

Ontogenetic changes in feeding habits of northern elephant seals revealed by $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analysis of growth layers in teeth

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ABSTRACT: Stable isotope analysis is useful for examining the feeding strategies of mammals. Isotopes in the annual deposition growth layers of dentine in teeth permit assessment of ontogenetic dietary shifts in individuals, because this metabolically inert tissue is not resorbed after deposition. Profiles of stable isotope ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) from the teeth of northern elephant seals *Mirounga angustirostris*, aged between 1 and 11 yr, were described from stranded individuals in San Benito and Magdalena Islands, Mexico, between 2000 and 2009. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values differed with age in each sex indicating shifts in feeding habits throughout life, as well as in their physiological condition. Although steady $\delta^{13}\text{C}$ values within each individual suggest fidelity to feeding grounds, differences in $\delta^{13}\text{C}$ values between males and females were likely a reflection of sexual segregation on the feeding areas, which begins during the juvenile stage. Mean $\delta^{15}\text{N}$ values of males (18.3‰) and females (18.2‰) would suggest that both groups feed at a similar trophic level; however a combination of differences in benthic versus pelagic and longitudinal foraging habitats would be masking real sex differences in trophic level, estimated for the species at 4.6. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values varied markedly between individuals of the same sex, suggesting the potential existence of diverse feeding strategies leading to a resource partitioning in this species. The alleged fetal growth layer has a $\delta^{15}\text{N}$ value 1.8‰ higher than the average of the adult female stage, suggesting that the fetal layer is more likely the product of the intensive nursing during the first month of life.

KEY WORDS: *Mirounga angustirostris* · Stable isotope · Dentine growth layer · Ontogenetic dietary shifts · Feeding strategy · Isotope fractionation

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INTRODUCTION

Sex differences in foraging behavior are common in species that exhibit extreme sexual dimorphism. Sexual selection for large size, leading to sexual dimorphism, has implications for resource accrual (Le Boeuf et al. 1993); when males are larger than females they must consume more energy to achieve and to maintain their larger size over females (Deutsch et al. 1994, Le Boeuf et al. 2000). Individual differences in physiological capacity and social orga-

nization also lead to diet specialization (Bolnick et al. 2003), which may involve differences in the type of prey consumed by each sex and changes in prey consumed during development (Lewis et al. 2006). Thus, alimentary niche partitioning is an incidental by-product of ontogeny (Bolnick et al. 2003).

One of the most sexually dimorphic mammals is the northern elephant seal *Mirounga angustirostris*, a species in which adult males obtain a mass up to 10 times greater than that of adult females (Deutsch et al. 1994). Based on differences in size, a male of 2500 kg

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has daily energy requirements 3 times greater than those of a female weighing 500 kg (Le Boeuf et al. 1993). The diving pattern of elephant seals suggests that the sexes consume different prey (Le Boeuf 1994, Le Boeuf et al. 2000). To the extent that this is so, competition for prey is predominantly intrasexual (Lee & Sydeman 2009), owing to spatial and temporal separation at sea. The reduced diving capacity of juveniles relative to adults (Le Boeuf et al. 1996), as well as the rapid development of differences in foraging strategies (Le Boeuf et al. 1993), has been observed during the first 2 yr of life (Le Boeuf et al. 1996). This suggests differential use of resources between juveniles of different ages and sexes.

Northern elephant seals make 2 foraging migrations per year, during which adult males and females go to different regions of the Northeastern Pacific Ocean with minimal overlap in range (Le Boeuf et al. 2000). Data from individuals instrumented at Año Nuevo, California, suggest that males feed primarily on benthic prey in focal foraging areas located along the continental shelf off Oregon and Washington in the southern part of the range to as far as the western Aleutian Islands to the northwest. Females, feed on patchily distributed, vertically migrating, epipelagic and mesopelagic prey distributed in mid latitude waters between 40° and 55° N and as far west as 172.5° E, hundreds to thousands of kilometers off the coast. Consequently, adult females are more variable in their feeding location than adult males while pursuing widely distributed prey (Le Boeuf et al. 2000), whose distribution varies with oceanographic characteristics and habitat structure (Hakoyama et al. 1994, Simmons et al. 2007).

The diet of northern elephant seals is dominated by epi- and mesopelagic species (*Octopoteuthis deletron*, *Histioteuthis heteropsis*, *Gonatopsis borealis*, *H. dofleini*, *Loligo opalescens*), benthic fishes (rockfish *Sebastes* sp., *Chilaria taylori*), demersal fish (Pacific hake *Merluccius productus*), crustaceans (pelagic red crab *Pleuroncodes planipes*), elasmobranchs (*Cephaloscyllium ventriosum*, *Hidrolagus colliei*) and cyclostomes (*Lamprreta tridentata*) (Condit & Le Boeuf 1984, Antonelis et al. 1994, Sinclair 1994). Nevertheless, studies of diet in this species are limited, coming primarily from scats collected at island rookeries and the stomachs of dead animals found along the coast. The scat or stomach sample analysis provides insight to only a small part of this species' feeding habits owing considerably to the great separation in time and space between feeding and sample collection.

Knowledge of the feeding habits of various species has been advanced using stable isotope analysis of

nitrogen and carbon ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively) in certain tissues. The $\delta^{15}\text{N}$ provides data on feeding behavior, trophic breadth and level (Post 2002, Bearhop et al. 2004), and the $\delta^{13}\text{C}$ on the foraging location (Walker & Macko 1999, Clementz & Koch 2001, Newsome et al. 2010). The isotopic composition of prey is reflected in predators' tissues. Isotopic differences follow from one level to another in the trophic web (DeNiro & Epstein 1978, Fry et al. 1984, Minagawa & Wada 1984, Hobson & Welch 1992), becoming enriched in the heavier isotope at each trophic level, from ~3 to 5‰ for $\delta^{15}\text{N}$ and from ~0.5 to 1‰ for $\delta^{13}\text{C}$ (Minagawa & Wada 1984, Hobson et al. 1996).

The geographical differences in the isotopic values of primary producers are reflected in the top predators (Burton & Koch 1999, Burton et al. 2001, Aurioles et al. 2006). Spatial isotopic gradients associated with latitude and distance from the coast in the northeastern Pacific have made it possible to indicate the general feeding sites of various species of marine mammals (Burton & Koch 1999, Burton et al. 2001, Kurle & Worthy 2002, Aurioles et al. 2006). Characteristically, $\delta^{13}\text{C}$ values from the base of the trophic plot increase with proximity to the coast. On the other hand, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values increase ~1‰ in accordance with decreases of ~4° and 5° of latitude, respectively (Burton & Koch 1999, Burton et al. 2001, Aurioles et al. 2006).

