

## USING CARBON AND NITROGEN ISOTOPE VALUES TO INVESTIGATE MATERNAL STRATEGIES IN NORTHEAST PACIFIC OTARIIDS

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### ABSTRACT

We examine the utility of stable carbon and nitrogen isotope variations to characterize the length of the nursing/lactation period and age at weaning for two northern Pacific otariid species, the northern fur seal (*Callorhinus ursinus*) and California sea lion (*Zalophus californianus*). We used two sampling strategies to measure ontogenetic trends in isotope value, and compared our results to observational data on the reproductive strategies used by these otariids. For *Zalophus*, we found evidence for  $^{15}\text{N}$  enrichment and  $^{13}\text{C}$ -depletion in bone collagen representing the first and second year of growth, which is consistent with the  $\sim 12$ – $14$ -mo weaning age in this population after a suitable turnover rate for bone collagen is considered. Analysis of individual tooth annuli from a different suite of *Zalophus* specimens suggests that half of the individuals were weaned at  $\sim 12$  mo of age, and half were dependent on milk for a portion of their second year. For *Callorhinus*, bone collagen for age classes that contain pre-weaned individuals were  $^{15}\text{N}$ -enriched, but values were significantly lower in specimens between 6 and 20 mo of age. These  $^{15}\text{N}$ -enriched values, presumably acquired during nursing between 0 and 4 mo of age in *Callorhinus*, were not present in specimens older than 12 mo of age. Thus complete bone collagen turnover in young-of-the-year occurs in 8–10 mo.  $^{15}\text{N}$  enrichment is evident in the first annulus of female *Callorhinus* individuals, but is not detectable in males. Analyses of *Callorhinus* tooth annuli show no ontogenetic trends in  $\delta^{13}\text{C}$  values. Our study indicates that nitrogen, and in some cases carbon, isotopes can be used to assess reproductive strategies in marine mammals. When coupled with accurate age estimates based on bone growth regressions, this isotopic technique can be applied to historical or fossil otariids to gain insight into the flexibility of maternal strategies within and across species.

Key words: northern fur seals, *Callorhinus ursinus*, California sea lions, *Zalophus californianus*, maternal strategy, stable isotopes, otariids, nursing behavior.

The use of natural variations in stable isotope ratios to study the diet and migration of animals and humans is an increasingly common approach in ecology and archaeology (Fogel 1997, Kelly 2000, Rubenstein and Hobson 2004). Quantitative estimates of dietary sources can be made using different tissues if appropriate "tissue-specific" fractionation factors are used to account for differences in metabolic and biosynthetic processes. In most cases, consumers are enriched in the heavy isotope ( $^{13}\text{C}$  or  $^{15}\text{N}$ ) relative to their diets. For similar tissues or whole bodies, the enrichment is roughly 1‰ for carbon and 3‰ for nitrogen. In the case of nitrogen isotopes, this strong  $^{15}\text{N}$  enrichment with trophic level has been attributed to excretion of nitrogenous waste (e.g., urea) that is enriched in  $^{14}\text{N}$  relative to the body. Trophic level enrichment in heavy isotopes has been used extensively to characterize terrestrial and marine food webs and to determine the major source(s) of prey/forage for both carnivores and herbivores in various ecosystems (Hobson *et al.* 1994, Michener and Schell 1994).

The isotopic fractionations associated with mother-to-offspring transfer of nutrients during pregnancy, lactation, and weaning are still poorly understood. Theoretically, if lactating mothers catabolize their own tissues to produce milk, their nursing offspring should have isotope values indicating that they are feeding a trophic level higher than their mother. Recent studies, however, contend that the model of increased trophic level of nursing offspring in relation to mother may be too simplistic (Jenkins *et al.* 2001), and the magnitude of animal-to-diet  $^{15}\text{N}$  enrichment is a function of the protein quality (Robbins *et al.* 2005) or the C:N ratio (*i.e.*, nitrogen concentration) of the diet (Pearson *et al.* 2003). With regard to our study, trophic level enrichment factors of  $\sim 2\text{‰}$ – $4\text{‰}$  are common for diets of fish or milk, which contain high-quality protein and have relatively high nitrogen concentrations in comparison to herbivorous diets.

For carbon isotopes, the trophic level prediction is complicated by the fact that milk has a high lipid content, and lipids are  $^{13}\text{C}$ -depleted relative to proteins (DeNiro and Epstein 1978). An animal that produces milk with a high lipid content, such as an otariid with milk that is  $\sim 30$ – $40$  wt% lipid (Costa 2002), feeds its young a food source with a relatively low  $\delta^{13}\text{C}$  value. There is no difference in  $\delta^{15}\text{N}$  value between lipids and associated proteins, so the consumption of milk rich in lipids would not affect the  $\sim 3\text{‰}$   $^{15}\text{N}$  enrichment. Besides the  $\sim 1\text{‰}$  trophic level enrichment discussed above, another factor that could potentially act to offset the "lipid effect" are differences in metabolic routing. Because collagen primarily represents a protein pathway, carbon sourced from milk protein will be preferentially routed into bone or tooth dentin collagen before carbon sourced from milk lipids, which is more likely to be assimilated into body lipids (*i.e.*, blubber) or consumed during energy-producing metabolic reactions. As mentioned above, however, otariid milk has a substantial lipid component ( $\sim 30$ – $40$  wt%), thus it is safe to assume that a considerable portion of milk lipid carbon is used to construct bone collagen or tooth dentin, especially during periods of rapid growth. After considering these factors, we predict that nursing offspring should have higher  $\delta^{15}\text{N}$  values, but either lower or higher  $\delta^{13}\text{C}$  values than their mothers, depending on the lipid content of the milk.

Most studies investigating these issues are from the archaeological literature, where nitrogen isotopes have been used to assess weaning age in modern and prehistoric

human populations. Controlled studies on modern humans have found that nursing young have keratin  $\delta^{15}\text{N}$  values  $\sim 2.5\text{‰}$  higher than their mothers, whereas neonates and weaned children have keratin  $\delta^{15}\text{N}$  similar to their mothers (Fogel *et al.* 1989, 1997). In prehistoric human populations, young age classes have higher  $\delta^{15}\text{N}$  values than associated adults, allowing researchers to constrain age at weaning in various archaeological and environmental contexts (Katzenburg *et al.* 1993, Schurr 1997, Wright and Schwarcz 1999, Fuller *et al.* 2003). In other mammals, nursing young always have higher  $\delta^{15}\text{N}$  values relative to those of their mother; however, fetal and neonate age classes may also have higher  $\delta^{15}\text{N}$  values than their mothers depending on the species (Balasse *et al.* 2001, Jenkins *et al.* 2001, Polischuk *et al.* 2001).

