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Diet of lactating South American sea lions, as inferred from stable isotopes, influences pup growth

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ABSTRACT

Serum and blood cell $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signals from 26 suckling pups of the South American sea lion from northern Patagonia were used as proxies of the composition of their mothers' diet to test the hypothesis that the foraging habits of the mother influence pup growth. Samples of primary producers and the female potential prey were analyzed to establish baseline isotopic values and to determine energy density. Pups were weighed to determine specific growth rate. Individual variability in female diet was large, probably as a consequence of dissimilarities in the foraging performance that depends on the individual's age, body size, and/or foraging skills. Growth of a pup was influenced by its mother's diet, as pups of females mostly relying on pelagic offshore prey were found to grow faster than those of females basing their diet on benthic coastal prey.

Key words: South American sea lion, *Otaria flavescens*, diet, growth rate, suckling pup, lactating females, stable isotopes.

The South American sea lion, *Otaria flavescens* (Shaw 1800), is the most abundant marine mammal occurring along the southern tip of South America (Cappozzo 2002). Males and females exhibit contrasting feeding habits (Koen Alonso *et al.* 2000, Campagna *et al.* 2001) due to their marked sexual dimorphism (Cappozzo 2002). In Northern Patagonia, stomach content analysis shows that the main prey in males is the offshore demersal Argentine hake, *Merluccius hubbsi*, whereas in females it is the coastal benthic red octopus, *Enteroctopus megalocyathus* (Koen Alonso *et al.* 2000).

However, time-depth recorders and satellite tracking have revealed high levels of variability in the foraging patterns of lactating females of South American sea lions from the same rookery (Werner and Campagna 1995, Thompson *et al.* 1998, Campagna *et al.* 2001), a phenomenon also reported for other sea lion species (Chilvers *et al.* 2005, Villegas-Amtmann *et al.* 2008). The available data indicate that some lactating females from northern Patagonia and the Falkland (Malvinas) Islands behave as benthic foragers whereas others behave as pelagic predators, in some cases even exploiting the offshore feeding grounds typically used by males (Werner and Campagna 1995, Thompson *et al.* 1998, Campagna *et al.* 2001).

Targeting coastal benthic prey might be especially convenient for lactating females as a way to reduce the intervals between suckling bouts (Trillmich 1990, Boyd *et al.* 1998, Costa 2008) and to keep females with their pups for longer periods to safeguard the pups from the risk of being killed by subadult males during the breeding season (Campagna *et al.* 1988, Werner and Campagna 1995). On the other hand, pelagic potential prey, whose highest abundance is recorded in offshore areas (Brunetti *et al.* 1998, Cousseau and Perrotta 2000), typically have a higher energy density than coastal benthic ones (Eder and Lewis 2005), although the increased travel cost may counteract the benefit of preying on offshore pelagic prey with a higher energy density (Costa 2008). Comparing the growth performance of sucking pups from females using contrasting foraging strategies is one of the available methods to investigate these trade-offs.

Although a combination of methods is often the best approach for dietary studies, diet determination based on stomach content or scat analyses is not appropriate for this study. This is because these methods provide only a single "snapshot" of the diet of each individual just before sampling and, hence, allow the study of feeding variability among well-defined *a priori* groups, like morphotypes, sexes, or age classes, but not that of individuals, unless through repeated sampling. However, repeated sampling of large animals for stomach content analysis is extremely difficult and being able to assign scats to particular individuals is highly unlikely in crowded rookeries. Stable isotope analysis offers a suitable alternative because the consumer tissues reflect those of their prey in a predictable manner over a long period of time (DeNiro and Epstein 1978, 1981).

The blood isotopic signal of suckling pups of pinnipeds and other mammals and that of their mothers are positively and linearly correlated, although some fractionation exists (Jenkins *et al.* 2001, Ducatez *et al.* 2008). Hence, the isotopic signal of pup tissues would reflect that of the mother's diet, after correcting the prey-to-predator and mother-to-pup fractionations. Pups of the South American sea lions enter water for the first time when they are about 3 or 4 wks old (Campagna 1985) and milk is their exclusive diet during at least their first 3 wks of life, although the whole lactation period lasts approximately one year (Ofstedal *et al.* 1987). Although reconstructing precisely the diet of lactating females of the South American sea lion from the isotopic values of pups' blood is impossible because the above reported fractionation factors have not been determined experimentally for this species, pup isotopic values can be used as proxies of the mother's diet if the isotopic landscape of the foraging grounds of females is properly described. Furthermore, as serum half-life in endotherms is 3–4 d and that of blood cells is 28–30 d (Hobson and Clark 1993, Hilderbrand *et al.* 1996), the isotopic signal of blood cells collected just after birth and that of serum collected 3 wks later can be used respectively as proxies of the female diet in late pregnancy and early lactation.

