

Stable isotopes of oxygen reveal dispersal patterns of the South American sea lion in the southwestern Atlantic Ocean

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Abstract

Stable isotopes of oxygen have been widely used to reconstruct paleotemperatures and to investigate the thermal environment of fishes and mollusks, but they have only occasionally been used as geographical markers in marine systems. As bone apatite grows at a constant temperature in marine mammals and food is the major source of water for these animals, particularly for pinnipeds, variations in the ratio of stable isotopes of oxygen ($\delta^{18}\text{O}$) of bone apatite will likely reflect changes in the $\delta^{18}\text{O}$ values of diet, and thus of the surrounding water mass, despite the potential confounding role of factors as the proximate composition of diet, sex and body size. Here, we used the $\delta^{18}\text{O}$ values in bone apatite to investigate whether adult males of South American sea lion (*Otaria byronia*), from three regions in southwestern Atlantic Ocean (Brazil, Patagonia and Tierra del Fuego in Argentina), used the same water masses to forage and whether differences exist in the water masses used by sea lions differing according to sex and developmental stage. Statistically significant differences were observed among the $\delta^{18}\text{O}_{\text{bone}}$ values of adult males from the three regions, with those from Patagonia more enriched in ^{18}O , as expected from the $\delta^{18}\text{O}_{\text{seawater}}$ values. These results revealed restricted dispersal movements of adult males between the three areas. On the other hand, adult males and females from Patagonia did not differ in average $\delta^{18}\text{O}_{\text{bone}}$ values, thus indicating the use of foraging grounds within the same water mass. Finally, the variability in the $\delta^{18}\text{O}_{\text{bone}}$ values of young of both sexes was much wider than the adults of the same sex from the same region, which suggests the existence of a juvenile dispersal phase in both sexes, although much shorter in females than in males. These results confirm the usefulness of stable isotopes of oxygen as habitats tracers in marine mammals.

Introduction

Recent advances in satellite telemetry have helped to fill the gap in our knowledge of animal movements, but these methods are expensive and tracking is often restricted to a few individuals for relatively short periods (Shillinger *et al.*, 2008). Biogeochemical markers such as stable isotopes lack the detailed resolution of satellite tags, but laboratory analyses are inexpensive and provide information integrated over known and predictable timescales that can be linked directly

to geographical regions if the isotopic landscape, or isoscape, has been previously reconstructed (Hobson & Wassenaar, 2008; Graham *et al.*, 2010).

Stable isotopes are known from several chemical elements and the relative abundance of two stable isotopes in a sample is usually expressed as a ratio between the heavy and the light isotope and compared with that ratio in a standard (Hobson & Wassenaar, 2008). Stable isotopes of carbon and nitrogen are among the most widely used biogeochemical markers in ecological studies (Koch, 2008) and maps describing the vari-

ability of their stable isotope ratios across entire oceans are available (Graham *et al.*, 2010). However, interpreting changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to track migrations is possible only if no major dietary shifts occur during migrations, which is not necessarily true for opportunistic predators.

Stable isotopes of oxygen have been widely used to reconstruct paleotemperatures (e.g. Schöne *et al.*, 2004) and to investigate the thermal environment of fishes (e.g. Jones & Campana, 2009) and mollusks (e.g. Soldati *et al.*, 2009), because carbonates in biominerals become enriched in ^{18}O as temperature declines (Kim & O'Neil, 1997; Soldati *et al.*, 2009). Nevertheless, most of the current variability in the ratios of stable isotopes of oxygen in the ocean is not linked to thermal gradients, but caused by the preferential evaporation of the light isotope and the preferential condensation of the heavy isotope (Bowen, 2010). Accordingly, a sharp contrast exists between $\delta^{18}\text{O}$ values in freshwater and seawater and well-defined latitudinal gradients of $\delta^{18}\text{O}$ values also exist from mid to high latitudes in most oceans (Bowen, 2010).