Stable isotope analysis in dentine is useful for evaluating developmental changes in diet of marine mammal species (Newsome et al. 2010). Teeth grow throughout life with annual deposition of dentine layers on the internal surface of the pulp cavity (Klevezal 1996). Like hair and vibrissae, dentine is metabolically inert and is not resorbed after deposition. Therefore, successive layers of different density (translucent and opaque) reflect the conditions under which they were secreted (Carrick & Ingham 1962). Thus, when annual groups of growth layers are sampled, they offer a summary of feeding history, migratory behavior, and fasting pattern of the individual (Hobson & Sease 1998, Newsome et al. 2006, Mendes et al. 2007).

Lactation period is also detected in ontogenetic, isotopic time series of sea mammals (Hobson & Sease 1998, Newsome et al. 2006, Niño-Torres et al. 2006, Mendes et al. 2007, Knoff et al. 2008, Newsome et al. 2009a). Theoretically, if the mother catabolizes her tissue to synthesize milk, the nursing pup, which is indirectly feeding on maternal tissue, will have isotope values indicating that it is feeding at a trophic level higher than that of its mother until it is weaned (Newsome et al. 2006). The ^{15}N -enrichment may be a function of the quality of protein (Robbins et al. 2005)

or the nitrogen concentration of the diet (Pearson et al. 2003), but the magnitude of these effects is species-specific (Jenkins et al. 2001). For $\delta^{13}\text{C}$, the effects of nursing are complicated because the milk has high lipid content and lipids are ^{13}C -depleted relative to proteins (DeNiro & Epstein 1978). Therefore one can observe a decrease in $\delta^{13}\text{C}$ values in pup tissues (Newsome et al. 2006).

In this study, we used stable isotope analysis of tooth growth layers of northern elephant seals to identify developmental changes in the diet of individuals, as well as to measure the differences in feeding behavior within and between the sexes. We examined the efficiency of this approach in order to characterize phocid lactation.

MATERIALS AND METHODS

We collected teeth from animals with known date of death on San Benito ($28^{\circ}18.37' \text{N}$, $115^{\circ}34.01' \text{W}$; $n = 24$) and Magdalena Islands ($24^{\circ}55.45' \text{N}$, $112^{\circ}13.50' \text{W}$; $n = 2$), Baja California Sur (BCS) (Fig. 1). San Benito is an elephant seal rookery and Magdalena is a stranding site, where several individuals marked at San Benito beached (D. Auriolles unpubl. data). The teeth were collected between 2000 and 2009 and are now in the osteological collection at CICIMAR (Colección Osteológica CICIMAR), La Paz, BCS, Mexico.

The sex of the specimens was determined by the degree of sexual dimorphism (Briggs & Morejohn 1975). From each individual a superior canine was extracted and aged in the laboratory by counting dentine growth layers (Scheffer 1950, Klevezal 1996). Dentine collagen was collected from each one of the growth layers and isotopic analysis was conducted to obtain an ontogenetic profile for each individual (Table 1). The age classes of each sex were defined according to Reiter & Le Boeuf (1991) and Clinton (1994). To examine the feeding strategies, most samples came from subadult and adult specimens (Magdalena: $n = 2$; San Benito: $n = 8$). The samples from 16 pups collected on San Benitos Islands were used only to measure the isotopic discrimination between adult females and pups.

Tooth sectioning and sampling

Each tooth was sectioned longitudinally with a low speed precision saw (Isomet-Buehler). The sections were polished with sandpaper until achieving a

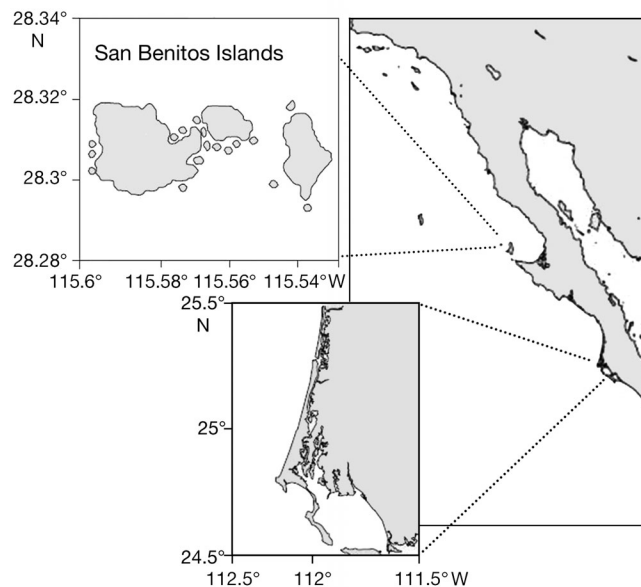


Fig. 1. Baja California Sur. Collection sites of northern elephant seal teeth: San Benito Islands ($28^{\circ}18.37' \text{N}$, $115^{\circ}34.01' \text{W}$) and Magdalena Island ($24^{\circ}55.45' \text{N}$, $112^{\circ}13.50' \text{W}$)

smooth finish. For better identification of growth layers (GLs), the polished tooth sections were treated with a formic acid solution, varying the concentration and time of exposure in accordance with the size and degree of tooth calcification, i.e. 10% and 1 h for teeth of juveniles and adults, or 5% and 30 min for the teeth of pups. The acid etched the surface between the GLs helping to distinguish their borderlines. We assumed this had no influence on the values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ because the exposed part of the tooth was a small fraction of the total sample (Hobson & Sease 1998, Newsome et al. 2006). We took dentine samples from each GL using a high-resolution micro-milling system (Merchantek) following a sampling path that was $\sim 160 \mu\text{m}$ wide and ~ 100 to $300 \mu\text{m}$ deep. Tooth dentine is composed of 65% hydroxyapatite and other minerals and 35% organic material, mainly collagenous protein (Klevezal 1996).

