Isotopic niche partitioning between two apex predators over time

Massimiliano Drago*,[1] (iD), **Luis Cardona[2], Valentina Franco-Trecu[3], Enrique A. Crespo[4,5], Damián G. Vales[4], Florencia Borella[6], Lisette Zenteno[2], Enrique M. Gonzáles[7]** and **Pablo Inchausti[1]**

[1]*Departamento de Ecología & Evolución, Centro Universitario Regional Este (CURE), Universidad de la República, Tacuarembó s/n, 20000 Maldonado, Uruguay;* [2]*IRBio y Departamento de Biología Evolutiva, Ecología y Ciencias Ambientales, Universidad de Barcelona, Av. Diagonal 643, 08028 Barcelona, España;* [3]*Departamento de Ecología & Evolución, Facultad de Ciencias, Universidad de la República, Iguá 4225, 11400 Montevideo, Uruguay;* [4]*Laboratorio de Mamíferos Marinos, Centro para el Estudio de Sistemas Marinos (CESIMAR - CCT CONICET-CENPAT), Blvd. Brown 2915, U9120ACD Puerto Madryn, Argentina;* [5]*Universidad Nacional de la Patagonia, Blvd. Brown 3600, U9120ACD Puerto Madryn, Argentina;* [6]*Investigaciones Arqueológicas y Paleontológicas del Cuaternario Pampeano (INCUAPA-CONICET), Universidad Nacional del Centro, Av. Del Valle 5737, B7400JWI Olavarría, Argentina; and* [7]*Museo Nacional de Historia Natural, 25 de Mayo 582, 11000 Montevideo, Uruguay*

Summary

1. Stable isotope analyses have become an important tool in reconstructing diets, analysing resource use patterns, elucidating trophic relations among predators and understanding the structure of food webs.

2. Here, we use stable carbon and nitrogen isotope ratios in bone collagen to reconstruct and compare the isotopic niches of adult South American fur seals (*Arctocephalus australis*; $n = 86$) and sea lions (*Otaria flavescens*; $n = 49$) – two otariid species with marked morphological differences – in the Río de la Plata estuary (Argentina – Uruguay) and the adjacent Atlantic Ocean during the second half of the 20th century and the beginning of the 21st century. Samples from the middle Holocene ($n = 7$ fur seals and $n = 5$ sea lions) are also included in order to provide a reference point for characterizing resource partitioning before major anthropogenic modifications of the environment.

3. We found that the South American fur seals and South American sea lions had distinct isotopic niches during the middle Holocene. Isotopic niche segregation was similar at the beginning of the second half of the 20th century, but has diminished over time.

4. The progressive convergence of the isotopic niches of these two otariids during the second half of the 20th century and the beginning of the 21st century is most likely due to the increased reliance of South American fur seals on demersal prey.

5. This recent dietary change in South American fur seals can be explained by at least two non-mutually exclusive mechanisms: (i) the decrease in the abundance of sympatric South American sea lions as a consequence of small colony size and high pup mortality resulting from commercial sealing; and (ii) the decrease in the average size of demersal fishes due to intense fishing of the larger class sizes, which may have increased their accessibility to those eared seals with a smaller mouth gape, that is, South American fur seals of both sexes and female South American sea lions.

Key-words: marine mammals, pinnipeds, stable isotopes, standard ellipse area, trophic ecology

Introduction

Eared seals (Pinnipedia, Otariidae) exhibit two contrasting morphologies and foraging tactics. Fur seals are slender, pelagic predators that primarily rely on their underfur for

thermoregulation, whereas sea lions are massive, benthic predators that rely primarily on a reduced surface-to-volume ratio and thick blubber for thermoregulation (Webber 2014). Molecular and morphological evidence indicates that a small body size and pelagic foraging are plesiomorphic features in otariids and that different lineages of sea lions evolved independently as fur seal

*Correspondence author. E-mail: m.drago@ub.edu

ancestors dispersed across the Southern Hemisphere from the family's centre of origin in the North Pacific in the late Miocene (Yonezawa, Kohno & Hasegawa 2009; Churchill, Boessenecker & Clementz 2014). Interestingly, only some of the otariids first arriving to the Southern Hemisphere evolved into modern sea lions, whereas those arriving more recently have retained the plesiomorphic fur seal morphology (Yonezawa, Kohno & Hasegawa 2009; Churchill, Boessenecker & Clementz 2014). This suggests that interspecific competition and character displacement have been major forces driving the morphologic evolution of eared seals.

The current structure of marine ecosystems is, however, rather different from that existing when sea lions and fur seals colonized the Southern Hemisphere. This is because of both the environmental changes that took place over the last 5 million years and the large and confounding effects of the relatively modern impact of human activities on the marine environment. The combination of whaling, sealing and overfishing has resulted in dramatic reductions in the world-wide abundance of marine megafauna during the past two centuries (Jackson *et al.* 2001), and the eared seals inhabiting the Southern Hemisphere have not been exempt from these impacts (Costa, Weise & Arnould 2006). In this scenario, the evolutionary pressures leading to character displacement in sympatric eared seals might have been progressively relaxed over the recent past. Moreover, fur seals may have benefited from competitive release due to a general slower recovery rate for sea lions in many regions, probably because of their delayed sexual maturity in comparison with fur seals (e.g. Grandi *et al.* 2010) and their higher sensitivity to changes in food availability (e.g., Lander *et al.* 2013), after the cessation of commercial sealing (Costa, Weise & Arnould 2006).

The South American sea lion (*Otaria flavescens*) and the South American fur seal (*Arctocephalus australis*) are the only eared seals that have historically coexisted along the coasts of South America and the Falkland Islands (Webber 2014). Although commercial sealing strongly reduced their populations everywhere, local populations of the two species have responded differently to the cessation of sealing during the second half of the 20th century. For instance, while the South American fur seal population in Uruguay has been increasing, that of the South American sea lion has continued to decline since sealing stopped (Franco-Trecu 2015). In addition, the recovery of South American fur seals has been faster than that of South American sea lions in the Falkland Islands (Baylis *et al.* 2015), but the opposite trend has been documented in northern Patagonia, where the recovery of South American sea lions has been faster than that of South American fur seals (Dans *et al.* 2004; Tunez, Capozzo & Cassini 2008).

South American fur seals currently have a more pelagic diet than South American sea lions in the southwestern part of the South Atlantic (Franco-Trecu, Aurioles-Gamboa & Inchausti 2014; Saporiti *et al.* 2016), and stable isotope ratios of zooarchaeological bone material suggest

that the same was also true during the second half of the Holocene (Saporiti *et al.* 2014b). Nevertheless, the contribution of demersal prey to the diet of South American fur seals in northern Patagonia is negligible but they represent 16–37% of the biomass consumed by fur seals in areas influenced by the Río de la Plata estuary (Uruguay-Argentina) (Naya, Arim & Vargas 2002; Franco-Trecu *et al.* 2013; Vales *et al.* 2014, 2015). Such a pattern might be related to the opposite trends in size of the sympatric populations of South American sea lions in these two regions, as the sea lion population is declining in both the Río de la Plata and the adjoining Atlantic (Franco-Trecu *et al.* 2015) while increasing in northern Patagonia (Grandi *et al.* 2012). Nevertheless, the Río de la Plata estuary (with the adjacent Atlantic Ocean coast) and northern Patagonia currently support very different ecosystems (Acha *et al.* 2004; Saporiti *et al.* 2015); therefore, differences in the diet of South American fur seals in these two regions might be because of other ecosystem attributes and deserve further research.

Furthermore, human activity has also modified resource availability for both species. Two species of fish are central to the diets of both South American fur seals and South American sea lions in the Río de la Plata estuary and the adjoining Atlantic ocean, and these are small (less than 15 cm total length) pelagic anchovies (*Anchoa marinii* and *Engraulis anchoita*; Family Engraulidae) and the much larger (up to 50 cm total length) demersal striped weakfish (*Cynoscion guatucupa*; Family Sciaenidae) (Naya, Arim & Vargas 2002; Szteren, Naya & Arim 2004; Suarez *et al.* 2005; Oliveira, Ott & Malbarba 2008). The overall biomass of small pelagic fishes has remained rather stable in the Río de la Plata and adjacent Atlantic Ocean during the recovery of the South American fur seal population in Uruguay (Madirolas, Hansen & Cabreira 2013). In contrast, the overall biomass of demersal fishes has decreased due to overfishing during the same period (Vasconcellos & Haimovici 2006; Ruarte & Perrotta 2007; Defeo *et al.* 2009). The impact of fishing has been particularly severe on sciaenid fishes, leading to a reduction in the total biomass (Ruarte & Perrotta 2007) and an increase in the relative abundance of the smaller size classes of the striped weakfish (Jaureguizar, Ruarte & Guerrero 2006; Villwock de Miranda & Haimovici 2007).

