), Botto et al. (2011) and Franco-Trecu et al. (2013a). Additional potential prey previously identified by stomach and scat analysis (Naya et al. 2000; Szteren et al. 2004, 2006; Suárez et al. 2005; Oliveira et al. 2008; Machado 2013) was collected. Samples were obtained from fishermen from Brazil (Santa Catarina and Rio Grande do Sul province) and northern Argentina (Buenos Aires province) in 2009 and 2010 (Fig. 1; Table 2). White dorsal muscle was sampled from fishes and mantle

from cephalopods. All samples were stored in a freezer at $-20\text{ }^{\circ}\text{C}$ until analysis.

Sex and age determination

Sex was determined based on the external morphology (presence of *baculum* bone) during sampling collection and eventually assessed according to secondary sexual characteristics of skull following Crespo (1984, 1988). Only males were considered for this study, due to the scarcity of females in the scientific collections. South American sea lions had previously been aged by counting growth layers in the dentine of the canines (assuming annual deposition) in thin ground sections or acid-etched highlighted teeth (Hohn 1980; Perrin and Myrick 1980; Crespo 1988). The life span of South American sea lions is around 20 years (Crespo 1988), and they become physiologically mature between 4 and 6 years, although mate for the first time when they are 9 years old or more (Crespo 1988; Grandi et al. 2010). Furthermore, skull growth stops at the age of 9 years (Drago et al. 2009b). Based on these data, South American sea lions 2–8 years old were considered juveniles and adolescents and those older than 8 years were considered adults. All the analysis was done independently for adults and for younger animals. Furthermore, individual age was included in the correlation analysis conducted for each age class.

Stable isotope analysis

Bone, dentine (all the layers for FURG samples) and muscle samples were thawed, dried in a stove at $60\text{ }^{\circ}\text{C}$ for 36 h and grounded into a fine powder using a mortar and pestle. Since lipids can bias the analyses by decreasing $\delta^{13}\text{C}$ values (DeNiro and Epstein 1977), they were removed from the samples using a sequential soak in a chloroform/methanol (2:1) solution (Bligh and Dyer 1959) and shaken with a rotator to accelerate the lipid extraction. Vibrissa was soaked in a chloroform/methanol (2:1) solution for 15 min in an ultrasonic bath. Any remaining residue on vibrissae was scrubbed off with a brush and the soaking process repeated. The samples were then dried again for 48 h at $60\text{ }^{\circ}\text{C}$. Vibrissae were cut into 3-mm-long consecutive sections starting from the proximal end and each section analyzed separately. This is because each section integrates diet during 1 month (Hirons et al. 2001; Cherel et al. 2009; Kernalégen et al. 2012) and the results will be used latter in a different study aiming to reconstruct monthly changes in the diet of sea lions (Zenteno, unpublished data). Here, only the average values of individual vibrissa were used, because they integrate approximately the same time span than bone (Riofrío-Lazo and Aurióles-Gamboa 2013).

Table 1 Stable isotope ratios (mean \pm SD) in dentine and bone tissue of male South American sea lions found dead along the coast of southern Brazil

Sample number	Sex	Tissue	Death (yr)	$\delta^{13}\text{C}$ (‰)	Suess corrected $\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Age (yr)
FURG837	♂	Tooth	1986	-10.7	-11.2	19.0	4
FURG839	♂	Tooth	1986	-12.7	-13.2	20.7	8
FURG913	♂	Tooth	1986	-11.0	-11.5	20.6	9
FURG824	♂	Tooth	1986	-11.7	-12.2	20.3	10
FURG826	♂	Tooth	1986	-11.4	-11.9	21.9	14
FURG1021	♂	Tooth	1986	-11.5	-12.0	21.8	15
FURG1016	♂	Tooth	1986	-12.5	-13.0	21.4	22
FURG1070	♂	Tooth	1987	-13.6	-14.1	20.5	5
FURG1088	♂	Tooth	1987	-13.8	-14.3	20.3	5
FURG1066	♂	Tooth	1987	-11.1	-11.6	20.9	7
FURG1063	♂	Tooth	1987	-11.6	-12.1	20.6	8
FURG1089	♂	Tooth	1987	-13.8	-14.3	20.3	8
FURG1084	♂	Tooth	1987	-10.8	-11.3	20.2	11
FURG1134	♂	Tooth	1987	-11.8	-12.3	20.4	13
FURG1202	♂	Tooth	1988	-13.7	-14.2	20.4	7
FURG1201	♂	Tooth	1988	-11.1	-11.6	21.6	14
GEMARS184	♂	Bone	1994	-12.3	-12.6	20.8	10
GEMARS193	♂	Bone	1994	-11.5	-11.8	21.2	10
GEMARS171	♂	Bone	1994	-10.6	-10.9	20.6	19
GEMARS268	♂	Bone	1995	-12.2	-12.5	20.9	5
GEMARS284	♂	Bone	1995	-12.4	-12.7	20.4	10
GEMARS288	♂	Bone	1995	-11.5	-11.8	21.2	12
GEMARS299	♂	Bone	1995	-11.9	-12.2	20.9	25
GEMARS343	♂	Bone	1996	-11.7	-12.0	20.6	4
GEMARS353	♂	Bone	1996	-12.7	-13.0	20.5	12
GEMARS358	♂	Bone	1996	-13.8	-14.1	20.0	21
GEMARS444	♂	Bone	1997	-12.2	-12.5	20.6	6
GEMARS434	♂	Bone	1997	-11.4	-11.7	20.6	8
GEMARS428	♂	Bone	1997	-11.5	-11.8	21.2	14
GEMARS517	♂	Bone	1998	-10.2	-10.4	20.0	5
GEMARS555	♂	Bone	1998	-12.7	-12.9	21.6	6
GEMARS523	♂	Bone	1998	-12.2	-12.4	21.4	10
GEMARS553	♂	Bone	1998	-11.0	-11.2	19.8	15
GEMARS516	♂	Bone	1998	-11.9	-12.1	21.2	18
GEMARS659	♂	Bone	2000	-11.1	-11.3	19.4	6
GEMARS642	♂	Bone	2000	-11.0	-11.2	20.1	10
GEMARS658	♂	Bone	2000	-11.7	-11.9	19.7	14
GEMARS667	♂	Bone	2000	-10.9	-11.1	19.1	15
GEMARS812	♂	Bone	2001	-12.4	-12.6	20.1	4
GEMARS809	♂	Bone	2001	-11.8	-12.0	20.9	12
GEMARS813	♂	Bone	2002	-13.4	-13.6	22.9	2
GEMARS868	♂	Bone	2002	-11.4	-11.6	20.1	12
GEMARS967	♂	Bone	2003	-12.4	-12.5	20.6	5
GEMARS1111	♂	Bone	2003	-12.2	-12.3	21.2	8
GEMARS1151	♂	Bone	2003	-12.1	-12.2	20.7	8
GEMARS992	♂	Bone	2003	-13.7	-13.8	20.6	11
GEMARS1040	♂	Bone	2003	-10.8	-10.9	20.4	12
GEMARS1060	♂	Bone	2003	-12.1	-12.2	20.8	12
GEMARS970	♂	Bone	2003	-11.9	-12.0	20.5	14
GEMARS1126	♂	Bone	2003	-11.4	-11.5	21.0	15

