Temporal variability in stable isotope ratios of C and N in the vibrissa of captive and wild adult South American sea lions *Otaria byronia*: More than just diet shifts

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Abstract

We analyzed the δ^{13}C and δ^{15}N values in the vibrissae of captive adult breeding South American sea lions (*Otaria byronia*) fed at a constant diet and then used this information to analyze the change in stable isotope values along the vibrissae from wild individuals. The overall diet-to-vibrissa discrimination factor of the captive animals was 3.0‰ ± 0.1‰ for δ^{13}C and 3.6‰ ± 0.1‰ for δ^{15}N, but the stable isotope ratios fluctuated periodically despite constant diet. The δ^{13}C and δ^{15}N values of the captive male declined at the end of the breeding season, whereas the δ^{13}C values of the female increased during the central part of pregnancy and the δ^{15}N values peaked during lactation. The δ^{13}C and δ^{15}N values of adult wild specimens also fluctuated periodically and vibrissae growth rate (0.15 mm/d in both sexes) was slightly lower than in captivity (0.17 mm/d), assuming an annual periodicity for oscillations. Similarities in the amplitude of the cycles of captive and wild males suggested that fasting was probably the main source of periodic variability in the δ^{15}N of wild males, whereas pregnancy and lactation were probably the main source of periodic variability for the δ^{13}C of wild females.

Key words: diet, inert tissue, *Otaria flavescens*, sea lion, stable isotopes, vibrissa.

Large air breathing predators are major components of marine food webs (Estes *et al.* 2011), but our understanding of their actual role is limited by our ability to reconstruct their diets. Most of the available dietary information is derived from the analysis of the stomach contents of dead stranded individuals (*e.g.*, Koen Alonso *et al.*

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2000), regurgitates of adults and chicks at nesting colonies (*e.g.*, Wilson *et al.* 2005) and scats collected at rookeries and haul-out sites (*e.g.*, Romero *et al.* 2011), but all these approaches have limitations. Seasonal bias is one of them, as most marine birds and pinnipeds can be accessed by researchers only during the breeding period.

Stable isotope analysis has become a standard technique in ecological studies during the past two decades (Rubenstein and Hobson 2004, West *et al.* 2006, Martínez del Rio *et al.* 2009) and is particularly well suited to address the foraging habits of cryptic stages (Reich *et al.* 2007). The basic assumption of the method is that stable isotope ratios in animal tissues integrate those in diet, plus a trophic discrimination factor (TDF) that is tissue, diet, and taxa specific (Caut *et al.* 2009). The time window integrated by each tissue depends on its turnover rate and ranges from a few days to several years, but metabolically inert tissues represent a consumer's diet at the time of deposition, and hence these tissues can be used as a timeline of the consumer's isotopic history. Accordingly, changes in stable isotope ratios across inert tissues have been used to reconstruct dietary changes throughout time in a variety of air-breathing marine predators (*e.g.*, Hobson and Schell 1998, Cardona *et al.* 2009, Cherel *et al.* 2009, Ramos *et al.* 2009, Borrell *et al.* 2013, Aguilar *et al.* 2014).

However, a number of physiological processes have been identified as potential sources of variability in the stable isotope ratios independent from diet changes, which might complicate the interpretation of the stable isotope record of inert tissues. Fasting, for instance, has strong influence on the stable isotope ratios of some vertebrates (Hobson *et al.* 1993, Polischuk *et al.* 2001, Fuller *et al.* 2005, but see Kempster *et al.* 2007, Gómez-Campos *et al.* 2011, Aguilar *et al.* 2014), and hence the cyclical changes in the stable isotope values of wild marine mammals interpreted as shifts in foraging grounds (*e.g.*, Cherel *et al.* 2009, Kernaléguen *et al.* 2012) could indeed reveal cyclical changes in food availability (Beltran *et al.* 2016, Matthews and Ferguson 2015, Vales *et al.* 2015). On the other hand, pregnancy has been reported to modify the nitrogen balance of females, and hence TDF (Fuller *et al.* 2004). Furthermore, the stable isotope values of milk differ from those of the mother's tissues (Jenkins *et al.* 2001, Habran *et al.* 2010, Cherel *et al.* 2015, Borrell *et al.* 2016), indicating that lactation might result in major changes in the stable isotope values of lactating females, although to our knowledge no experimental evidence exists. All this suggests that confounding factors associated with metabolically induced changes in TDF during the annual breeding cycle may exist in mammals and may interfere with the interpretation of stable isotope ratios in inert tissues.

Vibrissae have been used recently for analyzing temporal trends in the diet of otariids (Cherel *et al.* 2009; Lowther *et al.* 2011; Kernaléguen *et al.* 2012, 2015; Franco-Trecu *et al.* 2014; Baylis *et al.* 2015; Vales *et al.* 2015), as they have a rather constant growth rate (Hirons *et al.* 2001, Cherel *et al.* 2009) and laboratory experiments have reported negligible variation in δ^{13}C and δ^{15}N values along the vibrissa of captive otariids and phocids kept on a constant diet (Hobson *et al.* 1996, Hirons *et al.* 2001). However, nothing is known about the influence of the annual breeding cycle on the stable isotope ratios of vibrissa. The South American sea lion (*Otaria byronia*) is a highly dimorphic otariid (Sepúlveda *et al.* 2013) with males and females differing in reproductive and foraging strategies (Campagna 1985, Koen Alonso *et al.* 2000, Campagna *et al.* 2001, Drago *et al.* 2010). In this paper, we have analyzed the stable isotopes of carbon and nitrogen in the vibrissa of captive adult breeding South American sea lions fed a constant diet to (1) calculate the diet to vibrissa discrimination factor for this species and (2) test the hypothesis that physiological changes associated with the breeding cycle do not influence stable isotope ratios in vibrissae. We then

used this information to analyze the fluctuations of the stable isotopes of carbon and nitrogen in the vibrissae of wild adult breeding South American sea lions.

