Stable oxygen isotopes reveal habitat use by marine mammals in the Río de la Plata estuary and adjoining Atlantic Ocean

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Abstract

Detailed knowledge on habitat use by marine mammals is critical for understanding their ecological role and for adequate management. Here, we assess the habitat use patterns of thirteen species of marine mammals along the salinity gradient of the Río de la Plata estuary and the adjoining western South Atlantic Ocean, for which we use the ratios of stable oxygen isotopes in bone carbonate (apatite) as a proxy for salinity. Furthermore, we compare the results with those found from analysing the distance of the stranding site from the innermost point of the estuary. The overall evidence indicates that stable oxygen isotopes can be good tracers of habitat use, as they distinguish those species that regularly use inshore estuarine-coastal ecosystems from those that are restricted to offshore pelagic oceanic waters. In contrast, sampling location does not sufficiently characterize the species' habitat, since other factors can be involved in the animals being transported to stranding sites far from their typical habitats. In conclusion, the analysis of stable oxygen isotopes represents a useful and inexpensive approach to studying habitat use among marine mammals where salinity gradients exist.

Key-words: cetaceans, marine mammals, pinnipeds, stable oxygen isotopes, habitat preferences, stranding sites.

1. Introduction

Management decisions and conservation actions should ideally be informed by accurate ecological information regarding the structure and function of ecosystems (Franklin, 1993). However, accurate data about habitat use by many marine mammal species is often scarce or missing (Schipper et al., 2008). This is due to the logistical difficulties related to the sampling of marine mammals, especially elusive species that usually occupy off-shore areas and hence are virtually impossible to study in the wild (Moore et al., 2018). On the other hand, knowledge about habitat use by marine mammals is critical for understanding their ecological roles (Roman and Estes, 2018). Furthermore, this information is also important for understanding direct (e.g., bycatch) and indirect (e.g., competition) interactions with fisheries (Reeves, 2018; Reeves et al., 2003).

Often, most of the available information is derived from a few stranded individuals (Moore et al., 2018), which may represent a highly biased sample of the population. The location of the stranding site might be particularly misleading and may not represent an accurate characterization of the species' habitat if individuals become stranded far from their normal distribution as a result of transport by currents or being discarded by fishermen close to ports (Moore et al., 2018).

The application of stable isotope analysis has grown tremendously over the past 30 years and has become a key analytical method in ecological studies (Crawford et al., 2008; Layman et al., 2012; Newsome et al., 2010; Wolf et al., 2009). Stable isotopes are known from several chemical elements, but those of carbon and nitrogen are the most widely used biogeochemical markers for studying topics related to the trophic ecology of wild animals (Crawford et al., 2008; Layman et al., 2012; Newsome et al., 2010; Wolf et al., 2009). Stable carbon isotopes have been used widely as habitat tracers because pelagic and oceanic species are depleted in ^{13}C compared to benthic and coastal species (Michener and Lajtha, 2007). However, caution is advised when using stable isotopes of carbon to trace habitat preference, because changes in diet may confound the interpretation.

On the other hand, carbonates become enriched in ^{18}O as temperature declines (Kim and O'Neil, 1997); hence, stable isotopes of oxygen ($^{18}O/^{16}O$; $\delta^{18}O$) in mollusc shells have been widely employed to reconstruct the paleotemperature of aquatic ecosystems (Burgess et al., 2010; Schöne et al., 2004) and to study the thermal environment of fishes and molluscs (Soldati et al., 2009; Torniainen et al., 2017). 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 (Bowen, 2010). Fractionation during evaporation and precipitation of meteoric water results in freshwater environments that are typically ^{18}O-depleted relative to seawater (Bowen, 2010; Dansgaard, 1964; Gat, 1996; Trueman and Glew, 2019). Furthermore, as salinity and $\delta^{18}O$ in seawater are controlled by the same process, the overall variation in marine $\delta^{18}O$ values is positively and linearly correlated to salinity (Belem et al., 2019; Conroy et al., 2014; Delaygue et al., 2001; Pierre, 1999; Pierre et al., 1994; Pierre et al., 1991). This suggests that the stable isotopes of oxygen can be useful tracers of habitat use in marine species inhabiting areas with strong salinity gradients (Clementz et al., 2006; Clementz and Koch, 2001; Trueman et al., 2012; Wheatley et al., 2012; Zenteno et al., 2013). Accordingly, the ratio of stable oxygen isotopes can be used as a proxy for salinity in marine species moving between marine, brackish and freshwater ecosystems, since species inhabiting brackish or freshwater habitats should have lower $\delta^{18}O$ values of body water (Newsome et al., 2010; Roe et al., 1998). Despite this potential, only a few studies aiming to resolve the habitat preference of extant marine vertebrates have used stable oxygen isotopes (e.g., Clementz and Koch, 2001; Vighi et al., 2016; Vighi et al., 2014; Wheatley et al., 2012; Yoshida and Miyazaki, 1991; Zenteno et al., 2013).

In marine mammals, water is the largest oxygen supply, and its intake is the major avenue of flux into the body. Ingested water, which may account for as much as 70–80% of total flux into the marine mammal body, includes preformed water (i.e., water that is consumed in food or actively drunk) and seawater consumed incidentally when eating (Costa, 2018; Ortiz, 2001). Metabolic water, on the other hand, is that which results from the oxidation of organic molecules (Newsome et al., 2010). The proportion of water obtained from these sources varies widely among marine mammals (Costa, 2018; Ortiz, 2001); however, as these processes do not strongly fractionate stable oxygen isotopes, the fluxes should all have $\delta^{18}O$ values close to that of seawater (0‰V-SMOW) (Newsome et al., 2010; Soto et al., 2013). Therefore, the $\delta^{18}O$ value of body water is overall similar to that of environmental water, as its bioapatite phosphate and carbonate form in near isotopic equilibrium with environmental water (Newsome et al., 2010; Soto et al., 2013; Vander Zanden et al., 2016).