Table 1 continued

Sample number	Sex	Tissue	Death (yr)	$\delta^{13}\text{C}$ (‰)	Suess corrected $\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Age (yr)
GEMARS1189	♂	Bone	2004	−11.2	−11.3	19.8	12
GEMARS1303	♂	Bone	2008	−12.2	−12.2	20.3	15
GEMARS1323	♂	Bone	2009	−12.0	−12.0	20.7	9
GEMARS1345	♂	Bone	2009	−11.9	−11.9	20.9	11

Ratios corrected for the Suess effect are referenced to 2009

As bones and teeth samples contain a high concentration of inorganic carbon that may add undesirable variability to $\delta^{13}\text{C}$ (Lorrain et al. 2003), they were previously treated by soaking for 24 h in 0.05 N hydrochloric acid (HCl) to decarbonise them (Ogawa and Ogura 1997). Since acidification may modify $\delta^{15}\text{N}$ values (Bunn et al. 1995), samples were divided into two subsamples, one used to measure $\delta^{13}\text{C}$ values following acidification and the other to measure $\delta^{15}\text{N}$ values prior to acidification.

Approximately 0.3 mg of vibrissae, 0.4 mg of dentine, 0.8 mg of bone and 0.3 mg of white muscle from fish and mantle from cephalopods were weighed into tin capsules (3.3×5 mm), combusted at 900 °C and analyzed in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo Finnigan, Bremen, Germany). Atropine was used as a system check for elemental analyses. Samples were processed at Centres Científics i Tecnològics de la Universitat de Barcelona.

The abundances of stable isotopes, expressed in delta (δ) notation, were the relative variations of stable isotope ratios expressed as per thousand (‰) deviations from predefined international standards as:

$$\delta X = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000 \quad (1)$$

where X is ^{13}C or ^{15}N , and R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios in the sample and standard, respectively. The $\delta^{13}\text{C}$ standard was Vienna PeeDee Belemnite (VPDB) calcium carbonate, and $\delta^{15}\text{N}$ standard was atmospheric nitrogen (N_2). International standards (ammonium sulfate, potassium nitrate, glutamic acid for $\delta^{15}\text{N}$ and polyethylene, sucrose and glutamic acid for $\delta^{13}\text{C}$) were inserted after every 12 samples to calibrate the system and compensate for any drift over time. Precision and accuracy for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements were 0.1 and 0.3 ‰, respectively.

Suess effect correction

The content of ^{13}C in atmospheric CO_2 has decreased 0.022 per mil/year since 1960, due largely to fossil fuel burning (Francey et al. 1999; Indermühle et al. 1999). For that reason, we have corrected the original $\delta^{13}\text{C}$ values of the skeletal material shown in Table 1 to account for such a decrease and allow comparison among samples from different periods. All the corrected $\delta^{13}\text{C}$ values were referenced to 2009.

Stable isotope discrimination factors

The use of appropriate diet–tissue discrimination factors is one of the most important basic requirements when applying stable isotope mixing models to predict the dietary sources of a consumer and the trophic position relative to primary consumers (Newsome et al. 2010a). In pinnipeds, previous studies have assessed discriminating factors between diet and blood, skin and vibrissae (Hobson et al. 1996), but nothing is known about the diet-to-bone discrimination factor. Here, we calculated two discrimination factors using different approaches.

The first discrimination factor was calculated using previously published information about diet composition from northern Patagonia (Koen-Alonso et al. 2000), stable isotope ratios of potential prey from that area (Drago et al. 2010b) and stable isotope ratios in the bone of South American sea lions from the same area (Drago et al. 2009a). The second discrimination factor was calculated using previously published information about diet-to-vibrissa discrimination in marine mammals (Hobson et al. 1996; Newsome et al. 2010b) and the stable isotope ratios in paired samples of vibrissa and skull from the CENPAT scientific collection. This latter diet-to-bone fractionation was computed as follows:

$$\Delta_{(\text{bone} - \text{vibrissae})} + \Delta_{\text{Means Reference (vibrissae} - \text{diet})} = \Delta_{(\text{bone} - \text{diet})} \quad (2)$$

Data analysis

Data are presented as mean \pm standard deviation (SD), and significance was assumed at the 0.05 level. All statistical analyses were carried out with PASW Statistics (version 17.0 for Windows, SPSS). As long as the assumptions of normality (tested using Lilliefors's test) and homoscedasticity (tested using Levene's test) were met, parametric approaches (Pearson's correlation and ANCOVA) were used.

