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Stable carbon and nitrogen isotopes in multiple tissues of wild and captive harbor seals (*Phoca vitulina*) off the California coast

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ABSTRACT

Stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of serum, red blood cells (RBC), muscle, and blubber were measured in captive and wild northeast Pacific harbor seals (*Phoca vitulina richardii*) at three coastal California sites (San Francisco Bay, Tomales Bay, and Channel Islands). Trophic discrimination factors ($\Delta_{Tissue-Diet}$) were calculated for captive seals and then applied in wild counterparts in each habitat to estimate trophic position and feeding behavior. Trophic discrimination factors for $\delta^{15}N$ of serum (+3.8‰), lipid-extracted muscle (+1.6‰), and lipid-blubber (+6.5‰) are proposed to determine trophic position. An offset between RBC and serum of +0.3‰ for $\delta^{13}C$ and −0.6‰ for $\delta^{15}N$ was observed, which is consistent with previous research. Specifically, weaner seals (<1 yr) had large offsets, suggesting strong trophic position shifts during this life stage. Isotopic values indicated an average trophic position of 3.6 at both San Francisco Bay and Tomales Bay and 4.2 at Channel Islands. Isotopic means were strongly dependent on age class and also suggested that mean diet composition varies considerably between all locations. Together, these data indicate that isotopic composition of blood fractions can be an effective approach to estimate trophic position and dietary behavior in wild pinnipeds.

Key words: stable isotopes, harbor seal, *Phoca vitulina*, carbon, ^{13}C, nitrogen, ^{15}N, San Francisco Bay, Channel Islands, Tomales Bay, trophic discrimination.

The Pacific harbor seal (*Phoca vitulina richardii*) is a small pinniped that is common on the northeastern Pacific coast in temperate and arctic waters from Baja California to the Aleutian Islands (Bigg 1981, Carretta *et al*. 2001). Harbor seals are philopatric and undertake short and shallow dives in familiar foraging locations (Stewart *et al*. 1989). Past studies using scat and stomach-gut content analyses suggested they are opportunistic feeders, whose diet mainly consists of fish and cephalopods (Antonellis and Fiscus 1980). All past studies of the diet of this species along the California coast (Harvey 1989, Torok 1994, Kopec and Harvey 1995, Grigg 2008) have used traditional methods based on scat or stomach-content analysis (Tollit *et al*. 1997). These studies offer invaluable, detailed information on prey consumption, but quantitative assessment of diet using scat or stomach-content analysis is subject to well-known biases related to differential digestion and a narrow temporal window of assessment (*i.e.*, sampling the last few meals).

Stable isotope techniques of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in tissues are a powerful tool for studying animal ecology, especially for organisms in difficult to observe environments (Kelly 2000, Koch 2007, Newsome *et al*. 2010). Stable isotopes provide information on trophic level, source of diet, migratory patterns, and body condition. Trophic discrimination factors are the isotopic differences between predator and prey ($\Delta^{13}C_{Tissue-Diet} = \delta^{13}C_{PredatorTissue} - \delta^{13}C_{Diet}$, and similarly for $\Delta^{15}N_{Tissue-Diet}$), and they vary with tissue type and metabolic state. Predictable ^{15}N trophic discrimination factors of $+3‰$ to $+5‰$ between prey and predator allow identification of trophic level. The average ^{13}C trophic discrimination factor is smaller and less variable in marine food webs at $+0.5‰$ to $+1.1‰$. As a consequence, $\delta^{13}C$ values in top consumers often vary with factors that affect photosynthetic fractionation of carbon isotopes at the base of the food web (*e.g.*, CO_2, growth rate, plankton size, *etc*.), allowing them to be used as a proxy of foraging location. Stable isotope analysis offers a broader temporal integration of feeding behavior, expanding the horizon of analysis to weeks, months, or even decades, depending on the type of tissue chosen for analysis (Newsome *et al*. 2010). A great potential advantage of stable isotope studies is the ability to analyze different time frames by selecting tissues with different turnover rates. Despite the broad advantages of stable isotope approaches to understanding diet, harbor seal populations have not been examined closely in the northeast Pacific, with past work conducted solely in Alaskan waters (Hobson *et al*. 1997, Burton and Koch 1999).

Zhao *et al*. (2006) determined trophic discrimination factors in *captive, adult* seals in Alaska, which were needed to assess the feeding behavior of their wild counterparts. Hobson *et al*. (1996) and Lesage *et al*. (2002) combined values from several phocids (ringed, *Pusa hispida*; harp, *Pagophilus groenlandicus*; gray, *Halichoerus grypus*; and harbor seals) for red blood cells (RBC) and serum. Both $\Delta^{13}C_{RBC-Diet}$ and $\Delta^{15}N_{RBC-Diet}$ values ranged from $+1.5‰$ to $+1.7‰$. For captive adult harbor seals ($n = 3$), Zhao *et al*. (2006) estimated a $\Delta^{13}C_{Serum-Diet}$ value of $-0.6‰$ to $+1.7‰$ and $\Delta^{15}N_{Serum-Diet}$ value of $+3.9‰$ to $+4.6‰$, and similar values have been reported for wild Alaskan pinnipeds (Hobson *et al*. 1996, Kurle and Worthy 2001).

One goal of our study was to determine if harbor seals in California (CA) have Δ values similar to their northern counterparts. In addition, ours is the first study to examine blood, muscle, and blubber tissues in *young, growing* seals. Our primary objective was to apply a dual-isotope and dual-tissue approach (RBC *vs*. serum) to assess the trophic level of different groups of harbor seals off the California coast. We analyzed isotopes in rehabilitating, stranded weaner seals (<1 yr) as well as in wild

seals in different environments in northern and southern CA (*e.g.*, a coastal site with upwelling; open water islands experiencing upwelling and the Californian Current; an urbanized, impacted bay). For wild populations, blood and tissue samples were collected in the same seasonal time frame (spring after pupping, early summer before molting) at all locations to standardize physiological shifts associated with molting, breeding, or a switch in prey that might impact isotopic values (Fadely *et al*. 1998, Zhao *et al*. 2006). Finally, examining the tropic position and diet of these animals should provide information on local environmental conditions, acting as a "tape-recorder" by documenting shifts in specific abundance of prey (kelp- or plankton-derived carbon), sources of food, weaning time frames, and trophic dynamics, while in parallel, revealing how these environmental changes affect their body conditions (nutritional stress, anabolic *vs*. catabolic metabolism).

Materials and Methods

Captive and Wild Animal Sampling

From April to June 2007, blood samples were collected from recovering harbor seal pups at The Marine Mammal Center (TMMC), a rescue and rehabilitation center in Sausalito, CA, during routine exams. Tissue samples were only collected once per individual seal, either immediately before successful release or after death. Blood serum was retained by the TMMC for successfully rehabilitated animals. Both muscle and blubber samples were collected from most deceased pups that TMMC was not able to rehabilitate, and blood serum was collected for those that fed at least 1 mo before succumbing (refer to Table S1 for time in center, tissues collected, and isotope values).

All seals were fed formula (salmon oil) for about 1 wk or until healthy enough to eat thawed, ground Atlantic herring (*Clupea harengus*). Several batches of formula and herring were collected during this timeframe, and isotopic values were pooled to establish "food" baseline values for calculation of $\Delta_{\text{Tissue}-\text{Diet}}$ values. While this study was not designed to measure tissue-turnover times, assessment of tissue isotope values across the collection suite relative to time of arrival at TMMC suggests that, as expected, serum equilibrated the fastest (by day 20), while all other tissues equilibrated more slowly (days 35–40; Fig. S1). All animal sampling procedures were approved by TMMC and UCSC Institutional Animal Care and Use Committee Protocols.