Such regularities make stable isotopes of oxygen potential habitats tracers for marine species moving between marine and freshwater ecosystems and for species with distribution ranges spanning over marine regions differing in $\delta^{18}\text{O}$ values. However, stable oxygen isotopes have seldom been used to track the migrations of marine vertebrates (e.g. Yoshida & Miyazaki, 1991; Clementz & Koch, 2001; Coulson *et al.*, 2008; Ramos, González-Solís & Ruiz, 2009). A constant body temperature does not pose any actual limitation to the use of stable isotopes as tracers in marine mammal, as the $\delta^{18}\text{O}$ values of endotherms records dietary information and not body temperature (Koch, Fogel & Tuross, 1994; Bryant & Froelich, 1995; Kohn, 1996; Koch, 2008). Therefore, $\delta^{18}\text{O}$ values can be a useful tracer for investigating marine mammal habitats in areas where well-defined gradients exist.

South American sea lions, *Otaria byronia*, are widely distributed along both coasts of South America (Vaz-Ferreira, 1982). Genetic markers suggest female philopatry and male-mediated gene flow among populations in the southwestern South Atlantic Ocean (Szapkievich *et al.*, 1999; Freilich, 2004; Túnez *et al.*, 2007, 2010; Artico *et al.*, 2010 and Feijoo *et al.*, 2011). Tagging confirmed that young females often remain close to their breeding site (Thompson *et al.*, 1998; Campagna *et al.*, 2001), whereas adult males travel longer distances than females after the breeding season (Vaz-Ferreira, 1982; Rosas *et al.*, 1994; Giardino *et al.*, 2009). Nevertheless, nothing is known about the actual duration of the juvenile dispersal phase or the actual fidelity of adults to a particular stretch of coastline.

Stable oxygen isotopes could provide an insight into these issues because the $\delta^{18}\text{O}_{\text{seawater}}$ values of Patagonia are higher than those from southern Brazil and Tierra del Fuego (Bowen, 2010). Accordingly, the $\delta^{18}\text{O}_{\text{bone}}$ values in sea lions from those three regions should not reproduce the local pattern reported above for $\delta^{18}\text{O}_{\text{seawater}}$ values if sea lions dispersed over scales of more than 1000 km along the coastline of the southwestern Atlantic Ocean. Furthermore, the $\delta^{18}\text{O}_{\text{bone}}$ values in males and females from the same region would differ if males had larger dispersal areas than females along the coastline. Finally,

$\delta^{18}\text{O}_{\text{bone}}$ values would be similar across age classes of female sea lions from the same region if young females remained close to their natal site, but they would vary across age classes of male sea lions if young males dispersed longer distances from their natal sites before settlement as adults. This paper describes the variability of $\delta^{18}\text{O}_{\text{bone}}$ values in South American sea lions to test the three former hypotheses.

Material and methods

Sample collection

A total of 112 bone samples were collected from the skulls of sea lions found dead between 1978 and 2010 in three coastal zones of the southwestern South Atlantic coast of South America. Samples from southern Brazil (from 29°S to 31°S) came from the collection from the Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul at Imbé, Brazil (17 adult males). Samples from central and northern Patagonia coasts (from 40°S to 47°S) (here after Patagonia) came from the collection from the Centro Nacional Patagónico at Puerto Madryn, Argentina (39 males and 39 females covering all the developmental stages). Finally, samples from Tierra del Fuego (from 53°S to 54°S) were obtained from the collection from the Museo Acatushún de Aves y Mamíferos Marinos Australes, near at Ushuaia, Argentina (17 adult males). These three regions differed in salinity levels (Fig. 1a) and $\delta^{18}\text{O}_{\text{seawater}}$ gradients (Fig. 1b).