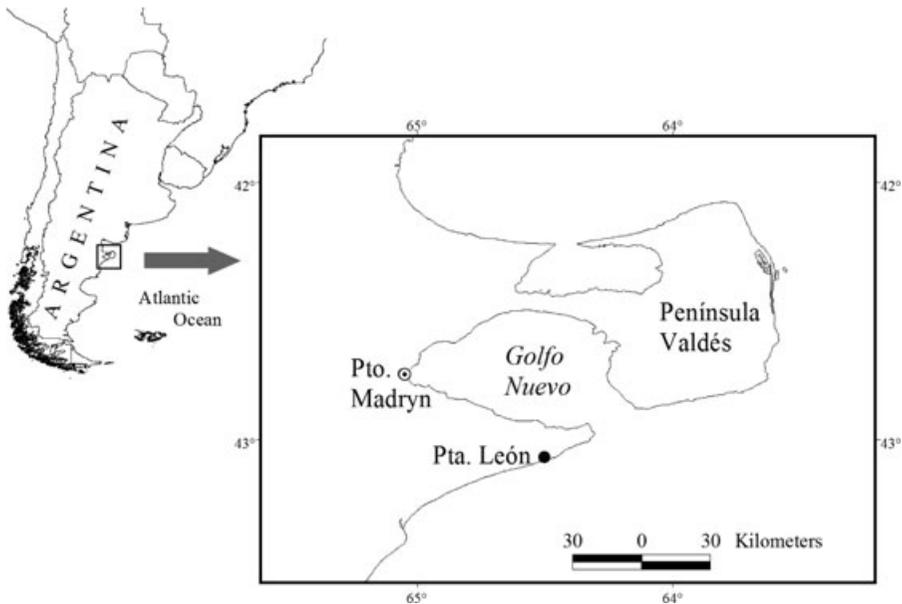


Figure 1. Study area.

In this article, carbon and nitrogen stable isotopes of blood from suckling pups were used to characterize the diet of female South American sea lions during late pregnancy and early lactation, and to test the hypothesis that contrasting foraging strategies of nursing females affect the growth of their pups.

METHODS

Study Area

This study was conducted in Punta León ($43^{\circ}06'S$, $64^{\circ}29'W$), a provincial reserve, which is located on the Atlantic coast of Argentina, 25 km south of Golfo Nuevo and about 80 km from the city of Puerto Madryn, in the Chubut Province (Fig. 1). This site was chosen because one of the most important breeding areas in Northern Patagonia and has among the highest rate of annual growth (Dans *et al.* 2004). During the last decade, the breeding area of Punta León has changed considerably, increasing both in number of pups and area of occupation, and new breeding areas have developed south of the traditional area (Dans *et al.* 2004, Grandi *et al.* 2008). These new breeding areas have a social structure different from the traditional one, as described by Campagna and Le Boeuf (1988), being higher in the proportion of juveniles of both sexes and of subadult males (Grandi *et al.* 2008).

Sampling

Sampling was carried out during the 2006 breeding season, extending from mid December to early February (Campagna 1985). Twenty six 1-wk-old pups (11 males

and 15 females) were captured at random using a noose pole (Gentry and Holt 1982). They were placed in a nylon bag and weighed on a 50 kg (± 0.25 kg) capacity spring balance; then they were sexed, bleach-marked (Campagna *et al.* 2001), and finally released close to their mothers after extracting about 2–5 mL of blood from the caudal gluteal vein in the lumbar region (Geraci and Lounsbury 1993). The entire operation took approximately 10–15 min for each pup. All pups were found to be readily accepted and nursed by their mother, and all of them survived to the end of the study. Two weeks later after the first sampling, the same pups were recaptured to extract a second blood sample and weighed again to calculate their specific growth rate (SGR).

The blood samples were centrifuged *in situ* at $4,000 \times g$ for 10 min without anti-clotting factors to separate serum and blood cells (Cunningham 2003). Anticlotting factors were not employed to avoid the alteration of the isotopic signal (Bosley and Wainright 1999). Serum and blood cells were stored in liquid nitrogen and, later, in a freezer at -20°C until they were used for stable isotope analysis.