Material and Methods

Captive Animals

Vibrissae from one adult male sea lion (Alvin) and one adult female (Demi) from the reproductive group kept in captivity at L'Oceanogràfic (Valencia, Spain) were collected on 22 February 2012. Alvin was 8 yr old and the only adult male in the group. He had been reproductively active during the three previous boreal summers, during which his food consumption from June to September was dramatically reduced. Demi was 11 yr old and was the only reproductive female in the group. She had given birth only twice: 6 July 2009 and 16 July 2010. Both individuals had been held on a constant diet of thawed herring (*Clupea harengus*) and capelin (*Mallotus villosus*) for at least 3 yr prior to isotopic analysis. The stable isotope values of the white muscle of the herring ($\delta^{13}C$ = –18.6‰ ± 0.3‰; $\delta^{15}N$ = 12.6‰ ± 0.5‰) and capelin ($\delta^{13}C$ = –20.6‰ ± 0.3‰; $\delta^{15}N$ = 11.7‰ ± 0.5‰) used to feed the captive sea lions had been previously reported by Drago *et al.* (2015). Vibrissae were collected during one of the health checks conducted by the veterinary staff of L'Oceanogràfic on 22 February 2012. Animals attended voluntarily to the health check and were unrestrained while vibrissae were cut at their base. The intradermal section of the vibrissa was not sampled.

Wild Animals

Vibrissae from five adult male and five adult female sea lions were collected from dead individuals stranded along 750 km of coastline in northern and central Patagonia (Fig. 1) from 2006 to 2011 (Table 1). The longest vibrissa was collected from each individual including the follicle, so the whole vibrissa was available for analysis. No manipulation of live wild animals occurred during this study as sea lions were found dead on beaches. The age of the sampled individuals had previously been assessed by counting growth layer groups in the dentine of the canines (assuming annual deposition) in thin ground sections or acid-etched highlighted teeth (Perrin and Myrick 1980, Crespo 1988), except in a few individuals (Table 1). In those cases, adulthood was assumed on the basis of body size. The life span of South American sea lions is around 20 yr and they become physiologically mature between 4 and 6 yr, although males mate for the first time when they are 9 yr old or more (Crespo 1988).

Stable Isotope Analysis

Vibrissae were hand washed with running water, dried and stored at ambient temperature until processing. Once in the laboratory, vibrissae were washed in a chloroform/methanol (2:1) solution and cleaned in distilled water for 5 min in an ultrasonic bath to eliminate any surface contaminants (Newsome *et al.* 2010). The entire vibrissae, from the most recent part synthesized at the base to the tip, were measured, dried in a stove at 60°C for 36 h and cut into 3 mm long consecutive sections starting from the proximal end (Cherel *et al.* 2009), each integrating 18 d (see Results for the growth rate).
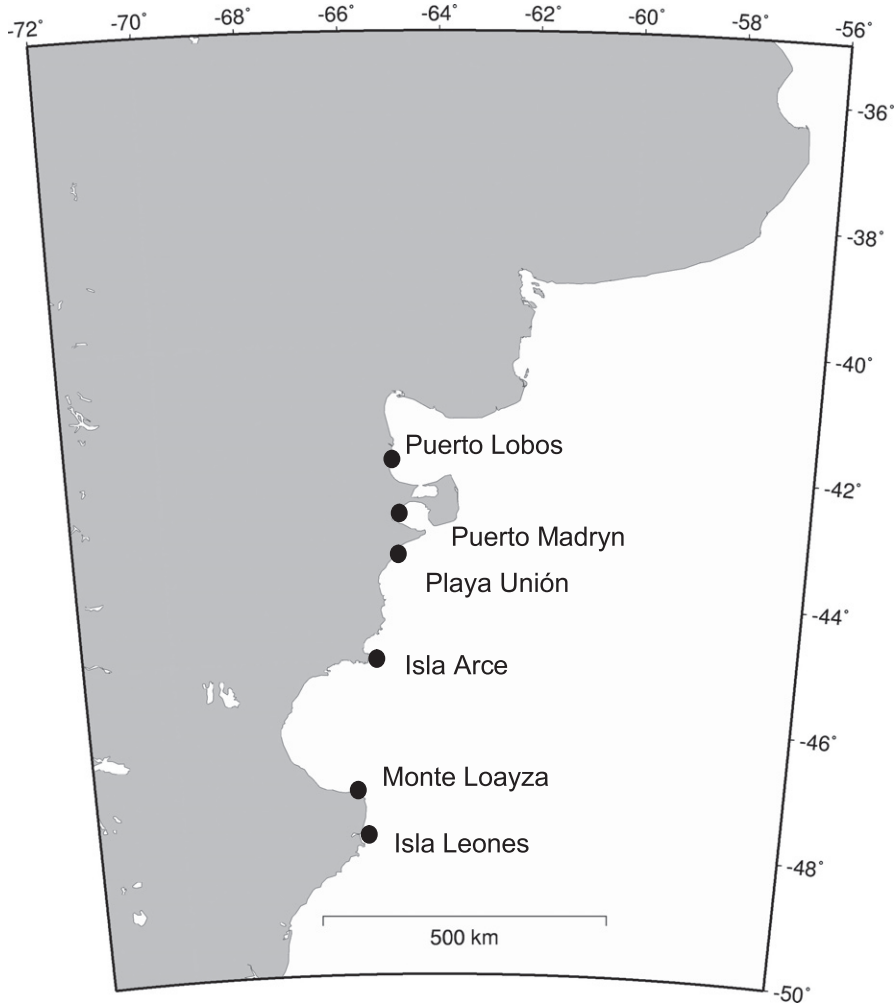
Figure 1. Map of northern and central Patagonia (Argentina) showing the sampling sites.