Two-way ANOVA was used to compare the stable isotope ratios of potential prey in southern Brazil and northern Argentina. Potential prey from Uruguay was not included in the analysis because only average and standard deviation values have been published (Franco-Trecu et al. 2013a). Temporal trends in the isotopic signal of the bones and teeth of South American sea lion were investigated

Table 2 Stable isotope ratios (mean \pm SD) of the potential prey species for South American sea lions off southern Brazil and northern Argentina

Sample			<i>n</i>	$\delta^{13}\text{C}$ (‰) (Mean \pm SD)	$\delta^{15}\text{N}$ (‰) (Mean \pm SD)
Southern Brazil					
Medium pelagic fishes					
<i>Cynoscion guatucupa</i>	Stripped weakfish	Sciaenidae	5	-16.5 ± 0.7	16.0 ± 0.6
<i>Macrodon atricauda</i>	King weakfish	Sciaenidae	5	-14.5 ± 0.7	17.0 ± 0.3
<i>Pomatomus saltatrix</i>	Bluefish	Pomatomidae	5	-16.6 ± 0.5	18.6 ± 0.7
<i>Trachurus lathami</i> ^a	Rough scad	Carangidae	9	-16.1 ± 0.1	16.1 ± 1.1
<i>Trichiurus lepturus</i>	Cutlassfish	Trichiuridae	5	-15.7 ± 0.3	16.6 ± 0.5
Small Pelagic Fishes					
<i>Engraulis anchoita</i> ^a	Argentine anchovy	Engraulidae	14	-16.5 ± 0.5	14.7 ± 0.5
<i>Sardinella brasiliensis</i> ^a	Brazilian sardine	Lupeidae	7	-17.6 ± 0.6	11.4 ± 1.0
Demersal Fishes					
<i>Genypterus brasiliensis</i> ^a	Pink cusk-eel	Ophidiidae	1	-16.1	14.7
<i>Menticirrhus americanus</i>	Southern kingfish	Sciaenidae	5	-15.4 ± 0.5	-16.0 ± 0.5
<i>Micropogonias furnieri</i>	White croaker	Sciaenidae	5	-15.7 ± 0.6	15.3 ± 0.4
<i>Paralichthys brasiliensis</i> ^c	Banded croaker	Sciaenidae	5	-14.2 ± 0.5	15.6 ± 0.6
<i>Polyprion americanus</i> ^a	Wreckfish	Polyprionidae	1	-15.7	17.7
<i>Prionotus nudigula</i> ^a	Bluewing searobin	Triglidae	9	-15.0 ± 0.5	16.9 ± 0.7
<i>Sparus pagrus</i> ^a	Red porgy	Sparidae	1	-15.9	15.5
<i>Umbrina canosa</i> ^a	Argentine croaker	Sciaenidae	10	-15.5 ± 0.3	16.6 ± 0.8
<i>Urophycis brasiliensis</i> ^a	Brazilian codling	Phycidae	11	-15.6 ± 0.5	16.5 ± 0.7
Demersal Pelagic Cephalopods					
<i>Illex argentinus</i>	Argentine short-finned squid	Ommastrephidae	5	-18.1 ± 0.2	10.0 ± 0.5
<i>Loligo paelei</i>	Atlantic longfin squid	Loliginidae	5	-17.6 ± 0.2	11.3 ± 0.5
Northern Argentina					
Medium Pelagic Fishes					
<i>Cynoscion guatucupa</i>	Striped weakfish	Sciaenidae	5	-17.2 ± 0.2	17.6 ± 0.3
<i>Merluccius hubbsi</i> (>30 cm)	Argentine hake	Merlucciidae	3	-18.0 ± 0.5	15.6 ± 0.4
<i>Pseudoperca semifasciata</i>	Argentine sandperch	Pinguipedidae	4	-16.5 ± 0.3	18.2 ± 0.4
<i>Trachurus picturatus</i>	Blue jack mackerel	Carangidae	5	-17.6 ± 0.3	16.9 ± 0.5
Small Pelagic Fishes					
<i>Engraulis anchoita</i>	Argentine anchovy	Engraulidae	5	-18.2 ± 0.3	15.5 ± 0.5
<i>Merluccius hubbsi</i> (<30 cm)	Argentine hake	Merlucciidae	2	-18.4 ± 0.1	14.8 ± 0.0
<i>Sorgentinia incisa</i>	Silverside	Atherinopsidae	5	-17.6 ± 0.3	15.5 ± 0.2
<i>Stromateus brasiliensis</i>	Butterfish	Stromateidae	5	-16.9 ± 0.6	17.4 ± 0.4
Demersal Fishes					
<i>Menticirrhus americanus</i>	Southern kingfish	Sciaenidae	5	-15.0 ± 0.9	19.1 ± 1.1
<i>Micropogonias furnieri</i>	White croaker	Sciaenidae	5	-16.0 ± 0.3	16.7 ± 0.5
<i>Prionotus nudigula</i>	Bluewing searobin	Triglidae	5	-17.0 ± 0.1	18.0 ± 0.6
<i>Paralichthys sp</i>	Flounder	Paralichthyidae	5	-16.7 ± 0.2	17.7 ± 0.2
<i>Raneya brasiliensis</i>	Banded cusk-eel	Ophidiidae	5	-16.2 ± 0.4	17.4 ± 0.6
<i>Sparus pagrus</i>	Red porgy	Sparidae	6	-16.3 ± 0.6	17.4 ± 0.5
Demersal Pelagic Cephalopods					
<i>Illex argentinus</i>	Argentine short-finned squid	Ommastrephidae	5	-17.5 ± 0.4	14.7 ± 0.5

^a Reference: Bugoni et al. (2010); ^b Reference: Franco-Trecu et al. (2013a); ^c Reference: Silva-Costa and Bugoni (2013)

using partial correlation coefficients controlling for ages. Although bone and dentine integrate dietary information over long periods, stranding year was used as a temporal

reference, without any attempt to calculate the central year of the time span integrated by each individual. $\delta^{13}\text{C}$ values were corrected for the Suess effect.

Finally, SIAR, a Bayesian mixing model Stable Isotope Analysis in R (Parnell et al. 2010) package for software R (R Development Core Team 2009), was used to assess the relative contributions of potential prey species to the diet of South American sea lion males dead stranded before 1990 and after 1999. There were two reasons for that partitioning. First, only dentine samples were available before 1989 and only bone samples were available since 1994 (Table 1). Second, fisheries operating in the adjoining Argentinean–Uruguayan Common Fishing Zone suffered major changes in the average trophic level of landings during the mid-1990s (Jaureguizar and Milessi 2008; Milessi and Jaureguizar 2013). Although the significance of those changes for the availability of potential prey for South American sea lions in southern Brazil remains unknown, the exclusion from the analysis of those specimens that lived during that period aims to control such a possible influence.