Eight potential prey species of female South American sea lions off Northern Patagonia, as previously identified by Koen Alonso *et al.* (2000), were collected to characterize their isotopic signal. The potential prey species were selected to balance fish and cephalopods from benthic and pelagic habitats (Table 1). Samples of seaweeds (*Codium vermilara* and *Undaria pinnatifida*) and phytoplankton (collective samples comprising diatoms and dinoflagellates), were also collected to determine their isotopic signal and hence to produce a complete and better description of the isotopic landscape off the Chubut province. The samples of the potential prey and primary producers (seaweeds and phytoplankton) were provided by local fishermen or collected by the staff of the Laboratory of Marine Mammals of CENPAT. The phytoplankton was collected with a 20 μm mesh-size plankton net and, once in the laboratory, filtered in a precombusted CF/G filter and processed for isotopic determination. All potential prey and primary producer samples were stored in a freezer at -20°C until analysis.

Growth

The SGR, relative to the period covering from the 7th to the 21st day of age, was calculated according to the following expression:

$$\text{SGR}(\%) = \{[\ln(W_{t+d}/W_{t0})/d]\} \times 100$$

where W_{t0} is the initial body weight of the pup, W_{t+d} is the final body weight after d days and d is the number of days.

Stable Isotope Analyses

Once thawed, samples were dried at 60°C and ground to a fine powder with mortar and pestle. Lipids were extracted with a chloroform/methanol (2:1) solution (Bligh and Dyer 1959) because they are depleted in ^{13}C in comparison with other molecules, thus confounding the results by decreasing the $\delta^{13}\text{C}$ signal (DeNiro and Epstein 1977). Phytoplankton was also treated with 0.05 M HCl to remove carbonates (Ogawa and Ogura 1997).

Table 1. Isotopic signal, energy density, and proximate chemical composition (mean \pm SD) of the main potential prey of the South American sea lion females analyzed in this study; (n_1) sample size for isotopic analysis, (n_2) sample size for energy density and proximate chemical composition analysis.

Scientific name	Common name	n_1	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	n_2	Energy density (KJ g ⁻¹ wet mass)	Ash (% wet mass)	Lipid (% wet mass)	Protein (% wet mass)
Fish									
<i>Paralichthys isocetes</i> ^(B)	Flounder	5	-15.9 \pm 0.4	18.0 \pm 0.6	5	5.7 \pm 0.8	3.7 \pm 0.5	5.9 \pm 1.1	13.5 \pm 1.4
<i>Raneya brasiliensis</i> ^(B)	Banded cusk eel	5	-15.3 \pm 0.6	18.8 \pm 0.5	2	4.6 \pm 0.4	3.4 \pm 0.9	2.7 \pm 0.3	13.5 \pm 0.2
<i>Engraulis anchoita</i> ^(P)	Argentine anchovy	5	-17.9 \pm 0.2	15.7 \pm 0.8	5	8.4 \pm 1.4	2.8 \pm 0.3	12.3 \pm 3.5	13.5 \pm 1.2
<i>M. hubbsi</i> ^(P)	Argentine hake	5	-17.7 \pm 0.6	15.9 \pm 0.5	5	5.1 \pm 0.3	2.7 \pm 0.1	5.0 \pm 0.7	12.5 \pm 0.5
Cephalopods									
<i>Enteroctopus megalocyathus</i> ^(B)	Red octopus	5	-14.6 \pm 0.7	18.9 \pm 0.9	2	4.3 \pm 0.3	1.8 \pm 0.3	4.2 \pm 0.1	11.1 \pm 1.6
<i>Octopus tehuelibus</i> ^(B)	Tehuelche octopus	5	-14.8 \pm 0.2	19.9 \pm 0.4	5	4.9 \pm 0.4	1.4 \pm 0.1	4.9 \pm 0.9	12.0 \pm 0.4
<i>Illex argentinus</i> ^(P)	Argentine shortfin squid	5	-17.0 \pm 0.6	13.7 \pm 0.8	5	5.3 \pm 0.3	1.1 \pm 0.3	6.5 \pm 0.3	11.0 \pm 1.1
<i>Loligo gahii</i> ^(P)	Patagonian squid	4	-17.6 \pm 0.4	15.7 \pm 0.6	4	5.6 \pm 0.3	1.3 \pm 0.3	6.2 \pm 0.7	13.1 \pm 0.4
^(B) Benthic prey		4	-15.1 \pm 0.6	18.9 \pm 0.8	4	4.9 \pm 0.6	2.6 \pm 1.1	4.4 \pm 1.3	12.5 \pm 1.2
^(P) Pelagic prey		4	-17.6 \pm 0.4	15.2 \pm 1.0	4	6.1 \pm 1.5	2.0 \pm 0.9	7.5 \pm 3.3	12.5 \pm 1.1