Age, sex and development stage determination

Sea lions obtained from these collections had previously been aged by counting growth layers in the dentine of the canines (after being decalcified in 5% formic or nitric acid and thick sections stained with hematoxylin (Crespo, 1988)). Sex was assessed according to secondary sex characteristics at the time of collection and measurements of the skull (Crespo, 1988). The life span of sea lions is around 20 years (Crespo, 1988), with females reaching adulthood at about 4 years of age and males mating for the first time when they are at least 9 years old (Grandi *et al.*, 2010). Based on these data, we established four developmental stages; young post-weaned and not yet sexually mature individuals between 1 and 5 years of age for males and between 1 and 3 years of age for females; early adult sexually mature individuals between 7 and 8 years of age for males and between 5 and 7 years of age for females; adult sexually mature individuals between 9 and 12 years of age for males and between 8 and 12 years of age for females and senile sexually mature individuals >13 years old for males and >12 years old for females. The main difference between early adults and adults is that the former can still grow in length, whereas the latter are thought to have ceased length growth.

Sample preparation

Each skull sample used for the isotopic analysis consisted of a fragment of turbinate bone from the nasal cavity, which was

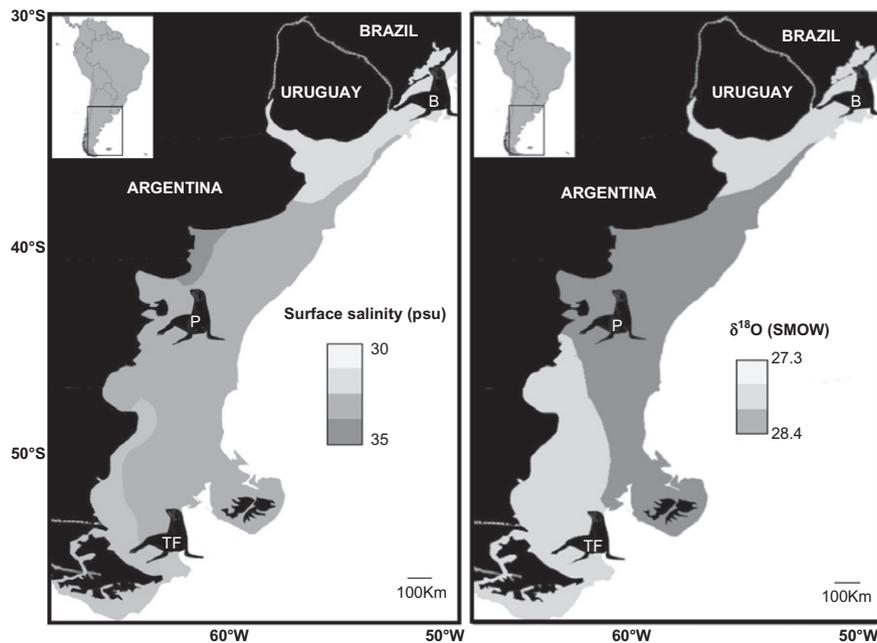


Figure 1 Map of southern South America, showing the study areas (B, southern Brazil; P, central and northern Patagonia; TF, Tierra del Fuego), sea surface salinity [left panel, according to Falabella *et al.* (2009)] and $\delta^{18}\text{O}_{\text{seawater}}$ values (right panel, according to Bowen, 2010).

ground with a mortar and pestle. Approximately 15 mg of sample powder were soaked with 30% hydrogen peroxide solution for 24 h, rinsed five times with deionized water, soaked for another 24 h in a solution of acetic acid (1M) buffered to pH~4.5 with 1M calcium acetate, rinsed again five times with deionized water and finally dried for 24 h (Koch, Tuross & Fogel, 1997).