SIAR estimates the probability distributions of multiple source contributions to a mixture while accounting for the observed variability in source and mixture isotopic compositions, dietary isotopic fractionation and elemental concentration. The model included prey species that were clumped into ecological groups: All the demersal fishes together, small pelagic fishes from Brazil, small pelagic fishes from Argentina, demersal pelagic cephalopods from Brazil and demersal pelagic cephalopods from Argentina. The species included in each group were selected according to previous studies analyzing stomach contents and scats from the region (Oliveira et al. 2008; Machado 2013; Naya et al. 2000; Szteren et al. 2004; Riet-Saprizza et al. 2012; Suárez et al. 2005), although they may not give full coverage of the diet due to seasonal biases in sampling. Data within each group fitted a normal distribution, as this is required by SIAR (Parnell et al. 2010). The model was run twice, using the two sets of fractionation factors obtained in this study.

Results

The stable isotope ratios of potential prey from northern Argentina and southern Brazil are shown in Table 2. Potential prey from northern Argentina was usually depleted in ^{13}C and enriched in ^{15}N when compared with the same species from southern Brazil (two-way ANOVA; $\delta^{13}\text{C}$: $F_{(11, 48)} = 37.41$, $P < 0.001$; $\delta^{15}\text{N}$: $F_{(11, 48)} = 32.15$, $P < 0.001$). However, the species–area interaction term was statistically significant in both cases ($\delta^{13}\text{C}$ $F_{(11, 48)} = 8.12$, $P < 0.001$; $\delta^{15}\text{N}$: $F_{(11, 48)} = 12.3$, $P < 0.001$), thus indicating that some species departed from that pattern. Nevertheless, demersal fishes from the two regions were more enriched in ^{13}C than any other group of potential prey and small pelagic fishes

from both regions were more depleted in ^{15}N than any other group (Fig. 2). For further analysis, prey was pooled into ecological groups differing in average stable isotope ratios: demersal fishes, medium-size pelagic fishes, small pelagic fishes from Brazil, small pelagic fishes from Argentina, demersal pelagic cephalopods from Brazil and demersal pelagic cephalopods from Argentina.

Paired samples of bone and dentine from adult South American sea lions did not differ in average $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{bone}} = -11.9 \pm 0.4 \text{‰}$; $\delta^{13}\text{C}_{\text{dentine}} = -12.0 \pm 0.5 \text{‰}$; paired t test; $t = 0.571$, $P = 0.574$, $n = 12$ for each tissue), but dentine was depleted in ^{15}N when compared with bone from the same individual ($\delta^{15}\text{N}_{\text{bone}} = 22.2 \pm 0.8 \text{‰}$, $\delta^{15}\text{N}_{\text{dentine}} = 21.4 \pm 0.6 \text{‰}$; paired t test; $t = 2.763$, $P = 0.011$, $n = 12$ for each tissue). Accordingly, only the $\delta^{13}\text{C}$ values from the whole data set can be considered to analyze temporal changes while analysis of $\delta^{15}\text{N}$ values had to be limited to the 1994–2009 period (bone samples).

When the whole data set of males South American sea lions older than 9 years was considered (years 1986–2009), stranding year and $\delta^{13}\text{C}$ values were uncorrelated (Fig. 3a; $\delta^{13}\text{C}$: partial correlation, $r = 0.038$, $N = 34$, $P = 0.834$) and the same was true for the juvenile and adolescent males (Fig. 3b; $\delta^{13}\text{C}$: partial correlation, $r = 0.332$, $N = 20$, $P = 0.165$). This result is unlikely to be an artifact of combining dentine and bone $\delta^{13}\text{C}$ values, not only because the absence of statistically significant differences above reported, but also because the variability of the $\delta^{13}\text{C}$ values was similar in the three decades (Table 3). The coefficient of variation was always $<10 \%$ of the mean, and $\delta^{15}\text{N}$ values were also uncorrelated when only the bone data set (1994–2009) was considered, both for adult males more than 9 years old (Fig. 3c; $\delta^{15}\text{N}$: partial correlation, $r = -0.201$, $N = 26$, $P = 0.336$) and juvenile males <8 years old (Fig. 3d; $\delta^{15}\text{N}$: partial correlation, $r = 0.219$, $N = 12$, $P = 0.519$). The variability of the $\delta^{15}\text{N}$ values was similar in the three decades (Table 3). The coefficient of variation was always $<10 \%$ of the mean.

The expected stable isotope ratios of the diet of South American sea lions from Northern Patagonia were $\delta^{13}\text{C} = -16.6 \text{‰}$ and $\delta^{15}\text{N} = 17.0 \text{‰}$ (Table 4), and the stable isotope ratios of male South American sea lions bone from the same area were $\delta^{13}\text{C} = -12.2 \pm 0.8 \text{‰}$ and $\delta^{15}\text{N} = 22.3 \pm 1.3 \text{‰}$. This resulted into a diet-to-bone discrimination factor of $4.4 \pm 0.8 \text{‰}$ for $\delta^{13}\text{C}$ and $5.3 \pm 1.3 \text{‰}$ for $\delta^{15}\text{N}$. Vibrissae of South American sea lions from northern Patagonia were depleted both in ^{13}C and ^{15}N relative to bone (mean $\delta^{13}\text{C}$: vibrissae = -13.1 ± 0.8 ; bone = -12.3 ± 0.8 ; mean $\delta^{15}\text{N}$: vibrissae = 21.2 ± 0.9 ; bone = 22.5 ± 1.5), which resulted into a diet-to-bone discrimination factor of $3.5 \pm 0.8 \text{‰}$ for $\delta^{13}\text{C}$ and $4.4 \pm 0.8 \text{‰}$ for $\delta^{15}\text{N}$ when combined with the published diet-to-vibrissa discrimination factors.