In addition, the $\delta^{18}O$ values of marine mammals show little within-population variability, presumably because the $\delta^{18}O$ values of body water experience little variation during the time window integrated by bone apatite, as well as because of little variability between individuals from the same populations (Clementz and Koch, 2001). The turnover rate of oxygen isotopes in bone apatite is poorly known, but complete bone apatite turnover in adult mammals is assumed to take several years (Ambrose and Norr, 1993; Schwarcz and Schoeninger, 1991).

The Río de la Plata is one of the largest estuarine systems in the world and profoundly affects the ecology of adjoining marine ecosystems (Acha et al., 2008; Miloslavich et al., 2011).The region is inhabited by a high diversity of marine mammals, including subpolar, subtropical and tropical species (Bastida et al., 2007; Miloslavich et al., 2011). Furthermore, these species occupy a wide variety of ecosystems, from shallow estuarine-coastal waters to deep pelagic oceanic waters. The skeletal material available in scientific and museum collections offers the opportunity to study habitat use by means of stable oxygen isotopes. Although they lack the detailed resolution of other methods such as satellite tagging, they provide a suitable and inexpensive alternative approach to studying habitat use.

In this paper, we assess the habitat preferences of marine mammals (pinnipeds and cetaceans) along the salinity gradient of the Rio de la Plata ecosystem, and we do so using the ratio of stable oxygen isotopes in bone carbonate (apatite) as a proxy for salinity. In addition, we compare the results with those obtained from analysing the distance of the stranding site from the innermost point within the estuary. Based on, first, the overall positive correlation between marine δ^{18}O values and salinity reported for the adjacent Atlantic Ocean (Belem et al., 2019; LeGrande and Schmidt, 2006; Pierre et al., 1991) and, second, the strong salinity gradient in the Rio de la Plata (Acha et al., 2008), we hypothesize that species which regularly use estuarine waters will have lower bone δ^{18}O values than species with distribution ranges that span across marine regions. Furthermore, we hypothesize that species which regularly use estuarine waters should become stranded mainly in the innermost part of the estuary if the stranding site is indicative of habitat use.

2. Methods

2.1. Study Area

The Río de la Plata estuary is located at 35ºS, between Uruguay and Argentina (Fig. 1); and the plume of the estuary expands to southern Brazil. It is one of the most important South American estuarine environments, and the waters under its influence are among the most productive in the world (Acha et al., 2008; Miloslavich et al., 2011). Due to the convergence of two major currents—the cold Falkland/Malvinas current and the warm Brazil current—combined with the massive freshwater run-off from the Paraná River, a unique system with extremely rich biodiversity is generated (Acha et al., 2004; Guerrero et al., 1997a; Miloslavich et al., 2011; Ortega and Martinez, 2007).

The Río de la Plata estuary is characterized by a salinity gradient that spans across some 300 km (Acha et al., 2008; Guerrero et al., 1997a), with three major zones that can be distinguished in Uruguayan coastal waters (Defeo et al., 2009; Guerrero et al., 1997a; Guerrero et al., 1997b): the inner estuarine zone (Zone 1) is characterized by salinity values of < 24.50; the outer estuarine zone (Zone 2) is characterized by salinity values of between 24.51 and 31.49; and the marine/oceanic zone (Zone 3) has values of > 31.50. The inner estuarine zone and the outer estuarine zone are influenced directly by the Río de la Plata discharge; whereas the marine/oceanic zone has a remarkable but variable oceanic influence (Fig. 1). Furthermore, a general increase in δ^{18}O values is observed from inshore (δ^{18}O = ~ -1 ‰) to offshore (δ^{18}O = ~ +2 ‰) marine environments in the nearby Atlantic Ocean (LeGrande and Schmidt, 2006; McMahon et al., 2013), although data for the estuary are few.

2.2. Sampling

A total of 362 bone samples were collected from the skulls of thirteen marine mammal species (Table 1): two otariid species (South American sea lion *Otaria flavescens* and South American fur seal *Arctocephalus australis*) and eleven odontocete species (the single pontoporiid Franciscana dolphin *Pontoporia blainvillei*; one ziphiid Cuvier's beaked whale *Ziphius cavirostris*; two phocoenids: the spectacled porpoise *Phocoena dioptrica* and Burmeister's porpoise *Phocoena spinipinnis*; and seven delphinids: the common dolphin *Delphinus delphis*, the long-finned pilot whale *Globicephala melas*, Risso's dolphin *Grampus griseus*, Fraser's dolphin *Lagenodelphis hosei*, the killer whale *Orcinus orca*, the false killer whale *Pseudorca crassidens*, and the bottlenose dolphin *Tursiops truncatus*). It should be noted that two subspecies of bottlenose dolphins inhabit the western South Atlantic Ocean: the common bottlenose dolphin *T. t. truncatus* (offshore ecotype) and Lahille's bottlenose dolphin *T. t. gephyreus* (coastal ecotype) (Costa et al., 2016). Based on their skull characteristics, all the bottlenose dolphins included in this study belong to the subspecies *T. t. gephyreus* (Costa et al., 2016; Wickert et al., 2016).

Every specimen included in the current study was found stranded and dead along the Uruguayan coastline or was incidentally caught by Uruguayan fishermen between 1940 and 2016 (Table 1 and Fig. 1). All the bone samples were collected from skulls in the scientific collection of the Museo Nacional de Historia Natural (MNHN) and the Facultad de Ciencias of the Universidad de la República (UdelaR) at Montevideo (Uruguay).