Wild seal blood samples were collected in May–June 2007 from northern and southern CA under NMFS Research Permit no. 555–1565. Males and females were sampled according to the following age classes based on their weight measurements (Bigg 1969): weaner (W), 1 mo–1 yr; yearling (Y), 1–2 yr; subadult (SA), 2–4 yr; and adults (A). All animals appeared in a healthy physical state (noticeable blubber layer and taut skin) before and after blood collection. The most northerly location, Tomales Bay (TB; 38°13.9′N, 122°58.1′W), is approximately 64 km north of San Francisco and adjacent to Point Reyes National Seashore. The site is a temperate estuary that, seasonally, receives advected water that experience oceanic and coastal upwelling. Our second northern capture site was in San Francisco Bay (SFB; 37°93.2′N, 122°41.9′W), centrally near Castro Rocks, which consist of six-rock clusters near the Richmond-San Rafael Bridge. This is the largest haul-out site in northern SFB, most likely due to being one of the few sites accessible during low tides

(Grigg 2008). SFB is an estuary-marine bay that is highly urbanized and impacted by agricultural runoff. Finally, samples were collected from seals in the Channel Islands (CI; 34°03.9′N, 120°37.4′W), in the northern islands of Santa Cruz Island, San Miguel Island, and Santa Rosa Island. The CI represent the most oceanic and least-anthropogenically disturbed location.

Blood samples were drawn from the epidural veinous sinus into nonadditive collection tubes, allowed to clot, and then centrifuged and separated into serum and RBC components, which were immediately frozen and archived at −80°C. In deceased seals, blood, pectoral muscle, and sternal axilla blubber tissue were collected during necroscopy and stored at −80°C. All samples were then lyophilized, homogenized, and stored in desiccator until ready for chemical treatment and isotopic measurements. Lipids were removed from blood (both serum and RBC), muscle, and blubber following the methods of Dobush *et al*. (1985), using petroleum ether in a Dionex Accelerated Solvent Extractor (Bannockburn, IL); samples were then dried under a fume hood for 1 h to evaporate residual solvent. For blubber samples, both the lipid-extracted material and the residual solvent containing lipids were dried and analyzed for $\delta^{13}C$ and $\delta^{15}N$ values.

Isotopic and Statistical Analyses

Nitrogen and carbon isotope analyses were conducted in the Stable Isotope Lab at the University of California, Santa Cruz on a Carlo Erba 1108 elemental analyzer (Lakewood, NJ) coupled to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer (San Jose, CA) (EA-IRMS). Homogenized, dried RBC, serum, and tissue components were weighed into tin capsules and then combusted and analyzed on the EA-IRMS system. EA-IRMS analytical precision using the standard Pugel, after calibrating for drift of instrument and mass variability, throughout six individual runs was ±0.1‰ for carbon and nitrogen ($n = 59$). Triplicate reproducibility between 25 blood samples after standard drift corrections was ±0.03‰ for carbon and ±0.06‰ for nitrogen. Stable isotopes are reported using standard delta (δ) notation in parts per thousand (‰) using the following equation: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1{,}000$, where X is ^{13}C or ^{15}N, and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for carbon was Vienna PeeDee Belemnite (PDB) and the standard for nitrogen was atmospheric N_2.

Trophic discrimination factors were calculated as the difference between tissue and fish feed ± error propagation. One-way analysis of variance (ANOVA) with Tukey-Kramer's pairwise comparisons was used to compare serum and RBC mean $\delta^{13}C$ and $\delta^{15}N$ values between sex, age classes, and among the three locations. Interaction tests were performed between location and sex, age and sex, and location and age. Isotopic differences were calculated for $\delta^{13}C$ and $\delta^{15}N$ between RBC and serum ($\Delta^{13}C_{\text{RBC-Serum}} = \delta^{13}C_{\text{RBC}} - \delta^{13}C_{\text{Serum}}$, and similarly for $\Delta^{15}N_{\text{RBC-Serum}}$) for all animals, locations, and interactions between location and age. The carbon and nitrogen isotopic differences between RBC and serum were tested using paired *t*-tests for all location and age combinations.

To estimate trophic level for wild seals, we use the following equation (Pauly *et al*. 1998):

$$\text{Trophic Level} = \left[\left(\delta^{15}N_{\text{Serum}} - \delta^{15}N_{\text{Fish}}\right)/\Delta^{15}N_{\text{Serum-Diet}}\right] + \left[\left(\delta^{15}N_{\text{Fish}} - \delta^{15}N_{\text{Plankton}}\right)/\Delta^{15}N_{\text{Fish-Diet}}\right] + 1, \quad (1)$$

Table 1. Stable carbon and nitrogen isotope values (mean ± SD) in tissues of successfully released (serum) and recently deceased (muscle/blubber) harbor seals, which were under care by TMMC for more than 1 mo. Discrimation factors ($\Delta_{\text{Tissue-Diet}}$; mean ± error propagation) were determined by difference between specific tissue and diet (fish feed). LE = lipid extracted.

Tissue	*n*	$\delta^{13}C$	$\Delta^{13}C_{\text{Tissue-Diet}}$	$\delta^{15}N$	$\Delta^{15}N_{\text{Tissue-Diet}}$
Serum	11	−18.0 ± 0.6	+1.5 ± 0.9	16.3 ± 0.4	+3.8 ± 0.5
Muscle, LE	3	−16.8 ± 0.9	+2.6 ± 1.2	14.1 ± 0.8	+1.6 ± 1.0
Muscle, bulk	4	−17.4 ± 0.5	+2.0 ± 0.9	14.4 ± 0.9	+1.9 ± 1.1
Blubber, lipid	3	−23.0 ± 0.5	−3.5 ± 0.9	19.0 ± 1.3	+6.5 ± 1.2
Blubber, LE	4	−18.3 ± 1.0	+1.1 ± 1.0	16.7 ± 1.1	+4.2 ± 1.7
Fish feed	4	−19.4 ± 0.6		12.5 ± 0.3	

where $\Delta^{15}N_{\text{Serum-Diet}} = 3.8‰$ (Table 1) and $\Delta^{15}N_{\text{Fish-Diet}} = 3.4‰$ (Post 2002) and 1 is added as plankton represents the first trophic level.

However, since we do not know $\delta^{15}N_{\text{Fish}}$, we assume

$$\left[\left(\delta^{15}N_{\text{Serum}} - \delta^{15}N_{\text{Fish}}\right)/\Delta^{15}N_{\text{Serum-Diet}}\right] = 1, \quad (2)$$

because the transfer from seal to fish represents ~1 trophic level. Consequently,

$$\text{Trophic Level} = \left[\left(\delta^{15}N_{\text{Fish}} - \delta^{15}N_{\text{Plankton}}\right)/\Delta^{15}N_{\text{Fish-Diet}}\right] + 2. \quad (3)$$

Finally, to remove $\delta^{15}N_{\text{Fish}}$, we note that

$$\Delta^{15}N_{\text{Serum-Diet}} = \delta^{15}N_{\text{Serum}} - \delta^{15}N_{\text{Fish}} = 3.8, \quad (4)$$

which after rearrangement and substitution results in the final equation

$$\text{Trophic Level} = \left[\left(\delta^{15}N_{\text{Serum}} - 3.8\right) - \delta^{15}N_{\text{Plankton}}/3.4\right] + 2. \quad (5)$$

The $\delta^{15}N_{\text{Plankton}}$ value varies depending on location: TB, 7.5‰ (Rau *et al*. 1998); SFB, 8‰ (Cloern *et al*. 2002); CI, 6.5‰ (Germain *et al.*, unpublished data).

Results

Stranded (Rehabilitated) Seal Tissue Isotope Values and Discrimination Factors

Mean tissue $\delta^{13}C$ and $\delta^{15}N$ values, $\Delta^{13}C_{\text{Tissue-Diet}}$, and $\Delta^{15}N_{\text{Tissue-Diet}}$ values in captive harbor seals are reported in Table 1 and Table S1. Isotopic values for serum from captive animals had stabilized on the TMMC diet by 18–30 d after arrival. Muscle and blubber appeared to stabilize between 41 and 60 d (stabilization assumed from proximity of data points to level-fitted line), with an exponential decrease to a constant value by day 40 for these tissues, except for lipid-extracted muscle (LE muscle), which was still equilibrating for $\delta^{13}C$ at day 60 (Fig. S1).

Trophic discrimination factors were only estimated for individuals that had equilibrated or were likely near equilibrium. Specifically, for serum, we only used values collected more than 30 d after arrival at TMMC, whether the animal was successfully

rehabilitated ($n = 10$) or succumbed ($n = 2$). For muscle and blubber, which were only collected from animals that were euthanized, only four individuals survived in captivity for 30–60 d. Hence, our sample size is small, and these animals may still have been equilibrating to TMMC diet at the time of death. Although Table 1 reports discrimination factors for a range of different tissues and treatments (which may be useful for other studies), the key values for our study of wild harbor seals are for serum ($\Delta^{13}C_{Serum-Diet} = +1.5 \pm 0.9‰$; $\Delta^{15}N_{Serum-Diet} = +3.8 \pm 0.5‰$).