Approximately 0.3 mg of serum, 0.25 mg of blood cells, 4.0 mg of homogenized seaweeds, 16.0 mg of the homogenized phytoplankton with filter, and 0.6 mg of white muscle from fish and of mantle from cephalopods were weighed in 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, Thermo Finnigan, Bremen, Germany). Atropine was used as a system check for elemental analyses.

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

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N , R_{sample} is the heavy-light isotope ratio of the sample ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$), and R_{standard} is the heavy-light isotope ratio in reference standards, which were the V-PDB (Vienna Pee Dee Belemnite) calcium carbonate for ^{13}C and the atmospheric nitrogen (air) for ^{15}N . International isotope secondary standards of known $^{13}\text{C}/^{12}\text{C}$ ratios, as given by the IAEA (International Atomic Energy Agency, Vienna, Austria), namely polyethylene (IAEA CH7, $\delta^{13}\text{C} = -31.8\text{‰}$), graphite (IAEA USGS24, $\delta^{13}\text{C} = -16.1\text{‰}$), and sucrose (IAEA CH6, $\delta^{13}\text{C} = -10.4\text{‰}$), were used for calibration at a precision of 0.2‰ . For nitrogen, international isotope secondary standards of known $^{15}\text{N}/^{14}\text{N}$ ratios, namely $(\text{NH}_4)_2\text{SO}_4$ (IAEA N1, $\delta^{15}\text{N} = +0.4\text{‰}$ and IAEA N2, $\delta^{15}\text{N} = +20.3\text{‰}$), and KNO_3 (IAEA NO₃, $\delta^{15}\text{N} = +4.7\text{‰}$), were used to a precision of 0.3‰ .

Energy Density

The proximate chemical composition (water, lipids, proteins, and ash contents) of eight potential prey species of sea lion females (Koen Alonso *et al.* 2000) was analyzed. Because carbohydrates are generally low in fish, and therefore their contribution to the energetic value is negligible (Sidwell *et al.* 1974, Craig *et al.* 1978), we did not measure their content in tissues. Once thawed, samples were weighed and dried at 100°C until a constant weight was reached. The moisture content was calculated by gravimetric difference between wet and dry mass (Eder and Lewis 2005). Dry samples were homogenized and a subsample burnt for 6 h in a muffle furnace at 600°C for ash determination (Doyle *et al.* 2007). Another subsample was processed to determine its nitrogen content by means of an elemental analyzer. This value was later multiplied by 5.8 to obtain the relative abundance of proteins in the dry material, as recommended by Gnaiger and Bitterlich (1984) for fish and other marine species. Lipids were extracted from a third subsample with a chloroform/methanol (2:1) solution (Bligh and Dyer 1959) and their content determined by the gravimetric difference between fat and non-fat dry mass (Gnaiger and Bitterlich 1984).

To calculate the energy provided by the lipids and proteins, we used a mean combustion equivalent of these compounds of 23.9 kJ g^{-1} for proteins and 39.5 kJ g^{-1} for lipids (Clarke *et al.* 1992).

Data Analyses

Prior to any statistical analysis, normality in data distribution was tested by Lilliefors's test, and their homogeneity of variances by Levene's contrast test.