Stable isotope analyses

Samples were analyzed for oxygen isotope ratios via a Carbonate Kiel Device III carbonate preparation system (Thermo Electron – Dual Inlet, Thermo Finnigan, Bremen, Germany) linked to a gas source mass spectrometer in the Scientific-Technical Services at the University of Barcelona. Approximately, 1.0 mg of bone powder was dissolved in 100% phosphoric acid at 70°C with concurrent cryogenic trapping of CO_2 and H_2O . The CO_2 was then admitted to the mass spectrometer for analysis. The measured isotope compositions were normalized to the NBS 19 calcite standard, with a value of $\delta^{18}\text{O} = -2.20\text{‰}$ relative to Vienna Pee Dee Belemnite (VPDB). Precision of replicate analyses for $\delta^{18}\text{O}$ was $\pm 0.06\text{‰}$ [standard deviation (SD)]. As $\delta^{18}\text{O}$ values in zoology are more commonly presented relative to Vienna Standard Mean Ocean Water (VSMOW), $\delta^{18}\text{O}$ values were converted from VPDB to VSMOW using the formula $\delta^{18}\text{O} (\text{VSMOW}) = [\delta^{18}\text{O} (\text{VPDB}) \times 1.03086] + 30.86$ (Koch *et al.*, 1997). The stable isotope abundances are expressed in delta (δ) notation in parts per thousand (‰), using the formula $\delta^{18}\text{O} = [({}^{18}\text{O}/{}^{16}\text{O})_{\text{sample}} \div {}^{18}\text{O}/{}^{16}\text{O}_{\text{standard}} - 1] \times 1.000$, where the standard is VSMOW.

Data analyses

Data are presented as mean \pm SD, unless otherwise stated. Normality was tested with the Lilleford test and homogeneity of variances with the Levene test. The Kruskal–Wallis test was used to compare the $\delta^{18}\text{O}_{\text{bone}}$ values of males from southern Brazil, Patagonia and Tierra del Fuego because the three datasets were heteroskedastic (Levene test, $W_{2,48} = 7.280$, $P = 0.02$). The Kruskal–Wallis test was followed by a *post hoc* nonparametric multiple comparisons test. The Student *t*-test was used to compare $\delta^{18}\text{O}_{\text{bone}}$ values of males and females from Patagonia and the Pearson regression analysis was used to test the hypothesis that $\delta^{18}\text{O}_{\text{bone}}$ values remained stable after adulthood. Significance was tested at $\alpha = 0.05$. All statistical analyses were performed with PASW Statistics (Version 17.0 for Windows, SPSS, version 17.0, Spain), except the nonparametric multiple comparison test, performed following Zar (1984).

Results

The bone of male South American sea lions from Patagonia was significantly enriched in ^{18}O when compared with that of males from southern Brazil and Tierra del Fuego (Fig. 2; Kruskal–Wallis test, chi-square = 6.210, d.f. = 2, $P = 0.045$ $n = 17$ for each region). *Post hoc* pairwise comparisons of the mean bone $\delta^{18}\text{O}$ values between regions revealed statistically significant differences between Brazil and Patagonia ($q = 4.295$, $P = 0.002$) and marginally significant differences between Patagonia and Tierra del Fuego ($q = 3.193$, $P = 0.070$), whereas differences between Brazil and Tierra del

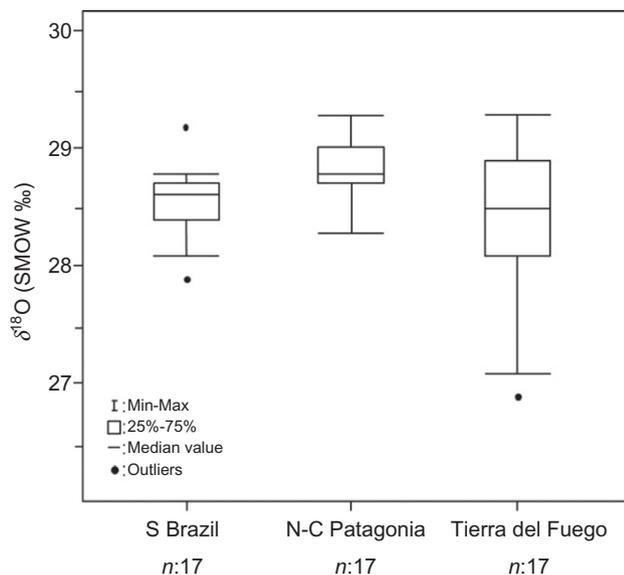


Figure 2 Boxplots of the $\delta^{18}\text{O}$ values in the bone of male South American sea lions, *Otaria byronia*, found dead on beaches in three regions along the coastline of the southwestern South Atlantic Ocean.