Wild Seal Tissue Isotope Values

Mean RBC and serum $\delta^{13}C$ and $\delta^{15}N$ values from 102 wild seals are reported in Table 2, along with the difference in the blood component values and the corresponding significance between RBC and serum (*P*-values). Over the entire data set, RBC $\delta^{13}C$ values averaged $-16.1 \pm 0.5‰$ (range, from $-14.7‰$ to $-18.4‰$), and $\delta^{15}N$ values averaged $16.6 \pm 0.7‰$ (range, 14.2‰ to 19.6‰), whereas serum $\delta^{13}C$ values averaged $-16.5 \pm 0.6‰$ (range, $-15.1‰$ to $-17.8‰$) and $\delta^{15}N$ values averaged $17.4 \pm 1.0‰$ (range, 15.8‰–20.9‰). Thus, RBC samples have greater $\Delta^{13}C$ (mean, $+0.3 \pm 0.1‰$; $P < 0.01$) and lesser $\Delta^{15}N$ values (mean, $-0.6 \pm 0.2‰$; $P < 0.01$) than the serum samples. This pattern was significant at all locations and was observed for all age/location groups with two exceptions: yearlings of TB and adults of SFB for which sample sizes were small ($n = 1$ and 4, respectively). Weaners were the only age classes that had $\Delta^{15}N_{RBC-Serum}$ values distinctly different from the mean value (Fig. 1A), whereas for $\Delta^{13}C_{RBC-Serum}$ values, all weaners and almost all SFB seals were different than the mean value (Fig. 1B). Consistent differences between the two blood components likely reflect mean differences in their amino acid composition (as different amino acids can have very different $\delta^{13}C$ and $\delta^{15}N$ values) (Macko *et al.* 1987, Kurle 2002). The differences in Δ values observed among age/sex/location groups may reflect the fact that these blood components have different turnover rates and thus, record slightly different time periods.

Because this is a relatively large data set with several variables (age, sex, location), we only report statistically significant observations. RBC and serum generally showed consistent trends compared to each other, although there were a few discrepancies. Mean location $\delta^{13}C$ values varied <1‰ and were highest for TB and lowest for SFB (Table 2, Fig. 2). Mean trophic position varied by 0.6 among locations, mostly attributed to weaners. Trophic position for adults, subadults, and yearlings varied by 0.2–0.3 within locations (TB 3.5–3.7, SFB 3.2–3.5, CI 3.9–4.2, $P < 0.01$, Table S2). At SFB and CI, weaners had the greatest $\delta^{15}N$ values (with trophic positions of 4.0 and 4.7, respectively), whereas yearlings had the lowest (with trophic positions of 3.4 and 3.9, respectively) (Table 2, Fig. 2, S2). There were significant isotopic interactions between age and location, and location and sex, depending on the blood fraction (ANOVA; significant interactions in bold in Table S2).

The interaction of $\delta^{15}N$ and $\delta^{13}C$ between age and location was significant ($P < 0.01$). In the RBC fraction, TB had the lowest $\delta^{15}N$ across age classes (the difference in $\delta^{15}N$ between age class and TB and CI were statistically significant and had $P < 0.01$, Table 2, and Fig. 3A), and yearlings had the lowest values among age classes (~15.0‰ compared to 16.0‰–17.0‰ for other age groups). At a given site, the most ^{15}N-enriched values always occurred in weaners, with an increase in trophic position by ~0.3 (or ~1.3‰), relative to adults. Serum $\delta^{15}N$ values at CI and SFB had significant differences between age classes ($P < 0.01$, Fig. 3B). CI exhibited a

Table 2. Wild harbor seal $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD) for each location for all age groups (ALL), interactions between locations and age groups, and the total mean of all 102 seals located in the final row. Sex (no. of F, no. of M) refers to the number of females and males in each category. Location and age abbreviations found in text (section Methods). $\Delta_{RBC-Serum}$ reports the difference between RBC and serum blood fractions for both $\delta^{13}C$ and $\delta^{15}N$ (‰), while significant differences are in bold. *P*-values of $\Delta_{RBC-Serum}$ are displayed using matched pairs analysis, where significant differences exist when $P < 0.05$. Bolded $\Delta_{RBC-Serum}$ values are when both the value is greater than the mean and significant.

Location	Age	Sex no. of F, no. of M	RBC		Serum		$\Delta_{RBC-Serum}$		$\Delta_{RBC-Serum}$		Trophic level*
			$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	*P*	$\delta^{15}N$ (‰)	*P*	
TB	ALL	24, 11	−15.9 ± 0.5	16.3 ± 0.6	−16.2 ± 0.7	16.9 ± 0.6	0.3	<0.01	−0.5	<0.01	3.6
	A	13, 6	−15.8 ± 0.5	16.5 ± 0.5	−16.1 ± 0.7	17.0 ± 0.6	0.3	0.04	−0.5	<0.01	3.7
	SA	8, 5	−16.0 ± 0.5	16.1 ± 0.3	−16.4 ± 0.5	16.7 ± 0.5	0.4	<0.01	−0.6	<0.01	3.6
	Y	2, 0	−16.4 ± 0.2	15.0 ± 1.1	−17.1 ± 0.5	16.3 ± 0.1	0.8	0.13	−1.3	0.37	3.5
	W	1, 0	−16.2	16.7	−15.6	16.3	−0.5	–	0.4	–	3.5
SFB	ALL	9, 8	−16.5 ± 0.7	16.8 ± 1.2	−16.9 ± 0.7	17.4 ± 1.3	0.5	<0.01	−0.7	<0.01	3.6
	A	3, 1	−16.7 ± 1.2	16.6 ± 1.6	−15.9 ± 0.2	16.0 ± 0.2	−0.7	0.22	**0.7**	0.02	3.2
	SA	2, 1	−16.5 ± 1.0	16.4 ± 0.7	−17.0 ± 0.2	16.8 ± 0.6	0.5	0.45	−0.4	0.02	3.5
	Y	1, 2	−16.3 ± 0.6	15.7 ± 0.1	−16.4 ± 0.8	16.4 ± 0.5	0.1	0.32	−0.7	0.11	3.4
	W	3, 4	−16.5 ± 0.3	17.6 ± 0.9	−17.4 ± 0.4	18.7 ± 0.8	**1.0**	<0.01	**−1.0**	<0.01	4.0
CI	ALL	29, 23	−16.1 ± 0.4	16.8 ± 0.5	−16.5 ± 0.4	17.9 ± 1.0	0.4	<0.01	**−1.0**	<0.01	4.2
	A	17, 13	−16.0 ± 0.4	16.8 ± 0.5	−16.4 ± 0.3	17.7 ± 0.6	0.3	<0.01	**−0.9**	<0.01	4.2
	SA	6, 4	−16.1 ± 0.4	16.7 ± 0.4	−16.6 ± 0.3	17.5 ± 0.5	0.5	<0.01	−0.8	<0.01	4.1
	Y	1, 2	−16.6 ± 0.1	16.0 ± 0.2	−16.9 ± 0.2	16.6 ± 0.6	0.3	0.19	−0.6	0.11	3.9
	W	5, 4	−16.0 ± 0.7	17.4 ± 0.3	−16.8 ± 0.7	19.5 ± 0.9	**0.8**	<0.01	**−2.1**	<0.01	4.7
Mean	ALL	62, 40	−16.1 ± 0.5	16.6 ± 0.7	−16.5 ± 0.6	17.4 ± 1.0	**0.3 ± 0.1**	<0.01	**−0.6 ± 0.2**	<0.01	3.8

*Trophic level = $[(\delta^{15}N_{Serum} - 3.8) - \delta^{15}N_{Plankton}/3.4] + 2$ using $\delta^{15}N_{Plankton}$ values of TB = 7.5‰, SFB = 8‰ and CI = 6.5‰, described in Methods.

