## **Short Note**

## Inferences on the Diet of the Eastern Pacific Harbor Seal (*Phoca vitulina richardii*) at the Southern End of Its Distribution: Stable Isotopes and Scats Analyses

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The Eastern Pacific harbor seal (Phoca vitulina richardii) occurs in the Eastern Pacific Ocean from central Baja California, México, to the Alaska Peninsula, and possibly the Aleutian Islands (International Union for Conservation of Nature [IUCN], 2008). This distribution includes islands off the western coast of the Baja California Peninsula such as Coronados, Todos Santos, San Martín, San Jerónimo, San Benito, Cedros, Natividad, Asunción, and San Roque (Gallo-Reynoso & Aurioles-Gamboa, 1984; Maravilla-Chavez & Lowry, 1999; Lubinsky, 2010). The maximum winter abundance (during the breeding season) in the west region of Baja California is about 5,000 individuals. Approximately 10% of this abundance corresponds to harbor seals found on Isla Natividad (Figure 1), a Mexican island that has one of the highest abundances and the lowest seasonal variation among harbor seal rookeries in Baja California, with around 400 individuals in spring and up to 560 individuals in winter (Lubinsky, 2010). The most recent winter estimate (2013) for the island reported around 700 individuals (Morales-Luna & Elorriaga-Verplancken, unpub. data). Because of these attributes, the colony in Isla Natividad should be a solid representation of this subspecies around its southernmost distribution in Baja California.

The Eastern Pacific harbor seal has a generalist diet, ingesting a high variety of prey from the nekton and benthos (Payne & Selzer, 1989). A technique used to identify prey composition is scat analysis (SA), which identifies prey species at different taxonomic levels from recognizable structures.

However, some of these structures can get degraded from digestion, and prey can be underestimated (Da Silva & Neilson, 1985); another limitation is the short amount of time represented by each scat. SA can be complemented by other studies such as stable isotope analysis of N and C (SIA) (Porras-Peters et al., 2008; Páez-Rosas & Aurioles-Gamboa, 2010), which has been proven to be a useful tool for feeding ecology research (e.g., Hobson et al., 1997; Elorriaga-Verplancken et al., 2013) since  $\delta^{15}N$  and  $\delta^{13}$ C increase predictably (3 to 5 and 0.5 to 2% per trophic level, respectively) from the base of the food web to the top predators (Minagawa & Wada, 1984). It is not possible to identify prey items based on SIA; however, by using  $\delta^{15}N$ , some aspects of trophic position, trophic breadth, and individual specialization can be inferred, while  $\delta^{13}$ C can be used to infer habitat use (Newsome et al., 2010; Elorriaga-Verplancken et al., 2013). Different studies based on this technique have used pups as indicators of their mothers' diets during lactation (e.g., Porras-Peters et al., 2008) since the pups' growth is the result of ingesting their mothers' milk, which reflects the prey ingested by adult females.

Most information on *P. v. richardii* comes from colonies in the United States and Canada; few studies have been done in México (Baja California), especially on sustained monitoring (Gallo-Reynoso & Aurioles-Gamboa, 1984; Maravilla-Chavez & Lowry, 1999; Lubinsky, 2010). The ecological contribution of the present study is based on two different approaches, providing evidence of habitat and resource use by



Figure 1. Location of Isla Natividad within a region that constitutes the southern end of the Eastern Pacific harbor seal (*Phoca vitulina richardii*) population

*P. v. richardii* at the southern end of its distribution, where there is not much knowledge in this regard. The population of this subspecies has fluctuated over the last 40 y (IUCN, 2008), so this and other studies are necessary in order to better understand its ecology and status in different time scales and in different areas of its whole distribution.