Fig. 2 Bivariate stable isotope ratios of prey and South American sea lion males from southern Brazil after correcting them with the indirect vibrissa–bone discrimination factor (a) and the direct discrimination factor (b) and their main prey from southern Brazil and northern Argentina. Bone and dentine samples are denoted by *circles* and *triangles*, respectively. *Open symbols* represent adult South American sea lions older than 9 years, and *solid symbols* represent adult South American sea lions younger than 8 years

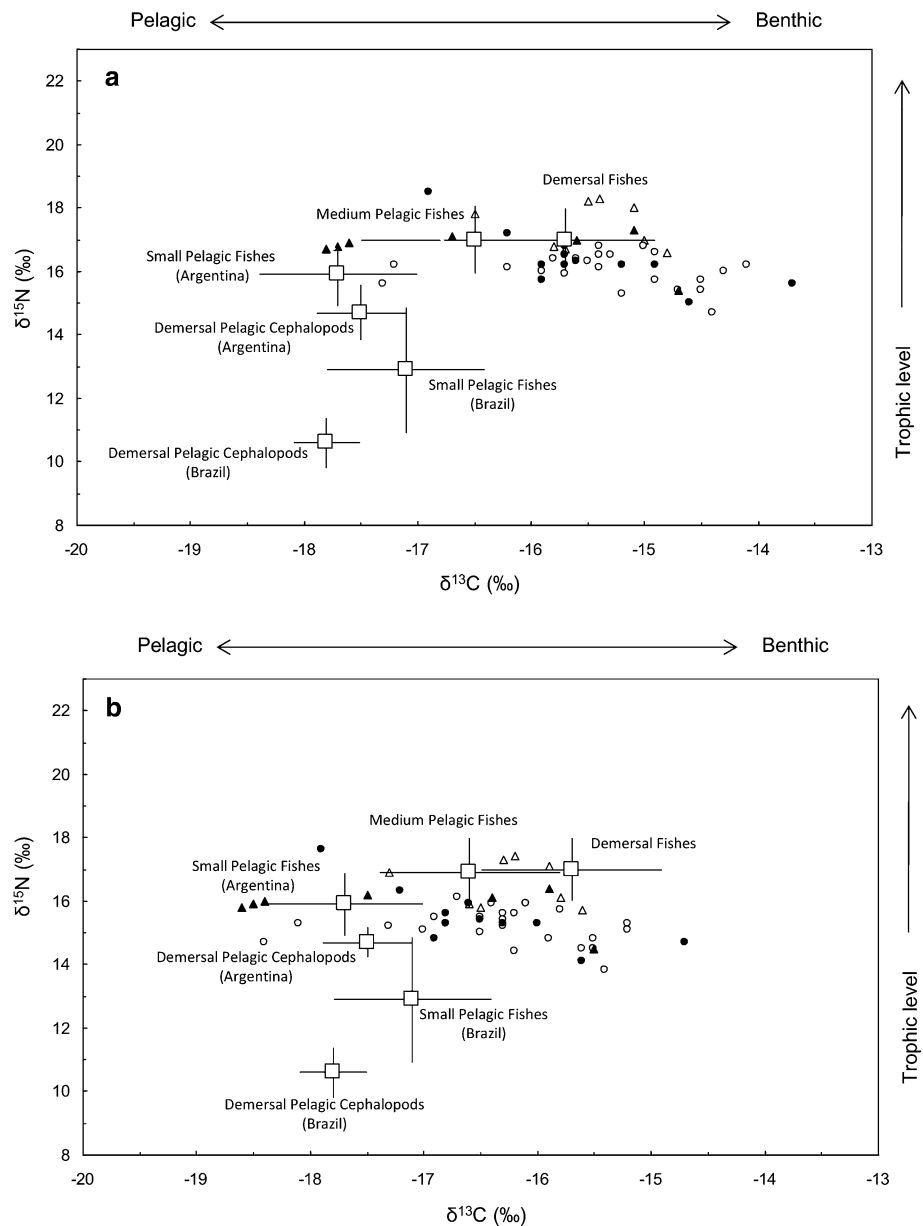


Figure 2 shows the position of potential prey and South American sea lions within the regional isoscape once the stable isotope ratios of the predator have been corrected for the Suess effect and diet-to-predator stable isotope discrimination. Most of the South American sea lion samples, independently on the tissue, were close to demersal prey when the indirect vibrissa–bone discrimination factor was used, although a few South American sea lion samples had stable isotope ratios consistent with pelagic foraging (Fig. 2a; Table 5). Conversely, the stable isotope ratios of South American sea lion samples were intermediate between those of demersal and medium-size pelagic prey when the direct prey–bone discrimination

factor was used, thus suggesting more mixed diets (Fig. 2b; Table 5).

The output of SIAR confirmed that demersal and medium-size pelagic fishes dominated the diet of South American sea lions older than 9 years during the whole considered period, although the actual proportions varied according to the discrimination factor used and the importance of medium-size pelagic fishes might have increased slightly after 1994 (Figs. 4, 5; Table 5). On the other hand, pelagic prey was always more relevant for the diet of juveniles males younger than 8 years than for adults, and no major dietary shift was observed during the period considered, although the actual proportion of pelagic and

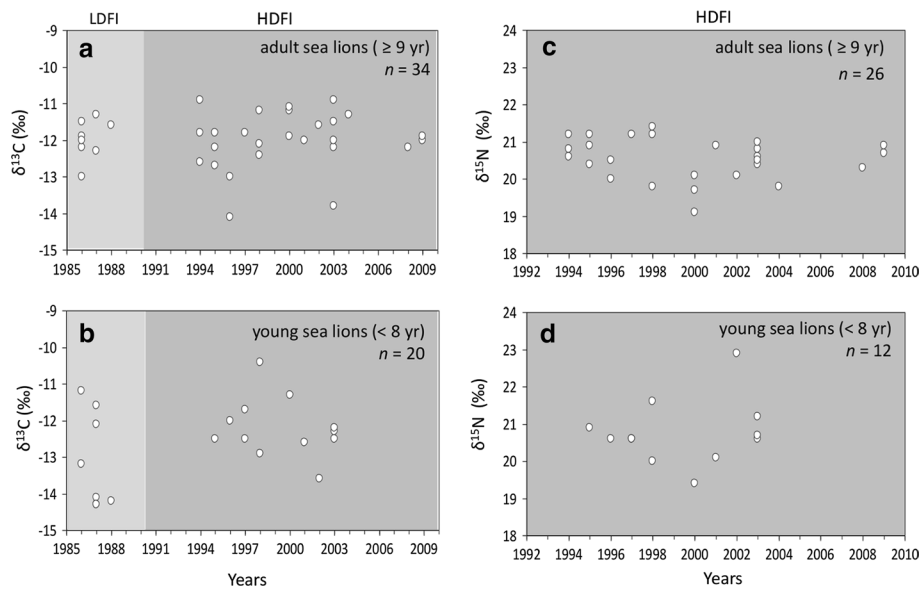


Fig. 3 Temporal changes in the ratios of stable isotopes of carbon and nitrogen in South American sea lions dead stranded along the coast of southern Brazil. The *lighter area* represents the period of low demersal fishing intensity (LDFI-years 1975–1989) and the *darker area* represents the period of increasing demersal fishing intensity (HDFI-years 1990–2010), accordingly by Haimovici (1998) and Milessi and Jaureguizar (2013). *Left panels* presents bone and