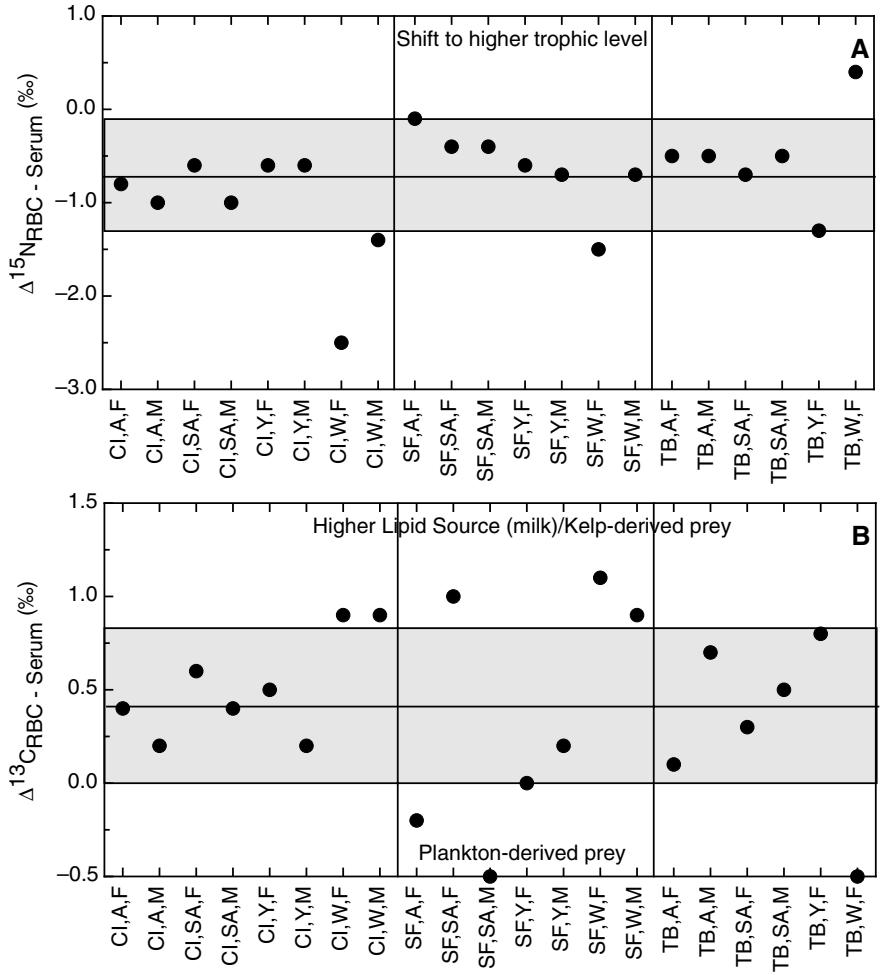

Figure 1. The difference in isotopic value between RBC and serum ($\Delta_{RBC-Serum}$) for (A) nitrogen and (B) carbon for each seal interaction (location and age and sex). The line is the mean difference, where the shaded area represents the SD for total error propagation between both blood fractions (0.4‰ for $\delta^{13}C$ and 0.5‰ for $\delta^{15}N$). Location and age abbreviations defined in text.

similar pattern to the RBC fraction, whereas SFB had greater weaner $\delta^{15}N$ values and relatively similar values among other age classes, and TB had a minor increase with age (though we have data for only one weaner).

For $\delta^{13}C$ values, the patterns in the RBC fraction were similar at TB and CI; weaners were slightly higher than yearlings, then values increased again from yearlings to ^{13}C-enriched adults, ($P > 0.01$ for age and location interaction, Fig. 3C). The $\delta^{13}C$ values in SFB were different from the other two sites. Values of $\delta^{13}C$ were lowest in weaners and decreased from younger adults to adult animals (Fig. 3C). In the serum fraction, TB and CI had a progressive increase in $\delta^{13}C$ values of

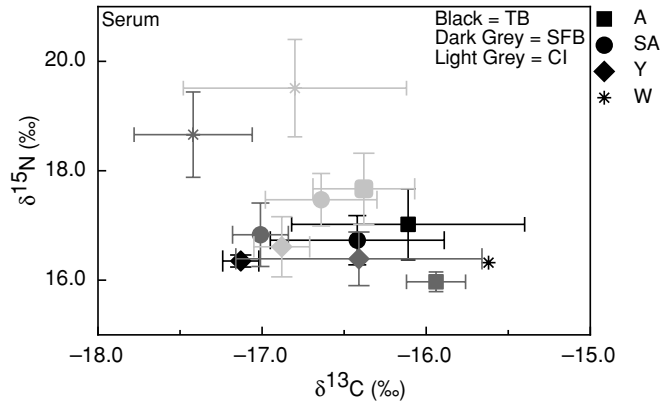

Figure 2. The means ± SD of $\delta^{13}C$ *vs.* $\delta^{15}N$ in serum of the four age classes (A, SA, Y, W) for each location (TB, SFB, CI). Location and age abbreviations defined in text. TB = black, SFB = dark gray, CI = light gray. A = squares, SA = circles, Y = diamonds, W = stars.

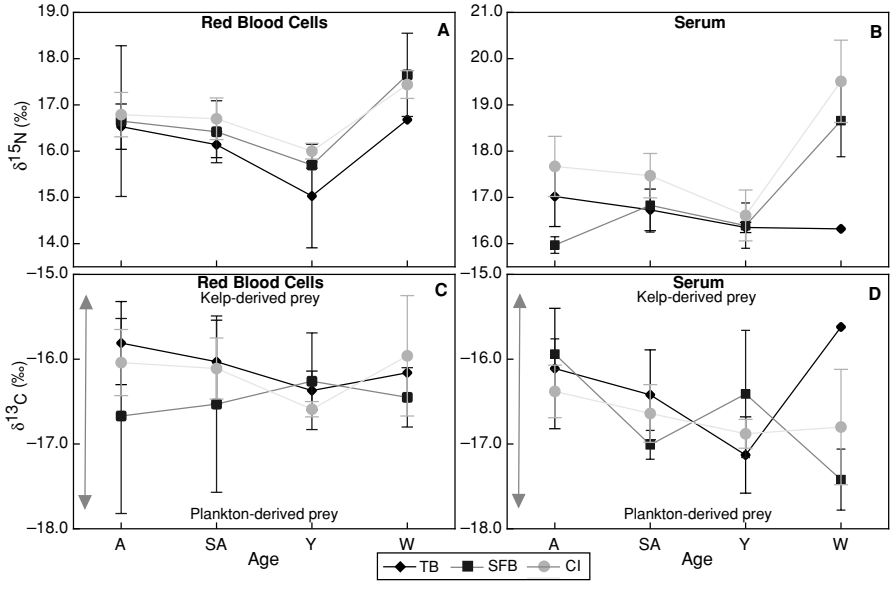

Figure 3. Significant interactions ($P < 0.05$, Table S2) between location and age for $\delta^{15}N$ (mean ± SD) in (A) RBC and (B) serum and for $\delta^{13}C$ (mean ± SD) in (C) RBC and (D) serum. As $\delta^{13}C$ approaches −10‰, it resembles kelp-derived carbon sources, whereas approaching −20‰ resembles plankton-derived. Location and age acronyms defined in text. TB = black diamonds, SFB = dark gray squares, CI = light gray circles.

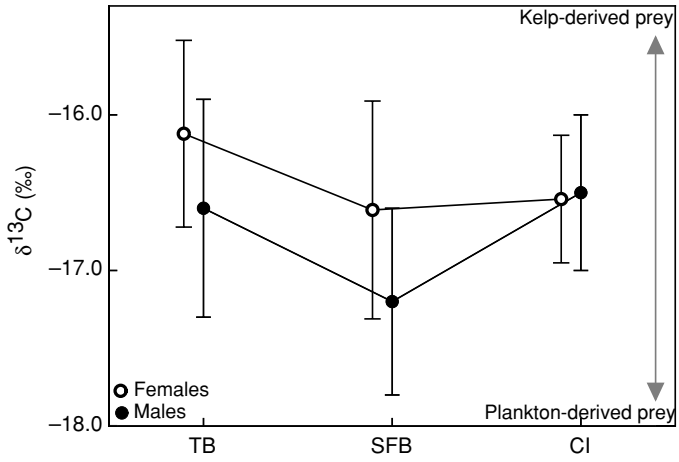

Figure 4. The significant interaction of mean $\delta^{13}C \pm SD$ values ($P < 0.05$, Table S2) between location and sex in serum. Since RBC displayed a consistent offset to serum at all locations ($\Delta_{RBC-Serum} \leq 0.3‰$), we only show serum. As $\delta^{13}C$ approaches $-10‰$, it resembles kelp-derived carbon sources, whereas approaching $-20‰$ resembles plankton-derived. Female = open symbols, Male = closed symbols.

yearlings to adult seals, consistent with RBC data (CI had $P < 0.05$ between age classes). SFB was again different, as the trend in serum was not consistent, with large swings between age classes (SFB had $P < 0.01$). For weaners, there was no consistent pattern of significant difference relative to other age classes among all three sites. (Fig. 3D).