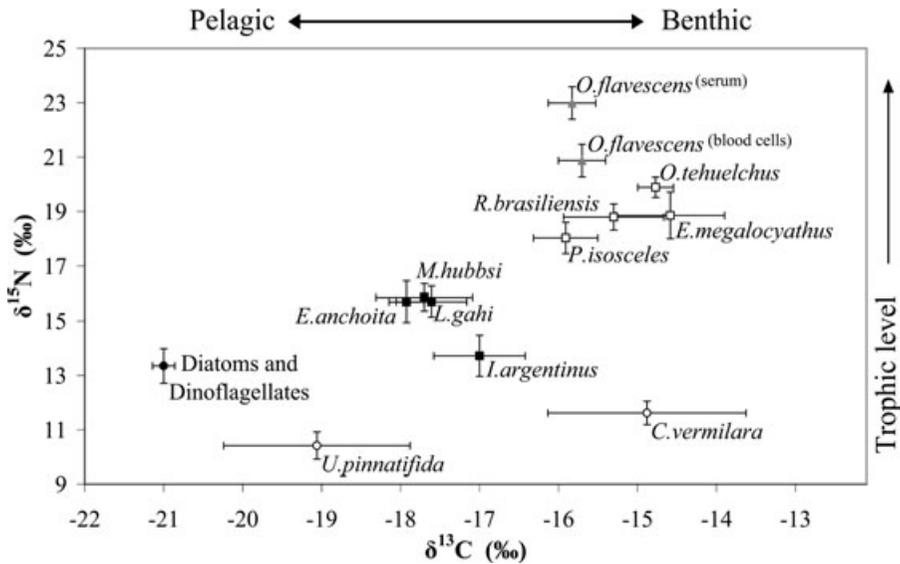


Figure 2. Bivariate isotopic signals (mean \pm SD) of the South American sea lion pups, the main potential prey of their mothers and the primary producers; (\blacktriangle) pups, (\square) benthic prey, (\blacksquare) pelagic prey, (\circ) seaweeds, (\bullet) phytoplankton. Sample size: $n = 5$ for all the species, except for the *O. flavescens* ($n = 26$ for each tissue), *L. gahi* ($n = 4$) and phytoplankton ($n = 2$; collective samples of diatoms and dinoflagellates).

Nested ANOVA was used to compare ash, lipid, and protein contents, the energy density and the isotopic signal ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in coastal benthic and offshore pelagic potential prey species.

The Student's t -test was used to investigate differences in the SGR and serum and blood cell isotope signals ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of male and female pups.

Pearson's parametric correlation coefficient was used to determine whether a relation existed between blood cell $\delta^{13}\text{C}$ and serum $\delta^{13}\text{C}$ and between blood cell $\delta^{15}\text{N}$ and serum $\delta^{15}\text{N}$. The same procedure was used to determine whether a relation existed between the pup SGR and the serum isotope signal of 3-wk-old pups ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$).

Data are always shown as mean \pm standard deviation (SD), unless otherwise stated. All statistical analyses were conducted with the SPSS 15 software package.

RESULTS

Figure 2 shows the isotopic signals of the local primary producers (phytoplankton and seaweeds) and the potential sea lion prey. The $\delta^{15}\text{N}$ increased with the trophic level, and the benthic primary producers were more enriched in ^{13}C than the pelagic ones. As a consequence, benthic potential prey were more enriched in ^{13}C , as well in ^{15}N , than pelagic ones (Table 1), as confirmed by nested ANOVA (Table 2).

Table 1 shows the energy density and the ash, protein and lipid contents of the potential prey analyzed. Nested ANOVA revealed that offshore pelagic potential prey had on average a higher energy density and lipid contents than coastal

Table 2. Summary of the results of nested ANOVA to test for differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the considered prey species within habitat (benthic *vs.* pelagic).

	SS	df	MS	F	P	r^2
$\delta^{13}\text{C}(\text{‰})$						
Model	64.201	7	9.172	35.067	< 0.001	0.888
Intersect	10,375.216	1	10,375.216	39,668.436	< 0.001	
Habitat	56.321	1	56.321	215.337	< 0.001	
Species (habitat)	7.708	6	1.285	4.912	0.001	
Error	8.108	31	0.262			
Total	10,463.580	39				
$\delta^{15}\text{N}(\text{‰})$						
Model	155.544	7	22.221	56.481	< 0.001	0.927
Intersect	11,295.582	1	11,295.582	28,711.302	< 0.001	
Habitat	129.188	1	129.188	328.372	< 0.001	
Species (habitat)	24.007	6	4.001	10.170	< 0.001	
Error	12.196	31	0.393			
Total	11,571.730	39				

benthic ones, but lower ash contents (Table 3). Statistically significant differences were also found in the protein contents of the considered species, but on average offshore pelagic and coastal benthic potential prey did not differ in protein contents (Table 3).

Pup sex had no statistically significant effect either on SGR or on serum and blood cells $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signals (Table 4). As a consequence, male and female pups were pooled for latter analyses. Figure 2 shows the isotopic values of the blood cells from the 1-wk-old pups and those of the serum from the 3-wk-old pups. The isotopic values of the serum from the 1-wk-old pups and those of the blood cells from the 3-wk-old pups are not shown, as they are expected to be influenced both by the diet just before parturition but also by the changes that might happen after parturition, thus obscuring interpretation.