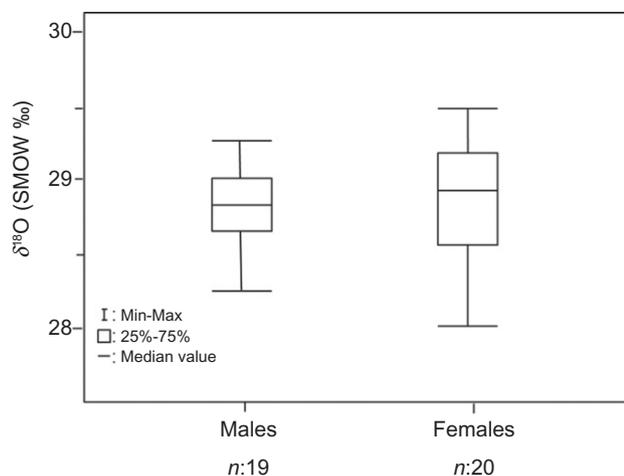


Figure 3 Boxplots of the $\delta^{18}\text{O}$ values in the bone of adult males and females South American sea lions, *Otaria byronia*, found dead in central and northern Patagonia.

Fuego were not statistically significant ($q = 0.490$, $P > 0.500$). These differences suggested limited exchange of individuals among the three regions.

Adult male and female sea lions from Patagonia did not differ in $\delta^{18}\text{O}_{\text{bone}}$ values (Fig. 3; $t = 0.861$, d.f. = 37, $P = 0.395$), indicating that they potentially used the same water masses for foraging. On the other hand, young animals of both sexes exhibited a much larger variability in $\delta^{18}\text{O}_{\text{bone}}$ values in comparison with the adults of the same sex (Fig. 4), as demonstrated by heteroskedasticity both among male (Levene test;

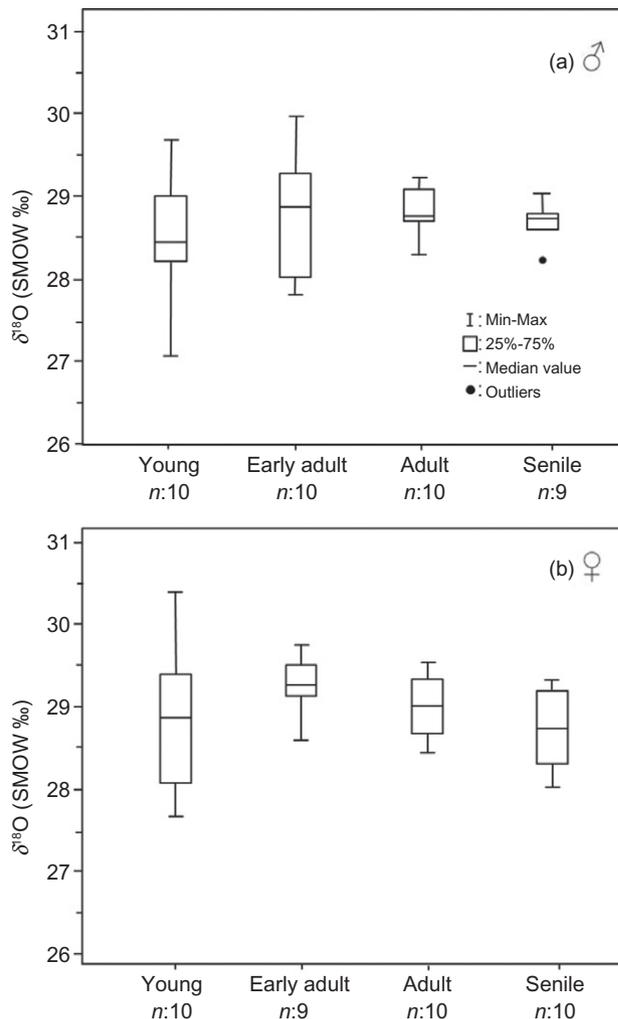


Figure 4 Boxplots of the $\delta^{18}\text{O}$ values in the bone of four developmental stages of South American sea lions, *Otaria byronia*, found dead in central and northern Patagonia. (a) Males; (b) Females.