Human ontogenetic carbon isotope trends are more complex. Studies of prehistoric human populations show no consistent pattern of difference in  $\delta^{13}\text{C}$  values between young and adult individuals. In some cases, nursing offspring have higher tooth dentin  $\delta^{13}\text{C}$  values than adults (Fuller *et al.* 2003); other studies show a high degree of inter-individual variability that makes it impossible to identify a consistent trend (Wright and Schwarcz 1999). In other mammal species, nursing and young age classes tend to have lower  $\delta^{13}\text{C}$  values than adults (Polischuk *et al.* 2001, Witt and Ayliffe 2001).

Isotopic studies of nursing and weaning in marine mammals have used ontogenetic series of bone elements or annuli in dentin from sectioned canine teeth. Analysis of tooth annuli in Steller sea lions (*Eumetopias jubatus*) shows that nursing young have higher tooth dentin  $\delta^{15}\text{N}$  values ( $\sim 2\text{‰}$ – $3\text{‰}$ ) and lower  $\delta^{13}\text{C}$  values ( $\sim 2\text{‰}$ ) than adults (Hobson and Sease 1998). Similarly, bones of pre-weaned northern fur seals (*Callorhinus ursinus*) from archaeological sites on the coast of California have bone collagen  $\delta^{15}\text{N}$  values  $3\text{‰}$  higher and  $\delta^{13}\text{C}$  values  $\sim 1\text{‰}$  lower than older age classes (Burton *et al.* 2001). As mentioned above, low  $\delta^{13}\text{C}$  values in nursing or young pinnipeds is most likely a result of consuming milk that is rich in  $^{13}\text{C}$ -depleted lipids.

The aim of this study is to explore the utility of carbon and nitrogen isotopes as proxies for nursing behavior in otariids through analysis of two species with very different weaning behaviors. Like most otariids, California sea lions (*Zalophus californianus*) have a relatively long lactation period that allows the pup to develop swimming and foraging skills before complete weaning (Kovacs and Lavigne 1992). Most pups are weaned by  $\sim 10$ – $12$  mo of age, but this appears to vary geographically; the age at weaning in Gulf of California populations is higher and more variable than in the southern California stock (Heath 1989). The weaning process is gradual and facultative nursing has been observed in the second or even third year in the Gulf of California (Francis and Heath 1991). The degree to which prolonged nursing plays a nutritional *vs.* social role in yearling/juvenile *Zalophus* has yet to be determined.

In contrast, northern fur seals (*C. ursinus*) adhere to a strict attendance and nursing strategy thought to be an adaptation to high latitude breeding (Gentry and Holt 1986, Gentry 1998). In the Pribilof Islands, the breeding season begins in late June and runs through mid-November ( $\sim 125$  d). During the breeding season, lactating females make regular foraging trips to sea that range from 4 to as many as 16 d, returning to the rookery to nurse their pups for an average of 2–3 d. In contrast to other NE Pacific otariids, northern fur seals undergo abrupt weaning at  $\sim 4$  mo of age, and young do not nurse beyond their first year of life. The only other otariid with a similar attendance and nursing strategy is the Antarctic fur seal (*Arctocephalus*

*gazella*), which has a slightly shorter nursing period, lasting only ~120 d (Doidge *et al.* 1986).

We present data on ontogenetic isotope trends from *Callorhinus* and *Zalophus* that were generated using two different sampling strategies: (1) a population-level (cross-sectional) approach using bulk bone collagen isotope values of specimens that were aged either with bone growth regressions or counts of annuli in canine teeth, and (2) an individual-level (longitudinal) approach where “life-history” records of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were produced by sub-sampling dentin annuli of canine teeth.

#### MATERIALS AND METHODS

All *Zalophus* bone elements were sampled from crania collected on Isla Santa Margarita, Baja California Sur (BCS) off the Pacific coast of Baja California. These specimens are archived at CICIMAR (La Paz, BCS) and were collected from individuals that stranded on the Isla Santa Margarita rookery over the past two decades. Unfortunately, detailed collection data were not recorded and there are no bone growth regressions for California sea lions. As a consequence, it is difficult to resolve age at a sub-annual resolution for this species. For each cranium, the number of annuli from a sectioned canine tooth was counted to produce annual bins (*i.e.*, 0–1, 1–2, 2–3 yr of age) that offered conservative annual-resolution age estimates. Once age had been accurately assessed, a small fragment of bone was removed from the mandible of each cranium using a low-speed cutting tool and stored in a glass scintillation vial for transport.

In addition, an upper canine tooth from selected Baja California *Zalophus* adults was removed for sectioning and sub-sampling on a Merchantek micromill system. Included in the *Zalophus* sample are four individuals from the Isla Santa Margarita rookery on the Pacific coast (the same rookery from which the *Zalophus* bone collagen ontogenetic series is derived), one individual from the Los Islotes rookery in the southern Gulf of California (IL-ZC02), and one individual sourced from the Isla Lobos rookery in the northern Gulf of California (ISL-ZC03). All *Zalophus* canines were opportunistically collected over the past two decades from stranded adult male individuals. None of the *Zalophus* canine specimens were derived from individuals that were used to construct the bone collagen ontogenetic series.

Canine teeth were cut in half longitudinally using a low-speed, water-cooled sectioning saw and polished using fine sandpaper (~600 grit). The inner surface of each tooth was etched lightly in 5% formic acid to help distinguish annuli. We assume that such treatment did not influence  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, since the surface portion of the tooth represents a small fraction of the total sample and all specimens were treated in a similar fashion.

*Callorhinus* bone collagen samples were acquired from archived specimens at the California Academy of Sciences (San Francisco, CA), Smithsonian Institution (Washington, DC), and National Marine Mammal Laboratory (Seattle, WA). All specimens were collected on rookeries in the Pribilof Islands over the past five decades and were either sacrificed specifically for ontogenetic studies (Scheffer and Wilke 1953) or were natural mortalities obtained opportunistically. Furthermore, all *Callorhinus* bone samples used in this study were selected from a list of specimens whose age

at death was determined by Etnier (2002). A small fragment of bone was removed (using pliers) from inside the cranial vault of each specimen and stored in a glass scintillation vial for transport. The age-at-death of each specimen was calculated in fractions of years, with an age of zero corresponding to the date of birth. Fur seals have a narrow interval during which pups are born, with virtually all of the births falling within a few weeks of the mean birthing date (Bartholomew and Hoel 1953, Peterson 1965, Trites 1992, York and Scheffer 1997, Gentry 1998), which ranges from July 3rd to 11th on rookeries in the Pribilof Islands, AK. Only *Callorhinus* specimens sourced from the Pribilof rookeries were analyzed, but it was impossible to determine the exact rookery of origin for each specimen. When locality information was not recorded or was ambiguous regarding population of origin, specimen age was rounded to the nearest whole month or whole year, depending on the specificity of collection information. We assume that collection date is synonymous with the date of death, since most of the specimens were directly harvested for commercial, subsistence, or scientific purposes.