tooth dentine $\delta^{13}\text{C}$ values from specimens older than 9 years (a) and younger than 8 years (b) stranded between 1986 and 2009. *Right panels* present bone $\delta^{15}\text{N}$ values from specimens older than 9 years (c) and younger than 8 years (d) stranded between 1992 and 2009. The $\delta^{13}\text{C}$ values were corrected for the Suess effect. See Table 2 for the original data

Table 3 Mean and standard deviation of the stable isotope ratios (mean \pm SD) of male South American sea lions from southern Brazil at three different periods

	1986–1988	1994–1998	2000–2009
$\delta^{13}\text{C}$ (‰)			
<9 years old	-12.6 ± 1.3	-11.7 ± 0.8	-12.3 ± 0.7
≥ 9 years old	-11.5 ± 0.5	-11.9 ± 0.9	-11.7 ± 0.7
$\delta^{15}\text{N}$ (‰)			
<9 years old	20.3 ± 0.6	20.7 ± 0.5	20.8 ± 1.9
≥ 9 years old	21.0 ± 0.7	20.8 ± 0.5	20.4 ± 0.5

Canine dentine was analyzed in 1986–1988 and bone hereafter

demersal prey depended on the fractionation factor used (Figs. 4b, d and 5b, d).

Discussion

South American sea lions have been reported as broad-spectrum predators (Aguayo and Maturana 1973; Koen-Alonso et al. 2000; Naya et al. 2000) and diet often overlaps, at least partially, with fisheries catch in most of their range (Koen-Alonso et al. 2000; Hückstädt and Antezana 2003; Oliveira et al. 2008; Romero et al. 2011; Riet-Sapiriza et al. 2012; Machado 2013). Nevertheless, they are

often considered to have a low vulnerability to the development of demersal fisheries because of a high trophic plasticity (Koen-Alonso et al. 2000; Müller 2004; Szteren et al. 2004). The data presented here confirm that adult male South American sea lions from southern Brazil had mixed demersal/pelagic diets through the study period and hence suggest that no major dietary changes happened since the 1980s.

Nevertheless, historical changes in the isotopic baseline may hinder the interpretation of retrospective studies on trophic level and food web structure (Casey and Post 2011), and thus, the interpretation of isotopic signals without relevant ecological data can be challenging. In this study, $\delta^{13}\text{C}$ values were corrected to account for the Suess effect (Francey et al. 1999; Indermühle et al. 1999), but reference samples from historical fish and invertebrates were not available, and hence, other sources of variation were not controlled. For instance, an increase in the arrival of sewage during the last decades might have enhanced primary productivity and simultaneously increased the $\delta^{15}\text{N}$ values of the coastal food web (Calvert et al. 1992; Wu et al. 1997). However, available evidence revealed no major changes in primary productivity in southern Brazil from 1998 to 2006 (Heileman and Gasalla 2008), and accordingly, no major change in the $\delta^{15}\text{N}$ baseline is expected, as both parameters are strongly correlated along the coasts of the southwestern Atlantic (Saporiti et al. 2014). In any

Table 4 Data used to compute the discrimination factor on the basis of published dietary information and bone stable isotope ratios of male South American sea lions off northern Patagonia, Argentina

Potential prey	Contribution to diet ^a (%)	Stable isotope ratios			Protein contents ^b (% wet mass)		Weighted factors
		n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	n		
<i>Engraulis anchoita</i>	0.9	5	-17.9 ± 0.2	15.7 ± 0.8	5	13.5 ± 1.2	0.01
<i>Genypterus blacodes</i>	3.7	–	–15.4	18.0	–	12.5	0.04
<i>Merluccius hubbsi</i>	56.0	5	-17.7 ± 0.6	15.9 ± 0.5	5	12.5 ± 0.5	0.57
<i>Paralichthys isósceles</i>	2.8	5	-15.9 ± 0.4	18.0 ± 0.6	5	13.5 ± 1.4	0.03
<i>Pseudoperca semifasciata</i>	12.3	–	–14.7	19.5	–	12.5	0.12
<i>Raneya brasiliensis</i>	3.0	5	-15.3 ± 0.6	18.8 ± 0.5	2	13.5 ± 0.2	0.03
<i>Enteroctopus megalocyathus</i>	10.0	5	-14.6 ± 0.7	18.9 ± 0.9	2	11.1 ± 1.6	0.09
<i>Octopus tehuelchus</i>	0.4	5	-14.8 ± 0.2	19.9 ± 0.4	5	12.0 ± 0.4	0.00
<i>Illex argentines</i>	8.8	5	-17.0 ± 0.6	13.7 ± 0.8	5	11.0 ± 1.1	0.08
<i>Loligo gahi</i>	2.0	4	-17.6 ± 0.4	15.7 ± 0.6	4	13.1 ± 0.4	0.02

^a Koen-Alonso et al. (2000); ^b Drago et al. (2010b)

Table 5 Relative contribution of demersal and pelagic prey to the diet of male South American sea lions