Here, we follow a historical approach (Drago *et al.* 2009b; Saporiti *et al.* 2014b; Zenteno *et al.* 2015a) in order to first look at changes over time in resource partitioning between South American fur seals and sea lions and, second, to ascertain whether current resource partitioning in the Río de la Plata estuary and adjacent Atlantic Ocean is the result of recent anthropogenic forcing caused by two types of changes: changes in the relative abundance of South American fur seals and sea lions; and changes in the availability of pelagic and demersal prey. Obviously, food availability may be influenced by multiple caused, including climate, fishing and watershed dynamics, but we consider fishing the main driver because of

above reported changes associated to fisheries development in the region.

The stable isotope ratios of carbon and nitrogen in the skeletal remains of specimens from the middle Holocene provide a historical reference for understanding resource partitioning before the occurrence of major anthropogenic changes in the study area. In addition, the stable isotope ratios in the skeletal material of both species (made available by scientific collections) offer the opportunity to reconstruct and compare the isotopic niches of both species during the 20th and 21st centuries. Our analysis evaluates whether resource partitioning has been stable over time or whether fur seals have only increased their consumption of demersal prey during the late 20th and early 21st centuries as a result of the following factors: the increase in their population; the decrease in the sea lion population; and changes in the size structure of their prey populations following the end of sealing and the development of demersal fisheries (Villwock de Miranda & Haimovici 2007; Franco-Trecu 2015; Franco-Trecu *et al.* 2015).

Materials and methods

STUDY AREA AND REGIONAL ISOSCAPE

The Río de la Plata estuary is located at 35°S, between Uruguay and Argentina (Fig. 1). The plume of the estuary expands to southern Brazil. The estuary is characterized by very strong vertical salinity stratification and a turbidity front (Acha *et al.* 2008).

Phytoplankton and detritus are the main sources of fixed carbon for the food web (Botto *et al.* 2011). South American fur seals and sea lions forage through the estuary and southern Brazil, but breeding rookeries are only in Uruguay (Ponce de León 2000) (Fig. 1). Both species primarily rely on anchovies (*Anchoa marinii* and *Engraulis anchoita*) and striped weakfish (*Cynoscion guatucupa*), although other species are also consumed (Naya, Arim & Vargas 2002; Szteren, Naya & Arim 2004; Oliveira, Ott & Malbarba 2008). Anchovies are pelagic, with a total length of less than 15 cm, and they forage on plankton; whereas striped weakfish, which can reach 50 cm in length, are demersal and forage on both pelagic and demersal fishes (Cousseau & Perrotta 2000). As a result, anchovies have lower $\delta^{13}C$ and $\delta^{15}N$ values than striped weakfish across the estuary and in southern Brazil (Vales *et al.* 2014; Drago *et al.* 2015). It should be noted that fishes from southern Brazil have usually lower $\delta^{13}C$ ratios and higher $\delta^{15}N$ ratios when compared with the same species from the Río de la Plata estuary (Vales *et al.* 2014; Drago *et al.* 2015). Nevertheless, demersal fishes of both regions have higher $\delta^{13}C$ ratios than any other group of potential prey, and small pelagic fishes from both regions have lower $\delta^{15}N$ ratios than any other group (Vales *et al.* 2014; Drago *et al.* 2015). Accordingly, a general increase in the consumption of demersal fish should produce a simultaneous increase in both the $\delta^{13}C$ and $\delta^{15}N$ values of eared seals.

SAMPLING

Ancient bone samples of South American sea lions (*n* = 5) and fur seals (*n* = 7) were collected at La Olla (Monte Hermoso), an archaeological site on the southern coast of the Buenos Aires

Fig. 1. Study area and sampling locations. Grey dots show modern breeding sites of South American sea lions and fur seals; dashed lines show the sampling location of modern sea lion and fur seal skulls; the black dot shows the sampling location of ancient sea lion and fur seal samples; the asterisk shows the sampling location of modern blue mussel samples.

Province in Argentina (Fig. 1), and they were dated as middle Holocene (c. 7300–6600 uncalibrated ^{14}C years before present) (Blasi, Politis & Bayón 2013). All ancient bone samples used for the isotopic analysis were obtained from the humeral bones of adult specimens of unknown sex. Furthermore, to avoid sampling the same specimen twice, samples were collected only from the right humerus. Adulthood was inferred from the degree of fusion in each humerus (Borella *et al.* 2013), and species were identified by humeral morphological traits (Borella *et al.* 2014).

Modern bone samples were collected from adult male and female South American sea lions ($n = 22$ males and $n = 27$ females) and fur seals ($n = 49$ males and $n = 37$ females), which had been stranded dead along the Uruguayan coast, respectively, between 1944 and 2012 and between 1946 and 2012 (Fig. 1). All modern bone samples were obtained from the skulls of the scientific collection of the Museo Nacional de Historia Natural (MNHN) and the Facultad de Ciencias of the Universidad de la Republica at Montevideo (Uruguay). The bone samples used for the isotopic analysis consisted of a small fragment of turbinate bone from the nasal cavity. The condylobasal length of each skull

was also measured, as it is highly correlated with standard length and body size in otariids (e.g. Rosas, Haimovici & Pinedo 1993); thus, it can be used as a proxy for body length (Drago *et al.* 2009a). In order to avoid any age-related bias, only sexually mature adult specimens were considered (Drago *et al.* 2009a). Individual age was assessed by counting dentine growth layers in canines (Laws 1952). The ages of the sampled sea lions ranged from 7 to 21 years for males and from 5 to 11 years for females; those of the fur seals ranged from 6 to 14 years for males and from 5 to 12 years for females. Sex was determined initially by the external morphology (e.g. presence of baculum bone in males) during sample collection and eventually by secondary sexual characteristics of the skulls from each species.

On the basis of the stranding year, each modern sea lion and fur seal was allocated to one of the three modern periods in the history of marine resource exploitation in Uruguay (Ponce de León 2000; Defeo *et al.* 2009; Franco-Trecu 2015) (Fig. 2). The first period (1944–1965) was characterized by intense exploitation of fur seals, no exploitation of sea lions and negligible fishing. During the second period (1971–1983), both otariid species were
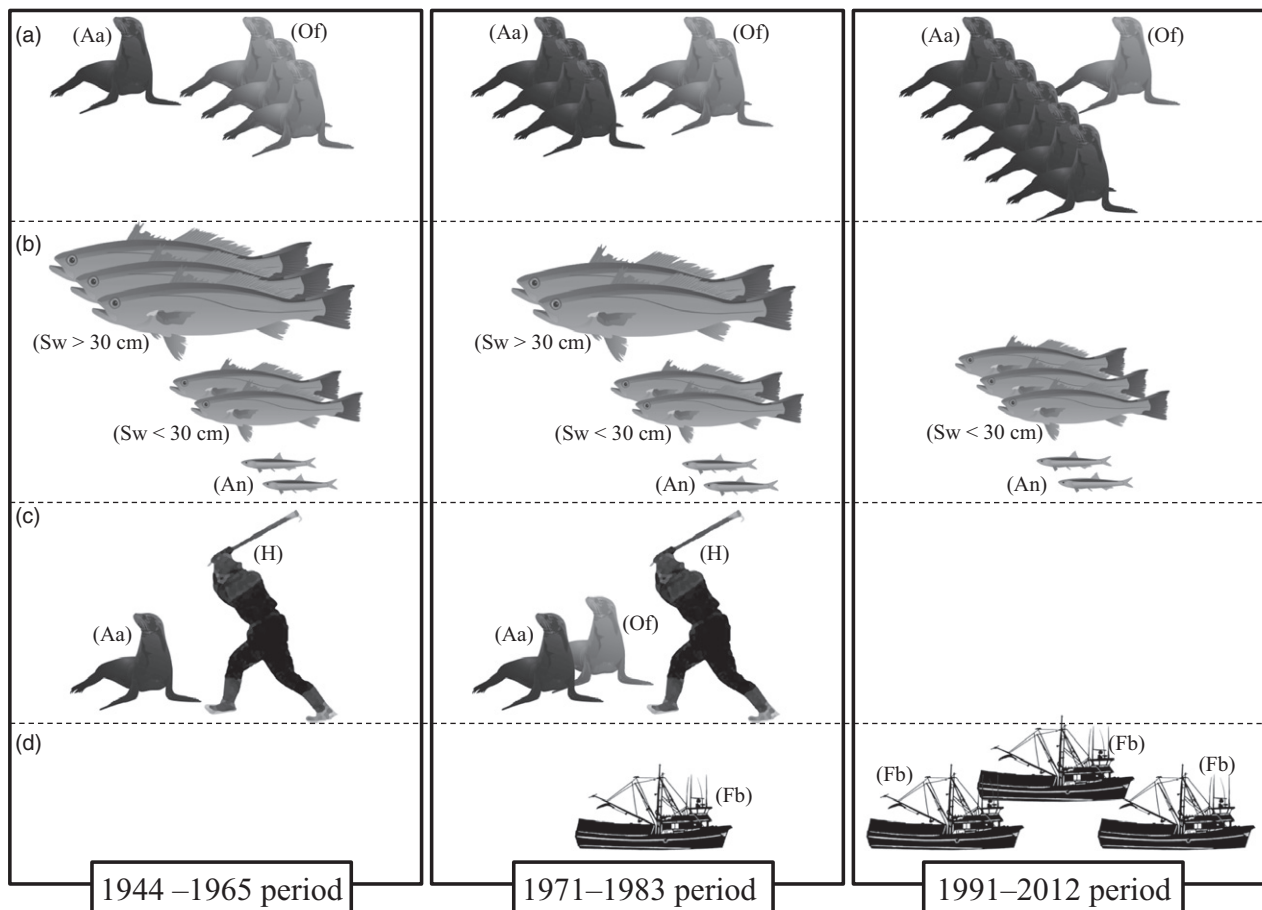