In order to meet our objective, 19 *P. v. richardii* pups, approximately 2 mo old, were captured at Isla Natividad to obtain hair samples to be analyzed isotopically. Hair samples from five dead adults were also collected, and 11 scats were collected on different beaches of the island. Hair samples were processed at the Chemistry Laboratory at CICIMAR-IPN (La Paz, BCS,

México). Samples were washed with distilled water and an acetone/hexane solvent (1:1), and were then homogenized in an agate mortar until a fine powder was obtained. Approximately 1 mg per sample was weighed using an analytic microbalance with a precision of 0.001 mg; samples were packed in  $8 \times 5$  mm tin capsules and sent to the Stable Isotope Laboratory at the University of California in Santa Cruz. The stable isotope proportion was represented using the delta ( $\delta$ ) notation, according to the equation proposed by DeNiro & Epstein (1978):

$$\delta^{15} N \text{ or } \delta^{13} C = 1000[(R_{sample}/R_{standard}) - 1]$$

where  $R_{sample}$  and  $R_{standard}$  are the molar quotients of the heavy isotopes over the light isotopes of the sample and standard, respectively. The standards internationally recognized for these elements are PeeDee Belemnite (PDB) for carbon, with a value of 0.011%, and atmospheric nitrogen (N<sup>2</sup>) for nitrogen, with a value of 0.004%. To determine the amplitude of the analyzed groups, we used the Bayesian method SIBER in the R package SIAR (R Development Core Team, 2008). This is a complementary analysis to niche determination using polygon areas, with measurements based on ellipses. Scats were placed in plastic vials with water and detergent for 12 to 48 h at the Fish Ecology Laboratory of CICIMAR-IPN. A set of sieves with different mesh sizes (2.0, 1.19, and 0.71 µm) were used to separate and collect fish otoliths and cephalopod beaks. To identify otoliths, we used as a reference the collection of the Fish Ecology Laboratory at CICIMAR-IPN as well as specialized literature (e.g., Fitch & Brownell, 1968). The importance of the different food items was quantified using the Index of Prey Importance (IIMPi) modified by García-Rodríguez & De La Cruz-Aguero (2011):

$$IIMPi = \frac{1}{U} \sum_{j=1}^{u} \frac{xij}{Xj}$$

where xij is the number of individuals of taxon *i* in scat *j*, *Xj* is the total number of individuals in scat *j* across all taxa, and *U* is the number of individuals in the sample. This index produces results ranging from zero to one, which are then converted into percentages. The trophic level was calculated using the Christensen & Pauly (1992) "TL" index, which identifies the trophic level of consumers:

$$TL = 1 + \left(\sum_{j=I}^{n} DC_{ij}\right) (TL_{i})$$

where DCij is the ratio of prey *i* in the diet of rookery *j*, *TLi* is the prey's trophic level, and *n* is the total number of prey species in the diet.

#### Isotopic Analysis Between Age Classes

The values of the N and C stable isotopes (average  $\pm$  SD) in hair of Eastern Pacific harbor seals were (1) pups:  $\delta^{15}N$ :19.1  $\pm$  0.3%,  $\delta^{13}C$ :-15.4  $\pm$  0.6%, and (2) adults:  $\delta^{15}N$ :19.3  $\pm$  0.6%,  $\delta^{13}C$ :-16.0  $\pm$  0.9%.

The SIBER analysis showed a low overlap (0.38) between *P. v. richardii* pups and adults. However, the results of this comparison should be

taken with caution due to the small adult sample size (n = 5), which also could have resulted in the large size of the ellipse in this size class (2.26)compared with pups (0.51) (Figure 2). There were no statistically significant differences between age groups ( $\delta^{15}$ N: Mann-Whitney U = 23, p > 0.05;  $\delta^{13}$ C: Mann-Whitney U = 26, p > 0.05), and  $\delta^{15}$ N variances were < 1 (pups = 0.1, adults = 0.3), which indicates that P. v. richardii may present a specialized behavior at the southern end of its distribution, according to criteria established by Bearhop et al. (2004), who state that a  $\delta^{15}N$  variance < 1 can be interpreted as such. These results are more indicative of pups since the sample size of this age group was larger. It should be mentioned that 15 out of 19 pups (79%) had  $\delta^{15}N$ values of between 18.8 and 19.4%, while 16 of 19 pups (84%) had  $\delta^{13}$ C values between -16.0 and -15.0%, supporting our insights about a probable specialized habitat and resource use by P. v. richardii based on stable isotopes.