*Callorhinus* adult canine specimens were derived from adult males and females stranded on Reef Rookery on Saint Paul Island, AK from 2000 to 2005. These specimens were collected as part of annual mortality studies collected under MMPA permit number 782-1708. None of the *Callorhinus* canine specimens were derived from individuals that were used to construct the bone collagen ontogenetic series. These specimens were prepared as described above for *Zalophus*.

For  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis, *Callorhinus* and *Zalophus* bone fragments were cleaned of soft tissue and demineralized in 0.5N hydrochloric acid (HCl) for ~12–15 h at 5°C. The resulting material was treated repeatedly with a chloroform/methanol/water (2:1:0.8) mixture to remove lipids and then lyophilized. To isolate collagen, powdered tooth dentin samples were demineralized in 0.1N HCl for 24–36 h at 5°C, and the resulting organic matter was vacuum concentrated. Dried bone collagen (~0.5 mg) and dentin samples (~1.5 mg) were sealed in tin boats and analyzed using a Costech elemental analyzer interfaced with a Finnegan Delta Plus gas source mass spectrometer (Department of Geological and Environmental Sciences, Stanford University). As a control for the quality of bone or dentin collagen, we measured the carbon-to-nitrogen (C/N) ratios of each sample to test the possibility that isotopic values were altered postmortem. In addition, since powdered tooth dentin samples were not lipid-extracted, we wanted to ensure that our samples did not contain any  $^{13}\text{C}$ -depleted lipids, which have much higher C/N ratios than associated proteins (DeNiro and Epstein 1978). The atomic C/N ratios of all bone collagen and tooth dentin samples are 3.2–3.4, well within the range that characterizes unaltered protein (Ambrose 1990).

Results are expressed as  $\delta$  values,  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = 1,000[(R_{\text{sample}}/R_{\text{standard}}) - 1]$ , where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratios of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon and atmospheric  $\text{N}_2$  for nitrogen. The units are expressed as parts per thousand or per mil (‰). Repeated measurements of a gelatin standard ( $n = 100$ ) yielded a standard deviation of  $<0.2\text{‰}$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Duplicate isotopic measurements were performed on ~20% of all unknown samples and yielded an average absolute difference of 0.2‰ for  $\delta^{13}\text{C}$  values and 0.3‰ for  $\delta^{15}\text{N}$  values.

Statistical tests were calculated using the software program JMP (v5.0). Differences in bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between the age classes discussed in the text were assessed using a one-way analysis of variance (ANOVA) followed by a *post hoc* Tukey pairwise comparison.

## RESULTS

*Northern Fur Seal Bone Collagen*

A total of 55 bone collagen samples were collected from male and female *Callorhinus* specimens estimated to be between 2 and 28 mo of age.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  results for the ontogenetic series are presented in Figure 1 and are segregated by sex. To characterize the ontogenetic trends that might be associated with weaning, these results are summarized in Figure 2, with specimens binned into 2–6, 6–8, 8–10, 10–12, 12–14, 14–20, and 20–28 mo age classes and plotted *vs.* mean  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values ( $\pm$  one standard deviation). Data for both sexes were used to calculate the means and standard deviations in Figure 2. There is a robust trend in  $\delta^{15}\text{N}$  value with age (Fig. 2A), with a significant decrease from 2–8 mo to 12–14 mo of age (ANOVA,  $F = 3.13$ ,  $P < 0.05$ ). There is also a significant difference in mean  $\delta^{15}\text{N}$  values between individuals 12–28 mo of age and adult males and females (Tukey,  $P < 0.05$ ; adult data from Burton and Koch 1999).

The  $\delta^{13}\text{C}$  values, in contrast, values show little to no trend through the ontogenetic sequence (Fig. 2B). The 2–6 and 6–8 mo age classes have lower mean  $\delta^{13}\text{C}$  values than older age classes, but these differences in mean values among age classes are not significant using a one-way ANOVA ( $F = 3.13$ , Fig. 2B).

Because our *Callorhinus* ontogenetic series contains individuals collected over the past five decades, we plotted bone collagen  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values of all individuals within a specific age group against collection year to test if there were temporal trends in isotope values not evident in our population-level approach. We found no correlation between carbon or nitrogen isotope value and collection year for any of the age groups.

*California Sea Lion Bone Collagen*

A total of 49 bone collagen samples were collected from male and female *Zalophus* mandibles estimated to be between 0 and 4 yr of age (Fig. 3). In addition, we sampled adult males and females ( $>7$  yr old;  $\sim 10$  per sex) collected from the Isla Santa Margarita rookery. The ontogenetic isotope curves for *Zalophus* are different than those for *Callorhinus*. The 0–1 and 1–2-yr-old age classes are  $^{15}\text{N}$ -enriched;  $\delta^{15}\text{N}$  values drop significantly in the 2–3-yr-old specimens. The differences in mean value among age classes are significant using a one-way ANOVA ( $F = 3.11$ ,  $P < 0.05$ ). The 0–1 and 1–2 yr age classes are not significantly different from one another, but are significantly different from the 2–3 and 3–4 yr age classes (Tukey,  $P < 0.05$ , Fig. 3A). Interestingly,  $\delta^{15}\text{N}$  values for the 2–3 and 3–4 yr age classes are not significantly different from those for adults.

The 0–1 and 1–2 yr age classes have lower  $\delta^{13}\text{C}$  values than the older age classes. Again, the differences in mean values among age classes are significant using a one-way ANOVA ( $F = 3.11$ ) followed by a Tukey pairwise comparison test ( $P < 0.05$ ). The 0–1 and 1–2 yr age classes do not differ significantly, but they are significantly different from the 2–3 and 3–4 yr age classes (Tukey,  $P < 0.05$ ; Fig. 3B). Furthermore,  $\delta^{13}\text{C}$  values for the 2–3 and 3–4 yr age classes are not significantly different from those for adults ( $>7$  yr old).