Stable isotope analysis

Collagen was obtained from the pulverized dentine samples by repeated demineralizing with aliquots of $\sim 0.05 \text{ ml}$ of hydrochloric acid (HCl, 0.1 N) for 8 to 12 h at 6°C . Then samples were rinsed with distilled water to eliminate the residual acid. Lipids were not extracted from dentine samples since this tissue does not contain appreciable quantities of ^{13}C -depleted

Table 1. *Mirounga angustirostris*. Age, sex, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ means (\pm SE), and collection site and year of subadult and adult individuals from which single teeth were analyzed. Isotopic data from the first growth layer (GL 0) is not included in the calculation of means. The C:N mass ratio of all samples ranged from 2.8 to 3.6 within the range for unaltered collagen. ID: identification code

Individual ID	Age class and sex	Formation of 1st growth layer (yr)	Age (yr)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	Collection	
						Year	Site
MG1MSd	Subadult male	1997	8	18.4 \pm 0.3	-12.9 \pm 0.2	2004	Magdalena
MG37M	Adult male	1999	11	18.3 \pm 0.2	-13.4 \pm 0.3	2009	Magdalena
SB2F	Adult female	2002	6	18.8 \pm 0.2	-13.7 \pm 0.1	2007	San Benito
SB3F	Adult female	2001	7	18.6 \pm 0.3	-13.3 \pm 0.2	2007	San Benito
SB4MSd	Subadult male	1995	6	18.8 \pm 0.0	-12.7 \pm 0.1	2000	San Benito
SB5F	Adult female	1991	11	16.7 \pm 0.2	-14.5 \pm 0.2	2001	San Benito
SB6M	Adult male	1992	9	18.41 \pm 0.2	-12.4 \pm 0.0	2000	San Benito
SB10F	Adult female	2001	8	18.4 \pm 0.1	-13.7 \pm 0.1	2008	San Benito
SB11M	Adult male	1992	9	17.7 \pm 0.3	-13.7 \pm 0.1	2000	San Benito
SB12M	Adult male	1992	9	18.0 \pm 0.2	-13.6 \pm 0.1	2000	San Benito

lipids (Hobson & Sease 1998, Newsome et al. 2006, 2009a). As a control, we measured the carbon to nitrogen (C:N) mass ratio of all samples, which ranged from 2.8 to 3.6, well within the range for unaltered collagen (DeNiro & Epstein 1978).

Of each dry sample, ~1 mg was sealed in 8 \times 5 mm tin capsules for isotopic analysis in an elemental analyzer, PDZ Europa ANCA-GSL, that was interfaced with a gas source mass spectrometer with a continuous flow rate, PDZ Europa 20-20 (Sercon) at the Stable Isotope Facility of the University of California at Davis. Results were determined using the following equation: $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ = $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R_{sample} and R_{standard} are the values of $^{15}\text{N}:^{14}\text{N}$ or $^{13}\text{C}:^{12}\text{C}$ ratio in the sample and standard, respectively. The standard for nitrogen was atmospheric N_2 and Pee Dee Belemnite (PDB) calcium carbonate for carbon. Results were calibrated with international standards (ammonium sulfate for $\delta^{15}\text{N}$; sucrose for $\delta^{13}\text{C}$). SD between the isotopic measurement trials was $<0.3\text{‰}$ for $\delta^{15}\text{N}$ and $<0.2\text{‰}$ for $\delta^{13}\text{C}$.

Estimate of trophic level

The trophic level (TL) was estimated using the algorithm of Vander Zanden & Rasmussen (1999): $\text{TL} = [(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}})/3.4] + 2.0$, where $\delta^{15}\text{N}_{\text{consumer}}$ and $\delta^{15}\text{N}_{\text{baseline}}$ are the $\delta^{15}\text{N}$ average values of northern elephant seals and the primary consumer *Calanus pacificus*, respectively. The copepod *C. pacificus* is a dominant species on the central California coast, Monterey Bay; its $\delta^{15}\text{N}$ value (9.9‰) was taken from Rau et al. (2003) with an assumed TL of 2.0. The 3.4 value is the average trophic enrichment along the food chain to top predators (Minagawa & Wada 1984).

Statistical analysis

The variability of isotopic values throughout each ontogenetic profile was determined with the coefficient of variation (CV). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the growth layers of the specimens were categorized by age and grouped by sex in such a manner that an individual adult or subadult provided information about its own earlier age classes. Differences in isotopic values between pups and the adult phase of females were tested by an analysis of variance (ANOVA) test. Differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between age classes, between sexes, and between individuals were assessed using linear mixed effects (LME) models. These models characterize individual variation relative to the mean of the population while considering the correlation between repeated measurements within and among individuals.

Data from each tooth were treated as a repeated measures set. Individual identity (ID) was treated as a random effect while site, gender and age classes were treated as fixed effects. Because environmental conditions differ from year to year and may differentially affect individuals according to age, we used 'Time' (calendar years) as a co-variable to remove differences owing to this factor. Models were run using the restricted maximum likelihood method. p-values were not corrected, so that we used a Fisher's least significant difference (LSD) test for multiple comparisons. The 'El Niño' effects were examined by regression analysis between the Oceanic Niño Index (ONI) and the isotopic values of the individuals. Statistical analyses were performed using SPSS version 19 and Statistica version 8. Statistical significance was assumed at $p < 0.05$.

RESULTS

The ontogenetic profiles of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for the dentine growth layer samples of each adult male and female are shown in Fig. 2. The $\delta^{13}\text{C}$ values showed little variation in most individuals. The $\delta^{13}\text{C}$ values varied between 0.3 to 3‰ and were more variable in males (average CV measured from dentine layer 1; $\text{CV}_A = 2.9\%$) than in females ($\text{CV}_A = 2.6\%$). In addition, some individuals (MG1MSd, MG37M, SB12M) showed a steep decrease in $\delta^{13}\text{C}$ values late in life. In all females and half of the males, the first growth layer (GL 0) had the lowest $\delta^{13}\text{C}$ compared with the subsequent layer. In both sexes, $\delta^{15}\text{N}$ values showed a similar interannual pattern which, however, varied more in males than females ($\text{CV}_A = 4.0\%$ in males and 3.3% in females). In almost all cases, the highest $\delta^{15}\text{N}$ values were found in GL 0 and the greatest difference (between 0.5 and 3.2‰) was between GL 0 and GL 1, with a difference greater in females (mean difference = 1.5‰) than in males (mean difference =

1.4‰). In most of the individual profiles, the $\delta^{15}\text{N}$ values varied widely in GLs, between 0.3 and 2.8‰, and there was no pattern evident between individuals.

The $\delta^{15}\text{N}$ value of the first growth layer (GL 0) in all individuals ($n = 26$; $19.4 \pm 1.0\%$, mean \pm SD) from San Benito and Magdalena, when compared with the GL value of just the adult phase of females ($n = 20$, obtained from 4 females; $\delta^{15}\text{N} = 17.6 \pm 1.0\%$), reveals a difference of 1.8‰, which is consistent with the statistically significant isotopic fraction between pups and mothers (ANOVA: $F_{1,44} = 37.77$, $p < 0.05$). In $\delta^{13}\text{C}$, the difference between the mean for pups and adult phase of females was 0.2‰, which was not statistically significant (ANOVA: $F_{1,44} = 0.45$, $p > 0.05$).