Approximately 0.3 mg of vibrissa were weighed into tin cups (3.3 × 5 mm), combusted at 900°C and analyzed in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo Finnigan, Bremen, Germany). Atropine was used as a system check for elemental analyses. Samples were processed at the Centres Científics i Tecnològics of the Universitat de Barcelona. The abundance of stable isotopes, expressed in delta (δ) notation, were calculated as the relative variation of stable isotope ratios expressed as per mil (‰) deviations from predefined international standards as:

$$\delta X = \left[\left(R_{\text{sple}}/R_{\text{stnd}}\right) - 1\right] \times 1,000$$

where X is ^{13}C or ^{15}N, and R_{sple} and R_{stnd} are the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the sample and standard, respectively. The $\delta^{13}C$ standard was Vienna PeeDee Belemnite

Table 1. Summary statistics of the vibrissae analyzed. Stable isotope ratios are reported as mean ± SD. Pto: Puerto; I: Isla, Mt: Monte.

Specimen	Sex	Sampling year and locality	Age (yr)	Vibrissa length (cm)	3 mm sections	Correlation between δ^{13}C and δ^{15}N		Fourier analysis Period of the major oscillation (3 mm sections)	
						r	P	δ^{13}C	δ^{15}N
Captive									
Alvin	♂	L'Oceanogràfic	8	14.4	48	0.744	<0.001	25	25
Demi	♀	L'Oceanogràfic	11	18.3	63	0.129	0.312	25	17
Wild									
OF 817	♂	2006-Pto.Lobos	9	15.8	53	0.792	<0.001	20	13
OF 1017	♂	2007- I.Leones	10	12.5	42	0.635	<0.001	25	25
OF 1117	♂	2011-Pto.Madryn	9	7.1	24	0.681	<0.001	13	13
OF 23	♂	2010-Mt.Loayza	Adult	10.8	36	0.439	0.007	20	20
OF 1100	♂	2010-Pt.Madryn	4+	14.2	47	0.319	0.029	17	17
OF 1081	♀	2010-I.Arce	Adult	7.1	24	0.756	<0.001	11	25
OF 1071	♀	2010-PlayaUnion	20	9.4	31	0.784	<0.001	17	14
OF 1128	♀	2011-Mt.Loayza	14+	11.6	39	0.693	<0.001	13	13
OF 1068	♀	2009-Pt.Madryn	6	12.8	43	0.360	0.018	14	11
OF 1138	♀	2011-Mt.Loayza	6+	10.2	34	0.434	0.010	17	25

(VPDB) calcium carbonate, and the δ^{15}N standard was atmospheric nitrogen (N$_2$). International standards (ammonium sulphate, potassium nitrate, and glutamic acid for δ^{15}N and polyethylene, sucrose, and glutamic acid for δ^{13}C) were inserted after every 12 samples to calibrate the system and compensate for any drift over time. Precision and accuracy for both δ^{13}C and δ^{15}N measurements were 0.1‰ and 0.3‰, respectively.

Data Analysis

Data are shown as means ± standard deviation (SD), unless otherwise stated. Pearson correlation was used to assess the significance of correlation between δ^{13}C and δ^{15}N values along individual vibrissae. The assumptions of normality and homogeneity of variance were checked using the Lilliefors and Leven tests, respectively. The Student t-test was subsequently used to compare the average period and amplitude of cycles in wild males and females.

The diet-to-vibrissa trophic discrimination factor (TDF) was calculated in captive sea lions as the offset between diet and vibrissae stable isotope ratios. According to the daily record of food consumption, Alvin's diet included 83.3% herring and 16.7% capelin, whereas Demi's diet included 68.7% herring and 31.3% capelin. The δ^{13}C and δ^{15}N values of Alvin's diet were –18.9‰ ± 0.2‰ and 12.5‰ ± 0.5‰, respectively and those of Demi's diet were –19.2‰ ± 0.3 ‰ and 12.3‰ ± 0.2‰, respectively. Vibrissa stable isotope ratios were expected to lay within the 95% confidence interval of the diet ratios if this was the only source of variability.

Fourier, or spectral, analysis was run with IBM SPSS Statistics 21 to determine the existence of periodic oscillations. Data were Fourier transformed using the default option in SPSS and smoothed using a Tukey-Humming time window ($n = 5$). This transformation allowed identifying waveforms in terms of trigonometric functions from the raw periodograms, and the spectral peaks revealed after Fourier transformation were assumed to represent periodicities in the time series (Warner 1998).