Similar to the *Callorhinus* ontogenetic series, the *Zalophus* series contains individuals collected over a relatively long-time period. Again, we tested for temporal trends in *Zalophus* isotope values using the method described in the preceding section and found no correlation between bone collagen  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values and collection year.

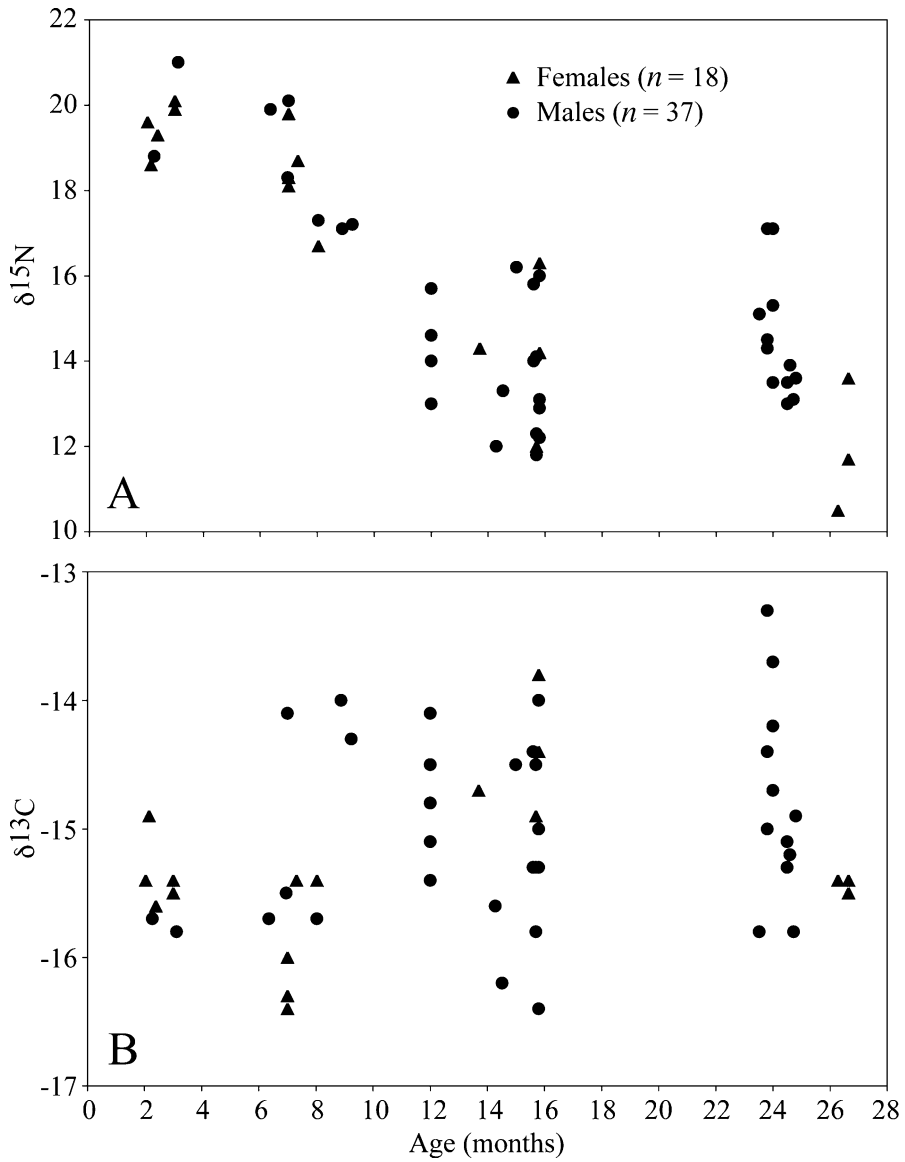


Figure 1.  $\delta^{15}\text{N}$  (A) and  $\delta^{13}\text{C}$  (B) nursing curves for male and female northern fur seals from rookeries on the Pribilof Islands.

*Individual Ontogenetic Patterns via Isotopic Analysis of Canine Tooth Annuli*

Figure 4, 5 present the results of the individual-level approach, in which we analyzed collagen derived from consecutive annuli in tooth dentin to provide an annual-resolution ontogenetic sequence from individual animals. We have randomly

