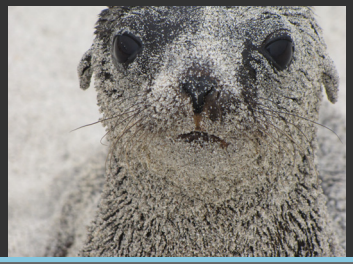
Examination of Ontogenetic Trends in Foraging Behaviors of California Sea Lions *(Zalophus californianus)* using Stable Isotope Analysis



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Objective: Use stable isotope analysis to investigate the development of foraging behaviors of the California sea lion

Abstract

During the first year of life, California sea lion *(Zalophus californianus)* pups undergo a gradual switch from a terrestrial phase marked by a complete nutritional reliance on their mother to an aquatic lifestyle where they forage independently in the marine environment. Their long maternal dependency period reduces the impact of weaning by providing opportunities for pups to develop diving and foraging skills while continuing to suckle. Stable isotope analysis has emerged as a method for assessing the foraging ecology of marine mammals, especially the nutritional transition associated with weaning. Here we used stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values to examine ontogenetic trends in foraging behaviors, and to determine at what age weaning occurs for California sea lions at San Miguel Island, California. $\delta^{13}C$ and $\delta^{15}N$ values were determined from 636 tissue samples (i.e. fur, red blood cells, plasma, and/or serum) collected from 290 sea lions of various ages during 2004 – 2007. Pre-weaned pups had higher mean $\delta^{15}N$ values by ~1.5‰ and lower mean $\delta^{13}C$ values by ~0.5‰ in comparison to adult females, which indicated that pups were feeding at a higher trophic level than older conspecifics and on a lipid-rich diet (i.e. milk, which is ^{13}C-depleted relative to proteins) prior to weaning. The most robust proxy for weaning was a significant decline in $\delta^{15}N$ values starting at ~8 to 9 months of age, and values of all blood components continued to gradually decline until ~11 to 17 months of age. Yearling $\delta^{15}N$ values were either slightly lower than pup or indistinguishable from adult values, depending on the tissue analyzed. Juvenile and adult sea lions had similar $\delta^{15}N$ values indicating that they were feeding at the same trophic level. Unlike $\delta^{15}N$, there did not appear to be a pronounced ontogenetic trend in $\delta^{13}C$ values, however patterns were consistent with current knowledge of movement patterns of California sea lions.

Methods

- Tissues with varying turnover rates were collected from California sea lions of various ages at San Miguel Island, California.
- Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values of tissues were determined using a mass spectrometer. Isotopic results are expressed as δ values, $\delta^{13}C$ or $\delta^{15}N = 1000^{*}\,[(R_{sample}/R_{standard})-1]$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively.
- Generalized additive models (GAMs) were used to describe changes in isotope ratios with age.
- Different GAMs also were fitted to consider the importance of tissue, sex, and year.
- ANOVA was used to quantify isotopic differences among age classes.

Discussion and Conclusions

- The ↓ in $\delta^{15}N$ and ↑ in $\delta^{13}C$ values of tissues from pre-weaned pups to weaned individuals reflected a switch from mother's milk to a piscivorous and cephalopod diet.
- By using tissues with a high turnover rate (e.g. plasma and serum), we were able to examine the foraging ecology of sea lions on a finer temporal scale compared to previous studies that used fur or teeth annuli; thus were able to discern a weaning signal.
- From examining $\delta^{15}N$ values, it appeared the onset of weaning begins ~ 8 mo of age; however, transition to an adult diet was gradual and some individuals were still suckling after their first birthday.
- Based on similar $\delta^{15}N$ values, it appeared that weaned individuals (esp. juveniles and adults) fed at the same trophic level. However, differences in $\delta^{13}C$ values indicated that individuals of different ages or sexes may be feeding in different habitats, which is consistent with what we know about the movement patterns of this species.