The Bayesian mixing model SIAR (Stable Isotope Analysis in R; Parnell *et al.* 2008) was used to assess the influence of potential physiological fluctuations on the output of mixing models used for diet reconstructions on the basis of stable isotope ratios. First, we used the values from all the sections of Alvin's and Demi's vibrissae to test the accuracy of SIAR in reconstructing their diets. Second, we used SIAR to reconstruct the diets of Alvin and Demi on the basis of stable isotope ratios in the 3 mm sections into the upper and lower δ^{15}N deciles.

<div align="center">RESULTS</div>

Captive Animals

Stable isotope ratios varied largely along the vibrissae of both specimens (Fig. 2). Most of the variability was within the limits expected according to the standard deviation of the stable isotope ratios of the diet, but the simultaneous decline in δ^{13}C and δ^{15}N at 1.5 cm, 8.1 cm, and 13.8 cm from the base of Alvin's vibrissa resulted in values well below those expected from diet variability and deserve a different explanation. The same was true for the peaks in δ^{13}C at 10.6 cm and 16.9 cm and the peaks in δ^{15}N at 6.3 cm and 11.4 cm from the base of Demi's vibrissa, when values were above those expected from diet variability.
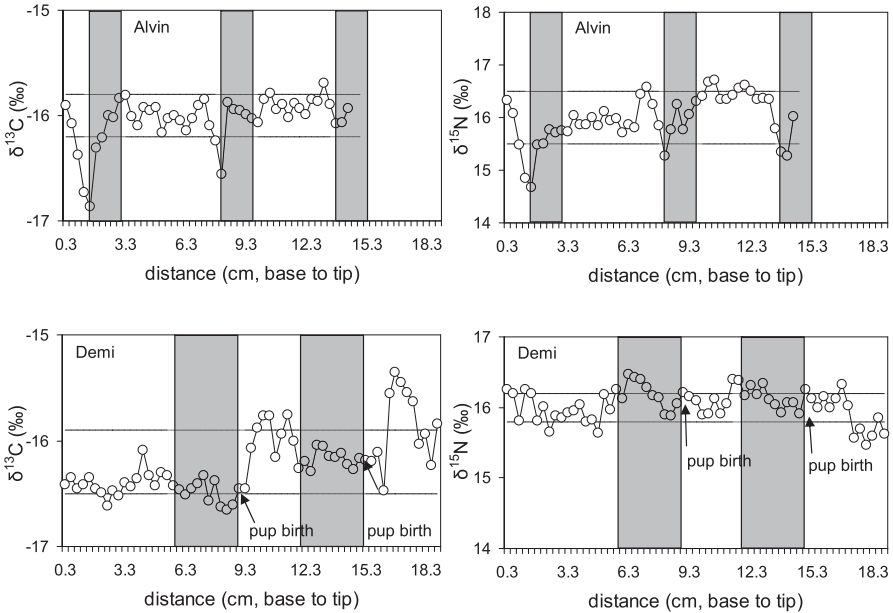
Figure 2. Profiles of $\delta^{13}C$ and $\delta^{15}N$ values along the vibrissa of an adult male (Alvin, top panels) and an adult female (Demi, bottom panels) South American sea lion kept in captivity and fed a constant diet. Shadow sections show the rutting period of Alvin in 2009, 2010, and 2011 (June to September) and the lactation period of Demi in 2009 and 2010 (July to January). Younger tissue is on the left and consistent growth rate is assumed. Horizontal lines show the 95% confidence interval of the mean values expected if diet was the only source of variability.

Synchronous changes in the $\delta^{13}C$ and $\delta^{15}N$ values of Alvin's vibrissa sections resulted in statistically significant correlation (Table 1). This was not true for the $\delta^{13}C$ and $\delta^{15}N$ values of Demi's vibrissa sections, as stable isotope ratios peaked at different times (Table 1). Nevertheless, Fourier analysis revealed the existence of two overlapping periodic oscillations for $\delta^{15}N$ and $\delta^{13}C$ in both Alvin and Demi (Table 1). Assuming an annual periodicity for those oscillations, the average vibrissae growth rate in Alvin and Demi was 0.17 ± 0.01 mm/d.

Peaks in the $\delta^{13}C$ values of Demi's preceded drops in the $\delta^{13}C$ and $\delta^{15}N$ values of Alvin by 2.7 ± 0.3 cm. According to the reported growth rate above, the peaks in the $\delta^{13}C$ values of Demi predated in 162.7 ± 20.9 d the drops in Alvin's $\delta^{13}C$ and $\delta^{15}N$ values. The length of the intradermal vibrissae was assumed to be 16.0 mm and it was supposed to have grown during the 96 d prior to sampling. If so, the first 3 mm section of the Alvin's and Demi's vibrissae integrated their diets from 97 to 115 d before the sampling date (22 February 2012), *i.e.*, from 30 October 2011 to 17 November 2011. Accordingly, the first drop in the $\delta^{13}C$ and $\delta^{15}N$ values of Alvin corresponded to the period from 19 August 2011 to 6 September 2011 and the two subsequent drops to late summer 2010 and late summer 2009, assuming annual periodicity.

Alvin had been in the rutting state from June to September every year from 2009 to 2011 and its daily rate of food ingestion decreased dramatically at that time.