Direct comparison between the isotopic values of the blood cells and the serum is not possible, as those tissues are known to differ in fractionation values (Kurlle 2002). However, the average $\delta^{15}\text{N}$ of both serum and blood cells from pups was higher than that of the potential prey and the average $\delta^{13}\text{C}$ of both serum and blood cells was intermediate among that of benthic and pelagic potential prey. This is consistent with a diet including a mixture of both pelagic and benthic potential prey, although with a certain level of individual variability (Fig. 2). The existence of a positive linear correlation between blood cell $\delta^{13}\text{C}$ and serum $\delta^{13}\text{C}$ ($r = 0.681$, $P < 0.001$) and between blood cell $\delta^{15}\text{N}$ and serum $\delta^{15}\text{N}$ ($r = 0.587$, $P = 0.002$) indicated that individual differences in the relative dietary importance of pelagic and benthic prey already occurred in late pregnancy (blood cell data) and were maintained in early lactation (serum data). Unfortunately, ignorance about the actual fractionation factors for the South American sea lion does not permit a precise identification of the consumed prey.

Pup SGR was negatively correlated with the serum signals of $\delta^{13}\text{C}$ ($r = -0.659$, $P < 0.001$) and $\delta^{15}\text{N}$ ($r = -0.535$, $P = 0.005$) (Fig. 3).

Table 3. Summary of the results of nested ANOVA to test for differences in the energy density and the proximate chemical composition (lipids, proteins, and ash contents) of the considered prey species within habitat (benthic *vs.* pelagic).

	SS	df	MS	F	P	r ²
Energy density						
Model	47.856	7	6.837	13.850	< 0.001	0.795
Intersect	858.217	1	858.217	1,738.595	< 0.001	
Habitat	10.373	1	10.373	21.013	< 0.001	
Species (habitat)	38.772	6	6.462	13.091	< 0.001	
Error	12.341	25	0.494			
Total	1,121.564	33				
Ash						
Model	43.401	7	6.200	57.646	< 0.001	0.942
Intersect	177.867	1	177.867	1,653.718	< 0.001	
Habitat	7.680	1	7.680	71.408	< 0.001	
Species (habitat)	37.984	6	6.331	58.859	< 0.001	
Error	2.689	25	0.108			
Total	230.690	33				
Lipid						
Model	221.526	7	31.647	12.830	< 0.001	0.782
Intersect	1,050.927	1	1,050.927	426.051	< 0.001	
Habitat	57.669	1	57.669	23.379	< 0.001	
Species (habitat)	166.784	6	27.797	11.269	< 0.001	
Error	61.667	25	2.467			
Total	1,663.568	33				
Protein						
Model	32.089	7	4.584	4.983	0.001	0.583
Intersect	4,511.675	1	4,511.675	4,904.215	< 0.001	
Habitat	0.152	1	0.152	0.165	0.688	
Species (habitat)	31.679	6	5.280	5.739	0.001	
Error	22.999	25	0.920			
Total	5,281.324	33				

Table 4. Summary of the results of the Student's *t*-test to test differences in the SGR (%) and serum and blood cell isotope signals ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; ‰) of male and female pups.

	Males (mean \pm SD)	Females (mean \pm SD)	<i>t</i>	df	<i>P</i>
SGR	1.1 \pm 0.4	0.9 \pm 0.4	1.270	24	0.216
Serum $\delta^{13}\text{C}$	-15.9 \pm 0.2	-15.7 \pm 0.3	-1.243	24	0.226
Serum $\delta^{15}\text{N}$	22.9 \pm 0.6	23.3 \pm 0.6	-1.595	24	0.124
Blood cells $\delta^{13}\text{C}$	-15.6 \pm 0.2	-15.7 \pm 0.4	0.545	24	0.591
Blood cells $\delta^{15}\text{N}$	20.8 \pm 0.4	20.9 \pm 0.7	-0.536	24	0.597

DISCUSSION

In recent years stable isotopes have become a standard tool for investigating trophic relationships of wild animals (Crawford *et al.* 2008). Their use is based on the assumption that carbon and nitrogen in the body of an animal come directly