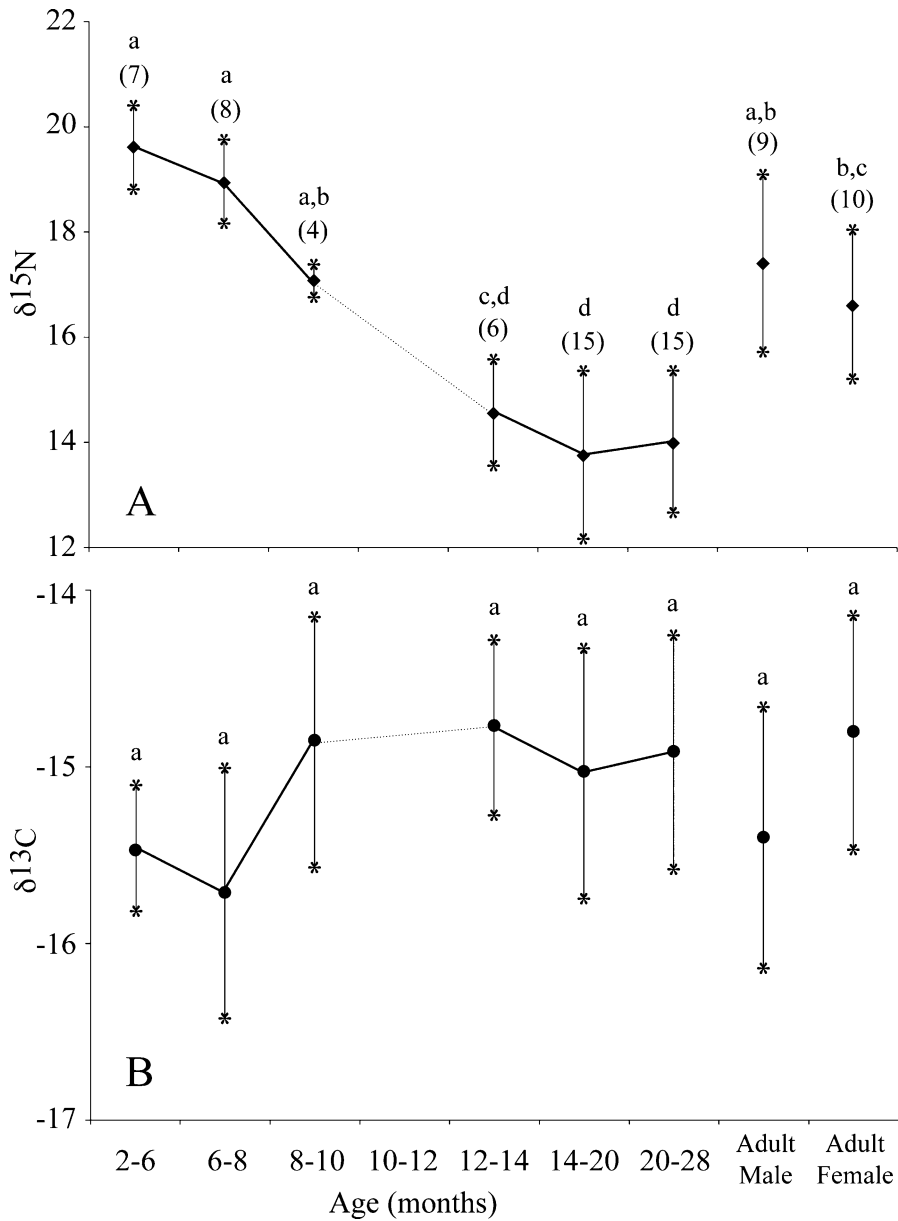


Figure 2. Mean  $\delta^{15}\text{N}$  (A) and  $\delta^{13}\text{C}$  (B) values for bone collagen from northern fur seals from the Pribilofs binned by age group. Error bars represent one standard deviation from the mean. The number of specimens in each age group is noted in parentheses. Mean values ( $\pm$  one standard deviation) for adult males and females are from Burton and Koch (1999). For  $\delta^{15}\text{N}$ , age groups not connected by the same lower-case letter are significantly different from one another based on a one-way ANOVA ( $F = 3.13$ ) followed by a Tukey pairwise comparison test ( $P < 0.05$ ). For  $\delta^{13}\text{C}$ , none of the age groups are significantly different from one another ( $P > 0.05$ ).



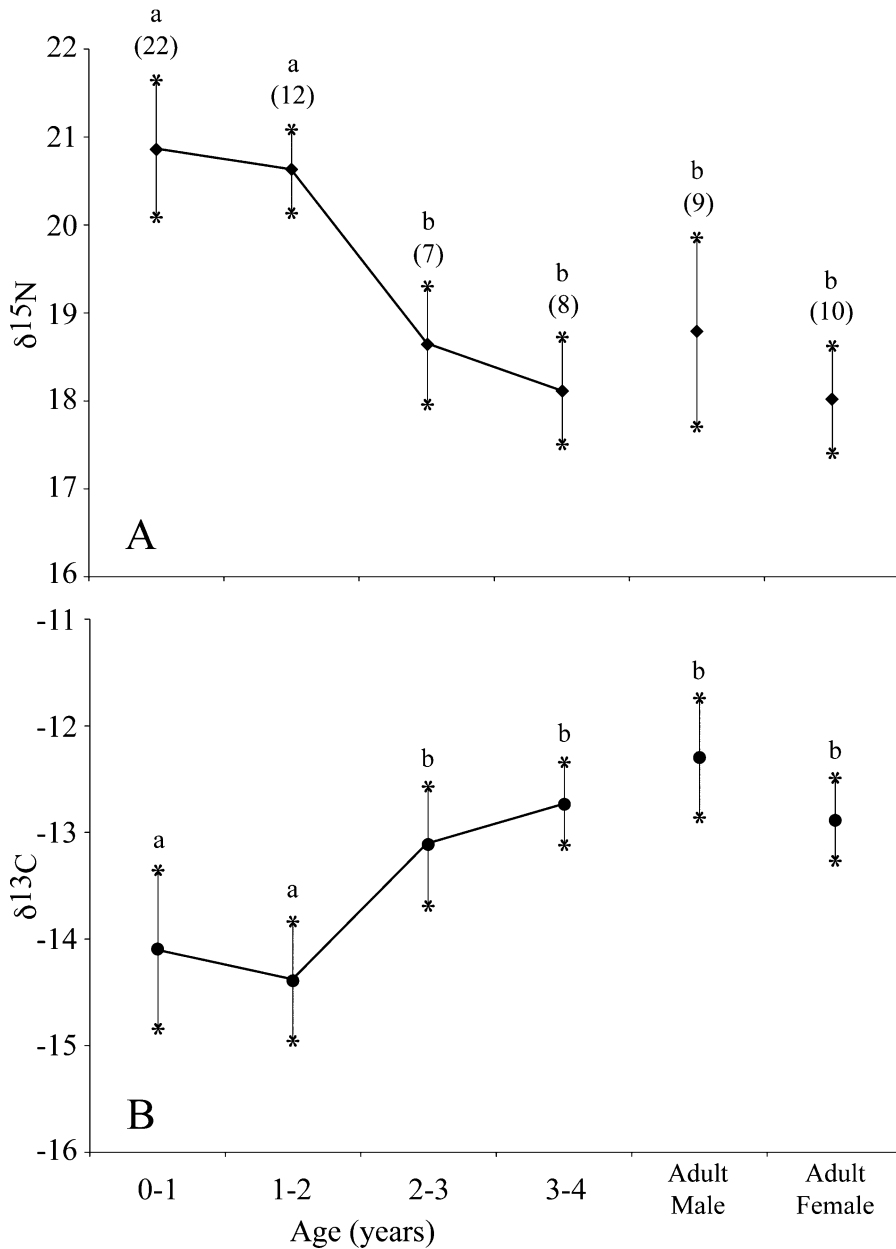


Figure 3. Mean bone collagen  $\delta^{15}\text{N}$  (A) and  $\delta^{13}\text{C}$  (B) values for Isla Santa Margarita California sea lion specimens binned by age group. Error bars represent one standard deviation from the mean. The number of specimens in each age group is noted in parentheses. For  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , age groups not connected by the same lower-case letter are significantly different from one another based on a one-way ANOVA ( $F = 3.11$ ) followed by a Tukey pairwise comparison test ( $P < 0.05$ ).