The bone samples of pinnipeds and cetaceans used for the oxygen isotopic analysis consisted, respectively, of a small fragment of bone from the nasal cavity (turbinate bone) and the maxilla. All the skulls from the South American sea lion, South American fur seal and Franciscana dolphin were determined to pertain to specimens that were either adult, physically mature or in the process of maturation. According to the lines of arrested growth in canine dentine, the ages of the sampled sea lions ranged from 5 to 16 years, whereas those of the fur seals ranged from 5 to 14 years (Laws, 1952). The age of the sampled Franciscana dolphins was unknown, but adulthood was inferred from standard length (ranging from 121 to 174 cm), according to size at sexual maturity (Botta et al., 2010; Danilewicz, 2003; Danilewicz et al., 2004). Standard length was recorded in the field during skull collection. The age and standard length of the specimens of the remaining cetacean species were unknown, so the condylobasal length of each skull was measured in order to consider only specimens of similar body size within each species and to avoid age-related bias. The condylobasal length ranged: from 45 to 48 cm for common dolphins; from 68 to 71 cm for long-finned pilot whales; from 39 to 44 cm for Fraser's dolphins; from 89 to 94 cm for killer whales; from 27 to 29 cm for spectacled porpoises; from 27 to 29 cm for Burmeister’s porpoises; from 61 to 65 cm for false killer whales; from 55 to 59 cm for Lahille’s bottlenose dolphins; from 89 to 95 cm for Cuvier's beaked whales; and 47 cm for the Risso's dolphin.

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

Additionally, the distance (km) to the inner part of the Río de la Plata estuary (see Fig. 1) along the Uruguayan coast was assessed for each individual sampling location in order to evaluate whether or not it can be used as a proxy for the species’ habitat preference. Distances were calculated using the measuring tool from QGIS (QGIS Development Team, 2018).

2.3. Stable Isotope Analysis

Bone samples were cleaned with distilled water, dried in a stove at 60 ºC for 36 h, and ground into a fine powder using a mortar and pestle. As the stable oxygen isotope analysis could be carried out only on the inorganic matrix, powder bone samples were treated by soaking them in 30% hydrogen peroxide (H_2O_2) for 24 h to remove any organic compounds. Furthermore, samples were then treated by soaking them in 1 M of calcium acetate–acetic acid buffer for another 24 h to remove any diagenetic carbonate. Finally, they were rinsed carefully with deionized (Milli-Q) water and dried for 24 h (Koch et al., 1997).

Approximately 1.0 mg of each processed sample of bone was weighed and analysed for oxygen isotope ratios using a Kiel III Carbonate Device preparation system (Thermo

Electron-Dual Inlet, Thermo Fisher Scientific, Bremen, Germany) linked to a model MAT-252 gas source mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Samples were 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. All analyses were performed at the Centres Cientifics i Tecnològics (CCiT-UB) 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. 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 (^{18}O), and ^{i}X is the lighter isotope (^{16}O) in the analytical sample and international measurement standard (Bond and Hobson, 2012); the international standard was the Vienna Pee Dee Belemnite (V-PDB) calcium carbonate. However, data were normalized using commercially available laboratory reference materials. Isotopic reference materials of known $^{18}O/^{16}O$ ratios, as given by the International Atomic Energy Agency (IAEA, Vienna, Austria), were the NBS-19 and NBS-18 calcite standard, with $\delta^{18}O$ values, respectively, of -2.20‰ and -23.2‰ relative to V-PDB. These two isotopic reference materials were employed once every six analysed samples in order to recalibrate the system and compensate for any measurement drift over time. The raw data were normalized by the multipoint normalization method based on linear regression (Skrzypek, 2013). The analytical precision of $\delta^{18}O$ tested by replicate analyses was ± 0.05‰ (standard deviation).

Because $\delta^{18}O$ values in animal studies are more commonly presented relative to the Vienna Standard Mean Oceanic Water (V-SMOW) index, $\delta^{18}O$ values were converted from PDB to SMOW according to the following equation (Koch et al., 1997):

$$\delta^{18}O\ (SMOW) = [\delta^{18}O\ (PDB) \times 1.03086] + 30.86$$

2.4. Data Analyses

Prior to statistical analyses, normality was tested by means of Lilliefors test, and homoscedasticity by means of Levene's test. Data were log-transformed to ensure normality. We checked the assumptions of the statistical models by carrying out the customary residual analysis.

First, we used linear models with year as a continuous explanatory variable and species as a categorical covariate to assess any temporal trend in the bone $\delta^{18}O_{smow}$ values of South American sea lions, South American fur seals and Franciscana dolphins. We selected those species because they covered a broad time window (i.e., from the 1940s to the 2010s) and sample size was large (Table 1). We started with the most complex model, which included the interaction between explanatory variables, and subjected it to sequential, stepwise simplification by deleting one term (whether it was an interaction or a main effect). Comparisons between successive steps of model simplification were performed by the Akaike information criterion (AIC) and selecting the model with the lowest AIC. The selected models were validated by residual analyses (Crawley, 2007). We refrained from including the sex of the species as a categorical covariate in the model because a previous exploratory analysis indicated that males and females did not differ over time in any of the three species. Therefore, we created additional linear models by pooling male and female data and also incorporating Franciscana dolphin individuals of unknown sex.

Second, we used nested-ANOVA with species nested within their presumed habitat (i.e., the habitat previously reported in the literature for the species; see Table 1) in order to compare the average $\delta^{18}O_{smow}$ and distance values among presumed habitats and species. We used the Scheffé test (because of uneven sample size) for post hoc comparisons. Furthermore, we applied the same data set to a linear mixed model using distance as a fixed effect and species as a random effect, which allowed us to assess whether a relationship existed between the bone $\delta^{18}O_{smow}$ values and the distance from the inner part of the estuary. The simplification and validation of the model was carried out as mentioned above, whereas the most parsimonious model was selected using the likelihood ratio test of models (Bolker, 2008). Only species with a sample size of ≥ 5 individuals were included in the statistical analyses.

Data were always reported as mean ± standard deviation (SD), unless otherwise stated. All statistical analyses were carried out using the free software R 3.6.1 (R Core Team, 2018).

3. Results

The final model adjusted for $\delta^{18}O_{smow}$ values over time showed a significant decrease for South American sea lions but not for South American fur seals or Franciscana dolphins (Table 2 and Fig. 2). The slope of the $\delta^{18}O_{smow}$ vs. time function for South American sea lions was also significantly larger than that of the other two species, which had similar slopes and did not differ in their average values (Table 2 and Fig. 2).