males: $W_{3,35} = 4.523$, $P = 0.009$) and female developmental stages (Levene test; $W_{3,35} = 3.145$, $P = 0.037$). The variability of the $\delta^{18}\text{O}_{\text{bone}}$ values of females decreased sharply at first maturity but remained high in males for several years after sexual maturity. Finally, after first maturity, a statistically significant though moderate decrease in the $\delta^{18}\text{O}_{\text{bone}}$ values occurred in females with age ($r^2 = 0.319$, $P = 0.001$), but not in males ($P = 0.946$).

Discussion

Previous studies using external tags have concluded that adult male South American sea lions may travel longer distances than females after the breeding season (Vaz-Ferreira, 1982; Rosas *et al.*, 1994; Giardino *et al.*, 2009) and may also exhibit a high degree of fidelity to haul-out sites on consecutive nonbreeding seasons (Giardino *et al.*, 2009). Nevertheless,

external tags do not last for a long time on sea lions (e.g. Oliveira, 2010) and resightings a few months after tagging are scarce (Giardino *et al.*, 2009). Similarly, satellite tags remain attached to sea lions for only a few months and have offered no information about interannual movements (Campagna *et al.*, 2001; Riet-Sapiriza *et al.*, 2012). As a consequence, the actual proportion of adult males moving to distant foraging grounds after the breeding season has remained unknown.

Stable isotope analysis offers an alternative approach, although a number of confounding factors should be considered. Firstly, bone samples come from dead stranded sea lions, which are likely biased for sex ratio and age distribution. However, such biases are not relevant for the hypothesis here to be tested because comparisons among areas were based only on adult males. Furthermore, recent research on dead stranded marine mammals and sea turtles has revealed that decomposition does not significantly modify the stable isotope ratios of soft tissues (Payo Payo *et al.*, 2013) and hence is not expected to have any impact on the $\delta^{18}\text{O}$ values of biominerals.

The existence of a second set of potential confounding factors is suggested by the water balance models developed for other mammals (Kohn, 1996; O'Grady *et al.*, 2012; Podlesak *et al.*, 2012). Sea lions obtain the water they need from food (Ortiz, 2001; Berta, Sumich & Kovacs, 2005). South American sea lions consume a diversity of prey species, differing widely in water and fat contents, but not in protein contents (Drago *et al.*, 2009b, 2010). Therefore, dietary changes between sexes and age classes (Drago *et al.*, 2009a) may result in differences in the relative contribution of metabolic and preformed water to the water supply of sea lions. As the metabolic water generated by food oxidation is ^{18}O -enriched relative to ingested water and preformed water in the diet (Newsome, Clementz & Koch, 2010) and the consumption of fat-rich prey declines with body size in sea lions (Drago *et al.*, 2009a), younger developmental stages and females might be more enriched in ^{18}O than older age classes and males, even if they forage in the same water mass.

Reproduction is another potentially confounding factor, not only because lactation results in a high water turnover rate in adult females, but also because male and females differ dramatically in haul-out time and foraging behavior during the breeding season. Adult male sea lions spend as much as 40 days in the beach during the reproductive season (Campagna, 1985) and they fast during that time (Campagna *et al.*, 2001). Conversely, females resume feeding as soon as they have been fertilized by males (Campagna & Leboeuf, 1988) and alternate foraging bouts with periods on the beach nursing pups (Campagna *et al.*, 2001). Fasting animals rely primarily on the metabolism of fat, and secondarily on protein, to maintain their water balance (Worthy & Lavigne, 1982), and hence fasting for long periods is expected to increase the $\delta^{18}\text{O}$ values of body water. Furthermore, males are exposed to high air temperatures during the breeding season, at least in Uruguay and northern Patagonia, which likely increase water loss through sweating (Khamas *et al.*, 2012).