For both blood components, an interaction of location and sex contributed significantly to differences in $\delta^{13}C$ values. CI and TB seals have similar $\delta^{13}C$ values for each sex, in both serum and RBC, whereas SFB seals were always depleted in ^{13}C compared to other locations (Fig. 4). The ^{13}C-depletion in SFB seals relative to seals compared to the other sites ($P < 0.05$) is greatest in males ($-0.9‰$ for RBC and $-0.6‰$ for serum) whereas ^{13}C-depletion in SFB females is much less ($-0.1‰$ for RBC and $-0.4‰$ for serum).

Discussion

Tissue Discrimination Factors from Stranded Seals

To date, only three controlled feeding studies have examined Δ values in harbor seals (Hobson *et al*. 1996, Lesage *et al*. 2002, Zhao *et al*. 2006), and all reported exclusively on *adults*, examining blood, hair, whiskers, or nails. We examined serum in growing, successfully rehabilitated pups that had been at TMMC for more than a month. In addition, we analyzed blubber and muscle tissue, from a small number of animals that were considered nutritionally healthy (*i.e.,* eating satisfactory proportions of fish feed for more than a month), but were unable to be released for a variety of physiological reasons (trauma, neurological, colitis, enteritis) and were euthanized (Table S1).

Isotopic incorporation into tissues occurs during growth and tissue breakdown and resynthesis (*i.e.*, turnover). It takes ~3 isotopic half-lives for a tissue to equilibrate fully to a new prey after a switch in diet. Tissues undergoing greater metabolic activity (serum, liver, fat) have shorter half-lives than tissues with lesser metabolic rates (brain, muscle, bone) (Tieszen *et al*. 1983). For example, plasma from American black bears (*Ursus americanus*) had a half-life of 4 d and fully incorporated isotopes from an altered diet within 10 d (Hilderbrand *et al*. 1996), and liver from gerbils had a similar half-life of 6.4 d (Tieszen *et al*. 1983). Previous work has indicated that isotopic equilibrium for seal serum occurs over similar time frame (Kurle 2002, Zhao 2002). Zhao *et al*. (2006) concluded that due to the turnover rate of serum, isotope values were able to record a shift in diet of less than 1 mo. In addition, younger, rapidly growing animals have a higher metabolic and tissue turnover rate from extremely active anabolism (Sakano *et al*. 2005). Thus, we are confident that serum had equilibrated after 30 d on TMMC rations and, therefore, that our Δ values are robust.

Our $\Delta^{15}N_{Serum-Diet}$ values of $+3.8 \pm 0.5‰$ and $\Delta^{13}C_{Serum-Diet}$ values of $+1.5 \pm 0.9‰$ are quite similar to trophic discrimination factors from studies of other phocids studies, where $\Delta^{15}N_{Serum-Diet}$ ranged from $+1.7‰$ to $+4.6‰$ and $\Delta^{13}C_{Serum-Diet}$ ranged from $-0.6‰$ to $+1.7‰$ (Hobson *et al*. 1996, Lesage *et al*. 2002, Zhao *et al*. 2006). $\Delta_{Tissue-Diet}$ values may vary with the macromolecular composition of diet or with the growth state of the animal. Trueman *et al*. (2005) found lower $\Delta^{15}N$ values in salmon undergoing intensive growth than those experiencing little to no growth, with no discernable impact on $\Delta^{13}C$ values. However, studies on rodents indicated that less than 10% of isotopic change is attributed to growth (West *et al*. 2001, MacAvoy *et al*. 2005). Other factors, such as nutritional stress and protein level of diet also can affect $\Delta_{Tissue-Diet}$ values (Sick *et al*. 1997, Zhao 2002). When animals ingest protein for calories at levels beyond that needed for maintaining nitrogen balance, they produce more nitrogenous waste (*e.g.*, urea), which is ^{15}N-depleted relative to body tissues and serum. As a consequence, $\delta^{15}N$ values are higher in serum, RBC, and body tissues than on low-protein diets (Sick *et al*. 1997). Correspondingly, higher protein diets (pollock) increase $\Delta^{15}N_{Serum-Diet}$ *vs*. lower protein diets, such as herring ($4.6‰$ *vs*. $3.9‰$; Zhao *et al*. 2006). For optimum growth, rehabilitating seals at TMMC were fed a mixture of ground or whole herring, which is a high-lipid/high-energy density fish, resulting in a mean $\Delta^{15}N_{Serum-Diet}$ value similar to that for herring in Zhao *et al*. (2006). For $\Delta^{13}C_{Tissue-Diet}$, because lipids are strongly ^{13}C-depleted, high-lipid diets are expected to yield lower $\Delta^{13}C_{Serum-Diet}$ values than low-lipid diets and may even be negative relatively. The similarity of our values with those previously reported for North Atlantic harbor seals also consuming herring ($\Delta^{15}N_{Serum-Diet} = +3.5‰$ and $\Delta^{13}C_{Serum-Diet} = +1.6‰$ after lipid corrections; Lesage *et al*. 2002) indicates that these values are relatively invariant when fed a high-lipid/high-energy prey. Because harbor seals require both high fat and high energy to sustain a long-term, reproductive life, we recommend using $\Delta^{15}N_{Serum-Diet}$ of $+3.8‰$ and $\Delta^{13}C_{Serum-Diet}$ of $+1.5‰$ to interpret wild trophic position in healthy populations.

Blubber is comprised of connective tissue and fat/lipid, where lipids are obtained from both diet and biosynthesis. For blubber, the $\Delta^{13}C_{Lipid-Diet}$ was $-3.5 \pm 0.9‰$. This is not surprising; lipids are often the most ^{13}C-depleted compound class in animals by $5‰$ to $10‰$ (DeNiro and Epstein 1977). After lipid-extraction, the $\Delta^{15}N_{Blubber-Diet}$ value for the residual lipid material (*e.g.*, blubber) was $+6.5 \pm 1.2‰$. However, the $\Delta^{15}N_{LE\ Blubber-Diet}$ component had a smaller value similar to the $\Delta^{15}N_{Serum-Diet}$. The $\Delta^{15}N$ differences between blubber samples could be related

to nitrogen composition, where the LE blubber fraction has very little nitrogen, less than half a percent, mostly likely as small enzymes or small polypeptide fragments derived from connective tissue, possibly affecting instrument analytical precision.

Although we believe serum and blubber had adequate times for isotopic equilibration to TMMC diet, lipid-extracted muscle $\delta^{13}C$ values had not stabilized, even for samples collected 60 d after the diet switch, whereas $\delta^{15}N$ values had stabilized. The $\delta^{13}C$ values only changed by $-0.5‰$ between days 20–40 ($n = 5$), yet decreased an additional 1.5‰ by day 60 ($n = 1$; Fig. S1). The $\Delta^{13}C_{LE,Muscle-Diet}$ value was $+2.6 \pm 1.2‰$, within error propagation of serum. The $\Delta^{15}N_{LE,Muscle-Diet}$ value was $+1.6 \pm 1.0‰$, however, slightly less than the value of $+2.4‰$ reported for another phocid ($n = 1$ harp seal; Hobson *et al*. 1996). This difference might be attributed to the age of the seals analyzed. Only weaners were sampled in this study, and these animals were growing rapidly, possibly reducing their flux of ^{15}N-depleted waste urine to form muscle tissue and to maintain higher rates of protein synthesis and catabolism. Regardless, a $\Delta^{15}N_{LE,Muscle-Diet}$ value of $+2.0‰$ appears to be a reasonable value for assessing trophic information in wild phocid populations.