We found a statistical difference in the $\delta^{18}O_{smow}$ values for the nine species of marine mammals included in the nested-ANOVA, both between presumed habitats ($F_{2,351}$ = 55.51, $p < 0.001$) and between species within the same presumed habitat ($F_{6,351}$ = 20.95, $p < 0.001$). Post-hoc tests revealed that presumed oceanic species were more enriched in ^{18}O than those in estuarine-coastal and continental shelf waters. Furthermore, the $\delta^{18}O_{smow}$ values of presumed continental shelf inhabitants were between those of presumed oceanic and estuarine-coastal species (Fig. 3). Within the group of presumed continental shelf inhabitants, the lowest $\delta^{18}O_{smow}$ values were observed in killer whales;

whereas, among the presumed estuarine-coastal species, Franciscana dolphins showed the highest $\delta^{18}O_{smow}$ values (Fig. 3).

Statistically significant differences were also observed in the distance of the stranding site from the inner part of the estuary, both among presumed habitats (nested-ANOVA; $F_{2,343} = 134.81$, $p < 0.001$) and among species within the same presumed habitat (nested-ANOVA; $F_{6,343} = 6.63$, $p < 0.001$). Post-hoc tests revealed that the distances of the oceanic species' stranding sites to the innermost part of the estuary were significantly smaller than those of any other species, while those of estuarine-coastal species were the largest (Fig. 4).

Finally, for the nine marine mammal species considered in the analyses, there was no statistically significant correlation between the bone $\delta^{18}O_{smow}$ values and the stranding site's distance from the inner part of the estuary (Table 3).

4. Discussion

The data reported here revealed that stable oxygen isotopes serve as powerful habitat tracers along salinity gradients. Conversely, stranding sites offer inconsistent information about habitat use because well-known ocean dwellers (Fraser's dolphin, long-finned pilot whale and Cuvier's beaked whale) become stranded mainly in the innermost part of the estuary. These inconsistencies may result from carcasses being transported by currents (Framiñan et al., 1999) or because fishermen discard bycatch close to ports. The latter is particularly obvious for the Franciscana dolphin, because 92% of specimens were incidentally caught at unknown locations and brought to port by fishermen from the Rocha Department. Furthermore, the aggregation of pinnipeds at rookeries and haul-outs may also result in localized stranding: 81% of South American sea lion specimens and 93% of South American fur seal specimens were found around Isla de Lobos and the Islas de Cabo Polonio, the two main rookeries of these species in Uruguay (Ponce de León, 2000) (Fig. 1).

The average $\delta^{18}O_{smow}$ values in bone tissue differed among species, with good agreement between presumed habitat and $\delta^{18}O_{smow}$ values. Lahille's bottlenose dolphin, the South American sea lion and the South American fur seal are known to use estuarine habitats in the western South Atlantic Ocean (Botta et al., 2012; Costa et al., 2016; Drago et al., 2017; Franco-Trecu et al., 2013; Franco-Trecu et al., 2019; González Carman et al., 2016; Lodi et al., 2016; Melo et al., 2010; Riet-Sapriza et al., 2013; Secchi et al., 2016), and all them had low $\delta^{18}O_{smow}$ values, which are consistent with extended use of the outer estuarine area.

It has been suggested that South American fur seals might forage mainly at the shelf break; hence, the distance to the shelf break could be a major determinant of rookery location (Tunez et al., 2008). However, scat analysis has revealed that South American fur seals in Uruguay feed primarily on coastal fishes associated with the Río de la Plata plume (Naya et al., 2002; Szteren et al., 2004). Furthermore, both the habitat modelling of tracking data (González Carman et al., 2016) and the $\delta^{18}O_{smow}$ values reported here are consistent with extended use of the outer estuary. South American sea lions also make intense use of the outer estuary, according to satellite telemetry data and habitat modelling (González Carman et al., 2016; Riet-Sapriza et al., 2013; Rodriguez et al., 2013); hence, their average $\delta^{18}O_{smow}$ value is close to that of fur seals. Habitat modelling suggests that the core habitats of both species largely overlap, but the South American fur seal's habitat is much larger than that of the South American sea lion, mainly due to intense use of both shelf habitats and deep penetration in the estuary (González Carman et al., 2016). This results in exposure to a broader salinity range, but the average salinity level of the population is similar to that of South American sea lions, while the two species also have similar $\delta^{18}O_{smow}$ values.

The Franciscana dolphin is endemic to the western South Atlantic Ocean and is only one of the basal dolphin species living in the marine environment, from the coast to the 50-m isobath (Crespo, 2018). The $\delta^{18}O_{smow}$ values reported here are consistent with previous studies finding that Franciscana dolphins more frequently roamed in marine rather than estuarine waters (Artecona et al., 2019; Drago et al., 2018; Franco-Trecu et al., 2019; Rodríguez et al., 2002).

Within our dataset, the group that maintain intermediate $\delta^{18}O_{smow}$ values (continental shelf group) includes the killer whale, the false killer whale, the common dolphin and Burmeister's porpoise. The killer whale is an apex marine predator preying on a great diversity of vertebrates and invertebrates, both inshore and offshore (Ford, 2018). In the Río de la Plata area and adjacent waters, killer whales roam along the continental shelf and make regular use of inshore waters (Botta et al., 2012; Iriarte, 2006; Ott and Danilewicz, 1998; Passadore et al., 2007). This is consistent with their reported $\delta^{18}O_{smow}$ values, which occupy an intermediate range between those of coastal and oceanic species of marine mammals. The false killer whale is considered to be predominantly an oceanic predator, but it is also known to forage over the continental shelf and in nearshore areas around tropical oceanic islands (Baird, 2018). The extended use of the continental shelf is consistent with our results (intermediate $\delta^{18}O_{smow}$ values, between those of well-known coastal and oceanic species) and with previous studies conducted in Río de la Plata and the western South Atlantic Ocean, which suggest that the species roam frequently over the continental shelf from the coast to the slope (Bisi et al., 2013; Botta et al., 2012; Koen Alonso et al., 1999; Pinedo and Rosas, 1989; Riccialdelli et al., 2010). Common dolphins are typical inhabitants of the lower shelf and the continental slope (Bisi et al., 2013; Botta et al., 2012; Melo et al., 2010) and, hence, have $\delta^{18}O_{smow}$ values lower than those of the oceanic species. Similar $\delta^{18}O_{smow}$ values were also observed in Burmeister's porpoise. Again, this is consistent with previous information, as Burmeister's porpoise inhabits continental shelf waters (Reyes, 2018; Riccialdelli et al., 2010), although a few incidental captures have been reported from coastal waters off Río de la Plata (Corcuera et al., 1994).