The specialization found in pups corresponds to that of their mothers since pups are a direct indicator of the maternal diet in a situation in which pups usually have a higher  $\delta^{15}N$ value ( $\sim 2.0$  to 2.5%) than adults. This is due to mothers catabolizing their own tissues (fat and muscle) to produce milk (Newsome et al., 2006; Elorriaga-Verplancken et al., 2013), resembling the relationship between predator and prey. The expected trophic enrichment of  $\delta^{13}C$  might not be observed due to the high percentage of lipids (~50% for *P. vitulina*; Bowen et al., 1994) in milk, which are depleted in <sup>13</sup>C and would result in pups having lower values than mothers during lactation (Newsome et al., 2006). In this study, we found no significant differences in  $\delta^{15}N$  or  $\delta^{13}C$  between pups and adults. The low adult sample size should not be ignored; however, we propose that our findings could be related to the time elapsed between the time of death of the adults and time of tissue collection. These individuals had been dead for at least 6 mo, so the isotopic signal of hair could presumably correspond to a time during or close to the summer of 2012, contrasting with information obtained for the pups' hair in the winter of 2013 (January to February). Isotopic variations during the year have been reported in other studies of pinniped vibrissae, which store feeding information at a seasonal scale that is the result of environmental changes that cause variations in the trophic baseline or the diet of individuals (Cherel et al., 2009). Our results could be reflecting these seasonal changes around Isla Natividad. However, more sampling should be done during the year, or tissue such as vibrissae should be used to have more accurate results around the baseline characteristics of this region.



**Figure 2.**  $\delta^{15}$ N and  $\delta^{13}$ C breadth and overlap (SIBER analysis) between harbor seal pups and adults in Isla Natividad, Baja California, México; large ellipse (adults) = 2.26; small ellipse (pups) = 0.51.

# Isotopic ( $\delta^{15}N$ and $\delta^{13}C$ ) Comparison with Other Pinniped Species Within the Region

The average hair values of P. v. richardii pups in our study are intermediate compared with those reported for the same tissue and age class (pups) of the Guadalupe fur seal (Arctocephalus townsendi) (~18.5%) and the California sea lion (Zalophus californianus) (~19.5%) in the San Benito Archipelago (~60 km to the NW of Isla Natividad), which are species categorized as mainly teutophagous and piscivorous, respectively (Pablo, 2009). Our  $\delta^{13}$ C value of pups was similar to the hair value of Z. californianus pups (~-15.9%), a species reported to be a mainly coastal consumer in that same study, implying a possible influence of coastal habitats on isotope values of P. v. richar*dii* in Isla Natividad. This is partially supported by Pablo (2009), who attributed a coastal component to P. v. richardii in the San Benito Archipelago, based on the  $\delta^{13}$ C signal (between ~-15 and -13%) of dental growth layers of three individuals.

### Scat Analysis

We admit that our scat sample may not be representative of the feeding habits of the whole P. v. richardii colony in Isla Natividad due to the small sample size. Results are a partial indicator of the trophic spectrum of this subspecies in the area. We found a total of 88 hard parts, such as fish otoliths and cephalopod mandibles, indicating a mainly piscivorous feeding habit near the coast based on the habitats reported for the different prey species (Froese & Pauly, 2003); this supports our SIA results. A total of 11 food items were identified, of which 10 were fish and one was an octopus (Table 1). The highest number of hard parts belonged to Achirus mazatlanus (23), followed by Strongy exilis (21), Octopus rubescens (14), and Sardinops sagax (11), while the eight remaining food items comprised 19 hard parts. The most frequently found item was Achirus mazatlanus (5), followed by Octopus rubescens, Sardinops sagax, and Strongy exilis, which were found in four