There was neither an effect of the years (Time treated as a co-variable) in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (LME: $F_{1,62.16} = 1.828$, $p > 0.05$ and $F_{1,67.07} = 0.27$, $p > 0.05$ respectively), nor in the interaction with age classes ($\delta^{15}\text{N}$: $F_{3,40.83} = 1.412$, $p > 0.05$; $\delta^{13}\text{C}$: $F_{3,49.89} = 0.367$, $p > 0.05$). Consequently, this co-variable was not used in the following models.

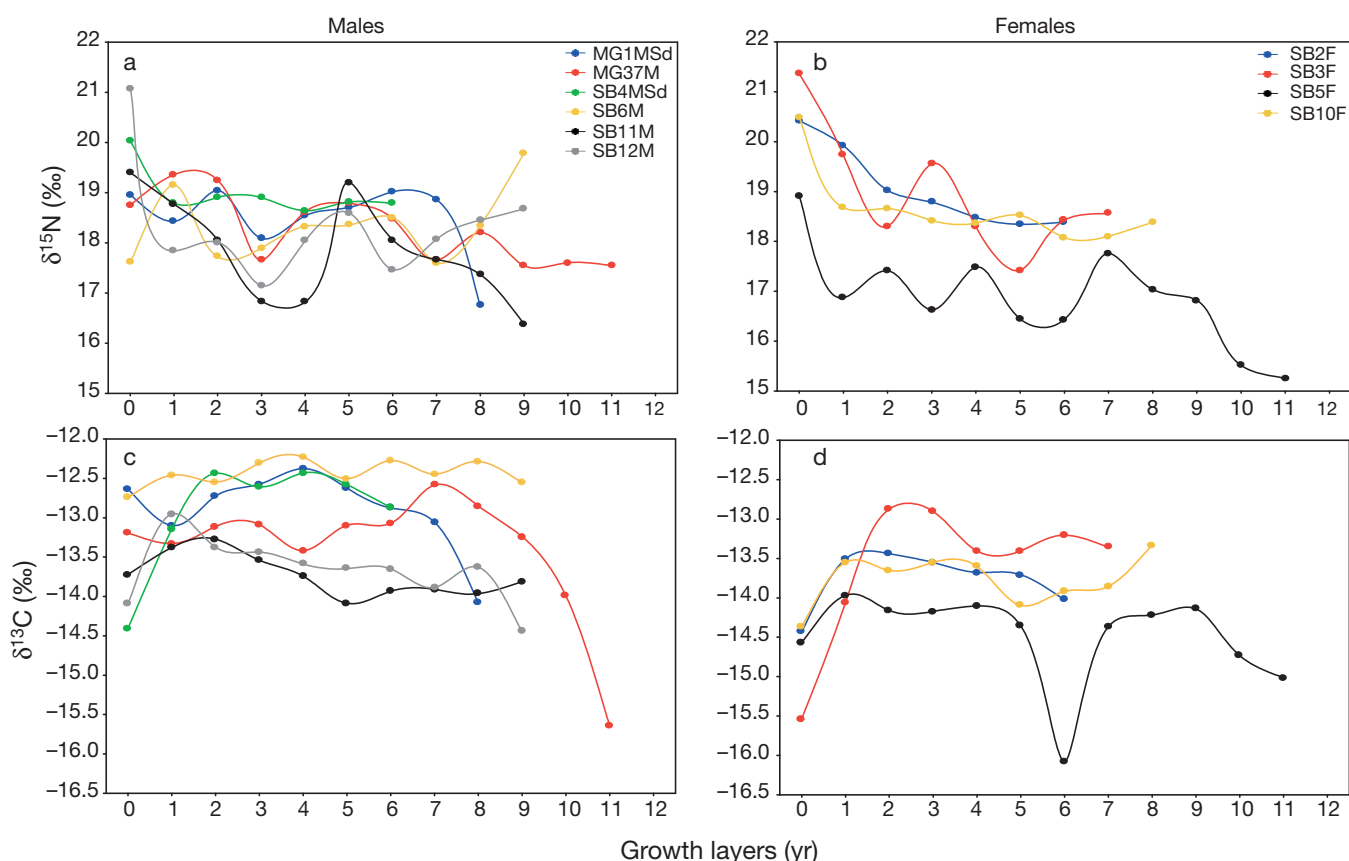


Fig. 2. *Mirounga angustirostris*. Ontogenetic profiles of (a,b) $\delta^{15}\text{N}$ and (c,d) $\delta^{13}\text{C}$ values of dental collagen of adult (a,c) male and (b,d) female individuals collected at San Benito (SB) and Magdalena (MG) Islands. Key: identification codes of individual seals, which indicate the collection site by the first 2 letters. The first x-axis value (0) at the left of each plot represents the first growth layer next to the enamel. See Table 1 for descriptions of individual seals