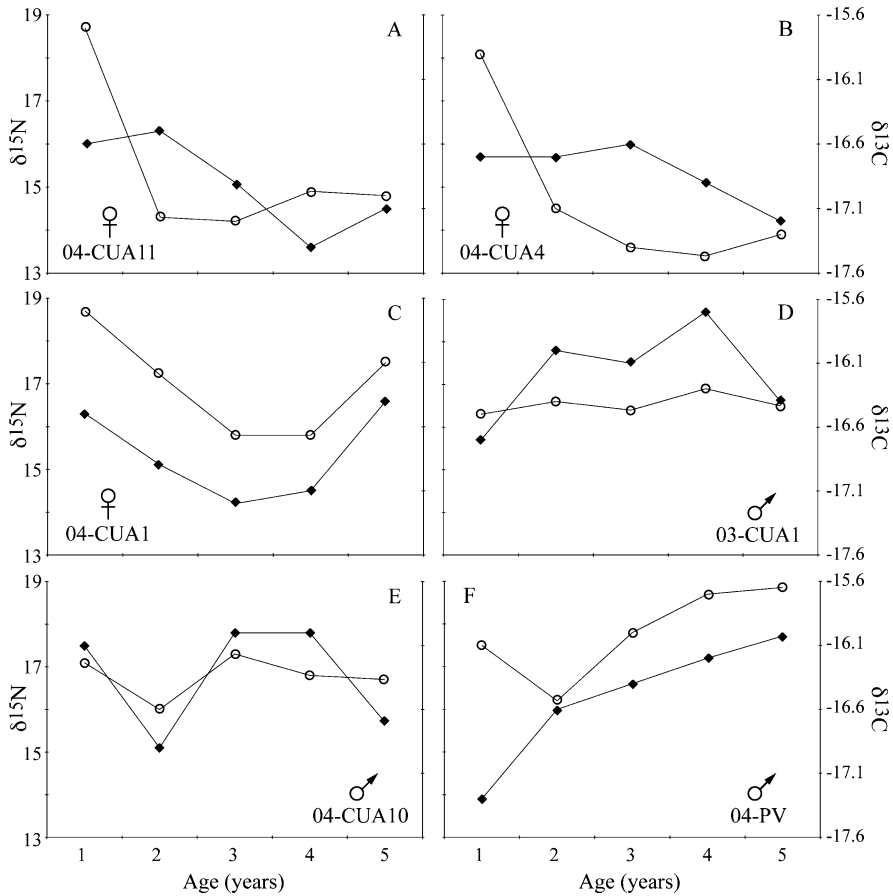


Figure 4. Early life history patterns of tooth dentin  $\delta^{15}\text{N}$  (open circles) and  $\delta^{13}\text{C}$  (diamonds) values for six northern fur seal individuals collected from the Pribilof Island rookeries. Sex and sample codes are noted for each specimen.

chosen six canine specimens of each species and have plotted carbon and nitrogen isotope values from annuli representing the first 5 yr of life.

#### Northern Fur Seal Tooth Annuli

Analysis of individual *Callorhinus* tooth dentin annuli reveal interesting ontogenetic and sex related patterns. First,  $\delta^{15}\text{N}$  values show considerable variation across annuli representing the first 5 yr of life, varying by as much as 5‰ in some specimens. Females tend to show greater amounts of inter-annual variability in  $\delta^{15}\text{N}$  values than males. Most of this variation in females relates to differences between the first annulus and subsequent annuli; the first annulus has  $\delta^{15}\text{N}$  values that are 2‰–4‰ higher than subsequent annuli. None of the male specimens show this pattern. Male  $\delta^{15}\text{N}$  values only vary by  $\sim$ 1‰–2‰ across annuli and none of the first annuli in male canines have considerably higher  $\delta^{15}\text{N}$  values than subsequent annuli.

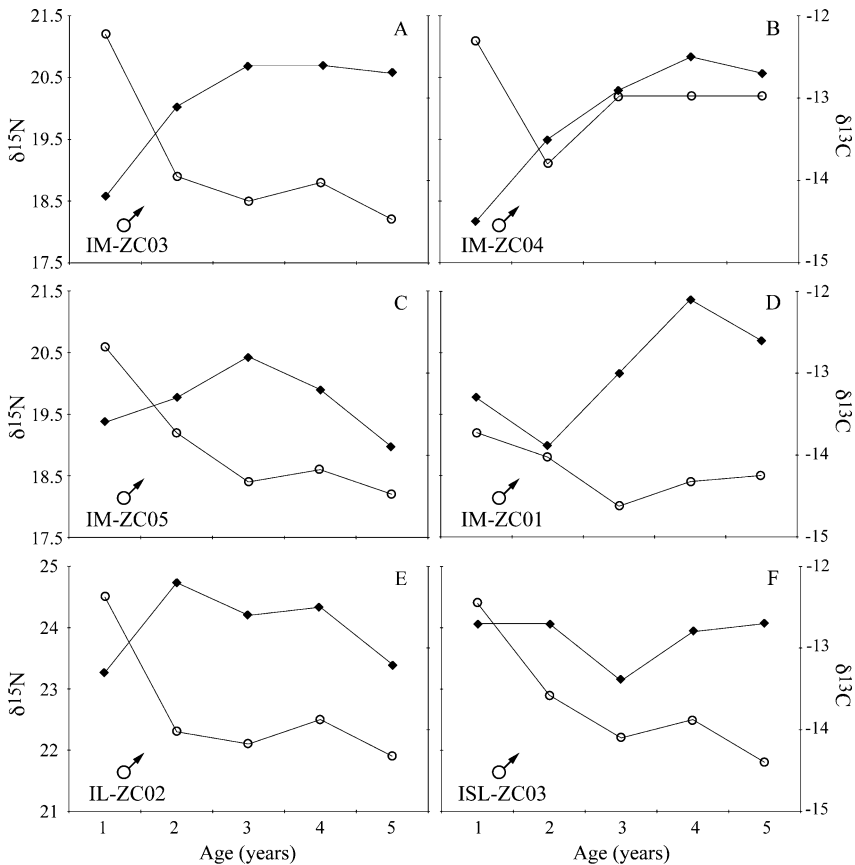


Figure 5. Early life history patterns of tooth dentin  $\delta^{15}\text{N}$  (open circles) and  $\delta^{13}\text{C}$  (diamonds) values for six male California sea lion individuals collected from rookeries in Baja California. Samples A–D are specimens from the Isla Santa Margarita rookery on the Pacific coast of BCS. Sample E was collected from Isla Lobos in the northern Gulf of California. Sample F was collected from the Islotes rookery in the southern Gulf of California. Sex and sample codes are noted for each specimen.