The oceanic group includes only Fraser's dolphin, which is characterized by the highest $\delta^{18}O_{smow}$ values. Again, this is consistent with previous information, as Fraser's dolphin is a well-known oceanic species inhabiting deep waters beyond the continental slope (Bisi et al., 2013; Botta et al., 2012; Sekiguchi et al., 1992).

The sample size of the remaining species was too small to conduct any robust statistical analysis, but most $\delta^{18}O_{smow}$ values were consistent with previous information about habitat preference. Risso's dolphin, the long-finned pilot whale, and Cuvier's beaked whale are well known oceanic species with a preference for the continental slope (Baird et al., 2008; Baumgartner, 1997; Bisi et al., 2013; Olson, 2018; Passadore et al., 2008; Riccialdelli et al., 2010; Santos et al., 2007; Schorr et al., 2014); and they have high $\delta^{18}O_{smow}$ values, similar to those of Fraser's dolphin. Very little is known about the spectacled porpoises, although they have been reported to inhabit oceanic waters (Goodall and Brownell Jr., 2018; Riccialdelli et al., 2010). This is consistent with the high $\delta^{18}O_{smow}$ values reported here.

Despite the strong agreement between presumed habitat and the actual $\delta^{18}O_{smow}$ values demonstrated here, caution is needed when using stable isotopes of oxygen as habitat tracers, because their behaviour in organisms is poorly understood. Metabolic routing, isotopic discrimination and environmental variability over time may influence the ratios of stable oxygen isotopes and therefore require special attention (Vander

Zanden et al., 2016). Here, we use $\delta^{18}O_{smow}$ as a proxy for salinity, but sea surface temperature (SST) might potentially confound the interpretation of results. However, this is unlikely for three reasons. First, apex predators act as ecological integrators, averaging isotopic fluctuations over time and dampening temporal variability in the environmental signal (Vander Zanden et al., 2016). Second, bone integrates environmental information over several years (Ambrose and Norr, 1993; Schwarcz and Schoeninger, 1991) and thus smooths any seasonal variations in water temperature. Third, the $\delta^{18}O_{smow}$ values in marine mammals have been expected to decrease since the 1940s as a result of global warming (Kim and O'Neil, 1997), if sea temperature was their main driver. However, a drop in $\delta^{18}O_{smow}$ values has been observed only in the South American sea lion, without any temporal trend in the $\delta^{18}O_{smow}$ values of South American fur seals and Franciscana dolphins. The drop in the $\delta^{18}O_{smow}$ values observed in the South American sea lion may indeed reveal a long-term contraction of this species' foraging grounds in Río de la Plata, and hence be independent of increasing SST.

The commercial harvest of South American sea lions during the first half of the 20th century resulted in the fragmentation of a formerly continuous distribution range in the western South Atlantic (Grandi et al., 2015; Rodriguez and Bastida, 1998). The populations breeding in Argentina has recovered and is currently expanding to new rookeries thanks to legal protection (Grandi et al., 2015). In contrast, the population breeding in Uruguay became isolated and experienced a negative demographic trajectory, which resulted in a small population size (Franco-Trecu et al., 2015). Historically, the foraging grounds of the South American sea lions breeding in Uruguay spanned from southern Brazil to northern Argentina (Rodriguez and Bastida, 1998; Rosas et al., 1994), but currently only a few South American sea lions occur in southern Brazil (Pavanato et al., 2013) and northern Argentina (Giardino et al., 2016), mainly during the winter months. Furthermore, only males are currently observed in southern Brazil (Pavanato et al., 2013), whereas females were observed there regularly until the mid-1980s (Rosas et al., 1994). Finally, satellite tracking and habitat modelling (González Carman et al., 2016; Riet-Sapriza et al., 2013; Rodriguez et al., 2013) confirm that the foraging grounds of South American sea lions in the Río de la Plata are currently centred in the outer estuary, close to the Isla de Lobos rookery, while they make only marginal use of other areas. Hence, the decline in the $\delta^{18}O_{smow}$ values observed in South American sea lions over the past 70 years might be related to the habitat contraction of this shrinking population, whose members— in a scenario of decreasing intraspecific competition—currently concentrate in the highly productive foraging ground around the outer estuary. If so, the $\delta^{18}O_{smow}$ values might have declined as a result of their reducing their use of less productive and more distant foraging grounds in southern Brazil and northern Argentina.

On these grounds, we feel that $\delta^{18}O_{smow}$ values in the other species of marine mammals considered here can be interpreted confidently as a proxy for habitat use along the salinity gradient, despite major knowledge gaps in metabolic routing, isotopic discrimination, and the integration time of stable oxygen isotopes in animals. The results reported here provide strong evidence that stable isotopes of oxygen offer a useful and inexpensive method for inferring habitat use in marine mammals and highlight the relevance of the bone material deposited in museums and other scientific collections.

Author contributions

- M.D., A.A. and L.C. conceived the ideas and designed the study.
- M.D., M.V., D.B. and E.M.G. performed the sampling and lab analysis.
- M.D. and L.C. analysed the data.
- M.D. and L.C. led the writing of the manuscript.
- All authors contributed critically to the drafts and gave final approval for publication.