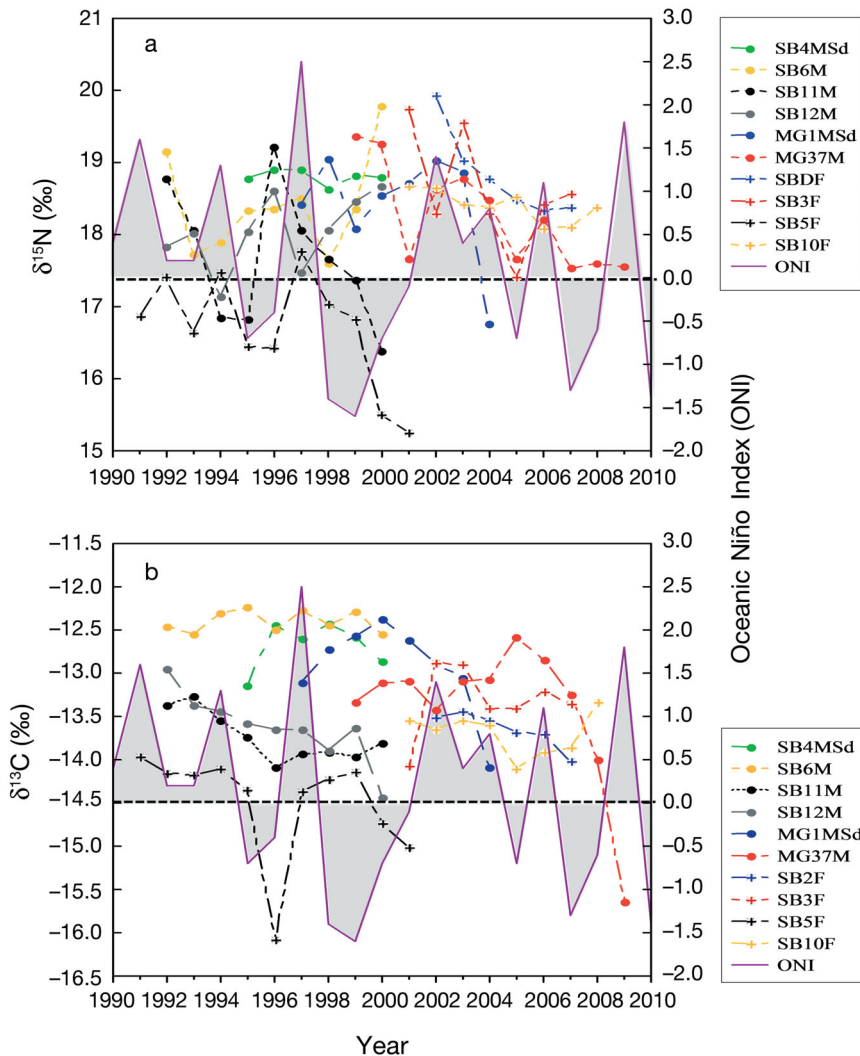


Fig. 3. *Mirounga angustirostris*. (a) $\delta^{15}\text{N}$ and (b) $\delta^{13}\text{C}$ values in relation to years and the Oceanic Niño Index (ONI). Each line represents a specific individual and begins at its year of birth. The first 6 individuals at the top of the key are males, the next 4 are females. The ONI is a 3 mo running mean of NOAA ERSST.v2 sea surface temperature (SST) anomalies in the Niño 3.4 region (5°N to 5°S , 120° to 170°W), based on the 1971 to 2000 base period. The ONI indicates the intensity of El Niño (positive values) and La Niña (negative values) events, which can be considered weak (with a 0.5 to 0.9 SST anomaly), moderate (1.0 to 1.4) or strong (≥ 1.5). See Table 1 for descriptions of individual seals

The relationship between the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the individual time series with the years and with the ONI is shown in Fig. 3. For comparisons, we refer only to the isotopic values of the 5 individuals (subadults and adults) who endured the strong 1997 El Niño year. The males SB11M and SB12M showed a reduction in their $\delta^{15}\text{N}$ signals (up to 1.2‰) during the strongest Niño year (1997), when both of them were 6 yr of age. The males SB4MSd and SB6M showed a variation of $<0.2\text{‰}$ in their $\delta^{15}\text{N}$ values when they were 3 and 6 yr of age, respectively, while

the female SB5F showed an increase of 1.3‰ in its $\delta^{15}\text{N}$ signal at 7 yr of age. Changes in $\delta^{13}\text{C}$ were $<0.2\text{‰}$ in the males (SB11M, SB12M, SB4MSd and SB6M), while the female SB5F increased its $\delta^{13}\text{C}$ signal in 1.7‰ from 1996 to 1997. Only 1 of 10 individuals (SB12M) showed a significant negative correlation between its $\delta^{15}\text{N}$ values and the ONI ($R^2 = 0.6$, $p < 0.05$) (Table 2). This indicates a reduction in $\delta^{15}\text{N}$ with the increase of the ONI. The higher the ONI values, the greater the intensity of El Niño event.

Between sites, the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were not statistically different (LME: $F_{1, 21.20} = 0.056$, $p > 0.05$ and $F_{1, 23.91} = 0.238$, $p > 0.05$, respectively), i.e. these animals come from the same region.

Isotopic variation among age classes

The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values from age classes in each sex were compared (Fig. 4, Table 3). In females, a significant difference in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ was found between the age classes (LME: $F_{2, 22.80} = 7.695$, $p < 0.05$ and $F_{2, 25.50} = 3.406$, $p < 0.05$, respectively). The Fisher's LSD test for multiple comparisons for $\delta^{15}\text{N}$ revealed that the pup group was statistically different from the juvenile females ($p = 0.009$) and adult females ($p = 0.001$), whereas for $\delta^{13}\text{C}$ significant differences were found between pups and juveniles ($p = 0.016$). For males, $\delta^{15}\text{N}$ values were not significantly different between the age groups (LME: $F_{3, 23.69} = 1.907$, $p > 0.05$), but in $\delta^{13}\text{C}$, statistical differences were found between juvenile and adult males (Fisher's LSD: $p = 0.037$).

Inter- and intra-sexual isotopic variation

No statistical differences in $\delta^{15}\text{N}$ were found between males and females (LME: $F_{1, 24.64} = 1.079$, $p > 0.05$), but in $\delta^{13}\text{C}$ the difference was significant (LME: $F_{1, 27.36} = 8.698$, $p < 0.05$). The isotopic values (mean \pm SD) calculated for males are: $\delta^{15}\text{N} = 18.3 \pm 0.8$, $\delta^{13}\text{C} =$

Table 2. *Mirounga angustirostris*. Linear regressions (R^2 values) between isotopic ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) values and Oceanic Niño Index (ONI) for each individual analyzed. Significant values ($p < 0.05$) in **bold**. The ONI values for each year were taken from the NOAA website. ID: identification code

Individual ID	ONI vs. $\delta^{15}\text{N}$	ONI vs. $\delta^{13}\text{C}$
SB4MSd	0.4	0.0
SB6M	0.0	0.1
SB11M	0.0	0.1
SB12M	0.6	0.1
MG1MSd	0.0	0.3
MG37M	0.0	0.2
SB2F	0.3	0.5
SB3F	0.0	0.2
SB5F	0.2	0.0
SB10F	0.0	0.0

-13.2 ± 0.7 , and for females: $\delta^{15}\text{N} = 18.2 \pm 1.3$, $\delta^{13}\text{C} = -14.0 \pm 0.7$.

The variance of the random interaction effect (individual, age classes nested in sex) was significant in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (Wald: $Z = 2.471$, $p < 0.05$ and $Z = 2.681$, $p < 0.05$ respectively) indicating differences among individuals of the same sex.

Isotopic values within each individual treated as repeated measures of an individual (residual variance) revealed statistical differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Wald: $Z = 5.338$, $p < 0.05$ and $Z = 5.472$, $p < 0.05$ respectively). The variance estimated for the random effect (between individuals: $\delta^{15}\text{N} = 0.567$; $\delta^{13}\text{C} = 0.262$) was larger than the residual variance (within individuals: $\delta^{15}\text{N} = 0.392$; $\delta^{13}\text{C} = 0.170$), indicating that most of the variability unaccounted for as fixed effects (site, gender, age) is due to inter-individual isotopic variation.