Fig. 2. Schematic representation of the three modern periods in the history of marine resource exploitation in Uruguay (Ponce de León 2000; Defeo *et al.* 2009; Franco-Trecu 2015). From 1944 to 1965, there was intense exploitation of fur seals, no exploitation of sea lions and negligible fishing. From 1971 to 1983, both species of eared seals were exploited, and bottom trawlers began to operate, targeting demersal fishes. Finally, from 1991 to 2012, there was no commercial hunting of eared seals, demersal fisheries had been fully developed, and the average sizes of striped weakfish and other demersal fish declined. (a) Relative abundance of the South American sea lion (Of) and fur seal (Aa) population; (b) Relative changes in the abundance and size distributions of the prey fish species over time (striped weakfish (Sw) >30 cm, striped weakfish (Sw) <30 cm and anchovies (An), top to bottom); (c) Commercial sealing (sea lion (Of), fur seal (Aa), hunter (H)); (d) Relative development of the industrial fishery (fishing boat (Fb)). Images (not drawn to scale) from OpenClip-Art.org (https://openclipart.org) and IAN/UMCES (http://ian.umces.edu/imagelibrary/).

exploited and bottom trawl fisheries were developed. Finally, commercial hunting of fur seals and sea lions ceased and bottom trawling fisheries were fully developed during the third period (1991–2012) (Fig. 2).

The stable isotope ratios of organisms cannot be directly compared over time because the isotopic baseline may be subject to temporal variations (Casey & Post 2011). Accordingly, we analysed the stable isotope ratios in the shells of modern mollusc, precisely blue mussel (*Mytilus edulis*) species, in order to reconstruct and account for changes over time in the stable isotope baseline. Unfortunately, there were no shell samples from the archaeological site. We used mollusc shells because the proteins that make up their organic matrix are encased within mineral crystals and thus preserved, hence offering a material suitable for reconstructing the changes in the isotopic baseline (Casey & Post 2011; Zenteno *et al.* 2015a).

The mussel shell samples going to 2014 ($n = 5$) were directly collected on the Uruguayan coast, whereas the ones collected from the same area (Fig. 1) in 1957 ($n = 5$) and 1988 ($n = 5$) were obtained from the scientific collection of the MNHN, where the shells had been preserved dry and without varnishing. We used this mollusc species not only because it was the only one dating back to different periods available in the scientific collection but also because it is thought to represent well the ecosystem baseline (see Discussion).

All bone and shell samples were stored dry at room temperature until analysis.

STABLE ISOTOPE ANALYSIS

Samples were cleaned in distilled water, dried in a stove at 60 °C for 36 h, and ground into a fine powder using a mortar and a pestle. The shell surfaces had been polished previously with sandpaper and a diamond wheel drill to eliminate any residue. As bone and shell samples contain a high concentration of inorganic carbon that may add undesirable variability to $\delta^{13}C$ (Lorrain *et al.* 2003), they were treated by soaking in 0·5 N (bone) and 1 N (shell) hydrochloric acid (HCl) for 24 h to decarbonise them (Newsome *et al.* 2006). Since HCl treatment adversely affects $\delta^{15}N$ (Schlacher & Connolly 2014), each sample was previously divided into two subsamples: one of them for carbon isotope analysis after decarbonation; and the other one for nitrogen isotope analysis without decarbonation. Furthermore, lipids were removed from bone samples by a chloroform-methanol (2 : 1) solution (Bligh & Dyer 1959), as they are depleted in ^{13}C compared with other molecules and may therefore lead to undesirable variability in $\delta^{13}C$ values (DeNiro & Epstein 1978). Nevertheless, given that chemical lipid extraction may affect $\delta^{15}N$ values due, *inter alia*, to the unintentional removal of amino acids (Ryan *et al.* 2012), we extracted lipids only from the subsamples for carbon isotope analysis and used non-extracted subsamples for nitrogen determination.

Approximately 1 mg of bone and 0·2–15·1 mg of shell were weighed into tin capsules and analysed by elemental analysis-isotope ratio mass spectrometry, specifically by means of a model FlashEA 1112 elemental analyzer (Thermo Fisher Scientific, Milan, Italy) coupled with a Delta C isotope ratio mass spectrometer (ThermoFinnigan, Bremen, Germany). All analyses were performed at the Centres Científics i Tecnològics of the University of Barcelona, Spain.

Stable isotope abundances are expressed in delta (δ) notation, with relative variations of stable isotope ratios expressed in per mil (‰) deviations from predefined international standards, and they were calculated as:

$$\delta^j X = [(^j X/^i X)_{sample}/(^j X/^i X)_{standard}] - 1$$

where $^j X$ is the heavier isotope (^{13}C or ^{15}N), and $^i X$ is the lighter isotope (^{12}C or ^{14}N) in the analytical sample and international measurement standard (Bond & Hobson 2012); international standards were the Vienna Pee Dee Belemnite calcium carbonate for the $\delta^{13}C$ value and atmospheric nitrogen (air) for the $\delta^{15}N$ value. However, data were normalized using commercially available laboratory reference materials. For carbon, isotopic reference materials of known $^{13}C/^{12}C$ ratios, as given by the International Atomic Energy Agency (IAEA, Vienna, Austria), were used for calibration at a precision of 0·05‰. These include polyethylene (IAEA CH_7, $\delta^{13}C = -32·1‰$), L-glutamic acid (IAEA $USGS_{40}$, $\delta^{13}C = -26·4‰$) and sucrose (IAEA CH_6, $\delta^{13}C = -10·4‰$). For nitrogen, isotopic reference materials of known $^{15}N/^{14}N$ ratios were used for calibration at a precision of 0·2‰. These include $(NH_4)_2SO_4$ (IAEA N_1, $\delta^{15}N = +0·4‰$ and IAEA N_2, $\delta^{15}N = +20·3‰$), L-glutamic acid (IAEA $USGS_{40}$, $\delta^{15}N = -4·5‰$) and KNO_3 (IAEA NO_3, $\delta^{15}N = +4·7‰$). All these isotopic reference materials were employed to recalibrate the system once every 12 samples were analysed in order to compensate for any measurement drift over time. The raw data were normalized by the multipoint normalization method based on linear regression (Skrzypek 2013). Furthermore, we also quantified the carbon to nitrogen (C/N) atomic ratio of each analysed sample as a control or proxy for the data quality (e.g. adequate lipid extraction or conservation status of the isotopic signal) of the bone collagen in both the modern and ancient samples (DeNiro 1985).

DATA ANALYSES

Prior to statistical analyses, normality was tested by means of the Lilliefors test, and homoscedasticity by means of the Levene test. Furthermore, we checked the assumptions of the statistical models by carrying out the customary residual analysis (see Appendix S1, Supporting Information) (Crawley 2007).

To evaluate changes in body size for each species and sex throughout the second half of the 20th century and the beginning of the 21st century, linear regression analysis was performed using the year of stranding and the condylobasal length of modern South American sea lion and fur seal skulls.