Despite all these potential sources of variability, the absence of statistically significant differences in the average $\delta^{18}\text{O}_{\text{bone}}$ values across sexes and developmental stages in the

sea lions from Patagonia suggest that these factors considered are actually minor sources of variability. Alternatively, diet might not vary across sexes and developmental stages, but this is unlikely considering the dietary information published to date (Koen-Alonso *et al.*, 2000; Drago *et al.*, 2009b). Hence, the regional variability in the $\delta^{18}\text{O}_{\text{seawater}}$ values stands as the most likely source of variability for the differences observed among males from Brazil, Patagonia and Tierra del Fuego. If so, the results reported here suggest that adult males are quite faithful to a particular coastal region for at least several years because the latitudinal differences observed in the $\delta^{18}\text{O}_{\text{bone}}$ values of males were in agreement with the latitudinal patterns of salinity (Guerrero & Piola, 1997) and $\delta^{18}\text{O}_{\text{seawater}}$ values in the region (Bowen, 2010). In addition, the rate of turnover of oxygen isotopes in hydroxyapatite is assumed to represent several years in large adult mammals (Schwarcz & Schoeninger, 1991; Ambrose & Norr, 1993).

Furthermore, the SD of the $\delta^{18}\text{O}_{\text{bone}}$ values in males was low in Brazil ($\pm 0.32\text{‰}$) and Patagonia ($\pm 0.26\text{‰}$), but much higher in Tierra del Fuego ($\pm 0.67\text{‰}$). When these figures are compared with the $\delta^{18}\text{O}_{\text{seawater}}$ gradients in each region (0.28‰ every 100 km from Rio de Janeiro to Rio de la Plata, 0.11‰ every 100 km from Rio de la Plata to central Patagonia and 0.08‰ every 100 km from central Patagonia to Tierra del Fuego; <http://data.giss.nasa.gov/o18data/>), males stranding in southern Brazil had foraged along approximately 230 km of coastline, those from Patagonia over 470 km of coastline and those from Tierra del Fuego over 1700 km of coastline (SD of $\delta^{18}\text{O}_{\text{bone}} = \pm 0.67\text{‰}$). Nevertheless, the high SD observed in Tierra del Fuego could be caused by a high degree of individual variability in the use of the relatively diluted channels of the Fuegian Archipelago and the saltier Atlantic waters (Guerrero & Piola, 1997).

Conversely, the absence of differences in the average $\delta^{18}\text{O}_{\text{bone}}$ values of adult males and females from Patagonia suggests that they share the same water mass year round, a conclusion that cannot be extrapolated to southern Brazil and Tierra del Fuego because only adult males were analyzed there due to the scarcity of female skulls in the museum collections from those areas. The scarcity of females for southern Brazil is because of the rarity of females in the local haul-out sites (Rosas *et al.*, 1994), but this is not true for Tierra del Fuego (Schiavini, Crespo & Szapkievich, 2004).

It should be noted that sharing a water mass does not necessarily mean using the same foraging grounds, because the values of $\delta^{18}\text{O}_{\text{seawater}}$ vary with latitude but not longitude over the continental shelf of southwestern South America (Bowen, 2010). Hence, animals using foraging grounds at a different distance from the coastline but at the same latitude will not differ in their $\delta^{18}\text{O}_{\text{bone}}$ values, which explain why adult male and female from Patagonia have similar $\delta^{18}\text{O}_{\text{bone}}$ values although females forage in more coastal areas than males (Campagna *et al.*, 2001).