Potential contributions	Discrimination factor			
	Indirect vibrissae–bone	Direct	Indirect vibrissae–bone	Direct
	Dentine/adult South American sea lions (≥ 9 years) 1986–1988		Bone/adult South American sea lions (≥ 9 years) 1994–2009	
Demersal fishes	0.56	0.37	0.42	0.29
Medium pelagic fishes	0.23	0.28	0.23	0.19
Small pelagic fishes (Brazil)	0.04	0.07	0.08	0.14
Small pelagic fishes (Argentina)	0.08	0.14	0.11	0.11
Demersal pelagic cephalopods (Brazil)	0.03	0.04	0.05	0.13
Demersal pelagic cephalopods (Argentina)	0.06	0.11	0.12	0.14
	Dentine/young South American sea lions (<8 years) 1986–1988		Bone/young South American sea lions (<8 years) 1994–2009	
Demersal fishes	0.33	0.16	0.35	0.17
Medium pelagic fishes	0.31	0.17	0.24	0.16
Small pelagic fishes (Brazil)	0.05	0.10	0.10	0.13
Small pelagic fishes (Argentina)	0.18	0.18	0.13	0.21
Demersal pelagic cephalopods (Brazil)	0.03	0.13	0.06	0.14
Demersal pelagic cephalopods (Argentina)	0.09	0.27	0.11	0.20

Results of SIAR mixing models applied with different discrimination factors, showing the mean proportion (%) of each probable source in diet of male South American sea lions

case, access to historical samples of potential prey will be extremely useful to be completely rule out changes in the stable isotope baseline during the period considered.

A second limiting factor is the existence of two tissues integrating dietary information over different time spans. Pinniped bone has been claimed to integrate dietary information throughout ~ 5 years, whereas canine dentine integrates dietary information through life (Riofrío-Lazo and Aurióles-Gamboa 2013). The difference is because bone is metabolically active and undergoes constant turnover,

whereas dentine is metabolically inert and new layers are settled throughout the life of the individual into the open pulp cavity of the canine teeth (Riofrío-Lazo and Aurióles-Gamboa 2013). However, the actual significance of these differences for diet reconstruction is probably limited to young individuals. Suckling pinniped pups are more enriched in ^{15}N than their mothers, whereas the relationship between suckling pups and their mothers is less clear for ^{13}C and may be species dependent (Ducatez et al. 2008; Drago et al. 2009b; Newsome et al. 2010a). The suckling

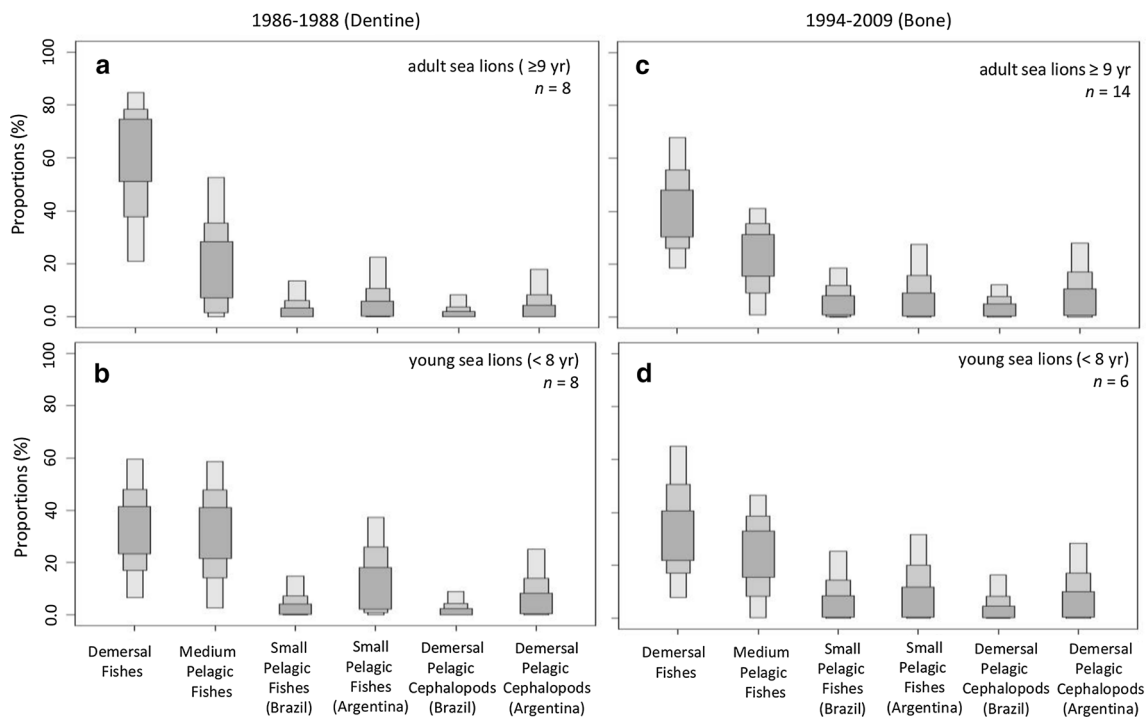


Fig. 4 Diet composition of male South American sea lions off southern Brazil according to SIAR mixing model and the indirect vibrissa–bone discrimination factor. The contribution of each prey to the diet is

shown with 95, 75 and 50 % credibility intervals. The $\delta^{13}\text{C}$ values of South American sea lions were corrected for the Suess effect, to allow comparison with modern preys. See Table 2 for the original data