One-way ANOVAs, followed by Tukey's *post hoc* tests, were used to compare the average $\delta^{13}C$ and $\delta^{15}N$ values of mussels from different sampling years and therefore to evaluate whether the isotopic baseline of the ecosystem changed over time. In case of changes, isotopic baseline correction factors were computed to allow the comparison of the $\delta^{15}N$ and $\delta^{13}C$ values of modern sea lions and fur seals from periods differing in isotopic baseline (i.e. the three periods for which mussel shells were available).

To compute the baseline correction factor, the average stable isotope ratios of mussel shells from a single sampling year were first subtracted from those of shells in the next, most recent sampling year; then, the result was divided by the number of years elapsed between the two consecutive sampling years. That informed us about the average annual rate of change in the isotopic baseline between the two consecutive sampling years. This procedure was chosen because the rate of change in the average
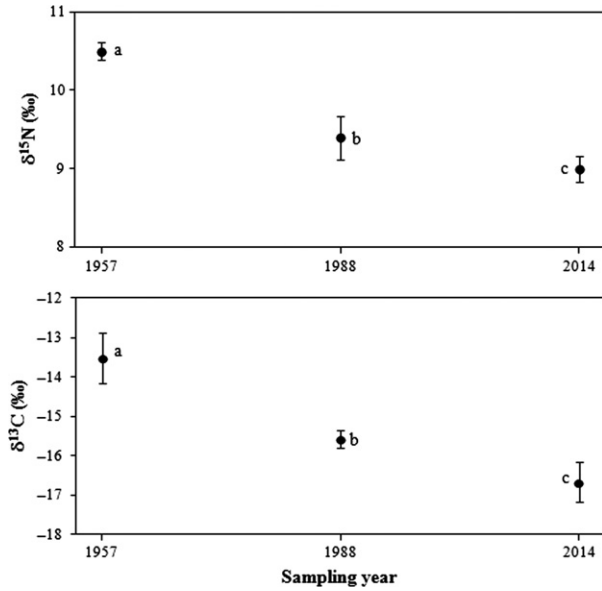
Fig. 3. Means and SD of the shell $\delta^{13}C$ and $\delta^{15}N$ values of blue mussels at different sampling years. Sampling years with different superscript (lower case letters) are statistically different in their mean values according to the Tukey post hoc test.

stable isotope ratios of mussel shells was not constant over time (see Results and Fig. 3). Second, a time-dependent correction was applied, specifically by considering the time elapsed from the death of each sea lion and fur seal specimen to 2014 and the rates of baseline change, which were computed from 1957 to 1987 and from 1988 to 2014. Because we could not reconstruct the isotopic baseline between 1944 and 1956 due to lack of shell samples, we used the estimated baseline correction factor for sea lions and fur seals from the adjacent time period (1957–1987). After verifying that there were temporal trends in $\delta^{13}C$ and $\delta^{15}N$ values of mussel shells (described in the Results), we decided to correct the bone isotope values of modern South American sea lions and fur seals (indicated with $\delta^{13}C_{cor}$ and $\delta^{15}N_{cor}$; see Table 1) over time by applying the following time-dependent correction factors: $0.0356‰$ for $\delta^{15}N$ and $-0.0666‰$ for $\delta^{13}C$ per year between 1944 and 1987; and $0.0153‰$ for $\delta^{15}N$ and $-0.0417‰$ for $\delta^{13}C$ per year since 1988.

We assessed the change in $\delta^{13}C_{cor}$ and $\delta^{15}N_{cor}$ values for modern sea lions and fur seals over time using a two-way ANCOVA, with the year as a continuous covariate and species and sex as categorical explanatory variables. We started with the most complex model, which included the triple interaction among explanatory variables, and subjected it to sequential, stepwise simplification by deleting the term that was furthest from being statistically significant; we then compared the models with and without the deleted term by using the likelihood ratio test (Crawley 2007) until obtaining the minimally adequate model for each isotope. Model selection was performed by the likelihood ratio test between models differing in one term, selecting the model with the lowest Akaike information criterion (AIC). These final statistical models of modern sea lions and fur seals were validated by residual analyses (see Appendix S1) (Crawley 2007).

Stable Isotope Bayesian Ellipses in R (SIBER) (Jackson *et al.* 2011) were used to estimate the isotopic niche width of modern South American sea lions and fur seals, once corrected their bone isotopic values in accordance with the isotopic baseline shifts, as

Table 1. Median and range (min; max) of the stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of ancient and modern South American sea lions (Of) and fur seals (Aa) during the middle Holocene (c. 7000 years before present) and the three modern periods. n_1; sample size for species; n_2; sample size for sex; $\delta^{13}C$ and $\delta^{15}N$; values not corrected for isotopic baseline shifts; $\delta^{13}C_{cor}$ and $\delta^{15}N_{cor}$; values corrected for isotopic baseline shifts

Period	Species	n_1	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N_{cor}$ (‰)	$\delta^{13}C_{cor}$ (‰)	Sex	n_2	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N_{cor}$ (‰)	$\delta^{13}C_{cor}$ (‰)
Holocene (c. 7000 yBP)	Of	5	22·6 (21·2; 24·0)	−12·1 (−12·8; −11·4)								
	Aa	7	19·7 (17·9; 21·7)	−13·2 (−13·9; −12·6)								
Modern (1944–1965)	Of	9	21·2 (20·4; 21·3)	−11·2 (−11·7; −10·6)	19·5 (18·5; 20·0)	−14·7 (−14·8; −14·1)	Male	4	21·2 (20·8; 21·2)	−11·2 (−11·4; −11·0)	19·5 (19·2; 20·0)	−14·5 (−14·7; −14·1)
							Female	5	21·0 (20·4; 21·3)	−11·1 (−11·7; −10·6)	19·1 (18·5; 19·8)	−14·7 (−14·8; −14·6)
	Aa	13	19·9 (18·5; 21·0)	−13·3 (−14·6; −12·4)	18·2 (17·1; 19·8)	−16·7 (−17·3; −15·6)	Male	10	19·9 (18·8; 21·0)	−13·5 (−14·6; −12·4)	18·3 (17·1; 19·8)	−16·5 (−17·3; −15·6)
							Female	3	19·7 (18·5; 20·2)	−13·2 (−13·2; −13·0)	18·0 (17·3; 18·3)	−16·7 (−17·1; −15·7)
Modern (1971–1983)	Of	10	21·3 (20·2; 22·9)	−11·7 (−12·8; −11·1)	20·4 (19·3; 21·9)	−13·9 (−14·9; −13·1)	Male	3	21·5 (21·0; 22·3)	−11·5 (−11·6; −11·1)	20·6 (20·1; 21·4)	−13·5 (−13·8; −13·1)
							Female	7	21·3 (20·2; 22·9)	−12·0 (−12·8; −11·1)	20·3 (19·3; 21·9)	−14·0 (−14·9; −13·2)
	Aa	38	18·8 (17·6; 20·0)	−14·0 (−14·8; −13·0)	17·8 (16·5; 19·0)	−16·0 (−16·9; −14·6)	Male	19	19·0 (17·6; 20·0)	−14·1 (−14·8; −13·0)	18·1 (16·5; 19·0)	−16·2 (−16·7; −14·6)
							Female	19	18·3 (17·8; 19·7)	−13·9 (−14·7; −13·1)	17·4 (16·8; 18·8)	−16·0 (−16·9; −15·2)
Modern (1991–2012)	Of	30	21·0 (19·0; 22·4)	−12·6 (−14·6; −11·2)	20·8 (19·0; 22·2)	−13·3 (−14·6; −12·2)	Male	15	21·1 (20·0; 22·4)	−12·1 (−13·2; −11·2)	21·0 (19·9; 22·2)	−13·7 (−13·5; −12·5)
							Female	15	20·6 (19·0; 21·4)	−12·1 (−13·2; −11·2)	20·4 (19·0; 21·3)	−12·7 (−13·5; −12·2)
	Aa	35	19·0 (17·8; 21·0)	−14·6 (−16·1; −13·8)	18·8 (17·7; 20·8)	−15·0 (−16·6; −14·1)	Male	20	19·6 (18·1; 21·0)	−14·3 (−15·2; −13·8)	19·4 (18·0; 20·8)	−14·7 (−16·0; −14·1)
							Female	15	18·7 (17·8; 19·7)	−14·8 (−16·1; −14·1)	18·6 (17·7; 19·6)	−15·1 (−16·6; −14·8)

well as to compare the isotopic niche space between both sexes and species in the three modern periods of the 20th and 21st century (see Fig. 2). This method is a Bayesian version of Layman metrics that can incorporate uncertainties such as sampling biases and small sample sizes into niche metrics (Jackson *et al.* 2011). Based on Markov-Chain Monte Carlo simulations, the SIBER approach obtains measures of uncertainty for constructing parameters of ellipses in a way similar to the bootstrap method. We used standard ellipse areas corrected for small sample size (SEA_C) to plot the isotopic niche of each species and sex within the isotopic space and to calculate the overlap between sexes and species. We also calculated the Bayesian standard ellipse areas (SEA_B) to obtain an unbiased estimate of the isotopic niche width with credibility intervals. We used these two approaches because they are complementary each other (Jackson *et al.* 2011).