Table 3. *Mirounga angustirostris*. Means (\pm SE) of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ from the dentine growth layers (GL) calculated for each age-sex class (ASC) of individuals collected at San Benito and Magdalena Islands. The GL values of the specimens were categorized by age and grouped by sex in such a manner that an individual adult or subadult provided information about its own earlier age classes. The number of GL samples is according to the age and number of individuals sampled in each ASC

Age-sex class	No. of GL samples	No. of ind. sampled	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Pups (GL 0)	26	26	19.4 ± 0.2	-14.2 ± 0.2
Juvenile females (GL 1–3)	12	4	18.5 ± 1.3	-13.6 ± 0.1
Juvenile males (GL 1–4)	24	6	18.3 ± 0.2	-13.0 ± 0.1
Adult females (>GL 3)	20	4	17.6 ± 0.2	-14.0 ± 0.2
Subadult males (GL 5–8)	22	6	18.3 ± 0.1	-13.2 ± 0.1
Adult males (>GL 8)	6	4	17.9 ± 0.5	-14.0 ± 0.4

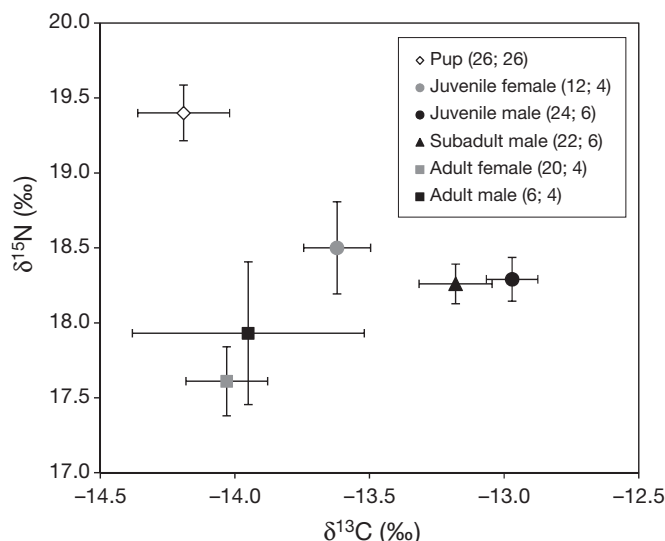


Fig. 4. *Mirounga angustirostris*. Means (\pm SE) of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in tooth collagen of individuals of different ages and sexes collected at San Benito and Magdalena Islands. The number of growth layers sampled (n_{GL}) and the number of individuals (n_{I}) from whom these samples were obtained for each age-sex class are shown in parentheses (n_{GL} ; n_{I})

Trophic level

Trophic level (TL) was estimated from the $\delta^{15}\text{N}$ average value of all specimens collected at San Benito and Magdalena using the algorithm of Vander Zanden & Rasmussen (1999). The TL estimated for northern elephant seals was 4.6, placing the species as a top trophic level predator in the northeastern Pacific Ocean.

DISCUSSION

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ontogenetic feeding patterns

In general, the teeth of northern elephant seals show large fluctuations in $\delta^{15}\text{N}$ during development, which may indicate changes in diet and physiological condition. Within-tooth fluctuations in $\delta^{15}\text{N}$ values were up to 3‰ of the mean value, which is similar to variation found in other studies (Newsome et al. 2006, 2009a, Mendes et al. 2007). Within an individual tooth, $\delta^{13}\text{C}$ values are more stable throughout development, indicating that the seals utilize the same general feeding areas year after year.

Females showed a decrease in $\delta^{15}\text{N}$ values from juvenile to the adult stage ($\sim 0.9\text{‰}$) and steady $\delta^{13}\text{C}$ values during age transition (Fig. 2). This could be the effect of positive nitrogen balance associated with pregnancy, which leads to a $\delta^{15}\text{N}$ drop as reported for the hair of human pregnant females by Fuller et al. (2004). This drop in $\delta^{15}\text{N}$ values has also been observed in a whisker of a single adult female northern elephant seal (Newsome et al. 2010). Males did not exhibit major shifts in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between juvenile and subadult stages (Fig. 2). However, $\delta^{15}\text{N}$ values tended to increase in 3 to 5 yr old individuals, when a peak in growth rate occurs in males (Clinton 1994).

With increasing protein accretion during periods of rapid and sustained growth, the consumer tissue-to-diet discrimination factor decreases (Focken 2001, Gaye-Siessegger et al. 2003, Trueman et al. 2005). Thus, the observed increase in $\delta^{15}\text{N}$ values in northern elephant seals during development may not be related to an increase in trophic level resulting from a change in diet, but rather a change in the diet-tissue discrimination factor associated with a decrease in growth rate. This effect is also observed in killer whale ontogenetic series (Newsome et al. 2009a).

Adult males, on the other hand, showed $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ mean values slightly lower than those of juvenile males (Table 3). This could be more a reflection of latitude differences in feeding sites. Geographic gradients in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of primary producers (or 'isoscapes') increase from offshore to nearshore habitats and from north to south latitudes (Graham et al. 2010). Both adult males and juveniles feed on coastal zones and on benthic prey (Hacker 1986, Sinclair 1994, Le Boeuf et al. 2000), thus the lower and significant differences in $\delta^{13}\text{C}$ values of adult males seems to reflect the more northerly foraging areas (in higher latitudes) for adults than juveniles. This is supported by observations of satellite-tracked animals (Le Boeuf et al. 1996, 2000).

The non-significant differences in $\delta^{15}\text{N}$ between adult and juvenile males would indicate that both groups were feeding on prey at a similar trophic level. The diet of juveniles and adults is similar (Sinclair 1994). Adult males, however, are >10 times heavier than juveniles males (Clinton 1994) and since size strongly correlates with trophic position in pelagic marine ecosystems (Jennings et al. 2001), adults are expected to feed on a higher trophic level than juveniles. The prey may be similar but of larger size. Moreover, since adults feed at higher latitudes than juveniles, where the $\delta^{15}\text{N}$ baseline is lower, their $\delta^{15}\text{N}$ signals are counterbalanced. There-

fore, the observed $\delta^{15}\text{N}$ values between adult and juvenile males are statistically indistinguishable.

The observed stability of $\delta^{13}\text{C}$ during development suggests that northern elephant seals repeatedly utilize the same feeding grounds, an extrapolation which is consistent with post-breeding and post-molt foraging migrations revealed by satellite tracking (Stewart & DeLong 1995, Le Boeuf et al. 2000), as also noted for southern elephant seals (Bradshaw et al. 2004). Site fidelity to feeding areas throughout life in highly variable environments may confer ecological benefits to individuals, such as familiarity with resources, their location and how they fluctuate (Greenwood 1980), even when energy gain is not consistently high in all years (Bradshaw et al. 2004). Le Boeuf et al. (2000) reported that adult males returned to the very same foraging locations 2 yr in a row taking the same migratory routes from the breeding rookery; adult females were much more variable in their location of foraging. Nevertheless, the site fidelity of females to feeding grounds is consistent during El Niño and non-El Niño years, though foraging success is poorer during warmer than normal years (Crocker et al. 2006).