Consequently, the periodic drops in $\delta^{13}C$ and $\delta^{15}N$ likely corresponded to the last part of the rutting period and resulted from reduced food consumption. Conversely, Demi's $\delta^{13}C$ values peaked during May and June 2009, 2010, and 2011, although the 2009 and 2010 peaks were higher and lasted for longer. It is worth noting that Demi gave birth in 2009 and 2010. Demi was pregnant but did not give birth in 2011, so the height and the duration of the peak is likely related to the development of the fetus. Finally, it is also worth noting that Demi's $\delta^{15}N$ values initially decreased after parturition to increase steadily during the following lactation period, when values were well above the 95% CI of the vibrissa average. In summary, $\delta^{13}C$ values peaked during the central part of pregnancy and $\delta^{15}N$ values peaked during lactation. This explains why $\delta^{13}C$ and $\delta^{15}N$ values were uncorrelated in Demi's vibrissa (Table 1).

According to the daily record of food consumption, Alvin's diet included 83.3% herring and 16.7% capelin, whereas Demi's diet included 68.75% herring and 31.25% capelin. The $\delta^{13}C$ and $\delta^{15}N$ values of Alvin's diet were $-18.9‰$ and $12.5‰$, respectively and those of Demi's diet were $-19.2‰$ and $12.3‰$, respectively. This resulted in an average offset between the $\delta^{13}C$ and $\delta^{15}N$ values in the vibrissae of the captive animals and those in their diets of $3.0‰ \pm 0.1‰$ for $\delta^{13}C$ and $3.6‰ \pm 0.1‰$ for $\delta^{15}N$, indicative of the diet to vibrissa TDF for South American sea lions.

SIAR accurately reconstructed Alvin and Demi's diets when all vibrissa sections were used (Fig. 3). However, SIAR largely overestimated the contribution of capelin to the diets of both adult sea lions when only the 3 mm sections in the lower $\delta^{15}N$ decile were considered. Bias was not so high when using the stable isotope ratios from the higher decile.
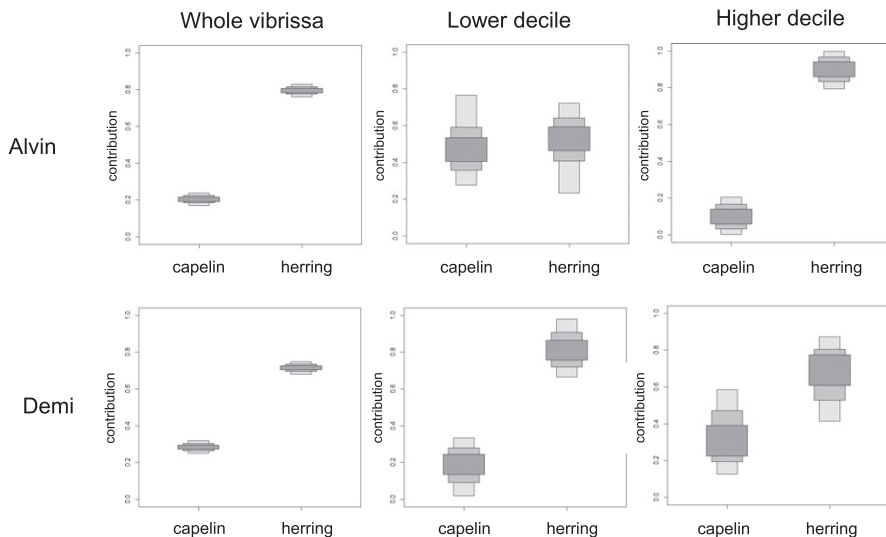

Figure 3. Feasible contribution of herring and capelin to the diet of two South American sea lions (Alvin and Demi) kept in captivity and fed a constant diet. Contributions were estimated by SIBER according to the stable isotope ratios of the whole vibrissae, the 3 mm segments more depleted in ^{15}N (lower decile) and the segments more enriched in ^{15}N (higher decile).

Wild Animals

The stable isotope values of vibrissae of the five adult males studied varied cyclically (Fig. 4, 5) and there was a significant correlation between $\delta^{13}C$ and $\delta^{15}N$, although it was weak in the youngest specimens (Table 1). The average period of the $\delta^{13}C$ cycles in males was 19.0 ± 3.4 sections and that of the $\delta^{15}N$ cycles was 17.6 ± 4.5 sections (Table 1), which were significantly shorter than the cycles observed in captive animals ($\delta^{13}C$: $t = 3.038$, df $= 4$, $P = 0.038$; $\delta^{15}N$: $t = 3.258$, df $= 4$, $P = 0.031$) and suggested a slightly slower growth rate of male vibrissae in the wild (0.15 ± 0.03 mm/d, assuming an annual periodicity for those oscillations). On the other hand, the amplitude of the stable isotope variation was larger than in captive individuals for the $\delta^{13}C$ values (mean: $1.6\%_0 \pm 0.23\%_0$, $t = 3.302$, df $= 4$, $P = 0.030$) but not for those of $\delta^{15}N$ (mean: $1.9\%_0 \pm 0.9\%_0$, $t = 2.000$, df $= 4$, $P = 0.116$).