We used the same procedures to define the isotopic niche space of the two otariid species during the middle Holocene and the three modern periods of the 20th and 21st century. However, in this latter case, the stable isotope values in the ancient and modern sea lion and fur seal bones were not corrected for isotopic baseline shifts; this is because the baseline could not be reconstructed for the Holocene due to a lack of shell samples for this period. For this reason, we only calculated the isotopic width and overlap of the two otariid species, respectively, in the Holocene period and in each of the three modern periods, to assessed if overall isotopic width of niches, overlap and trophic relationships (i.e. the relative positions of species niches in the isotopic space) between the two species have changed over time.

Data are always shown as mean ± standard deviation (SD) unless otherwise stated. All statistical analyses were carried out using the free software R (R Core Team 2013), and all functions for SIBER analyses were contained in the library SIAR (Stable Isotope Analysis in R) (Parnell *et al.* 2010).

Results

Linear regression analysis showed that the stranding years and the condylobasal length were unrelated for either South American sea lions (males: $n = 22$, $r^2 = 0.05$, $P = 0.91$; females: $n = 27$, $r^2 = 0.07$, $P = 0.10$) or South American fur seals (males: $n = 49$, $r^2 = 0.03$, $P = 0.11$; females: $n = 37$, $r^2 = 0.02$, $P = 0.78$). These results suggest that body size has remained approximately constant over the past century in both species and sexes.

The C/N atomic ratio of both modern and ancient bone samples ranged from 2·9 to 3·6, agreeing with the theoretical range that characterizes unaltered bone collagen protein (DeNiro 1985). One-way ANOVA indicated that the average $\delta^{13}C$ and $\delta^{15}N$ values of mussel shells significantly differed among the three sampling years considered ($\delta^{13}C$: $F_{2,12} = 51.722$, $P < 0.001$; $\delta^{15}N$: $F_{2,12} = 69.158$, $P < 0.001$), thus revealing temporal changes in the isotopic baseline of the ecosystem. Post hoc Tukey tests revealed significant differences between each of the sampling years, as the average $\delta^{13}C$ and $\delta^{15}N$ values of mussel shells collected in 1957 were significantly higher than those of the mussel shells collected in 1988 and 2014, and the average $\delta^{13}C$ and $\delta^{15}N$ values of mussel shells collected in 1988 were significantly higher than those of the mussel shells collected in 2014 (Fig. 3).

Accordingly, we corrected the bone isotope values of modern South American sea lions and fur seals, as described in the data analyses section (Table 1).

We found that $\delta^{13}C_{cor}$ increased over time at different rates in males and females of the two species ($P < 0.001$; Table 2 and Fig. 4). The slope of the time-$\delta^{13}C_{cor}$ function for male South American sea lions was significantly smaller than those of male and female South American fur seals and female South American sea lions, which had similar slopes but differed in their average values (Table 2 and Fig. 4). The final model adjusted for $\delta^{15}N_{cor}$ showed a significant increase over time and a similar slope for both sexes in the two species ($P < 0.001$, Table 2 and Fig. 4). Nevertheless, males of both species always had higher average $\delta^{15}N_{cor}$ than females, and the average $\delta^{15}N_{cor}$ of sea lions was significantly higher than that of fur seals (Table 2 and Fig. 4).

The estimated Bayesian ellipses (calculated without correcting for isotopic baseline shifts) of South American sea lions were usually larger than those of fur seals in the middle Holocene and during the second half of the 20th century and the beginning of the 21st century (Table 3). Furthermore, the Bayesian ellipses of the two species did not overlap at all in any period (Fig. 5). Finally, the trophic relationship between the two species viewed as the relative positions of species niches in the isotopic space, did not change over time (Fig. 5).

The estimated Bayesian ellipse areas (calculated after correcting for isotopic baseline shifts) of modern South American sea lion males were larger than those of females in the three different modern periods (Table 4). Furthermore, there was an increase in the Bayesian ellipse surfaces for both sexes from the oldest to the most recent modern period, when the two sexes did not overlap at all (Table 4 and Fig. 6). On the other hand, the Bayesian ellipse areas of modern fur seal males were larger and encompassed that of females in almost all periods (Table 4 and Fig. 6). Moreover, although the isotopic niches of the two species did not overlap in any of the three modern periods, the distance between the ellipses of the two species were much more reduced in the most recent modern period (Fig. 6) and resembled that during the middle Holocene (Fig. 5).

Discussion

Our results showed that South American fur seals and South American sea lions inhabiting the Río de la Plata and adjacent Atlantic coast occupied distinct isotopic niches during the middle Holocene and that the same pattern of isotopic niche segregation was true for most of the second half of the 20th century and the beginning of the 21st century (Fig. 5). However, both species increased their consumption of high trophic level, demersal prey – likely striped weakfish – over the past 70 years (Fig. 4) and the distance between their isotopic niches has decreased and resembles that observed during the middle Holocene (Figs 5 and 6).

Table 2. Linear models (two-way ANCOVA) for bone $\delta^{13}C_{cor}$ and $\delta^{15}N_{cor}$ values of modern South American sea lions and fur seals over time. Estimates and their statistical significance (*P*-values in brackets) are shown for each variable. The levels of reference for the two categorical variables (Sex and Species) are female and the South American fur seal respectively. Sex × year and year × species are the differences in the slopes between the level of reference of each variable and the other group (males for Sex and South American sea lion (Of) for Species). Year is analysed as a continuous variable and its value is the slope for the group of reference of each categorical variable. The three-way interaction (Species × Sex × slope) reflects that the species-specific slopes differ between sexes for the two species. For each stable isotope, we show the AIC (Akaike Information Criterion) of the initial statistical model (including up to the three way interaction among explanatory variables) and the final, minimally adequate model (in bold) obtained after stepwise model selection. Model selection was performed by the likelihood ratio test between models differing in one term, selecting the model with the lowest AIC (i.e. the most parsimonious model)

Stable Isotope	Model	Intercept	Sex (male)	Species (Of)	Year	Sex × year	Sex × species	Year × species	Species × sex × year	ANOVA	AIC
δ13C	**δ¹³C_cor ~ Sex × year × species**	**−53·95 (<0·001)**	**−33·51 (<0·001)**	**−30·52 (<0·025)**	**0·019 (<0·001)**	**0·017 (<0·001)**	**87·41 (<0·001)**	**0·016 (<0·016)**	**−0·044 (<0·001)**		**233·49**
	δ¹³C_cor ~ Sex × year + species + Sex × year + year × species + Sex × species	−75·63 (<0·001)	2·61 (<0·79)	12·64 (<0·22)	0·03 (<0. 001)	−0·001 (<0·81)	−0·78 (<0·001)	−0·005 (<0·31)	−	21·11 (<0·001)	252·25
δ15N	δ¹⁵N_cor ~ Sex × year × species	−23·10 (0·084)	−10·85 (0·53)	12·58 (0·50)	0·021 (<0. 001)	0·006 (0·50)	−6·60 (0·80)	−0·005 (0·58)	0·003 (0·81)		323·42
	δ¹⁵N_cor ~ Sex × year + species + Sex × year + Sex × species + year × species	−21·54 (0·06)	−13·45 (0·30)	9·47 (0·47)	0·019 (<0·001)	0·007 (0·28)	−0·26 (0·35)	−0·003 (0·58)	−	0·056 (0·81)	321·48
	δ¹⁵N_cor ~ Sex × year + species + Sex × year + Sex × species	−17·85 (0·057)	−14·77 (0·25)	2·13 (<0·001)	0·018 (<0·001)	0·007 (0·23)	−0·27 (0·32)	−	−	0·302 (0·58)	319·80
	δ¹⁵N_cor ~ Sex × year + species + Sex × year	−18·10 (0·05)	−13·35 (0·29)	2·00 (<0·001)	0·018 (<0·001)	0·007 (0·27)	−	−	−	0·987 (0·32)	318·83
	δ¹⁵N_cor ~ Sex + year + species	**−25·45 (<0·001)**	**0·55 (<0·001)**	**2·01 (<0·001)**	**0·02 (<0·001)**	−	−	−	−	**1·187 (0·278)**	**318·06**