During El Niño years, female northern elephant seals may feed on the cephalopods they typically consume but may also pursue alternative prey (Crocker et al. 2006). The most abundant prey during the strong 1982–1983 El Niño event was the red crab *Pleuroncodes planipes*, which usually is not common in the females' diet (Trillmich & Ono 1991). Switching from preferred prey to other less energetically rich prey that is more available in the same foraging area may also contribute to the observed $\delta^{15}\text{N}$ variation in the individual profiles.

Nevertheless, $\delta^{15}\text{N}$ values were not statistically different between the years covered in this study. Moreover, there was not a clear relationship between the isotopic values in the individual time series and the El Niño impacts (represented by the Oceanic Niño Index). Food web shifts associated with El Niño events, could not be detected by the stable isotopes in dentine tissue because of the wide intra-individual variability and the incorporation of ecological equivalents into the diet of individuals during these anomalous years. However, the environmental changes (extrinsic variability) should not be disregarded as factors that cause variation in the isotopic values throughout the life of individuals.

Only 1 of 10 individuals showed a significant negative correlation between its $\delta^{15}\text{N}$ values and the Oceanic Niño Index. Although this suggests that El Niño impacts could be detected by the $\delta^{15}\text{N}$ values in

the growth layers in teeth of elephant seals, there is a high degree of uncertainty in the interpretation of these results because this relation was not found in other individuals.

Foraging ecology

Differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between the sexes indicate the use of different feeding strategies. Significant differences in $\delta^{13}\text{C}$ indicate sexual segregation on the feeding grounds. Taking the isoscapes into account (Graham et al. 2010), males exhibit higher $\delta^{13}\text{C}$ values (range = -13.88 to -12.5), which are associated with coastal feeding on the continental shelf, while in females the range of $\delta^{13}\text{C}$ values (-14.73 to -13.85) indicates foraging in coastal as well as oceanic zones. These $\delta^{13}\text{C}$ ranges are also consistent with females foraging in middle latitudes and males foraging in mid to high latitudes, as suggested by Burton & Koch (1999).

The diving pattern of males suggests that they feed on benthic prey (Le Boeuf et al. 2000) while that of females is consistent with consuming primarily pelagic prey (Antonelis et al. 1994). The principal foraging area of adult females is the open ocean along the North Pacific Convergence; however, a small fraction of females may forage near coastal zones for part of their migration, overlapping with male foraging routes (Le Boeuf et al. 2000, Simmons et al. 2007). Males are more reliable than females in their migration routes and in returning to the same distal feeding sites in coastal areas (Le Boeuf et al. 2000).

The lack of significant differences in $\delta^{15}\text{N}$ between males and females would suggest that both groups were feeding on prey at similar trophic levels. This is consistent with studies reporting that the diets of males and females are similar (Antonelis et al. 1994, Sinclair 1994). However, because adult males are 3 to 10 times heavier than adult females (Deutsch et al. 1994), they are expected to feed at a higher trophic level on larger individuals of the same prey species than females as size strongly correlates with trophic position (Jennings et al. 2001).

Although males may feed at a higher trophic level, their $\delta^{15}\text{N}$ values are counteracted or masked by the isoscapes depending on the location where they are feeding. If baseline nitrogen signatures change with foraging location, isotopic differences will be more reflective of habitat or location than trophic level differences because the same prey will have different isotope signatures among these areas (Vander Zanden et al. 2010). Therefore, the slightly lower mean

$\delta^{15}\text{N}$ value observed in females (18.2) compared to that of males (18.3) is assumed to reflect longitudinal differences in foraging location (i.e. females forage further offshore than males) rather than latitudinal differences. Otherwise, males should have lower mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than females.

Individual foraging strategies that are maintained during development are suggested by our results. Individuals can be split into distinct groups according to their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (Fig. 5): (1) some males (SB6M, SB4MSd and MG1MSd) showed a coastal feeding strategy; (2) at least 1 female (SB5F) displayed open ocean feeding or feeding in high latitudes; and (3) both males (SB12M, SB11M, MG37M) and females (SB2F, SB3F, SB10F) foraged in coastal-oceanic zones but at different latitudes. These 3 foraging groups have been reported during migration with satellite-tracked animals (Hakoyama et al. 1994, Le Boeuf et al. 2000, Simmons et al. 2007).

In female northern elephant seals, different foraging strategies were also determined by combined satellite-tracking data and isotopic values of vibrissae (Hassrick 2011). According Hassrick (2011), the female SB5F could have foraged in the Alaska Downwelling province (ALSK), while the remaining females could have been feeding in the California Current province in the eastern North Pacific. Both are coastal provinces but ALSK is located in high latitudes. This could explain the lower isotopic values of the female SB5F and reflect latitudinal rather than longitudinal differences.

In northern elephant seals the different foraging and migration strategies displayed by individuals suggest resource partitioning, as also noted for southern elephant seals (Lewis et al. 2006). Individuals may balance consumption of preferred or abundant prey, while at the same time maintaining the flexibility to switch prey when scarcity prevails (Trillmich & Ono 1991), diversifying their diet in times of resource scarcity (Antonelis & Fiscus 1980).

We found little variance in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between individuals ($<0.7\%$), which suggests a specialist isotopic predator strategy (Bearhop et al. 2004, Newsome et al. 2007) for northern elephant seals. This assumes a variance of 1% to be a significant biological difference (Jaeger et al. 2009, Páez-Rosas & Aurióles-Gamboa 2010). Nevertheless, all variation factors, such as seasonal changes in the isotopic composition of food sources, shifts in diet-tissue fractionation patterns related to food quality, and potential isotopic variation resulting from temporal changes in consumer physiological condition (Bearhop et al. 2004, Newsome et al. 2009b), must be considered