The $\delta^{13}C$ and the $\delta^{15}N$ values of wild females also varied cyclically in phase (Fig. 5; Table 1). The average period of $\delta^{13}C$ cycles was 14.4 ± 2.6 sections and the average period of $\delta^{15}N$ cycles was 17.6 ± 6.8 sections. These figures were significantly shorter than the cycles observed in captivity for $\delta^{13}C$ ($t = 9.089$, df $= 4$, $P = 0.001$) but not for $\delta^{15}N$ ($t = 0.196$, df $= 4$, $P = 0.854$) and the period of the $\delta^{15}N$ values suggested a growth rate of female vibrissae in the wild similar to that of males (0.15 ± 0.06 mm/d, assuming an annual periodicity for those oscillations). The amplitude of the stable isotope variation was larger than in captive individuals for $\delta^{15}N$ (mean: $2.4\%_0 \pm 0.3\%_0$, $t = 11.068$, df $= 4$, $P = <0.001$) but not for the $\delta^{13}C$ values (mean: $2.1\%_0 \pm 0.4\%_0$, $t = 2.361$, df $= 4$, $P = 0.078$).

DISCUSSION

The use of stable isotope ratios in vibrissa to infer diet changes relies on the existence of suitable TDF values and the proper understanding of the sources of variability of stable isotope ratios along vibrissae. Here, we provide for the first time an estimate of TDF for the vibrissa of the South American sea lion, which are similar to the values reported previously for the vibrissa of other pinnipeds (Beltran *et al.* 2016) but differ from the values reported for other tissues of the South American sea lion (Drago *et al.* 2015). The growth rate of vibrissa estimated here was also similar to that reported by Hirons *et al.* (2001) for captive Steller sea lions (*Eumetopias jubatus*), although individual variability was large. Furthermore, we report for the first time broad variations in the $\delta^{13}C$ and $\delta^{15}N$ values of adult captive otariids fed a constant diet, in sharp contrast with previous research (Hobson *et al.* 1996, Hirons *et al.* 2001).

The $\delta^{13}C$ and $\delta^{15}N$ values of the captive South American sea lion male dropped during the rutting season, the $\delta^{13}C$ values of the captive female increased during the central part of pregnancy and her $\delta^{15}N$ values increased during lactation beyond the limits expected from the variability in the stable isotope ratios of the diet. The $\delta^{13}C$ and $\delta^{15}N$ values of adult wild specimens also fluctuated periodically and the amplitude of the $\delta^{15}N$ cycles of males and the $\delta^{13}C$ cycles of females did not differ from those of captive animals, although the vibrissae grew more slowly. This suggests that fasting associated to the rutting state was probably the major source of variability in the $\delta^{15}N$ values of wild males and that pregnancy and lactation were probably the main sources of periodical variability in the $\delta^{13}C$ values of wild females. The amplitude of the oscillations of the $\delta^{13}C$ cycles of wild males and the $\delta^{15}N$ cycles of wild
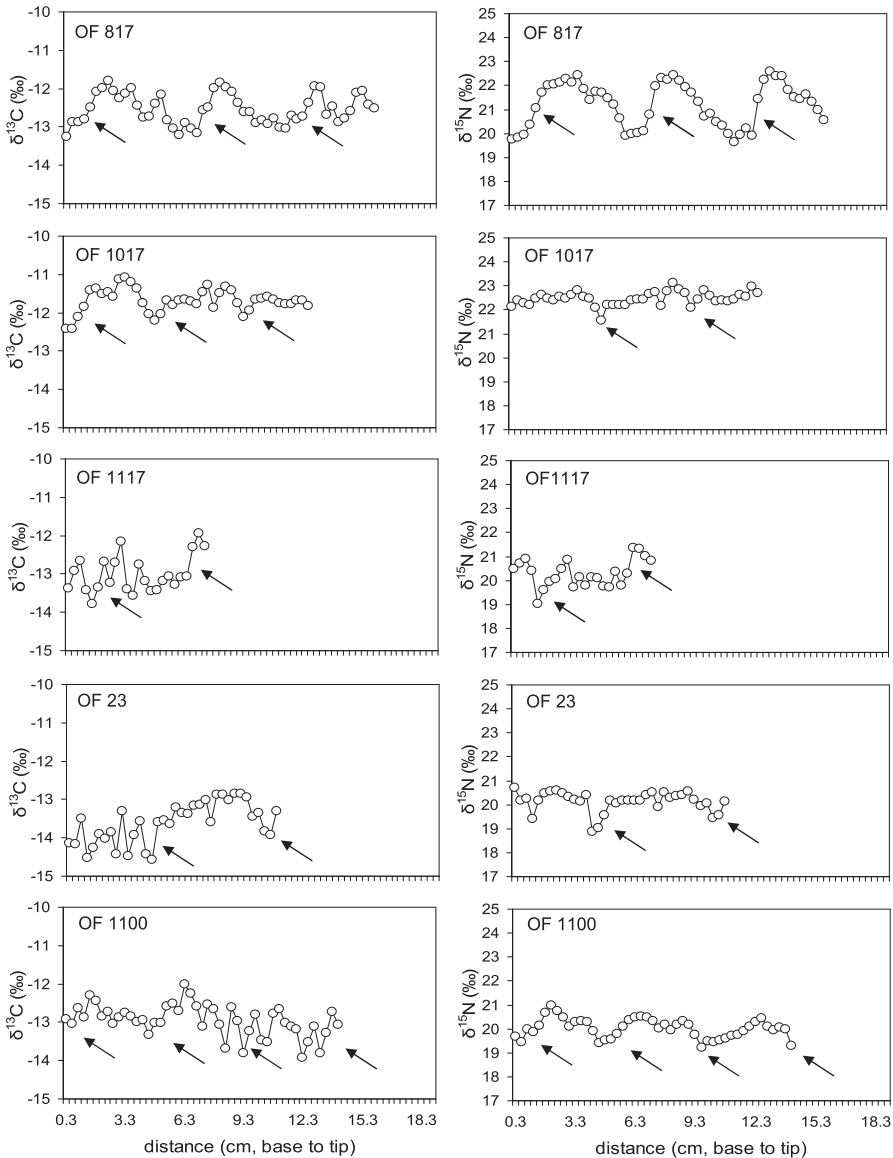
Figure 4. Profiles of δ^{13}C and δ^{15}N values along the vibrissae of five wild adult male South American sea lions from Patagonia. Younger tissue is on the left and consistent growth rate is assumed. Arrows show drops in stable isotope ratios tentatively assigned to the breeding season on the basis of simultaneous drops of δ^{13}C and δ^{15}N values.