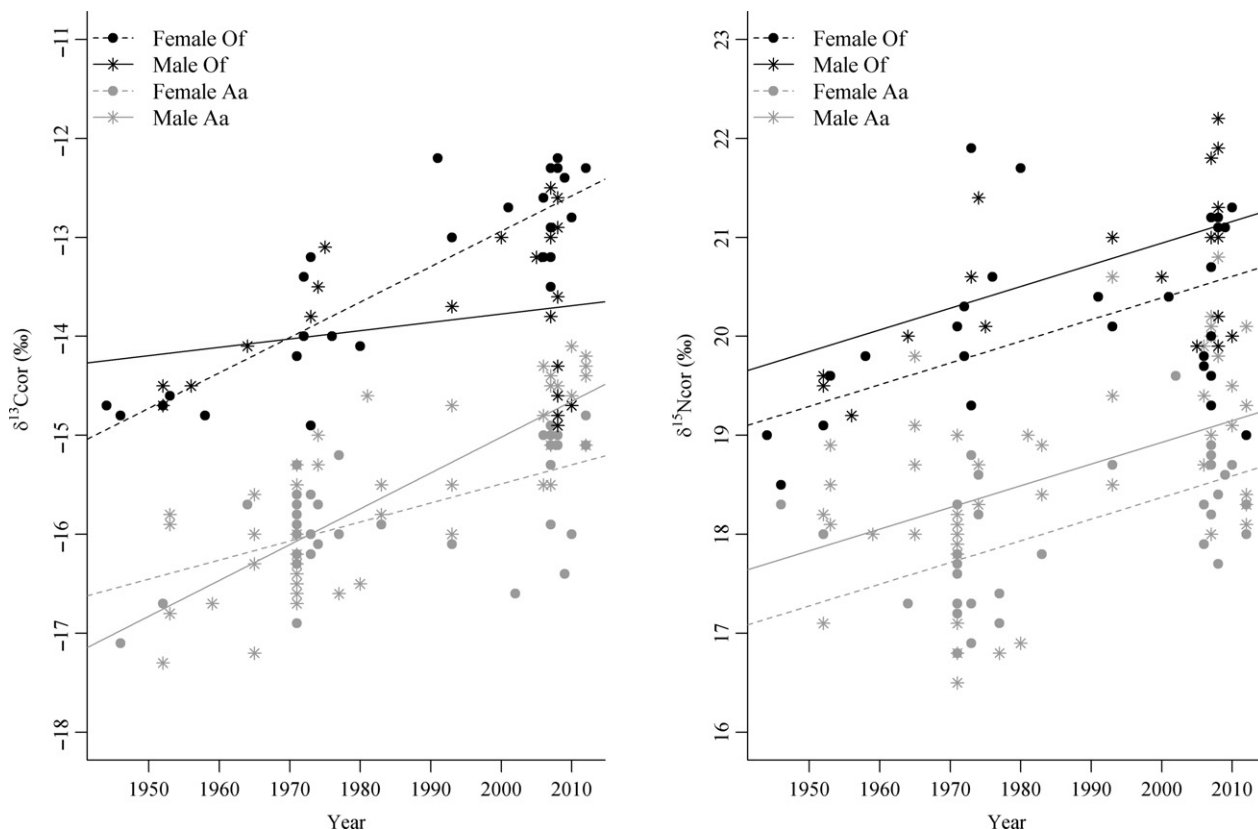**Fig. 4.** Trends of $\delta^{13}C_{cor}$ and $\delta^{15}N_{cor}$ values for both sexes of modern South American sea lions (Of) and fur seals (Aa) over time, once corrected in accordance with the isotopic baseline shifts, showing the best fitted lines for each sex and species (see Table 2).

Table 3. Bayesian standard ellipse areas (SEA_B) and their respective 95% credibility intervals (CI) of ancient and modern South American sea lions (Of) and fur seals (Aa) during the middle Holocene (c. 7000 years before present) and the three modern periods. The isotopic overlap areas between species are not shown because the two species did not overlap in any of the periods (see isotopic niche areas in Fig. 5)

Period	Species	SEA_B (‰²)	95% CI (‰²)
Holocene (c. 7000 yBP)	Of	3·17	1·03–6·08
	Aa	3·03	1·30–5·90
Modern (1944–1965)	Of	1·04	0·46–1·75
	Aa	1·68	0·88–2·64
Modern (1971–1983)	Of	1·83	0·85–3·05
	Aa	1·21	0·85–1·61
Modern (1991–2012)	Of	2·53	1·68–3·45
	Aa	1·65	1·14–2·22

Similar patterns of isotopic niche segregation between the two species were also reported for the second half of the Holocene in northern Patagonia and Tierra del Fuego (Saporiti *et al.* 2014b). Thus, ancient and historical South American fur seals foraged more pelagically (lower $\delta^{13}C$ values) and at a lower trophic level (lower $\delta^{15}N$ values) than contemporary sympatric South American sea lions everywhere in the Southwestern Atlantic. This pattern of trophic segregation is consistent with the smaller body size and mouth gape of South American fur seals as compared with those of South American sea lions (Segura *et al.* 2015; Saporiti *et al.* 2016), as South American fur seals consume, on average, smaller anchovies and striped weakfish than sympatric South American sea lions do (Szteren, Naya & Arim 2004).

However, the isotopic niche of South American fur seals from the Río de la Plata and the adjacent Atlantic coast progressively converged to that of the South American sea lion niche during the period 1991–2012 (Fig. 6), although without actually overlapping it (Franco-Trecu, Aurioles-Gamboa & Inchausti 2014; this study). This progressive convergence of the isotopic niche was concurrent with the increasing trend of the South American fur seal population and the decreasing trend of the South American sea lion population in Uruguay (Franco-Trecu 2015), as well as the reduction in the average size of striped weakfish (Villwock de Miranda & Haimovici 2007). Hence, recent changes in resource partitioning between the two species might be promoted by the changes in the Río de la Plata ecosystem caused by sealing and bottom trawling.

Our interpretation of these findings relies on two major assumptions: First, the organic matrix of mollusc shells is a reasonable proxy for the changes in the ecosystem's isotopic baseline over time, and the stable isotope ratios in the top predator's bones show correlated temporal changes (Casey & Post 2011). If so, changes in the
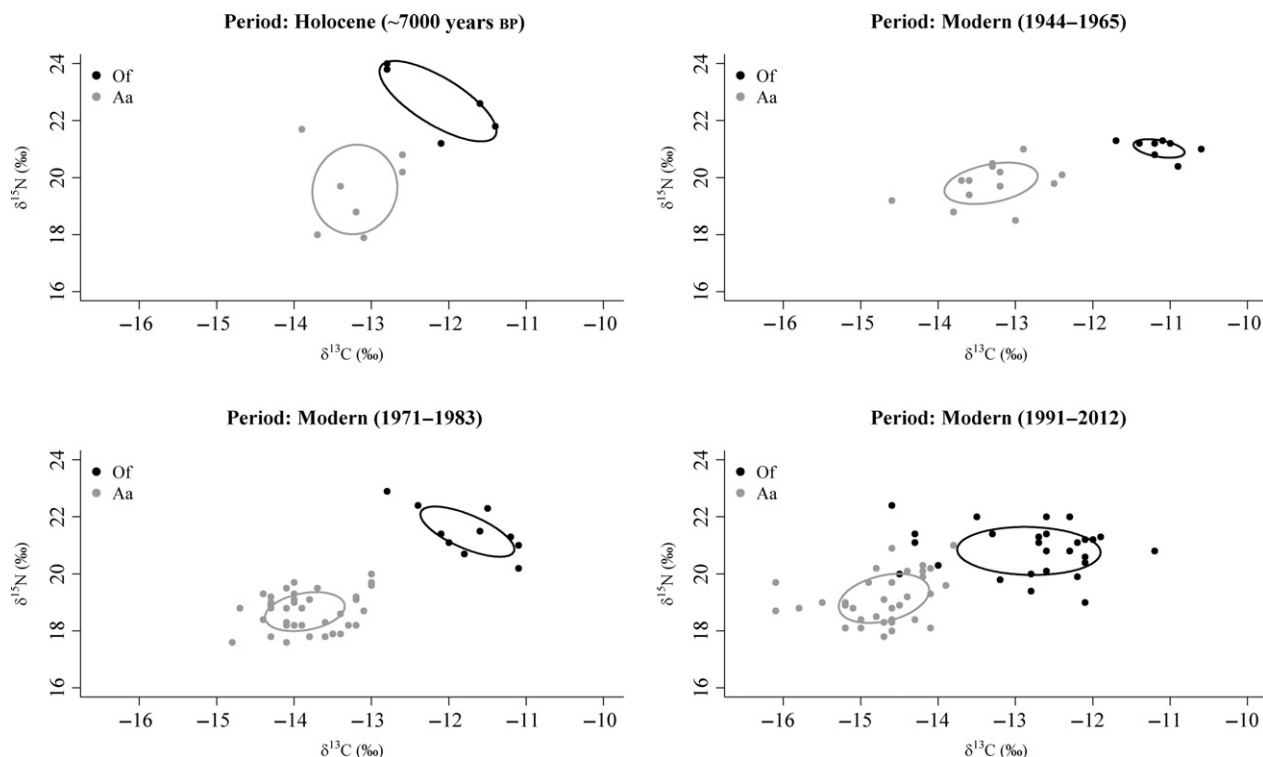
Fig. 5. Isotopic niche areas (calculated with SEA$_C$) of ancient and modern South American sea lions (Of) and fur seals (Aa), respectively, during the middle Holocene (c. 7000 years before present) and the three modern periods (see Table 3 for the ellipse area and credibility interval values). Stable isotope values in the ancient and modern bones of both species were not corrected for isotopic baseline shifts.