Declarations of interests

None.

Data statement

The authors declare that all data from this study will be made available in a public repository at the time of publication.

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Table 1. Mean and standard deviation of the stable isotope values ($\delta^{18}O_{smow}$) in the bone of the thirteen marine mammal species considered in this study. n_1: sample size for species; n_2: sample size for sex; Unknown: individuals of unknown sex; Habitat: habitat preference for each species from the Río de la Plata and adjacent Atlantic Ocean waters (*), northwestern Atlantic Ocean (†), southwestern Atlantic Ocean (§), northeastern Atlantic Ocean (‖), southeastern Atlantic Ocean (-), northeastern Pacific Ocean (‡); Evidence: methodology used in the corresponding sources to establish habitat preference for the species.

Scientific name	Common name	Sampling range (yr)	n_1	$\delta^{18}O_{SMOW}$(‰)	Sex	n_2	$\delta^{18}O_{SMOW}$(‰)	Habitat	Evidence	Source
Pinnipeds										
Otaria flavescens	South American sea lion	1944–2012	47	27.6 ± 0.9	Female Male	27 20	27.6 ± 0.8 27.6 ± 1.0	Estuarine-Coastal (*)	Isotopic data ($\delta^{13}C$), Tracking data, Bycatch data	(Drago et al., 2017; Franco-Trecu et al., 2019; González Carman et al., 2016; Riet-Sapriza et al., 2013; Rodriguez et al., 2013)
Arctocephalus australis	South American fur seal	1946–2012	86	28.0 ± 0.5	Female Male	37 49	27.9 ± 0.5 28.0 ± 0.5	Estuarine-Coastal (*)	Isotopic data ($\delta^{13}C$), Tracking data, Bycatch data	(Drago et al., 2017; Franco-Trecu et al., 2019; González Carman et al., 2016)
Cetaceans										
Tursiops truncatus gephyreus	Lahille's bottlenose dolphins	1947–2016	18	27.5 ± 1.0	Male Unknown	5 13	27.5± 0.7 27.5 ± 1.1	Estuarine-Coastal (*)	Sighting data, Isotopic data ($\delta^{13}C$), Stomach data, Genetic data, Morphological data	(Botta et al., 2012; Costa et al., 2016; Lodi et al., 2016; Melo et al., 2010; Oliveira et al., 2019; Secchi et al., 2016)
Pontoporia blainvillei	Franciscana dolphin	1953–2015	129	28.6 ± 0.8	Female Male Unknown	45 57 27	28.4± 0.6 28.7± 0.8 28.9 ± 0.9	Estuarine-Coastal (*)	Stomach data, Isotopic data ($\delta^{13}C$), Genetic data, Bycatch data	(Artecona et al., 2019; Costa-Urrutia et al., 2012; Franco-Trecu et al., 2019; Rodríguez et al., 2002)
Orcinus orca	Killer whale	1968–1988	5	27.9 ± 1.2	Female Male Unknown	2 2 1	28.3 ± 0.2 28.5 ± 0.3 25.8	Continental shelf (*)	Isotopic data ($\delta^{13}C$), Stomach data, Sighting data	(Botta et al., 2012; Iriarte, 2006; Ott and Danilewicz, 1998; Passadore et al., 2007)
Pseudorca crassidens	False killer whale	1960–2016	17	28.6 ± 0.4	Female Male	3 7	28.5± 0.4 28.6 ± 0.6	Continental shelf (*) (§)	Stomach data, Isotopic data ($\delta^{13}C$)	(Bisi et al., 2013; Botta et al., 2012; Koen Alonso et al., 1999;

					Unknown	7	28.6 ± 0.3			Pinedo and Rosas, 1989; Riccialdelli et al., 2010)
Delphinus delphis	Common dolphin	1994–2012	6	29.1 ± 0.5	Female	2	29.2 ± 0.5	Continental shelf (*)	Isotopic data ($\delta^{13}C$), Stomach data	(Bisi et al., 2013; Botta et al., 2012; Melo et al., 2010)
					Unknown	4	29.0 ± 0.5			
Phocoena spinipinnis	Burmeister’s porpoise	1968–2008	12	29.1 ± 0.4	Female	1	29.1	Continental shelf (§)	Bycatch data, Isotopic data ($\delta^{13}C$)	(Corcuera et al., 1994; Reyes, 2018; Riccialdelli et al., 2010)
					Male	5	28.9 ± 0.4			
					Unknown	6	29.2 ± 0.2			
Grampus griseus	Risso's dolphin	1985	1	29.2	Unknown	1	29.2	Continental shelf (*) (†) (§)	Isotopic data ($\delta^{13}C$), Sighting data	(Baumgartner, 1997; Bisi et al., 2013; Passadore et al., 2008; Riccialdelli et al., 2010)
Phocoena dioptrica	Spectacled porpoise	1945–2016	3	29.1 ± 0.8	Male	1	28.3	Oceanic (§)	Isotopic data ($\delta^{13}C$)	(Riccialdelli et al., 2010)
					Unknown	2	29.6 ± 0.4			
Lagenodelphis hosei	Fraser's dolphin	1991–2001	31	29.5 ± 0.5	Female	8	29.6 ± 0.4	Oceanic (*) (-)	Stomach data, Isotopic data ($\delta^{13}C$)	(Bisi et al., 2013; Botta et al., 2012; Sekiguchi et al., 1992)
					Male	2	29.9 ± 0.4			
					Unknown	21	29.4 ± 0.5			
Globicephala melas	Long-finned pilot whale	1940–1978	3	29.9 ± 0.6	Male	1	30.5	Oceanic (*)	Sighting data	(Passadore et al., 2008)
					Unknown	2	29.6 ± 2.4			
Ziphius cavirostris	Cuvier's beaked whale	1960–1969	3	30.4 ± 0.6	Female	1	31.0	Oceanic (‖) (‡)	Stomach data, Tracking data, Dive data	(Baird et al., 2008; Santos et al., 2007; Schorr et al., 2014)
					Male	1	30.6			
					Unknown	1	29.7			

Table 2. Linear model for the bone $\delta^{18}O_{smow}$ values of South American sea lions (Of), South American fur seals (Aa) and Franciscana dolphin (Pb) over time. Estimates and *p-values* (in brackets) are shown for each variable. In bold, we show the model selected according to the Akaike Information Criterion (AIC).