Common name	Species	Habitat	# structures	Frequency	TL	IMP (100%)
Mazatlan sole	Achirus mazatlanus	Demersal	23	5	3.2	25.04
P. red octopus	Octopus rubescens	Benthic	14	4	3.8	18.17
California needlefish	Strongylura exilis	Pelagic-neritic	21	4	4.5	16.36
Longfin sanddab	C. xanthostigma	Demersal	9	3	3.6	14.65
Sardine	Sardinops sagax	Pelagic-neritic	11	4	2.4	10.00
Pacific sierra	Scomberomus sierra	Pelagic-neritic	1	1	4.5	9.09
California lizardfish	Synodus lucioceps	Reef-associated	5	3	4.5	3.02
Fringed flounder	Etropus crossotus	Demersal	1	1	3.3	1.01
Threadfin sculpin	Icelinus filamentosa	Demersal	1	1	3.5	1.01
Inshore sand perch	Diplectrum pacificum	Demersal	1	1	4.0	0.83
Mexican lampfish	Triphotorus mexicanus	Pelagic-neritic	1	1	3.3	0.83
Total			88	11*		100

Table 1. Prey composition obtained via scat analysis in P. v. richardii in Isla Natividad, Baja California, México

**Note:** TL = trophic level; IMP = Importance Index \*Number of scats

scats, respectively. Citharichthys xanthostigma and Synodus lucioceps were found in three scats, while the remaining items were found in only one scat (Table 1). According to the IIMPi, the most important items were Achirus mazatlanus, followed by Octopus rubescens and the fishes Strongy exilis, *Citharichthys* xanthostigma, Sardinops sagax, and Scomberomus sierra. These prey species made up 93% of the diet (Table 1). The identified prey in our work differ from other studies of P. v. richardii from other latitudes. There are other important prey reported for harbor seals in California, Washington, and Alaska such as the yellowfin goby (Acanthogobius flavimanus), shiner surfperch (Cymatogaster aggregate), Pacific staghorn sculpin (Leptocottuss armatus), Pacific sand lance (Ammodytes hexapterus), coho salmon (Oncorhynchus kisutch), pollock (Theragra calcograma), capelin (Mallatus villosus), cephalopods (squids and octopus), and crustaceans, among other species (Pitcher, 1980; Gibble, 2011; Luxa & Acevedo-Gutiérrez, 2013). These and other studies, as well as ours, report a diet dominated by fish prey; we also report a high frequency of an octopus prey, an item that is present in the *Phoca*'s diet from regions like the Gulf of Alaska (Pitcher, 1980). Our SA also suggests a benthic-demersal behavior in the harbor seals from Isla Natividad, an insight that is also reported for other more northern latitudes (e.g., Payne & Selzer, 1989). Further ecosystem analyses are required to determine if this diet composition around our study area is proportional to the prey availability or if harbor seals are feeding selectively.

Based on the number of identified prey we infer that the real trophic spectrum of the Isla Natividad colony is probably larger, supporting previous reports of this species' generalist diet (Pitcher,

1980; Payne & Selzer, 1989). This contradicts the specialist behavior found using SIA. The different results found with each technique could be due to two factors that might not be mutually exclusive: (1) the time window varies significantly between techniques (days for scats vs 2 to 3 mo for hair) and (2) SIA can show a certain myopia and underestimate the real trophic breadth of a consumer group, especially if the diet is composed of a high number of items with similar trophic position, giving as a result a low  $\delta^{15}N$  variability, but this does not mean that the predator used a narrow set of resources. The same thing can be seen for habitat use and  $\delta^{13}$ C values (Newsome et al., 2007). Most food items identified with SA had a high trophic level, from 3.5 to 4.5 (Froese & Pauly, 2003), giving as a result a trophic level of around 4.9 for sampled harbor seals, categorizing them as secondary-tertiary consumers (Mearns et al., 1981). This value was higher than that of California sea lions and Guadalupe fur seals (4.1 for both species) in the San Benito Archipelago, both of which were calculated using the same method (Pablo, 2009). However, the scat sample size in that study was higher than ours, so we recommend increasing the sample size to determine with more certainty the diet structure of this pinniped subspecies in this region.

This study represents one of the few contributions on the feeding habits of *P. v. richardii* in México at the species' distribution limit. We present a case of how two approximations can complement each other to infer consumer diets, obtaining results that could eventually be used to increase knowledge of this subspecies at higher latitudes. It is necessary to keep sampling *P. v. richardii* over time as well as the other components of the trophic web, such as prey and other elements at the base of the web, increasing in this way the relationships that can be established to better understand the trophic ecology of this harbor seal.

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