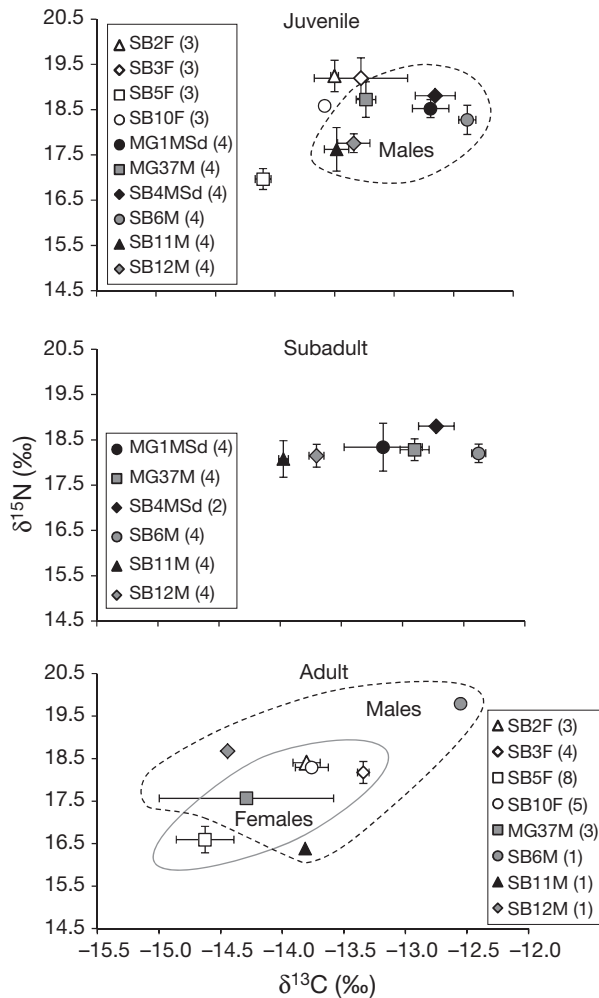


Fig. 5. *Mirounga angustirostris*. Individual means (\pm SE) of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of dental collagen of seal teeth collected at San Benito (SB) and Magdalena (MG) Islands. The values are shown for (white symbols) females and (gray and black symbols) males at 3 development stages (juvenile, subadult, adult). The collection site is indicated by the first 2 letters in the identification (ID) code shown in the key. The number of growth layers sampled is shown beside the ID code. Three groups are distinguished by feeding strategy: (1) Nearshore feeding individuals (SB6M, SB4MSd, MG1MSd): $\delta^{15}\text{N}$ range = 18.7 to 18.3, $\delta^{13}\text{C}$ range = -12.9 to -12.4; (2) Offshore or high latitude feeding (SB5F): $\delta^{15}\text{N}$ range = 16.9 to 16.5, $\delta^{13}\text{C}$ range = -14.3 to -14.7; (3) Nearshore-offshore feeding but in different latitudes (SB12M, SB11M, MG37M, SB2F, SB3F, SB10F): $\delta^{15}\text{N}$ range = 18.7 to 17.9, $\delta^{13}\text{C}$ range = -13.4 to -13.7

before this approach can be a good proxy measure to estimate trophic niche breadth.

Trophic level

The overall trophic level for adult northern elephant seals is estimated as 4.6, similar to the value

estimated for southern elephant seals (Cherel et al. 2008). In this study, we used the mean isotopic discrimination of Minagawa & Wada (1984). This did not include the exact discrimination information for each trophic level that varies with prey and type of prey tissue (Lesage et al. 2001). Nevertheless, in subtracting the isotopic fraction of 3‰ in the diet and dental collagen (Schoeninger & De Niro 1984) from the mean value of $\delta^{15}\text{N}$ of northern elephant seals (18.7‰), the approximate $\delta^{15}\text{N}$ value of its prey was estimated. This value, ~15.6‰, corresponds with that of some prey species in northeastern Pacific that commonly constitute the seal's diet (Antonelis et al. 1994), such as Pacific hake *Merluccius* sp., rockfish *Sebastes* spp., and brown catshark *Apristurus brunneus* (Bosley et al. 2004, Camalich 2011).

Evaluation of lactation

In most profiles examined, the principal isotopic discrimination occurred between GL0 and GL1, corresponding to the transition from pup to yearling. In northern elephant seals the pup phase lasts 26 to 28 d, during which pups are nursed exclusively on their mother's milk (Le Boeuf et al. 1973). Elephant seal milk reaches a composition of >54% lipids (Le Boeuf & Ortiz 1977, Ortiz et al. 1984) and enables pups to triple their body weight before weaning (Le Boeuf et al. 1989).

The first dental growth layer represents fetal life demarcated by the birth line, which in pinnipeds is present in both deciduous (DT) and permanent teeth (PT) because the formation begins *in utero* (Klevezal 1996). Dual dentition may develop simultaneously (Stewart et al. 1998, Meyer & Matzke 2004). In *Mirounga leonina*, the DT are replaced by PT that erupt, on average, during the first 8 to 10 d after birth (Carrick & Ingham 1962).

Permanent teeth of elephant seals (*Mirounga* spp.) have a complex organization of growth layers (Klevezal & Stewart 1994). Carrick & Ingham (1962) indicated that in *M. leonina*'s canines the primary dentine layer represents the last stage of fetal life and the first after birth, where one observes the birth line and the weaning line as fine bands of dense dentine. Nevertheless, we observed a distinct broad translucent layer adjacent to the enamel upon examining the longitudinal section of teeth.

Initial formation of elephant seal permanent teeth *in utero* has not been reported. Nevertheless, small portion of the dentine is likely deposited in the first layer before birth and a large mineral contribution is

deposited in most of the hypercalcified dentine during lactation. The latter should be reflected by enrichment in ^{15}N owing to lactation.

We found an enrichment of 1.8‰ in $\delta^{15}\text{N}$ between dentine layers corresponding to the pup and adult female age classes. Pups fed at a higher trophic level than adult females, who remobilize their body tissues to synthesize milk. Despite containing milk with a higher percentage of lipids, which are ^{13}C -depleted, the pups showed similar $\delta^{13}\text{C}$ values to those of adult females. Habran et al. (2010) determined that the nitrogen isotope fractionation between mother and offspring is tissue- and time-specific. They estimated an isotope fractionation of 1.1‰ in the $\delta^{15}\text{N}$ of serum of lactating mothers and pups of northern elephant seals, while the values of $\delta^{13}\text{C}$ of pups and mothers were very similar. This indicates the $\delta^{13}\text{C}$ values cannot always be used to evaluate reproductive strategies in marine mammals (Newsome et al. 2006). However, this may be primarily a function of the nursing period. The longer that a pup suckles, the greater the chance that a ^{13}C -depleted milk signal will be observed and probably occurs in the same way for $\delta^{15}\text{N}$ (S. D. Newsome pers. comm.).

Isotopic discrimination between adult females and pups has been observed in dentine layers and bones of some pinnipeds. In general, species that have short nursing periods, such as northern fur seals *Callorhinus ursinus*, southern elephant seals and northern elephant seals, show a less dramatic maternal effect between pups and mothers (Hobson & Sease 1998, Habran et al. 2010, Martin et al. 2011, this study) than in species that nurse for longer periods, such as California sea lions *Zalophus californianus* or Steller sea lions *Eumetopias jubatus* (Newsome et al. 2006, York et al. 2008). Our results demonstrate the efficiency of evaluating lactation in phocids by sampling the primary dentine layer and using $\delta^{15}\text{N}$ as the measure of this physiological condition.

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