females was larger than that observed in captivity, thus indicating that diet shifts might overlap and reinforce the fluctuations caused by physiological changes. On the other hand, it should be noted a large individual variability in the period of the oscillations of wild individuals, likely because of differences in the growth rate of vibrissa.
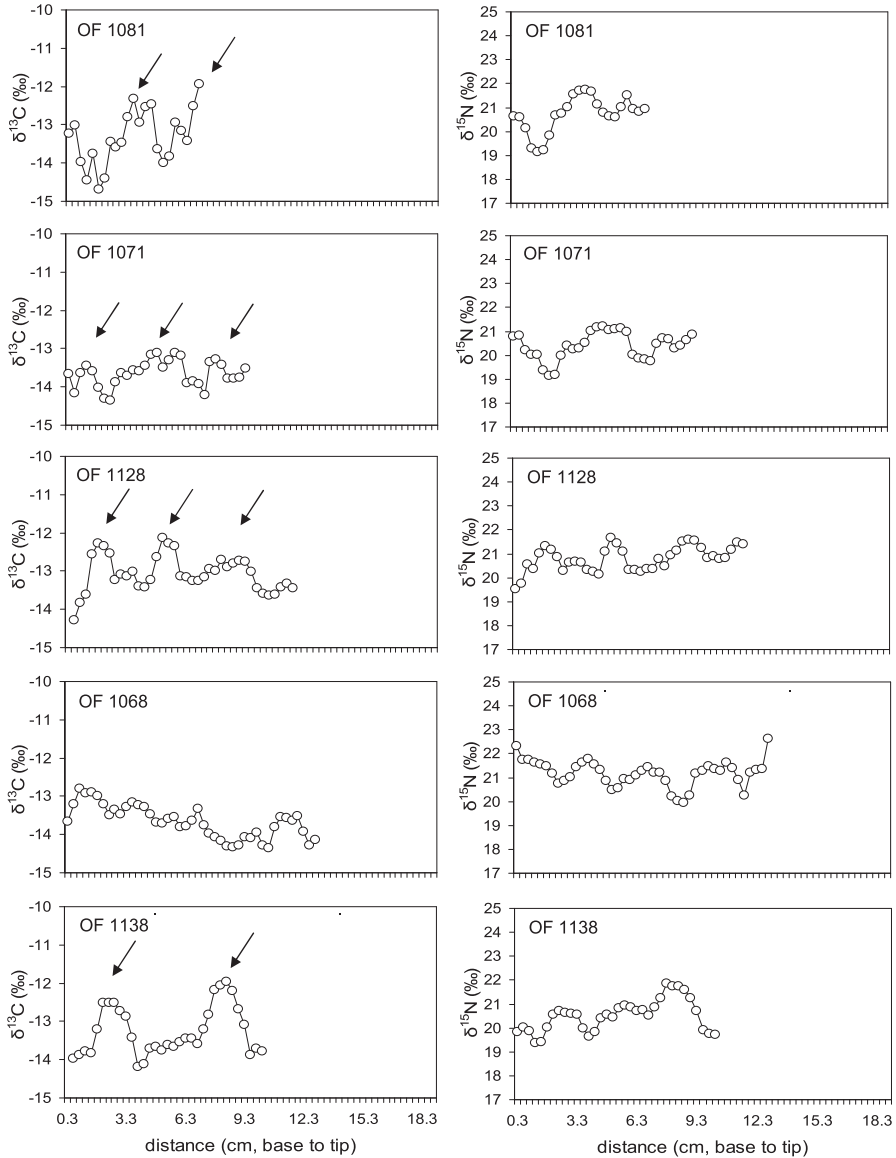
Figure 5. Profiles of $\delta^{13}C$ and $\delta^{15}N$ values along the vibrissae of five wild adult female South American sea lions from Patagonia. Younger tissue is on the left and consistent growth rate is assumed. Arrows show the peaks of $\delta^{13}C$ tentatively assigned to the central phase of pregnancy.

Similar variability has been reported for the growth rate and the period of the oscillations of baleen plates (Aguilar et al. 2014).

Wild male South American sea lions fast for as long as 40 d during the breeding season on the austral summer (Campagna 1985) and the captive male studied here also reduced food intake dramatically during the boreal summer months. Research on

birds suggests that fasting results in no change in $\delta^{13}C$ values but in an increase in $\delta^{15}N$ values due to protein mobilization and subsequent preferential excretion of ^{14}N (Hobson et $al.$ 1993). However, further research has revealed that, in marine mammals, fasting results in no change in stable isotope ratios (Gómez-Campos et $al.$ 2011) or in a decrease in both $\delta^{13}C$ and $\delta^{15}N$ (Polischuk et $al.$ 2001, Aguilar et $al.$ 2014, Matthews and Ferguson 2015, Vales et $al.$ 2015).The reasons for these contrasting results are not fully understood, but the high volume of fat reserves in marine mammals that mitigate the need for protein mobilization (Gómez-Campos et $al.$ 2011) and the possible capacity to recycle nitrogen to avoid osmotic unbalance during fasting have been invoked as possible explanations (Aguilar et $al.$ 2014).