isotopic baseline can be accounted for and baseline-corrected samples from different periods can be meaningfully compared. Although eared seals do not eat mussels, the latter are informative about changes in the stable isotope baseline of the Río de la Plata ecosystem. This is because mussels are suspension feeders, and stable isotope ratios in mussels match those in particulate organic matter (POM) along the coasts of the Southwestern Atlantic Ocean (Saporiti *et al.* 2015). Furthermore, POM of both terrestrial and estuarine/marine origin is the major source

of fixed carbon for the food web in Río de la Plata, with a negligible contribution from benthic macrophytes because of the high water turbidity (Acha *et al.* 2008; Botto *et al.* 2011; Saporiti *et al.* 2015). Second, although the topology of the prey community within the $\delta^{13}C$-$\delta^{15}N$ space may have changed over time, neither the depletion in $\delta^{13}C$ of pelagic prey compared with demersal prey nor the increase in the $\delta^{15}N$ values with trophic position may have changed, since these relationships are general across ecosystems (Post 2002).

Table 4. Bayesian standard ellipse areas (SEA$_B$) and their respective 95% credibility intervals (CI) for both sexes of modern South American sea lions (Of) and fur seals (Aa) during the three modern periods. Isotopic overlap areas between sexes within each species were calculated with SEA$_C$, and the respective percentage of overlap surface for each sex was estimated from overlap areas (see isotopic niche areas in Fig. 6)

Period	Species	Sex	SEA$_B$ (‰^{2})	95% CI (‰^{2})	Overlap area (‰^{2})	% Overlap area for sex
1944–1965	Of	Male	2·00	0·51–4·14	0·046	15·6
		Female	1·75	0·57–3·39		26·6
	Aa	Male	1·92	0·90–3·18	0·076	6·1
		Female	3·01	0·62–6·69		34·9
1971–1983	Of	Male	2·90	0·62–6·36	0·295	22·9
		Female	2·25	0·86–4·00		22·0
	Aa	Male	1·65	0·97–2·40	0·509	39·0
		Female	1·05	0·62–1·54		69·4
1991–2012	Of	Male	2·37	1·31–3·61	0·000	0·0
		Female	1·40	0·77–2·15		0·0
	Aa	Male	1·65	1·01–2·38	0·086	6·2
		Female	1·32	0·72–2·01		9·3

Accurately identifying and accounting for shifts in the isotope baseline is one of the major problems when using stable isotope data to track dietary changes over long time spans (Casey & Post 2011). Here, we analysed the stable isotopes in the shells of mussels, because their $\delta^{13}C$ and $\delta^{15}N$ values are expected to provide information about shifts in the isotopic baseline (Post 2002), which include not only changes in primary productivity (Saporiti *et al.* 2014a) but also the modification of the $\delta^{13}C$ values due to the Oceanic Suess effect following the Industrial Revolution (Keeling 1979). This is because any effect of anthropogenic CO_2 on the $\delta^{13}C$ composition of marine phytoplankton would be transmitted to their immediate consumers (i.e. mussels) and to the entire food web, eventually reaching higher trophic levels. Therefore, the organic matter trapped in the mineral matrix of mollusc shells was used to identify and correct for shifts in the isotope baseline (Casey & Post 2011; Zenteno *et al.* 2015a).

By combining this approach with the analysis of a dataset spanning a time window longer than that used in previous researches on the region (Vales *et al.* 2014; Zenteno *et al.* 2015b), it has been possible to detect the changes in the isotope niches of South American fur seals and sea lions, which are likely related to the effect of anthropogenic activities in the ecosystem. Nevertheless, assessing changes in the stable isotope baseline is challenging, and it has been only partially achieved in this study for several reasons. First, no mussels were available from La Olla

archaeological site and hence we could not obtain a baseline correction to compare middle Holocene eared seals with modern ones. Second, mussels from the second half of the 20th century and the beginning of the 21st century were collected from a single locality and hence may reflect local conditions and not be representative of the overall oceanographic conditions along the coast. Third, mussels were collected at only three discrete time periods, and environmental conditions may have changed in between. Finally, only five individual mussels were analysed at each time period. Small sample size is a usual limitation when using zooarchaeological and museum material, and little can be done to overcome this unless new material becomes available.

With the caution required by small sample size, isotopic data from mussel shells revealed a decrease in the $\delta^{13}C$ and $\delta^{15}N$ values of the food web baseline throughout the second half of the 20th century and the beginning of the 21st century. The Suess effect may account for some of the change in $\delta^{13}C$ values over time, but it cannot account for all of it and certainly not for the change in $\delta^{15}N$ values. We believe that the most likely explanation for these changes is the increase in the arrival of freshwater/terrestrial POM (associated with shifts in agricultural practices ranging from cattle ranching to soybean cultivation) upstream from the study area, as freshwater/terrestrial POM in Río de la Plata tributaries is typically depleted in $\delta^{13}C$ and $\delta^{15}N$ compared with phytoplankton from the
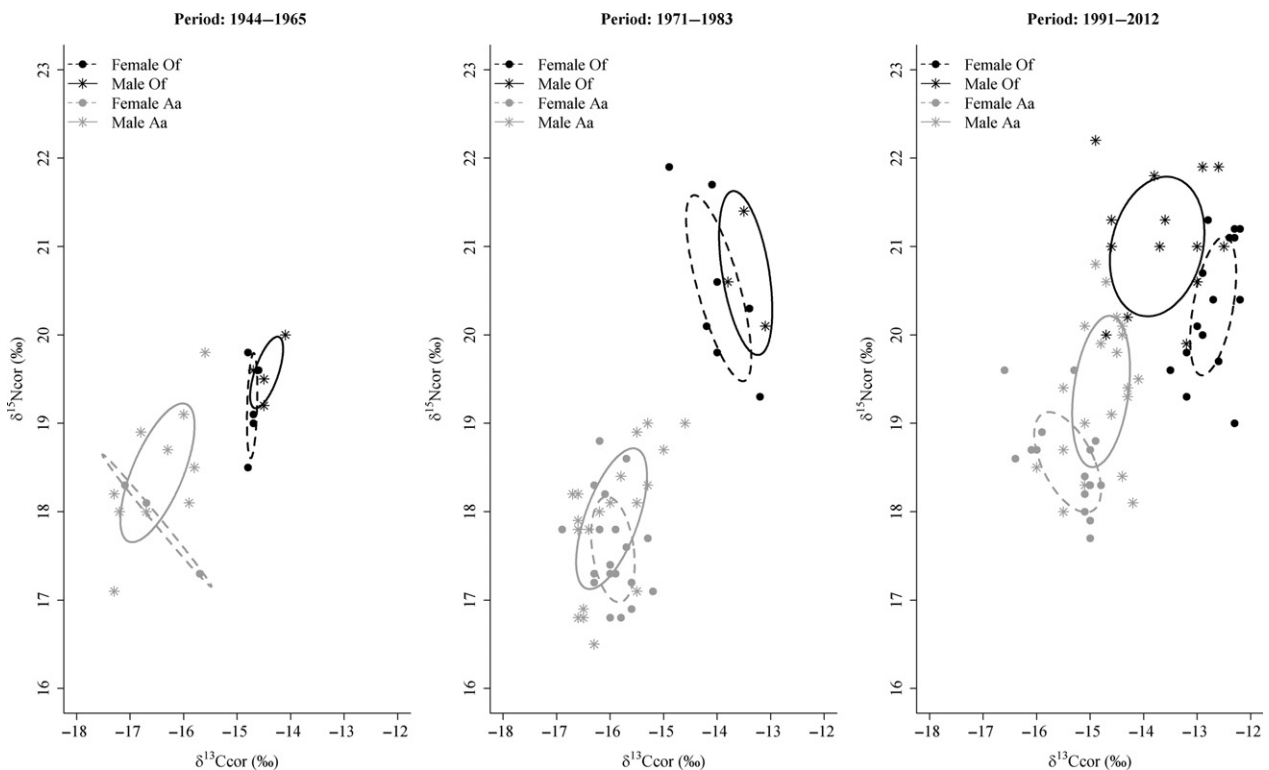

Fig. 6. Isotopic niche areas (calculated with SEA$_C$) for both sexes of modern South American sea lions (Of) and fur seals (Aa) during the three modern periods (see Table 4 for the ellipse area, credibility interval and overlap area values). To allow comparison, original stable isotope values in the bone of both species were corrected ($\delta^{13}C_{cor}$ and $\delta^{15}N_{cor}$) in accordance with the changes in the isotopic baseline.

estuary (Botto *et al.* 2011; Marchese *et al.* 2014; Saporiti *et al.* 2015).