Wild Seal Tissue Isotope Values

Stable isotope analysis has been used to assess the diets of pinnipeds off CA, Oregon, and Washington (Hobson *et al*. 1997, Hobson and Sease 1998, Burton and Koch 1999, Burton *et al*. 2001, Hirons *et al*. 2001, Kurle and Worthy 2001, Hobson *et al*. 2004, Aurioles *et al*. 2006, Newsome *et al*. 2006, Kurle and Gudmundson 2007). Yet, there are few isotopic studies of harbor seals from the northeast Pacific, and these were on the Bering Sea and Gulf of Alaska (Burton and Koch 1999, Hirons *et al*. 2001). Comparisons to seal populations in central and southern CA may be particularly useful in understanding harbor seal ecology in highly urbanized areas such as SFB, where animals may have to travel greater distances to obtain adequate nutrition due to the poor quality or quantity of prey.[1]

The significant differences in mean $\delta^{15}N$ values among sites (TB $> 0.5‰$ to SFB in serum, and TB $> 1‰$ to CI in serum; Table 2) may reflect differences in $\delta^{15}N$ values at the base of the food web or differences in trophic structure. Planktonic $\delta^{15}N$ values vary depending on the source of nitrogen to the oceanic system and the extent of uptake. For example, if the massive deep ocean nitrate pool ($\delta^{15}N \sim +5‰$) is the source of nitrate in the photic zone (and if this nitrate is completely exhausted by planktonic uptake), then plankton will have a value similar to the pool. In highly productive regions (with suboxic waters at depth), denitrification can enrich the ^{15}N in the upwelled nitrate to $\sim$15‰ to 20‰, strongly enriching plankton (Altabet *et al*. 1999). Freshwater runoff also has ^{15}N-enriched nitrate in urbanized areas. In areas or seasons where other nutrients limit production, phytoplankton can be ^{15}N-depleted relative to the nitrate pool due to incomplete nitrate utilization. Finally, as zooplankton and fish are typically ^{15}N-enriched by 3‰ to 4‰ with each trophic transfer, longer food webs have greater $\delta^{15}N$ values in top consumers (*e.g.*, Montoya 2007).

To calculate trophic level for each location and age class, $\delta^{15}N$ values at the base of the food web were assessed using values for particulate nitrogen from the literature (described in Methods). TB, a temperate estuary with coastal upwelling, obtains

[1]Personal communication from California Department of Fish and Game, Bay-Delta Region, 4001 N. Wilson Way, Stockton, CA, May 2009.

its nutrients primarily from suboxic and deep nitrate entrained in the California Current, thus, its plankton values are about +7.5‰ (Rau *et al*. 1998). In SFB, an urbanized estuary highly influenced by terrestrial runoff, particulate $\delta^{15}N$ values averaged +8.0‰, ranging from 5.0‰ to 10.6‰ due to variations in freshwater and tidal inputs (Cloern *et al*. 2002, Huntington and Boyer 2008). CI, the most oceanic of all the sites, are strongly influenced by the California Current and seasonal upwelling. It has the lowest $\delta^{15}N$ values averaging about 6.5‰ (Germain *et al*., unpublished data, samples collected in 1999), but values ranging from 6.9‰ to 8‰ were reported for the late 1980s (Altabet *et al*. 1999). Given these baseline $\delta^{15}N$ values, harbor seals fed at trophic levels ranging from 3.2 to 4.7, averaging 3.6 in TB and SFB and 4.2 in CI (Fig. S2). This suggests seals in the CI consume higher trophic level organisms, presumably feeding on more open-ocean than coastal prey (*i.e.*, squid, larger fish). Open-ocean fish tend to feed on longer food webs, where phytoplankton are the base of the food web, followed by microzooplankton, macrozooplankton, small fish, medium fish, large fish, then seals, resulting in an overall greater $\delta^{15}N$ value. In coastal systems, the food web is shorter, generally going from plankton to zooplankton to medium-sized fish to seals (*e.g.*, Montoya 2007).

Changes in habitat, food sources, metabolic, and physiological parameters affect stable carbon isotope ratios. The $\delta^{13}C$ data support the inferences above trophic level, assuming source $\delta^{13}C$ values of plankton range from −19‰ to −22‰ in CI and TB (Rau *et al*. 1998), and values of −17‰ to −27‰ in SFB (Cloern *et al*. 2002). These values are mainly influenced by changes in carbon source (*i.e.*, kelp-based *vs*. plankton-based prey). Kelp $\delta^{13}C$ values (−12‰ to −14‰) are much higher than those found in plankton (Page *et al*. 2008). Therefore, if a seal was feeding at a trophic position of 4.0 relative to plankton-derived prey, its $\delta^{13}C$ values would be about −16‰ to −19‰, similar to what was measured (Fig. 2). If feeding on eelgrass or kelp-derived prey, instead of plankton-derived, we would expect much higher $\delta^{13}C$ values of −5‰ to −11‰ (TB eelgrass ranges from −8‰ to −10‰ and CI kelp from −12‰ to −14‰; Fourqurean *et al.* 1997, Page *et al*. 2008). Overall, harbor seals from all coastal CA locations are feeding at similar trophic levels, preferring to consume prey around a trophic position of 3.

The differences between age classes for each location is particularly noteworthy, especially the weaners from SFB and CI, who are ^{15}N-enriched relative to the adults and subadults (Table 2, Fig. 2). The weaners in these locations are still obtaining the majority of their nutrition from their mother's milk and are ^{15}N-enriched by about 0.7 trophic level. Pups nurse for approximately 1 mo before they begin capturing and feeding on crustaceans and fish. Because they are unable to swim to the depths and distances of their older counterparts, they are restricted to feeding on prey that is nearby and easily obtained, making them susceptible to any potential food deficiencies within their constrained home range. Yearlings consume at the lowest trophic level, and while not drastically lower than the older seals, they are consistently lower by 0.2, presuming they feed on smaller prey (benthic, crustaceans, anchovies). In contrast, the seals in TB only vary in trophic position by 0.2 among all age classes, indicating all seals consumed similar prey items.

In this study, we determined a $\Delta^{15}N_{RBC-Serum}$ of -0.6 ± 0.2‰ for wild seals (Table 2), which is related to differences in amino acid compositions, and trophic level calculations varied less than 0.3 for all categories, except TB weaners and SFB adults. When $\Delta^{15}N_{RBC-Serum}$ values for an age/sex/location group fall within the range of error propagation, it suggests that any differences between the blood fractions are due to biochemical pathways. However, in several cases, the $\Delta^{15}N_{RBC-Serum}$ value

was outside of this range (outside of gray area, Fig. 1A), indicating a change in trophic level over the different time frames represented by these tissues (weeks for serum, months for RBC). At all locations, the weaners had a shift in diet. Specifically CI and SFB weaners switched from lower trophic level (indicated by RBC values) to higher trophic level (indicated by serum values), obtaining their entire diet from their mother's milk compared with *in utero*. This implies the isotopic incorporation experienced *in utero* is similar to that for other maternal tissues (including milk protein), whereas a weaner consuming these tissues as milk is a trophic level higher. The opposite trend was observed in the single weaner sampled in TB. This animal had likely transitioned from mother's milk to the lower trophic-level prey consumed by yearlings, since it had a serum $\delta^{15}N$ value identical to yearlings from TB (Table 2).

Carbon differences mainly arise from a change in the source of diet, whether a shift from milk to prey, a shift from open-ocean to coastal prey, or a shift from kelp-derived to plankton-derived prey. Once again, most $\Delta^{13}C_{RBC-Serum}$ values fall within the propagation of error (Fig. 1B), except for the weaners and almost all the seals in SFB (excluding the yearlings). The weaners in CI and SFB had positive $\Delta^{13}C_{RBC-Serum}$, implying their current diet was composed of lipid-rich milk (with a lower $\delta^{13}C$), whereas the TB weaner had likely transitioned to a fish and invertebrate diet (though its $\delta^{13}C$ value did not match that of yearlings). The differences in adults and subadults in SFB were likely caused by shifts in prey among benthic or pelagic sources. Harbor seals tend to forage within 5 km of their place of birth, but a small percentage are thought to travel further (Lander *et al*. 2002), which suggests either greater variability or lesser density of prey in SFB prey. It is possible these older seals are venturing further distances outside of SFB to obtain adequate nutrition, which has been observed by individual harbor seals preferring particular foraging and habitat ranges (*i.e.*, more open-ocean; Nickel 2003).