Model	Intercept	Species (Of)	Species (Pb)	Year	Species (Of)*Year	Species (Pb)*Year	AIC
$\delta^{18}O_{SMOW}$ ~ species * year	**14.329 (0.06)**	**44.016 (<0.001)**	**11.307 (0.32)**	**0.006 (0.07)**	**-0.022 (<0.001)**	**-0.005 (0.35)**	**567.16**
$\delta^{18}O$ ~ species + year	29.897 (<0.001)	-0.361 (<0.001)	0.673 (<0.001)	-0.001 (0.69)			577.24
$\delta^{18}O$ ~ species	27.959 (<0.001)	-0.365 (<0.01)	0.675 (<0.001)				575.39

Table 3. Linear mixed model relating the $\delta^{18}O_{smow}$ values of South American sea lions, South American fur seals, Lahille’s bottlenose dolphins, Franciscana dolphins, killer whales, false killer whales, common dolphins, Burmeister's porpoises and Fraser's dolphins to distance from the inner part of the estuary (fixed effect) and species (random effect). Estimates and *p-values* (in brackets) are shown for each variable, as well as the standard deviation (SD) of the random effect.

Model	Intercept	Distance	SD random effect
$\delta^{18}O_{SMOW}$ ~ distance+(1|species)	28.12 (<0.001)	0.12 (0.61)	0.65

Figure 1. Study area and sampling locations. The dashed lines show the sampling locations of the skulls corresponding to thirteen marine mammal species along the Uruguayan coast; the black symbol ∗ denotes the most internal and external stranding sites in the Río de la Plata estuary for the marine mammals included in this study; the black solid circles show the breeding sites of South American sea lions and South American fur seals; the grey dashed lines delineate the boundaries between the zones with different salinity: inner estuarine zone (Zone 1), outer estuarine zone (Zone 2) and Marine/Oceanic zone (Zone 3). Sea surface salinity values are reported in practical salinity units (psu).

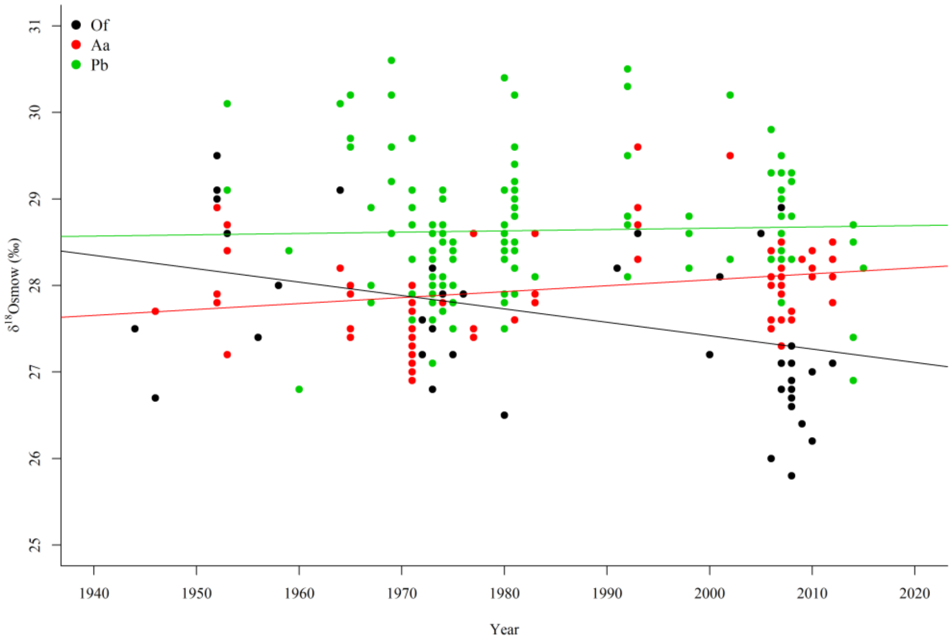

Figure 2. Trends in bone $\delta^{18}O_{smow}$ values for South American sea lions (Of), South American fur seals (Aa) and Franciscana dolphins (Pb) over time, showing the best fitted lines for each species (see Table 2).