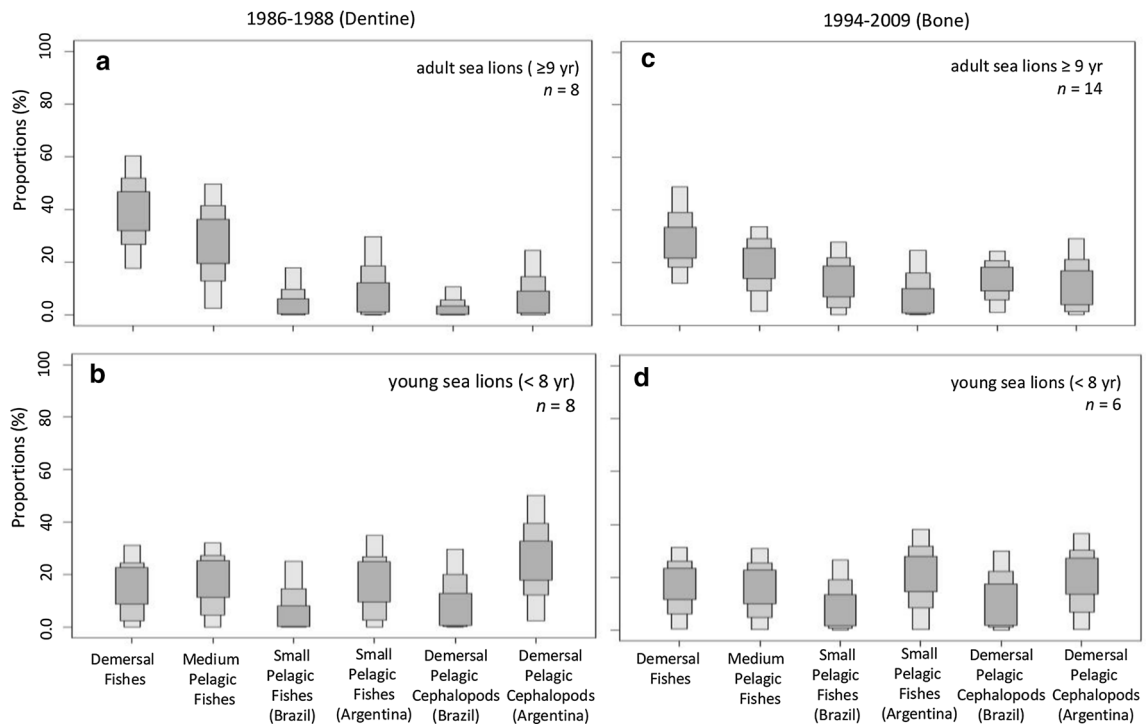


Fig. 5 Diet composition of male South American sea lions off southern Brazil according to SIAR mixing model and the direct bone discrimination factor. The contribution of each prey to the diet is shown

with 95, 75 and 50 % credibility intervals. The $\delta^{13}\text{C}$ values of South American sea lions were corrected for the Suess effect, to allow comparison with modern preys. See Table 2 for the original data

signal remains forever in the dentine formed during the first year of life, but fades from bone after 1 or 2 years due to tissue turnover (Drago et al. 2009b; Newsome et al. 2010a). Accordingly, the dietary reconstructions using dentine and bone from individuals older than 2 years may lead to different conclusions about trophic level. However, the impact of the suckling signal on the overall stable isotope ratio of dentine decreases as new layers are settled and is expected to have a negligible impact on adults, where represents <1/9 of dentine.

Independently of these obscuring factors, the results here reported reveal a remarkable dietary stability of both adults and juveniles during 30 years, although there is a high level of individual variability during the whole time span of the study, independently on the age class and tissue considered. There are at least two possible causes for such variability. First, South American sea lions forage over a wide area including southern Brazil, Uruguay and northern Argentina (Rodríguez et al. 2013), and prey from those regions is known to differ in their stable isotope ratios (Abreu et al. 2006; Bugoni et al. 2010; Botto et al. 2011; Franco-Trecu et al. 2013a; this study). We are uncertain about the actual foraging area used by each individual and for how long they foraged off southern Brazil, but stable isotopes of oxygen suggest some individual differences in the foraging grounds used (Zenteno et al. 2013). Second, the existence of different individual foraging strategies cannot be excluded, as the stable isotope ratios of some adult males are closer to those of midsize pelagic fishes than to those of demersal ones. Nevertheless, stomach content analysis (Oliveira et al. 2008; Machado 2013) and stable isotope analysis (this study) agree in identifying demersal fishes as the staple food of South American sea lions in southern Brazil. Scat analysis indicates that females breeding in Uruguay also forage primarily on demersal fishes, at least during the breeding season (Riet-Saprizza et al. 2012).

There are at least two non-excluding explanations for the intense use of demersal prey by adult South American sea lions, despite of the high abundance of pelagic prey in the study area. Firstly, a selection based on prey size, as benthic prey is usually larger than pelagic prey. Secondly, a preference for benthic prey would be explained by their more sedentary behavior (Womble and Sigler 2006) and the permanent motion of pelagic prey (Gende and Sigler 2006). The first hypothesis is supported by the larger size of the demersal prey consumed by South American sea lions when compared with that of pelagic prey (Szteren et al. 2004; Riet-Saprizza et al. 2012), although pelagic prey has a higher energy density (Drago et al. 2009a).

Demersal fishes also had a central role in the diet of juvenile and adolescent males, but small and medium pelagic fishes represented the bulk of their diet. Ontogenic dietary changes in pinnipeds are often related to somatic growth and

the associated improvement in diving performance (Gentry et al. 1986; Horning and Trillmich 1997; Costa et al. 2004). South American sea lions are not an exception, and they dive deeper (Rodríguez et al. 2013) and increase the consumption of demersal prey as they grow older (Drago et al. 2009b). This was also the pattern observed in the present study and suggests that the scats from unknown individuals with a high proportion of small pelagic fish (Naya et al. 2000; Szteren et al. 2004) likely represent the diet of juvenile and adolescent South American sea lions.

The dietary stability of the South American sea lion *Otaria flavescens* in southern Brazil is opposite to the dietary changes reported from northern and central Patagonia, where South American sea lions have increased the consumption of pelagic prey since the 1970 (Koen-Alonso et al. 2000; Drago et al. 2009a; Romero et al. 2011), in parallel to the development of the bottom trawling fishery but also to the increase in the South American sea lion population resulting from legal protection (Drago et al. 2009a). On the contrary, the population of the South American sea lion is decreasing in Brazil, Uruguay and northern Argentina (Páez 2006). This suggests that the per capita availability of demersal prey for the South American sea lion may have declined in northern Patagonia but remained stable in southern Brazil during the last three decades, which may explain why diet changed dramatically in the former (Drago et al. 2009a) but remained stable in the latter (this study). On the contrary, franciscana dolphins and sea birds from northern Argentina and southern Brazil have shifted diets during the past three decades (Pinedo 1994; Secchi et al. 2003; Crespo and Hall 2002; Bugoni 2008), which suggest species-specific responses to environmental changes, probably linked to differences in body size and diving performance (Páez-Rosas et al. 2012).

In conclusion, the results reported here do not support a major dietary shift for male South American sea lions during the past three decades in southern Brazil, opposite to the pattern reported in other top predators in the region which may be related to differences in body size and population dynamics (Drago et al. 2011). Certainly, females have not been considered in this study, but recent published information based on scat analysis and stable isotopes suggests a diet very similar to that of males (Riet-Saprizza et al. 2012; Franco-Trecu et al. 2013b).

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