Significant Interactions between Location and Age Class and Location and Sex

The most significant $\delta^{15}N$ interactions existed between locations and age classes (Fig. 3). It is important to note the striking similarities among these interactions from RBC, whereas the patterns in serum differ among location, confirming the value of obtaining tissues recording different timeframes. Location isotopic values were dependent upon seal age class, especially in the serum fraction (Table S2). In RBC $\delta^{15}N$ values, the pattern is consistent at all locations, with highest values in weaners, intermediate values in adults to subadults, and the lowest values in yearlings (Fig. 3A). This suggests that the yearling seals are feeding at the lowest trophic levels, and that seals begin feeding on higher level organisms as they mature (Oates 2005). In serum, this pattern still holds true for CI, but TB exhibited comparable $\delta^{15}N$ values of ~16‰–17‰ for all age classes, with *lowest* values and, thus, trophic feeding found in the single weaner (Fig. 3B). The weaners in SFB and CI, however, were the most ^{15}N-enriched, suggesting weaners have yet to wean completely from their mother's milk. SFB weaners are ^{15}N-enriched relative to the adults by ~2.5‰ (rather than a full trophic step of ~3.8‰), which implies they recently fed on prey instead of milk. CI weaners are greater in $\delta^{15}N$ by ~2.0‰ than the adults, and they are likely in the process of tissue equilibration to their diet (Fig. 3B). The low $\delta^{15}N$ value for adult SFB seals relative to serum from other adults and subadults indicates that they recently consumed lower trophic-level prey (crab, anchovies).

With respect to $\delta^{13}C$ interaction effects, RBC patterns in TB and CI follow each other closely, with yearlings exhibiting the lowest $\delta^{13}C$ values, but they only differentiate by 0.6‰ overall (Fig. 3C). However, yearlings in SFB have greater

$\delta^{13}C$ compared with other age classes, possibly due to a greater proportion of their diet coming from kelp-derived prey *vs*. plankton-derived prey. Once again, serum had greater variability among age classes (1.5‰ overall), with TB following the RBC pattern (Fig. 3D). Serum from CI seals barely shows any isotopic differences, indicating all age classes are all feeding on prey derived from similar sources. Both adults and yearlings in SFB are ^{13}C-enriched by about +1.0‰ compared with the subadults and weaners, which confirms the conclusion by ^{15}N of adults consuming more benthic/kelp-derived organisms than other locations (Page *et al*. 2008). Similar source and trophic interactions are most likely the main reason for age class differences in minimally disturbed locations such as TB and CI, where SFB is more influenced by allochthonous sources (*i.e.*, coastal *vs*. open-ocean).

Another significant $\delta^{13}C$ difference was the interaction between location and sex for both RBC and serum (Table S2, Fig. 4). Since RBC displayed a consistent offset to serum at all locations ($\Delta_{RBC-Serum} \leq 0.3$‰), we only describe the serum fraction. It appears females are physically constrained in both bays (SFB and TB), probably due to obligatory proximity to weaners, whereas the males can venture to more distant locations to consume higher-lipid/higher-energy prey (lower $\delta^{13}C$ values). Male and female seals in CI feed essentially on similar prey organisms, as they exhibit comparable $\delta^{13}C$ values.

Conclusions

In summary, blubber, muscle, and other tissue $\Delta_{Tissue-Diet}$ values still need more comprehensive analysis for pinnipeds, especially from animals fed for a longer period on a consistent diet, since we were unable to collect such data from our short-term study of captive mammals under marine mammal protocols. Our $\Delta_{Tissue-Diet}$ values will be useful in interpreting long-term isotopic records of wild seals in light of trophic-level changes or shifts in location. For future work, we recommend using a robust multispecies approach to investigate trophic structure, with $\Delta^{15}N_{Tissue-Diet}$ value of +3.8‰ and +2.0‰ for serum and muscle, respectively. Harbor seals along coastal CA are feeding at varying trophic levels, ranging from 3.6 to 4.2, mostly impacted by age. Weaners are still equilibrating isotopically after consuming mother's milk and yearlings are feeding at a slightly lower trophic position than adults and subadults in spring. Also, this study stresses the value of obtaining tissues that record different time frames to evaluate shifts in diet and trophic structure. Future studies with compound-specific amino acid isotope analysis will allow researchers to simultaneously determine $\delta^{15}N$ and $\delta^{13}C$ values at the base of food web and exact trophic level of the forager. This more detailed compound-specific isotope analysis may be a powerful tool to assess nutritional status in marine mammals, especially seals under extreme anabolic or catabolic conditions.

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Literature Cited

Altabet, M. A., C. Pilskaln, R. Thunell, C. Pride, D. Sigman, F. Chavez and R. Francois. 1999. The nitrogen isotope biogeochemistry of sinking particles from the margin of the eastern North Pacific. Deep-Sea Research 46:655–679.

Antonellis, G. A., and C. H. Fiscus. 1980. The pinnipeds of the California Current. CALCOFI Reports 21:68–75.

Aurioles, D., P. L. Koch and B. J. Le Boeuf. 2006. Differences in foraging location of Mexican and California elephant seals: Evidence from stable isotopes in pups. Marine Mammal Science 22:326–338.

Bigg, M. A. 1969. The harbour seal in British Columbia. Fisheries Research Board of Canada Bulletin no. 172. 33 pp.

Bigg, M. A. 1981. Harbour seal, *Phoca vitulina* and *Phoca largha*. Pages 1–27 *in* S. H. Ridgway and R. J. Harrison, eds. Handbook of marine mammals: Seals. Volume 2. Academic Press, New York, NY.

Burton, R. K., and P. L. Koch. 1999. Isotopic tracking of foraging and long-distance migration in northeastern Pacific pinnipeds. Oecologia 119:578–585.

Burton, R. K., J. J. Snodgrass, D. Gifford-Gonzalez, T. Guilderson, T. Brown and P. L. Koch. 2001. Holocene changes in the ecology of northern fur seals: Insights from stable isotopes and archaeofauna. Oecologia 128:107–115.

Carretta, J. V., J. Barlow, K. A. Forney, M. M. Muto and J. Baker. 2001. U.S. Pacific marine mammal stock assessments: 2001. U.S. Department of Commerce, NOAA Technical Memorandum, NOAA-TM-NMFS-SWFSC-317. 284 pp.

Cloern, J. E., E. A. Canuel and D. Harris. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of San Francisco Bay estuarine system. Limnology and Oceanography 47:713–729.

DeNiro, M. J., and S. Epstein. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261–263.

Dobush, G. R., C. D. Ankney and D. G. Krementz. 1985. The effect of apparatus, extraction time, and solvent type on lipid extractions of snow geese. Canadian Journal of Zoology 63:1917–1920.

Fadely, B. S., J. M. Castellini and M. A. Castellini. 1998. Recovery of harbor seals from EVOS: Condition and health status. Exxon Valdez Oil Spill Restoration Project Final Report (97001). Alaska Department of Fish and Game, Anchorage, AK.

Fourqurean, J. W., T. O. Moore, B. Fry and J. T. Hollibaugh. 1997. Spatial and temporal variation in C:N:P ratios, δ15N, and δ13C of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA. Marine Ecology Progress Series 157:147–157.

Grigg, E. K. 2008. Environmental predictors of habitat use patterns of Pacific harbor seals (*Phoca vitulina richardii*) in an urbanized estuary. Ph.D. dissertation, University of California, Davis, CA. 113 pp.

Harvey, J. T. 1989. Assessment of errors associated with harbour seal (*Phoca vitulina*) faecal sampling. Journal of Zoology 219:101–111.

Hilderbrand, G. V., S. D. Farley, C. T. Robbins, T. A. Hanley, K. Titus and C. Servheen. 1996. Use of stable isotopes to determine diets of living and extinct bears. Canadian Journal of Zoology 74:2080–2088.

Hirons, A. C., D. M. Schell and B. P. Finney. 2001. Temporal records of $\delta^{13}C$ and $\delta^{15}N$ in North Pacific pinnipeds: Inferences regarding environmental change and diet. Oecologia 129:591–601.

Hobson, K. A., and J. L. Sease. 1998. Stable isotope analyses of tooth annuli reveal temporal dietary records: An example using Steller sea lions. Marine Mammal Science 14:116–129.

Hobson, K. A., D. M. Schell, D. Renouf and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: Implications for dietary

reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Sciences 53:528–533.