There are no clear patterns in the  $\delta^{13}\text{C}$  data from annuli. Overall, there is a smaller amount of variation in  $\delta^{13}\text{C}$  values across annuli (1‰–2‰) in comparison to  $\delta^{15}\text{N}$  values. Only one specimen (male; Fig. 4F) has lower  $\delta^{13}\text{C}$  values in the first annulus in comparison to subsequent annuli. The other specimens show little variation across annuli with no clear ontogenetic patterns.

#### California Sea Lion Tooth Annuli

All the *Zalophus* canine specimens included in this study show the same general  $\delta^{15}\text{N}$  pattern, with relatively high values in year 1 followed by a decrease of  $\sim 3\%$  in some individuals in year 2, and a stabilization of values in all specimens by year 3. In some cases (Fig. 5C, F),  $\delta^{15}\text{N}$  values for the second annulus are intermediate in

comparison to values for years 1 and 3. In addition, there is a considerable difference in the range of  $\delta^{15}\text{N}$  values between individuals from Isla Santa Margarita on the west coast of Baja California (Fig. 5A–D) and animals from Gulf of California rookeries (Fig. 5E, F).  $\delta^{15}\text{N}$  values for Gulf of California individuals are  $\sim 3\text{--}4\%$  higher than values for animals from the Pacific coast of Baja California.

Tooth dentin  $\delta^{13}\text{C}$  patterns in *Zalophus* are more complex. Overall, there is less variation in  $\delta^{13}\text{C}$  values in comparison to  $\delta^{15}\text{N}$  values. Furthermore, there is no difference in  $\delta^{13}\text{C}$  values for animals from the Pacific Ocean (Isla Santa Margarita, Fig. 5A–D) and the Gulf of California (Fig. 5E, F). For half of the individuals sampled, the first year has the lowest  $\delta^{13}\text{C}$  value in comparison to subsequent annuli. In some cases, however,  $\delta^{13}\text{C}$  values are relatively uniform throughout the time series and fluctuate less than 1‰ from years 1 to 5.

## DISCUSSION

Overall, the ontogenetic trends in the *Callorhinus* and *Zalophus* bone and tooth annuli series are consistent with the known isotope enrichments related to trophic level and lipid content among the diets of pre-weaned pups, weaned juveniles, and adults. Pre-weaned pups feed one trophic level higher than their mothers, because females remobilize their body tissues to synthesize milk. As a consequence, pups are  $^{15}\text{N}$ -enriched by  $\sim 3\%$  relative to their mothers. Because pinniped milk is extremely rich in  $^{13}\text{C}$ -depleted lipids in comparison to the piscivorous diet of weaned animals, pre-weaned pups have lower  $\delta^{13}\text{C}$  values than older individuals (though not significantly so in *Callorhinus*). A more detailed evaluation of the ontogenetic isotope patterns requires that we consider the differences in time-resolution of the bone series for each species, the differences in turnover rates for the two tissues analyzed, and other sources of variation in the isotope compositions of pinnipeds not specifically related to weaning.

The *Callorhinus* ontogenetic bone series has sub-annual time resolution and in the first year of life, rapid growth leads to rapid remodeling and turnover. Weaning occurs at 4 mo of age. Weaning is signaled by the slight drop in  $\delta^{15}\text{N}$  values between the 2–6 and the 6–8 mo age classes; values continue to decline until the nursing signal is completely diluted by bone turnover by the 10–12 mo age class. If *Callorhinus* begin to ingest solid food shortly after weaning and we assume that recently weaned animals consume similar prey types as juveniles between 1 and 2 yr of age, it takes  $\sim 8$  mo for the  $\delta^{15}\text{N}$  weaning signal to be completely stripped from bone collagen by turnover. Furthermore, *Callorhinus* between 12 and 28 mo of age have lower bone collagen  $\delta^{15}\text{N}$  values than adults, most likely because these juveniles feed at a lower trophic level than adults.

The  $\sim 2\text{--}3$  mo age control available for the *Callorhinus* bone collagen series allows us to accurately determine the age at which weaning occurs in this species. Furthermore, the significant drop in trophic level after weaning is only seen in  $\delta^{15}\text{N}$  values of the ontogenetic series (Fig. 1A, 2B). There is no robust trend in  $\delta^{13}\text{C}$  values from either the ontogenetic bone series (Fig. 1B, 2B) or sectioned canine specimens (Fig. 4). It is difficult to assess why the ontogenetic trend in  $\delta^{13}\text{C}$  values is so much less robust than the  $\delta^{15}\text{N}$  trend. It may be due to the difference in the relative magnitude of the signal.  $\delta^{15}\text{N}$  values vary by greater than 5‰ from the 3–6 to 12–14 mo age class, whereas  $\delta^{13}\text{C}$  values only vary by  $\sim 2\%$  over this age range.

Previous studies have shown that Gulf of California *Zalophus* are weaned at ~12 mo of age (Aurióles-Gamboa 1989). While we have no data on the subject, in theory, growth rates should decrease with age, especially in otariids such as *Zalophus*, where before weaning, nursing animals begin to supplement their diet with solid foods that have a lower caloric content than milk (Aurióles-Gamboa 1989, Costa 2002). By extension, bone collagen turnover rates may be slightly slower for juvenile *Zalophus* older than 12 mo of age in comparison to 5–12-mo-old *Callorhinus*. Assuming bone collagen completely turns over on the order of 10–12 mo for *Zalophus* yearlings, milk is a major dietary component for Isla Santa Margarita juveniles up to and perhaps slightly beyond their first birthday.

Other than indicating that weaning is not very rapid in *Zalophus* in comparison to *Callorhinus*, isotopic analysis of *Zalophus* bone ontogenetic series provides a coarse estimation of weaning age. This is partly due to the lack of sub-annual resolution in age control for *Zalophus*, and will continue to be an issue until more known-age individuals can be measured and adequate bone regressions can be constructed for this species. The low resolution in age control may also contribute to the apparent similarity in mean carbon and nitrogen isotope values for *Zalophus* juveniles and adults. In *Callorhinus*, juveniles and adults have different  $\delta^{15}\text{N}$  values. We suspect these reflect differences in the trophic level of prey consumed by each age group, but spatial separation in foraging habitat for adults and juveniles, which can create isotopic differences in similar prey types in different locations, cannot be ruled out for either species. Spatial effects are more likely to contribute to the isotopic differences seen among *Callorhinus* age groups because the species is more migratory than *Zalophus* and spatial gradients in isotope values at the base of the food web are more pronounced in the North Pacific and southern Bering Sea than in the California Current.