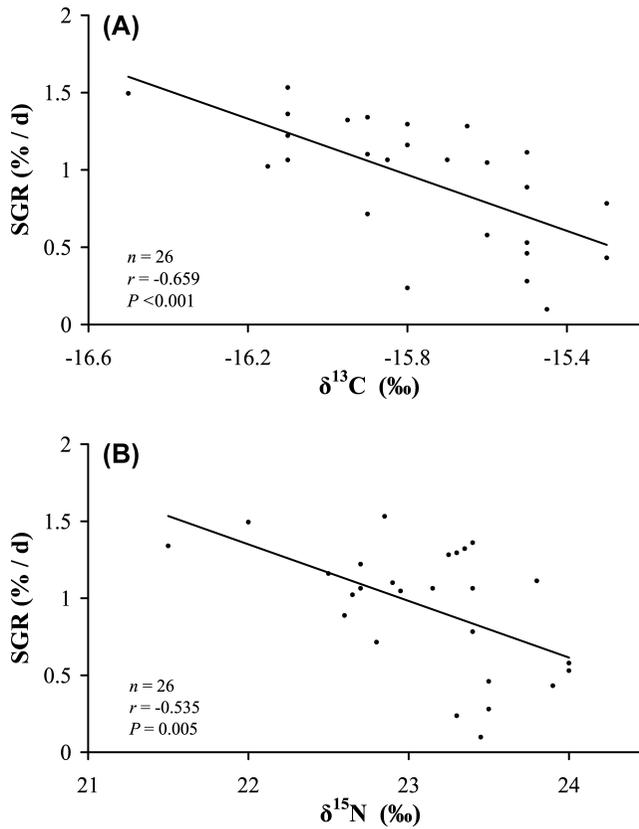


Figure 3. Correlation between carbon (A) and nitrogen (B) isotopic signal of serum from 3-wk-old pups and their SGR.

from its food and, as a consequence, the tissue isotopic signal can be used to ascertain the relative importance of feeding sources that differ in signal (DeNiro and Epstein 1978, 1981). Milk is the only food of suckling pups and hence the isotopic signal of pup blood is expected to reflect the diet of the mother, although modified due to prey-to-predator and female-to-offspring fractionation (Jenkins *et al.* 2001).

Prey-to-predator fractionation factors in pinniped blood cells range from 1.7‰ to 4.1‰ for the $\delta^{15}\text{N}$ and from 1.2‰ to 1.7‰ for the $\delta^{13}\text{C}$, whereas those of serum range from 3.9‰ to 5.2‰ for the $\delta^{15}\text{N}$ and from 0.2‰ to 0.6‰ for the $\delta^{13}\text{C}$, depending on the species and the nutritional status of the experimental individuals (Hobson *et al.* 1996, Kurlle 2002). The only published female-to-offspring fractionation factors for pinnipeds refer to whole blood and are 0.3‰ for the $\delta^{13}\text{C}$, and 1.3‰ for the $\delta^{15}\text{N}$ (Ducatez *et al.* 2008). In the South American sea lion, neither the prey-to-predator and female-to-offspring enrichment rates nor the tissue turnover have been determined in controlled experiments and hence stable isotope data cannot be used to infer diet composition at the species level (but see Hückstädt *et al.* 2007). Nevertheless, individual differences in the isotopic values of pups are expected to reflect individual differences in the diet of their mothers, thus allowing comparison among individuals in the absence of other confounding factors.

Fasting is known to modify the isotopic signal of some tissues in endothermic vertebrates (Hobson *et al.* 1993, Cherel *et al.* 2005, but see Kempster *et al.* 2007). The pups of the South American sea lions fast from 1 to 10 d between suckling bouts, while females forage at sea (Campagna and Le Boeuf 1988, Campagna *et al.* 2001). However, the data published on the effects of fasting in the isotopic signal of the blood of endotherms indicate only a slight increase of the $\delta^{15}\text{N}$ of plasma (0.70‰) and a much lower increase of the $\delta^{15}\text{N}$ of blood cells (0.24‰) after 25 d of fasting (Cherel *et al.* 2005). In this scenario, it is unlikely that a few days difference in the length of pup fasting may explain the observed variability in the $\delta^{15}\text{N}$ of pup serum (from 21.5‰ to 24.0‰).