Hobson, K. A., J. L. Sease, R. L. Merrick and J. F. Piatt. 1997. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. Marine Mammal Science 13:114–132.

Hobson, K. A., E. H. Sinclair, A. E. York, J. R. Thomason and R. E. Merrick. 2004. Retrospective isotopic analyses of Steller sea lion tooth annuli and seabird feathers: A cross-taxa approach to investigating regime and dietary shifts in the Gulf of Alaska. Marine Mammal Science 20:621–638.

Huntington, B. E., and K. E. Boyer. 2008. Evaluating patterns of nitrogen supply using macroalgal tissue content and stable isotopic signatures in Tomales Bay, CA. Environmental Bioindicators 3:180–192.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27.

Koch, P. L. 2007. Isotopic study of the biology of modern and fossil vertebrates. Pages 99–154 *in* R. Michener and K. Lajtha, eds. Stable isotopes in ecology and environmental science. 2nd edition. Blackwell Publishing, Boston, MA.

Kopec, A. D., and J. T. Harvey. 1995. Toxic pollutants, health indices, and population dynamics of harbor seals in San Francisco Bay, 1989–1992. Moss Landing Marine Laboratories Technical Publications 96-4. 300 pp (available at http://aquaticcommons.org/1855/1/MLML_TP_96-4.pdf)

Kurle, C. M. 2002. Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: Applications for studying the foraging ecology of wild otariids. Canadian Journal of Zoology 80:902–909.

Kurle, C. M., and C. J. Gudmundson. 2007. Regional differences in foraging of young-of-the-year Steller sea lions *Eumetopias jubatus* in Alaska: Stable carbon and nitrogen isotope ratios in blood. Marine Ecology Progress Series 342:303–310.

Kurle, C. M., and G. A. J. Worthy. 2001. Stable isotope assessment of temporal and geographic differences in feeding ecology of northern fur seals (*Callorhinus ursinus*) and their prey. Oecologia 126:254–265.

Lander, M. E., J. T. Harvey, K. D. Hanni and L. E. Morgan. 2002. Behavior, movements, and apparent survival of rehabilitated and free-ranging harbor seal pups. Journal of Wildlife Management 66:19–28.

Lesage, V., M. O. Hammill and K. M. Kovacs. 2002. Diet-tissue fractionation of stable carbon and nitrogen isotopes in phocid seal. Marine Mammal Science 18:182–193.

MacAvoy, S. E., S. A. Macko and L. S. Arneson. 2005. Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. Canadian Journal of Zoology 83:631–641.

Macko, S.A., M. L. Fogel, P. E. Hare and T. C. Hoering. 1987. Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. Chemical Geology 65:79–92.

Montoya, J. P. 2007. Natural abundance of ^{15}N in marine planktonic ecosystems. Pages 176–207 *in* R. Michener and K. Lajtha, eds. Stable isotopes in ecology and environmental science. 2nd edition. Blackwell Publishing, Boston, MA.

Newsome, S. D., P. L. Koch, M. A. Etnier and D. Aurioles-Gamboa. 2006. Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. Marine Mammal Science 22:556–572.

Newsome, S. D., M. T. Clementz and P. L. Koch. 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science 26:509–572.

Nickel, B. A. 2003. Movement and habitat use patterns of harbor seals in the San Francisco Estuary, California. M.A. thesis, San Francisco State University, San Francisco, CA. 134 pp.

Oates, S. M. 2005. Survival, movements, and diet of juvenile harbor seals along central California. MS thesis, San Jose State University, Moss Landing, CA. 220 pp.

Page, H. M., D. C. Reed, M. A. Brzezinski, J. M. Melack and J. E. Dugan. 2008. Assessing the importance of land and marine sources of organic matter to kelp forest food webs. Marine Ecology Progress Series 360:47–62.

Pauly, D. A., A. W. Trites, E. Capuli and V. Christensen. 1998. Diet composition and trophic levels of marine mammals. ICES Journal of Marine Science 55:467–481.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83:703–718.

Rau, G. H., C. Low, J. T. Pennington, K. R. Buck and F. P. Chavez. 1998. Suspended particulate nitrogen $\delta^{15}N$ versus nitrate utilization: Observations in Monterey Bay, CA. Deep-Sea Research Part II 45:1603–1616.

Sakano, H., E. Fujiwara, S. Nohara and H. Ueda. 2005. Estimation of nitrogen stable isotope turnover rate of *Oncorhynchus nerka*. Environmental Biology of Fishes 72:13–18.

Sick, H., N. Ross, E. Saggau, K. Hass, V. Meyn, B. Walch and N. Trugo. 1997. Amino acid utilization and isotope discrimination of amino nitrogen in nitrogen metabolism of rat liver in vivo. Journal of Nutritional Science 36:340–347.

Stewart, B., S. Leatherwood, P. Yochem and M. P. Heide-Jorgensen. 1989. Harbor seal tracking and telemetry by satellite. Marine Mammal Science 5:361–375.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.

Tollit, D. J., M. J. Steward, P. M. Thompson, G. J. Pierce, M. B. Santos and S. Hughes. 1997. Species and size differences in the digestion of otoliths and beaks: Implications for estimates of pinniped diet composition. Canadian Journal of Fisheries Aquatic Science 54:105–119.

Torok, M. L. 1994. Movements, daily activity patterns, dive behavior, and food habits of harbor seals (*Phoca vitulina richardsi*) in San Francisco Bay. M.S. thesis, California State University, Stanislaus, CA. 88 pp.

Trueman, C. N., R. A. R. McGill and P. H. Guyard. 2005. The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals. An experimental study with Atlantic salmon (*Salmo salar*). Rapid Communications in Mass Spectrometry 19:3239–3247.

West, G. B., J. H. Brown and B. J. Enquist. 2001. A general model for ontogenetic growth. Nature 413:628–631.

Zhao, L. 2002. Tracing amino acid metabolism of harbor seals (*Phoca vitulina*) using stable isotope techniques. Ph.D. dissertation, University of Alaska, Fairbanks, AK. 211 pp.

Zhao, L., D. M. Schell and M. A. Castellini. 2006. Dietary macronutrients influence ^{13}C and ^{15}N signatures of pinnipeds: Captive feeding studies with harbor seals (*Phoca vitulina*). Comparative Biochemistry and Physiology–Part A 143:469–478.

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Supporting Information

The following supporting information is available for this article online:

Figure S1. $\delta^{13}C$ and $\delta^{15}N$ equilibration times for serum, muscle (Bulk and Lipid-extracted [LE]), and blubber (Lipid and LE). Each data point represents an individual seal, which had either died or was successfully released between days 0 to 93 from admittance date to TMMC (refer to Table S1). Seals were fed salmon oil ($\delta^{13}C = -21.7‰$, $\delta^{15}N = 5.4‰$) for the first week, and then switched to ground herring for the remainder of time ($\delta^{13}C = -19.4‰$, $\delta^{15}N = 12.5‰$).

Figure S2. Stable carbon *vs.* nitrogen in blood serum of harbor seals for all locations. Refer to legend for symbol description. TB = Tomales Bay (black), SFB = San Francisco Bay (dark gray), CI = Channel Islands (light gray), A = Adult > 4 yr (diamond), SA = Subadult 2–4 yr (square), Y = Yearling 1–2 yr (star), W = Weaner 1 mo–1 yr (triangle).

Table S1. $\delta^{13}C$ and $\delta^{15}N$ of all tissues (serum, muscle, blubber) for captive, rehabilitating seals at TMMC (LE = Lipid Extracted). Tissue was collected once, on final day in center either due to successful release, death, or euthanization (R, D, E). Discrimination factors ($\Delta_{Tissue-Diet}$) were determined from seals at TMMC for greater than 30 d (bolded). Health (1 = emaciated, 7 = obese). Refer to Table S2 for specific tissue isotopic equilibrium graphs after grouping values from seals released or deceased over varying time frames in TMMC.

Table S2. ANOVA statistical analysis of interaction effects between sex, location, and age were used for both cell fractions (RBC and serum) on $\delta^{13}C$ and $\delta^{15}N$. Degrees of freedom (df), sum of squares, *F*-Ratios, and *P*-values are given for each test. Significant interactions are those with $P < 0.05$ (bolded) and are presented in Figures 3 and 4. Both RBC and serum are discussed to determine temporal changes in trophic-level feeding behavior in specific groupings.