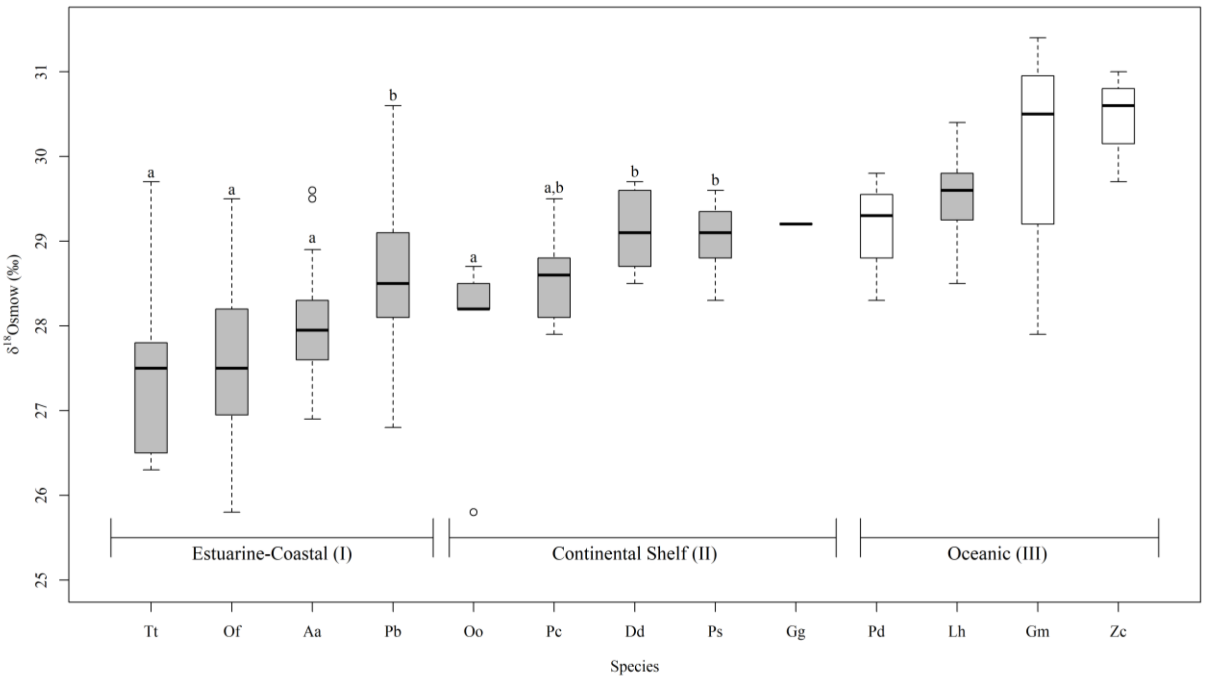

Figure 3. Boxplots of the $\delta^{18}O_{smow}$ values in the bones of the thirteen marine mammal species considered in this study. The species denoted by white boxes were not included in the statistical analyses due to sample sizes of < 5 individuals. Habitats with different roman numerals and species within each habitat, indicated by different lowercase letters, are statistically different in their mean values according to the Scheffé post-hoc test using nested ANOVA. Boxes represent the first and third quartiles, lines the median, and vertical bars indicate the 95% confidence interval. Species: Lahille’s bottlenose dolphins (Tt), South American sea lions (Of), South American fur seals (Aa), killer whales (Oo), Franciscana dolphins (Pb), false killer whales (Pc), common dolphins (Dd), Burmeister's porpoises (Ps), Risso’s dolphins (Gg), spectacled porpoises (Pd), Fraser's dolphins (Lh), long-finned pilot whales (Gm), and Cuvier’s beaked whales (Zc).

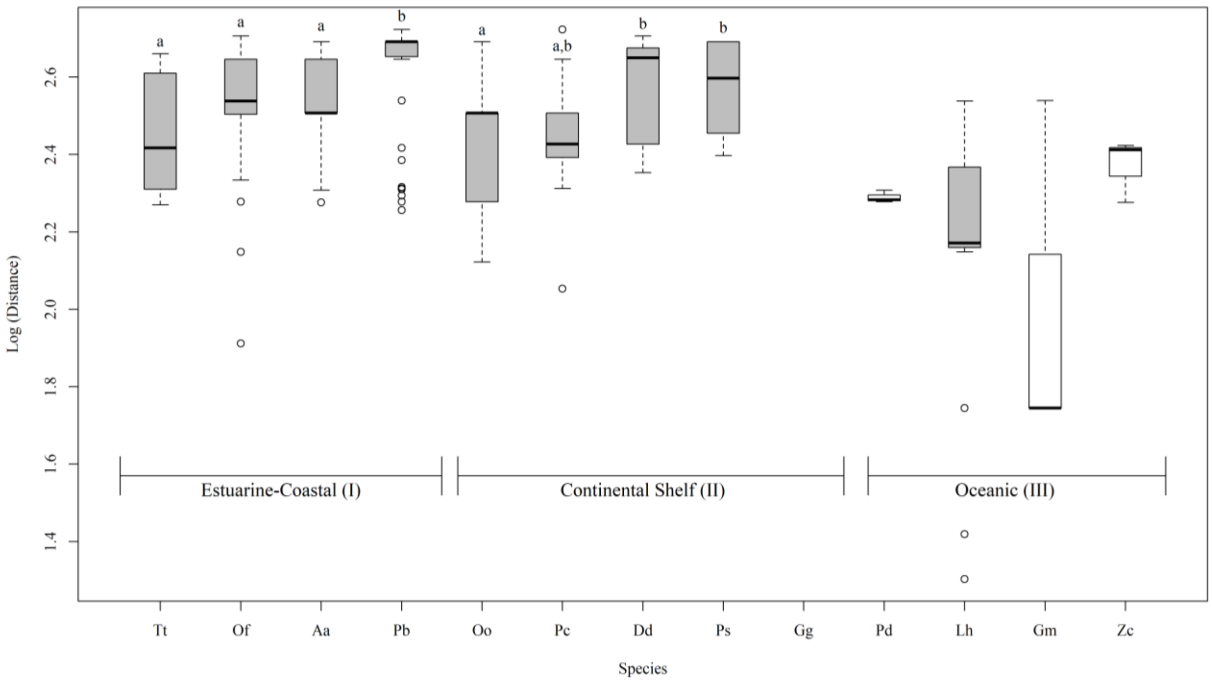

Figure 4. Boxplots of the distance from the inner part of the estuary for the thirteen marine mammal species considered in this study. The species denoted by white boxes were not included in the statistical analyses due to sample sizes of < 5 individuals. Habitats with different roman numerals and species within each habitat, indicated by different lower case letters, are statistically different in their mean values according to the Scheffé post-hoc test using nested ANOVA. Boxes represent the first and third quartiles, lines the median, and vertical bars indicate the 95% confidence interval. Species: Lahille’s bottlenose dolphins (Tt), South American sea lions (Of), South American fur seals (Aa), killer whales (Oo), Franciscana dolphins (Pb), false killer whales (Pc), common dolphins (Dd), Burmeister's porpoises (Ps), Risso’s dolphins (Gg), spectacled porpoises (Pd), Fraser's dolphins (Lh), long-finned pilot whales (Gm), and Cuvier’s beaked whales (Zc).