The ontogenetic series from tooth annuli were sampled to yield an annual time-resolution. Primary dentin does not experience extensive remodeling and turnover, but protein turnover from other body pools releases previously deposited amino acids to the circulating pool in the body that are fixed in continuously deposited tissues like dentin and hair (Ayliffe *et al.* 2004). At present, we cannot provide a firm quantitative estimate of the extent to which this process would “smooth” isotopic transitions recorded in tooth annuli, but it seems likely that the bulk of the isotopic equilibration to a new diet occurs in 1 or 2 mo (Ayliffe *et al.* 2004).

In the tooth annuli series from *Callorhinus* canines, only females record a drop in  $\delta^{15}\text{N}$  values between the first and second year of growth; males show no clear ontogenetic patterns in dentin  $\delta^{15}\text{N}$  values. The lack of a nursing signal in the first annulus of male *Callorhinus* canine specimens may be the result of differences in growth rates between young males and females. Male *Callorhinus* canine teeth tend to be larger than female teeth (Huber 1994), and they have thicker annuli. For males, the nursing signal deposited in the first 4 mo of life may be offset by a “larger” (*i.e.*, thicker) foraging signal in the subsequent 8 mo because male growth rates are faster in comparison to females. In this study, we typically used a 600–700  $\mu\text{m}$  thick carbide drill bit to sample the entire first annulus of female teeth and used an 800–1,000  $\mu\text{m}$  drill bit for male specimens. This difference in thickness could account for the lack of a nursing signal in male specimens if the extra material in male teeth is deposited post-weaning. Overall, ontogenetic series of isotope values from *Callorhinus* tooth annuli are a less reliable and accurate indicator of weaning age in comparison to the sub-annual age control available for the bone ontogenetic series.

Differences in the isotopic trends from the *Zalophus* bone ontogenetic series in comparison to those from tooth annuli highlight differences in turnover rates of these respective tissues. As mentioned above, extensive remodeling and turnover does not occur in primary dentin, thus  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from tooth annuli represent an ontogenetic series with true annual resolution. For *Zalophus*,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values from tooth annuli (Fig. 5) suggest that several individuals were weaned slightly beyond their first birthday. For these individuals, isotope values of the annuli representing the second year of growth are intermediate to values of the first and third annuli. In contrast, there is no difference in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the 0–1 and 1–2 yr age classes from the ontogenetic bone series, with a significant shift from the 1–2 to 2–3 yr age class. Coupling results from teeth and bone suggests that complete bone collagen turnover occurs on the order of 10–12 mo for yearling *Zalophus*, a slightly slower turnover rate in comparison to young *Callorhinus*. It appears that milk is an important source of nutrition for these *Zalophus* individuals into their second year; however, the majority of the second annulus was deposited while the animal was nutritionally dependent on solid food, characterized by lower  $\delta^{15}\text{N}$  and higher  $\delta^{13}\text{C}$  values in comparison to milk. Whether these animals nurse facultatively throughout their second year or are dependent on milk for only the first few months of their second year is difficult to assess. It is likely that these individuals were completely weaned as yearlings (~12–14 mo), and spent most of their second year foraging on their own, with little nutritional help from their mother. As mentioned above, it is common to observe young juveniles (12–14 mo old) suckling from an adult female that does not appear to have a pup, especially early in the summer breeding season. In a 2004 population census of eight rookeries in the Gulf of California, 3.1% of the juveniles counted (most likely yearlings) were observed nursing from an adult female (Table 1). If such a proportion of juveniles are still consuming milk in a single and rapid scan of each rookery, the percentages we observe likely represent a conservative estimate, and the actual number of nursing juveniles must be higher. Overall, isotopic analysis of tooth dentin provides a better assessment of weaning age in *Zalophus* in comparison to bone collagen due to the lack of sub-annual age control for ontogenetic bone series and differences in turnover rates in teeth *vs.* bone.

A more comprehensive study of *Zalophus* individuals from various rookeries across its breeding range may allow for the identification of geographical variation in reproductive strategies and help quantify the relative importance of milk in the diets of pups and young juveniles from different populations that forage in different

Table 1. Percent of suckling juveniles observed during a census in July 2004 of eight *Zalophus* rookeries in the Gulf of California.

Rookery	Juveniles	Suckling	(%)
San Jorge	382	16	4.1
Isla Lobos	192	4	2.1
Il Granito	132	5	3.8
Los Cantiles	97	4	4.1
Los Machos	77	1	1.3
El Partido	84	1	1.2
El Rasito	86	3	3.5
S. P. Martir	171	8	4.6
Mean ( $\pm$ standard deviation)			3.1( $\pm$ 1.4)

oceanographic settings that dictate the abundance and availability of prey. Furthermore, future studies that aim to further refine the isotopic enrichment associated with nursing in otariids should attempt to compare isotope values of otariid milk with those of maternal diets under controlled captive conditions, and characterize the isotope composition of tissues that are relatively easy to collect during the course of field-based studies on wild populations.

### Conclusions

- The switch in trophic level and lipid-content of diet associated with weaning in otariids can be traced *via* nitrogen, and sometimes carbon, isotope values in ontogenetic series of bone collagen and tooth dentin.
- For *Callorhinus*, bone collagen  $\delta^{15}\text{N}$  values provide the best estimate of weaning age because high-resolution bone regressions have been generated for several elements, allowing for sub-annual age control. For *Callorhinus* from the Pribilof Islands, bone collagen  $\delta^{13}\text{C}$  values do not record the switch in lipid-content of diet associated with weaning.
- For *Zalophus*, tooth dentin  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values provide an estimate of weaning age at an annual resolution since no high-resolution bone regressions currently exist for this species. Both carbon and nitrogen isotope values of bone collagen, however, can be used to approximate weaning age in *Zalophus* after consideration of appropriate bone collagen turnover rates.
- For *Callorhinus* young-of-the-year, bone collagen turnover occurs on the order of  $\sim 8$  mo. Because *Zalophus* weans at an older age ( $\sim 10$ – $14$  mo), when growth rates are relatively slower, bone collagen turnover occurs on the order of 10–12 mo.
- When coupled with sub-annual age estimates based on morphological measurements, bone collagen  $\delta^{15}\text{N}$  values can be used to determine the length of the nursing period, or approximate age at weaning, in otariids. Ultimately, this technique can be used to assess intra- and inter-specific age at weaning across breeding ranges or to identify changes in maternal strategies through time by analyzing bone elements found in archaeological and/or paleontological sites.

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