In contrast, the average $\delta^{18}\text{O}_{\text{bone}}$ values of young specimens of both sexes collected in northern Patagonia did not differ either from those of the adult and senile individuals, but were more variable. This was also true for early adult males, but not for early adult females. Although the $\delta^{18}\text{O}_{\text{bone}}$ values of

young specimens integrate the values of hydroxyapatite after weaning with those deposited in uterus and during lactation, the latter signals decays after weaning and vanish totally after several months (Newsome *et al.*, 2010). The high variability observed in the $\delta^{18}\text{O}_{\text{bone}}$ values of juveniles reveals the presence in the same region of individuals with contrasting foraging histories during the years previous to death which for young specimens could result from differences in age and the persistence of the pre-weaning signals in the youngest animals. This is because suckling mammals are expected to be enriched in the heavier isotopes as compared with their mothers; however, suckling signal decays after weaning (Newsome *et al.*, 2010). Although this pattern is well established for nitrogen (Newsome *et al.*, 2010) and some of the young individuals analyzed here were young enough to exhibit traces of the suckling signal in their nitrogen stable isotope ratios (Drago *et al.*, 2009a), experimental evidence supporting the existence of a suckling signal for oxygen stable isotope ratios in bone is ambiguous (Williams, White & Longstaffe, 2005; Kirsanow & Tuross, 2011). However, no trace of the suckling signal is expected to remain in early adult males (Drago *et al.*, 2009a) and hence the high SD observed in that group, when compared with that of older males, should have a different explanation.

Actually, the range of $\delta^{18}\text{O}_{\text{bone}}$ values observed in young specimens of both sexes and in early adult males greatly overlapped with the range of $\delta^{18}\text{O}_{\text{bone}}$ values reported for adult males across the study area, which suggests that young specimens of both sexes disperse before settlement along most of the range of the species in the southwestern Atlantic Ocean, from Uruguay to Tierra del Fuego. This is in sharp contrast with the dispersal pattern of young Steller sea lions (*Eumetopias jubatus*) in the North Pacific, where only males conducted long distance (>500 km) movements, although the range of round trip distance of juveniles increases with age in both sexes (Raum-Suryan *et al.*, 2004).

Genetic markers suggested philopatry for females, but not for males, which will disperse over much larger ranges and will be responsible for gene flow (Szapkievich *et al.*, 1999; Freilich, 2004; Túnez *et al.*, 2007, 2010; Artico *et al.*, 2010 and Feijoo *et al.*, 2011). The sudden decreases in the variability of the $\delta^{18}\text{O}_{\text{bone}}$ values of females observed after adulthood cannot be explained by bone remodeling (Schwarcz & Schoeninger, 1991; Ambrose & Norr, 1993) and suggest that females come back to their natal regions for settlement. Conversely, the SD of the $\delta^{18}\text{O}_{\text{bone}}$ values of males decreases more slowly as they grow older at a rate consistent with the expected apatite turnover, suggesting that males do not necessarily come back to their natal areas for settlement. Comparing the SD of $\delta^{18}\text{O}_{\text{bone}}$ values observed in young male sea lions from Patagonia ($\pm 0.81\text{‰}$) and the gradient of $\delta^{18}\text{O}_{\text{seawater}}$ values above reported for that region suggests that young males originated from a 2000 km stretch of coastline. Nevertheless, the duration of the juvenile dispersal phase is much longer in males (8 years) than in females (3 years), as suggested by the sudden decline of the variability of the $\delta^{18}\text{O}_{\text{bone}}$ after adulthood in females but not in males. Therefore, the overall evidence indicates that both males and females may disperse over long

distances as juveniles, but females do not settle far from their natal region although males can, as previously reported for other pinnipeds (e.g. Burg, Trites & Smith, 1999; Hoffman *et al.*, 2006; González-Suárez *et al.*, 2009).

In conclusion, the results are consistent with information from genetic markers indicating population isolation by distance and male-mediated gene flow, but suggest that once they become reproductively active, early adult males settling far away from their natal rookeries are the ones responsible for gene flow. Furthermore, these results demonstrate that stable isotopes of oxygen represent a useful and inexpensive approach to the study habitat use and dispersal patterns in marine mammals, and particularly highlight the importance of the bone material deposited in museums and other scientific collections as a source of samples.

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