$\delta^{15}N$ — **Trophic level (TL)**
- Change predictably w/TL
- ~ 2-5‰/TL in marine mammal foraging studies

$\delta^{13}C$ — **Geographic location**
- Alter little with TL; base of food web info
- Nearshore > offshore; benthic > pelagic, etc.
- ~ 0.5-2‰/TL in marine mammal diets

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Results

- Tissue samples collected from 290 California sea lions (Table 1).
- The first two GAMs provided the best fit of the data (Table 2).
- $\delta^{15}N$ began to ↓ at 8 mo of age for blood components and 20 mo for fur (Fig. 1).
- $\delta^{13}C$ values generally ↑ with age, but patterns were highly variable and not consistent (Fig. 2).
- $\delta^{15}N$ values differed among age classes (F=342.61, P<0.05; Fig. 3). Post-hoc comparisons indicated differences between: (1) juveniles & adults with other age classes, for all tissues; (2) pups with other age classes, for all blood components.
- $\delta^{13}C$ values differed among age classes (F=42.74, P<0.05; Fig. 3). Post-hoc comparisons indicated: (1) pups had lower $\delta^{13}C$ values compared to older age classes except for yearlings when fur and RBCs were used; (2) adults had higher $\delta^{13}C$ fur values compared to younger age classes.

Age Class	Sex	Fur	Plasma	RBC	Serum
Pup (< 1 yr)	Female (n=122)	103	32	30	60
	Male (n=103)	82	36	36	62
	Unknown (n=3)	3	2	2	3
Yearling (1 - 2 yr)	Female (n=8)	8	6	6	8
	Male (n=2)	2	2	1	2
Juvenile (2 - 4 yr)	Female (n=17)	17	14	12	16
	Male (n=1)	1	0	0	1
Adult (≥ 4 yr)	Female (n=23)	23	16	15	19
	Male (n=10)	10	0	0	0

Table 1. Number of samples for stable isotope analysis obtained from California sea lions at San Miguel Island, CA or Shilshole Bay, WA (adult male only) during 2004-2007.

Generalized Additive Model	GCV score	
	X = $\delta^{15}N$	X = $\delta^{13}C$
gam1 = gam(X ~ s(Age * Tissue) + Tissue)	0.181	0.156
gam2 = gam(X ~ s(Age * Tissue) + Tissue + Sex)	0.182	0.155
gam3 = gam(X ~ s(Age) + Tissue + Year)	0.200	0.173
gam4 = gam(X ~ s(Age) + Tissue + Sex)	0.202	0.188
gam5 = gam(X ~ s(Age) + Tissue)	0.202	0.190

Table 2. GAMs fitted to the $\delta^{15}N$ or $\delta^{13}C$ measurements of California sea lion tissue samples. s indicates a smooth function applied to the variable, and Age*Tissue means that the smooth function of age differed by tissue type. Model selection was based on minimum generalized cross validation (GCV).

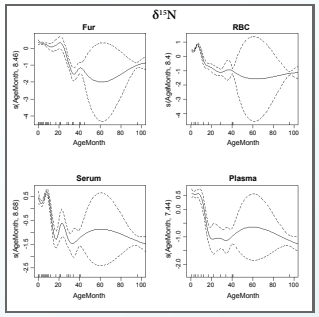

Figure 1. Plots of GAMs of $\delta^{15}N$ by age for each tissue type collected from California sea lions. X-axis denotes age (in mo) and Y-axis denotes relative distance from the mean. Dashed lines (---) indicate variation. Vertical ticks on the abscissa represent actual ages at which animals were sampled.

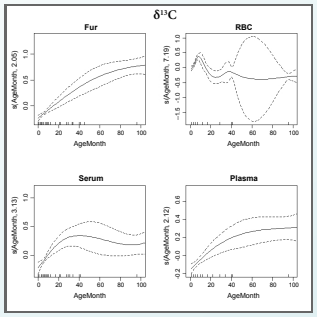

Figure 2. Plots of GAMs of $\delta^{13}C$ by age for each tissue type collected from California sea lions. X-axis denotes age (in mo) and Y-axis denotes relative distance from the mean. Dashed lines (---) indicate variation. Vertical ticks on the abscissa represent actual ages at which animals were sampled.

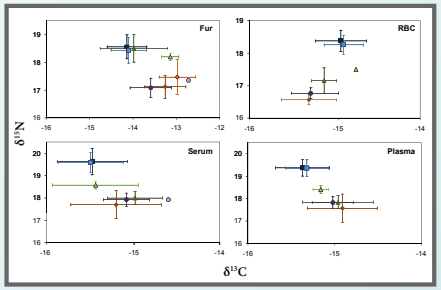

Figure 3. The relationship between mean $\delta^{13}C$ and $\delta^{15}N$ of fur and blood tissues collected from California sea lions of various age classes and sexes. Error bars represent ±1 S.D.

Acknowledgments

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