Most importantly, Cherel *et al.* (2005) reported no effect of fasting on the $\delta^{13}\text{C}$ of blood cells and a decrease (−0.71‰) of the $\delta^{13}\text{C}$ of plasma after 25 d. If the variability of the serum $\delta^{13}\text{C}$ of the pups studied had been caused by differences in the length of the fasting period between suckling bouts, the serum $\delta^{13}\text{C}$ of the pups would be expected to decrease as the length of the fasting period increased and the growth rate decreased. However our findings indicate that the serum $\delta^{13}\text{C}$ of the pups increased as the SGR of the pups decreased. Finally, the isotopic signal of blood cells, mostly synthesized one or 2 mo before parturition (Hobson and Clark 1993, Hilderbrand *et al.* 1996) and hence independent of the length of the fasting between suckling bouts, revealed a degree of variability similar to that of serum. This similarity confirms that the observed variability in the isotopic signal of serum is unrelated to the variability in the length of the foraging excursions of the females. Actually, the correlation existing between the isotopic signal of serum and that of blood cells indicates that females differed in their diet well before parturition and that the relative position of each female within a pelagic-benthic gradient remained unchanged after parturition.

The reason for such intrapopulation variability probably reflects dissimilarities in the individual foraging performance, which depends on age and/or body size. Pinnipeds with a larger body are physiologically more capable of exploiting benthic prey, as their skill at diving deeper is greater than those of smaller size, which results in sharp interspecific differences in foraging patterns (Costa 1991). Recently, Chilvers *et al.* (2006) and Villegas-Amtmann *et al.* (2008) also reported intraspecific differences in the diving behavior of lactating females of the New Zealand sea lion (*Phocarctos hookeri*) and Galapagos sea lion (*Zalophus wollebaeki*) caused by differences in body mass, with the lighter females feeding at shallower sites. Moreover, diving skills in pinnipeds are progressively acquired through their lifespan: dive duration, dive depth, traveling distance and swimming speed, all of them increase with age (Horning and Trillmich 1997, Bekkby and Bjørge 2000, Chilvers *et al.* 2005, 2006). Thus, it is also possible that the diet of females is strongly dependent on the age of individuals.

Whatever the reason for such variability, the data reported here indicate that pup growth was influenced by female diet. Pups of females that rely mostly on pelagic offshore prey were found to grow faster than those of females that feed on benthic coastal prey, as revealed by more positive $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the latter. A similar relationship was also observed in Patagonia in Magellanic penguins, *Spheniscus magellanicus* (Forero *et al.* 2002a, b). Our results also confirm that the energy density of pelagic offshore potential prey is higher than that of potential benthic coastal prey and that the protein content in both groups is similar, thus leading to a higher nutritional value of the potential pelagic offshore prey. However, these results do not demonstrate that a higher growth rate of pups of females that feed on pelagic

offshore prey is a direct consequence of a more nutritious diet, as other factors might be involved.

A faster growth rate of pups would be advantageous in this species only if it was not offset by an increase in pup mortality rate, which is likely to happen if the females are forced to abandon pups for a long period to carry out distant foraging trips (Soto *et al.* 2006). Actually, an increase in trip duration is thought to be the least preferred strategy for nursing otariids to overcome reductions in food availability (Costa 2008). Conversely, the high costs associated with benthic foraging in otariids (Costa and Gales 2003, Costa *et al.* 2004, Chilvers *et al.* 2006) and the low quality of coastal benthic prey (this study) can be counterbalanced by the improved protection of pups and more frequent feeding derived from shorter foraging trips.

Unfortunately, there is no information on the differential mortality of pups as a function of coastal or offshore feeding by females in the studied rookery, but Campagna *et al.* (2001) found that, despite the fact that lactating females traveling farther away swam faster than those feeding closer to the coast, trip length was significantly correlated with the distance traveled. Furthermore, in the Peruvian population of the South American sea lion, pup mortality rate rises dramatically when reduced food availability forces females to increase their foraging trip length (Soto *et al.* 2004, 2006).

Whatever the case, the strong influence of the consumption of pelagic prey on the growth rate of pups in the Patagonian population of South American Sea lions also has bearing on its conservation. Currently, the sea lion population is about one-third of its estimated original size and it is recovering steadily (Dans *et al.* 2004). This might indicate that it does not experience a shortage of high-quality food despite the fact that *M. hubbsi* has been heavily exploited by fishing (Koen Alonso and Yodzis 2005). This is the main pelagic species in the diet of South American sea lions in the region (Koen Alonso *et al.* 2000). Hence, a further reduction in the abundance of this species or increased exploitation of the currently underexploited stocks of anchovy and squid (Koen Alonso and Yodzis 2005) may reduce the average growth rate of the pups and hinder the long-term recovery of the population, particularly if the goal is to reach the abundance levels recorded at the beginning of the 20th century.

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