After accounting for shifts in the baseline, stable isotope ratios in the collagen of eared seals strongly suggest a steady increase in the consumption of demersal prey by both species and sexes during the second half of the 20th century and the beginning of the 21st century, which is consistent with the high proportion of demersal prey, particularly striped weakfish, in the scats of modern eared seals from Uruguay (Naya, Arim & Vargas 2002; Franco-Trecu *et al.* 2013). Nevertheless, the rate of change was not the same in every species and sex; as a result, the isotopic niche of male South American fur seals approached that of male South America sea lions.

Modifications in the patterns of oceanic circulation over the study period are unlikely to explain the observed changes in the isotopic niches. The Buenos Aires province coastline was located several kilometres westward during the middle Holocene (Codignotto & Aguirre 1993; Ponce *et al.* 2011), which allowed a southward extension of the Brazilian current and higher salinity levels than today (Aguirre 1993; Aguirre *et al.* 2011). This historic shift in the influence of the Brazilian current may have altered the marine primary productivity in the study area (Toledo *et al.* 2008; Nagai *et al.* 2010), such that the structure of the marine food web and the diet of the eared seals may have differed from modern ones. However, South American fur seals foraged more pelagically and at a lower trophic position than South American sea lions, both in the middle Holocene as well as during most of the second half of the 20th century and the beginning of the 21st century (Fig. 5). This, therefore, suggests that resource partitioning between the two species was largely insensitive to the changes in the pattern of oceanic circulation reported above.

Actually, changes in resource partitioning between South American fur seals and sea lions became evident only during the 1991–2012 period, when increased fishing efforts resulted in major changes in prey availability and the end of commercial sealing resulted in the demographic recovery of South American fur seals (but not of South American sea lions) in the study area. The overall biomass of small pelagic fishes, mainly the anchovies *Anchoa marini* and *Engraulis anchoita*, remained rather stable in the Río de la Plata and the adjacent Atlantic Ocean during that period (Madirolas, Hansen & Cabreira 2013). On the other hand, the biomass of some demersal fishes decreased significantly, as they were the target of increased fishing efforts during the 1991–2012 period (Vasconcellos & Haimovici 2006; Ruarte & Perrotta 2007; Defeo *et al.* 2009). The striped weakfish was one of the most impacted species, not only because its total biomass declined but also because the size structure of the population shifted towards smaller body sizes between the 1970s and the 1990s (Jaureguizar, Ruarte & Guerrero 2006; Ruarte & Perrotta 2007; Villwock de Miranda & Haimovici 2007).

Changes in the relative abundance of pelagic and demersal species and the size structure of fish populations after the development of the bottom trawl fishery have probably modified the diet of predatory fishes and the structure of the entire food web in the Río de la Plata and the adjacent Atlantic Ocean over the last 50 years. Although the total biomass of striped weakfish decreased during the 1990s, the shift towards small size classes may have increased its availability to both species of ear seals, as they capture primarily striped weakfish measuring 20–30 cm in length (Szteren, Naya & Arim 2004). This process parallels the one that is invoked to explain the increased consumption of juvenile hake by South American sea lions in northern Patagonia, which occurs despite a decrease in the overall hake biomass (Drago *et al.* 2009b). A reduction in the average individual size of demersal fishes might have been particularly beneficial for those eared seals with a small/medium mouth gape, – that is, South American fur seals of both sexes and female South American sea lions (Saporiti *et al.* 2016) – but not for male South American sea lions. This could explain why the rate of temporal change in the $\delta^{13}C_{cor}$ values was smaller in male South American sea lions than in any other group (Fig. 4). Conversely, the dietary change reported here for South American fur seals has been more intense in males than in females (Fig. 4), probably because their larger body size and broader mouth gape make them able to benefit from an increased availability of relatively large juvenile demersal fishes (Segura *et al.* 2015; Saporiti *et al.* 2016). It is worth noting that skull size has remained stable in both species and sexes throughout the second half of the 20th century and into the 21st century, suggesting that the above reported changes did not result from changes in body size or mouth gape.

Stable isotope ratios suggest that South American sea lions of both sexes also increased their consumption of demersal prey in the Río de la Plata estuary during the second half of the 20th century and the beginning of the 21st century, but simultaneously the isotopic overlap between the two sexes decreased (Fig. 6). Previous research in northern Patagonia had also revealed an inverse relationship between the size of the South American sea lion population and its consumption of demersal prey, as well as a decreasing overlap in the isotope niche of the two sexes as population size decreased (Drago *et al.* 2009b). Thus, intraspecific competition could be one major determinant of the South American sea lion diet. Furthermore, a stronger use of coastal-demersal food resources by individuals of both sexes of the South American fur seals could have recently led to greater pressure on South American sea lion females. Given the rapid recovery of the South American fur seal population since commercial sealing ceased in the early 1990s (Franco-Trecu 2015) and the progressive resource depletion by coastal fisheries in the study area (Defeo *et al.* 2009), the increasing overlap in the foraging areas could have

constrained South American sea lion females to feed in areas closer and closer to the shore. The latter would explain why South American sea lion females showed the most pronounced increase in $\delta^{13}C_{cor}$ values (Figs 4 and 6), as coastal-demersal resources in the Río de la Plata estuary and adjacent Atlantic Ocean are enriched in both $\delta^{13}C$ and $\delta^{15}N$ compared to pelagic prey (Franco-Trecu *et al.* 2012).

Conversely, South American sea lion males had the smallest variation in their trophic habits over the last 70 years (Fig. 4). The progressive removal of larger prey by coastal fisheries would truncate the upper end of prey sizes available for consumption by South American sea lion males. Given that trophic level is correlated with body size (Segura *et al.* 2015), the observed increase in the $\delta^{15}N_{cor}$ values of South American sea lion males over time (Fig. 4) would seem to be in contradiction with the depletion of larger individuals by coastal fisheries in the study area. The well-documented interaction between the South American sea lion and coastal (Szteren & Páez 2002) and industrial fisheries (Franco-Trecu V., unpublished data), makes us believe that the $\delta^{15}N_{cor}$ increase in male sea lions over time is more likely due to their consumption of fishes from the nets and hooks deployed by coastal fisheries. Nevertheless, given the declining population trend of this species in Uruguay and the dispersal of juvenile males to Brazil and Argentina (Vaz-Ferreira 1982; Rosas *et al.* 1994), sea lion males might continue consuming large-bodied fish gathered from the coastal fisheries without necessarily leading to a stronger conflict with fishermen.

In conclusion, the overall evidence suggests that: (i) the South American fur seal and sea lion were clearly segregated in the isotopic space 7000 years ago, and this was also true during the second half of the 20th century and the beginning of the 21st century; (ii) differences in body size and mouth gape between species and sexes resulted in differential access to resources and explain segregation in the isotopic niche, although (iii) the isotopic niches of both species have tended to converge during the last decade due to a reduction in the size range of available prey, which is a result of increased fishing efforts on demersal fishes and, perhaps, the declining numbers in the sea lion population.

Authors' contributions

M.D., L.C., V.F.T. and P.I. conceived the ideas and designed methodology, analysed the data and led the writing of the manuscript; M.D., E.A.C., D.G.V., F.B., L.Z. and E.M.G. collected the data. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.5b1cc (Drago *et al.* 2017).

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Supporting Information

Details of electronic Supporting Information are provided below.

Appendix S1. Residual analyses for isotopic data of eared seals and mussels.