Whatever the actual physiological explanation, the cyclical drops of $\delta^{13}C$ and $\delta^{15}N$ observed in the vibrissa of the captive male here studied, together with the fact that the lowest values were observed at the end of the fasting period, strongly support the hypothesis that fasting indeed causes dramatic drops in the $\delta^{13}C$ and $\delta^{15}N$ values of marine mammals. Physiological changes associated with the breeding cycle are also the most likely reason for the periodic drops in $\delta^{15}N$ observed in wild males, because the amplitude of the oscillations did not differ from that observed in captivity. Nevertheless, dietary changes cannot be ruled out in some individuals, because the variability in the $\delta^{13}C$ values along individual vibrisae of wild males was larger than that observed in captivity. For instance, the $\delta^{13}C$ and $\delta^{15}N$ values of male OF 23 revealed two major drops compatible with fasting during the breeding season, but after the most recent drop, the values of $\delta^{13}C$ fluctuated widely and frequently, probably indicating prey shifts. Likewise, the $\delta^{15}N$ values of male OF1100 exhibited three well-defined oscillations, but only the two most recent ones were associated with synchronous oscillations of the $\delta^{13}C$ values. Furthermore, the $\delta^{13}C$ values fluctuated largely along the oldest part of the vibrissa, probably indicating prey shifts.

In contrast to males, female South American sea lions alternate nursing and feeding bouts during the period of pup rearing (Campagna et $al.$ 2001) and are expected to rely primarily on diet to produce milk. In the otariids studied to date, milk protein is consistently depleted in both ^{13}C and ^{15}N as compared to the blood of lactating females (Cherel et $al.$ 2015), and this is also true for the whole milk of a diversity of other income breeders (Jenkins et $al.$ 2001). This suggests that lactation should result in the enrichment of female's tissues in ^{13}C and ^{15}N as lactation progresses. Enrichment in ^{13}C is expected to be particularly intense in lactating female otariids, as they produce milk with high fat contents and thus depleted in ^{13}C when compared with female tissues (Newsome et $al.$ 2006). The situation is different in female phocids and other capital breeders, which fast through lactation (Polischuck et $al.$ 2001, Ducatez et $al.$ 2008, Habran et $al.$ 2010, Borrell et $al.$ 2016), probably because fasting and lactogenesis have opposing effects of the stable isotope ratios of the female.

Consistent with the predictions for otariids discussed above, the $\delta^{13}C$ and $\delta^{15}N$ values of the captive female South American sea lion studied here increased slightly from parturition to weaning in the two lactation cycles monitored. But pregnancy had an even larger impact on the $\delta^{13}C$ values of the female than lactation, which resulted in the absence of correlation between $\delta^{13}C$ and $\delta^{15}N$ values when the whole vibrissae was considered. The five adult wild female South American sea lions here studied exhibited periodic oscillations of $\delta^{13}C$ and $\delta^{15}N$ along their vibrissa, as well as the females studied by Baylis et $al.$ (2015) in the Falkland/Malvinas Islands. As reported above, the amplitude of the $\delta^{13}C$ cycles in the wild females reported here matched that observed in captivity, and hence could be explained by the physiological changes associated to the breeding cycle. However, the amplitude of the $\delta^{15}N$

cycles was much larger than that caused by physiological changes, and could be related to the shift from pelagic/offshore prey to demersal/onshore prey after parturition (Drago *et al.* 2010). Such a shift could also explain why the amplitude of the oscillation of the δ^{15}N values of females exploiting coastal foraging grounds off the Falkland/Malvinas Islands are much larger than those of females exploiting offshore grounds (Baylis *et al.* 2015).

Nevertheless, inferring dietary information from the variations in the stable isotope ratios along the vibrissae of South American sea lions is not straightforward. For instance, the magnitude of the changes in the δ^{13}C and the δ^{15}N values of captive sea lions is equivalent to the difference between large hake (*Merluccius hubbsi*) and banded cusk eel (*Raneya brasiliensis*), two major prey for wild South America sea lions in northern Patagonia (Drago *et al.* 2010). This makes it almost impossible to discriminate such a dietary shift from the effects of the breeding cycle. This is well illustrated by the biases observed in the reconstruction of the diet of Alvin and Demi using SIAR, revealing large biases when the most ^{15}N-depleted 3 mm sections are considered. Thus TDF values computed for the whole vibrissa are unsuitable to infer diet at times when the physiological status of the individual is altered.

In conclusion, both dietary shifts and the physiological processes related to the breeding cycle may influence the stable isotope values in the vibrissae of otariids and probably other metabolically inert tissues. Thus, caution is needed when interpreting variability solely as evidence of dietary or habitat shifts and further studies in captivity with sexually active adults are necessary to improve our understanding of the role of the breeding cycle of TDF. Unfortunately, such studies are limited by the impossibility of keeping more than one adult male in each reproductive group and the uncommon presence of many species at zoos.

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