Trophic ecology of Mexican Pacific harbor seal colonies using carbon and nitrogen stable isotopes

Maricela Juárez-Rodríguez[1], Gisela Heckel[1]*, Juan Carlos Herguera-García[2], Fernando R. Elorriaga-Verplancken[3], Sharon Z. Herzka[4], Yolanda Schramm[5]

1 Departamento de Biología de la Conservación, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Baja California, México, **2** Departamento de Ecología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Baja California, México, **3** Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Departamento de Pesquerías y Biología Marina, La Paz, Baja California Sur, México, **4** Departamento de Oceanografía Biológica, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Baja California, México, **5** Facultad de Ciencias Marinas, Universidad Autónoma de Baja California, Ensenada, Baja California, México

* gheckel@cicese.mx

Abstract

There is limited information that provides a comprehensive understanding of the trophic ecology of Mexican Pacific harbor seal (*Phoca vitulina richardii*) colonies. While scat analysis has been used to determine the diet of some colonies, the integrative characterization of its feeding habits on broader temporal and spatial scales remains limited. We examined potential feeding grounds, trophic niche width, and overlap, and inferred the degree of dietary specialization using stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in this subspecies. We analyzed $\delta^{13}C$ and $\delta^{15}N$ on fur samples from pups collected at five sites along the western coast of the Baja California Peninsula, Mexico. Fur of natal coat of Pacific harbor seal pups begins to grow during the seventh month in utero until the last stage of gestation. Therefore pup fur is a good proxy for the mother's feeding habits in winter (~December to March), based on the timing of gestation for the subspecies in this region. Our results indicated that the $\delta^{13}C$ and $\delta^{15}N$ values differed significantly among sampling sites, with the highest mean $\delta^{15}N$ value occurring at the southernmost site, reflecting a well-characterized north to south latitudinal ^{15}N-enrichment in the food web. The tendency identified in $\delta^{13}C$ values, in which the northern colonies showed the most enriched values, suggests nearshore and benthic-demersal feeding habits. A low variance in $\delta^{13}C$ and $\delta^{15}N$ values for each colony (<1‰) and relatively small standard ellipse areas suggest a specialized foraging behavior in adult female Pacific harbor seals in Mexican waters.

Introduction

The concept of trophic ecology is based on understanding the structure of feeding relationships among all trophic levels [1]. In addition, it is of great importance to ascertain the

ecological role of top predators, such as the Pacific harbor seal (*Phoca vitulina richardii*), as they exert top-down control on lower trophic levels [2]. These interactions affect the structure, dynamics, and energy flux within a community or ecosystem [3, 4]. The harbor seal interacts both with members of the same subspecies and with other predators, which may result in competition for resources or niche partitioning, as it has been reported in other pinnipeds [5, 6].

The North Pacific harbor seal is distributed from Japan to Mexico [7], with its southernmost distribution recorded on nine islands and archipelagos, as well as on the west coast west of the Baja California Peninsula [8, 9]. Total counts based on photographs taken during aerial surveys conducted in 2009 yielded 4,862 individuals during the pupping season [9], representing only approximately 1.4% of the total abundance of the subspecies in the North Pacific, which is estimated at 375,100 individuals [10]. The annual life cycle of the harbor seal comprises foraging at sea and hauling out to breed, molt and rest, although the timing (start, peak, end, and duration) of these behaviors may vary depending on the latitude at which the colonies are located [11]. In Mexican colonies, seals start hauling out in mid-winter (late December-February) to give birth and breed [12], with pupping generally lasting from late December to the beginning of April, and longer in the southern than in the northern colonies of Mexico (*e. g.*14 weeks on San Roque Island and nine on Todos Santos Island, respectively) [12, 13]. Consequently, different degrees of synchronization occur among adult females from different colonies, whose lactation period lasts for three to four weeks [13]. Contrary to other seal species which fast during the breeding season (*e.g.* the northern elephant seal, *Mirounga angustirostris*) [14], the harbor seal tends to forage near its pupping and molting sites, no more than 45 km from shore [15].

Based on analysis of both scat and stomach contents, previous studies have suggested that the Pacific harbor seal is a generalist consumer with a high rate of consumption that depends on the seasonal and local abundance of benthic and pelagic prey (fish and cephalopods) [16–18]. While these traditional analyses enable prey identification up to a species-level taxonomic resolution, they are limited in that they correspond to a short time frame, that is, since the individual's most recent foraging trips. Moreover, there is also the potential for misidentification or underestimation of prey due to the digestive process. In addition, prey items may be underestimated because the haul-out sites where scat is collected are only located in nearshore areas, so prey captured in offshore areas may not be in collected samples [19, 20]. Little is known regarding the trophic ecology of the Pacific harbor seal colonies along the Baja California Peninsula. Although there is a wide composition of prey species in the colonies studied here, the harbor seal tends to feed mostly on two to five items at each site, leading to the conclusion that this subspecies is a specialist consumer [21–24].

Due to the large temporal and spatial scales of their ecological niche, it is challenging to study the diet of marine mammals. Stable isotopes analyses (SIA) is a powerful tool for dietary reconstruction of predators [25, 26]. SIA provides information on assimilated prey over different time scales, depending on the tissue selected [27–29]. Tissues with a higher metabolic rate, *e.g.* blood, tend to have shorter integration times, while non-metabolic tissues such as fur, nails, and teeth retain the isotopic composition that reflects the food assimilated at the time of formation [26, 30–33]. The stable isotope ratio of carbon (δ^{13}C) provides information on predator foraging areas and can be employed to determine primary producer sources in a trophic web. This is based on differences in isotopic fractionation due to taxon-specific photosynthetic pathways, variations in the isotopic composition of inorganic carbon caused by geochemical processes, and the differences between pelagic and benthic producers [31, 34]. Carbon isotope ratios of carbon sources at the base of the food web tend to display a gradient toward lighter values between coastal habitats and offshore regions, as well as in lower and higher latitudes, and when comparing pelagic and benthic food sources [34–36].

The stable isotope ratio of nitrogen ($\delta^{15}N$) is a proxy for trophic position, due to the predictable 2.5–3.4‰ ^{15}N-enrichment occurring among each subsequent trophic level [36–38]. Tissue-to-diet isotope discrimination varies because of differences in metabolic routing of dietary components from tissue to tissue, animal physiology, and the nutritional quality of the individual's diet [39, 40]. The $\delta^{13}C$ and $\delta^{15}N$ values observed in consumers are also influenced by the $\delta^{13}C$ and $\delta^{15}N$ values observed at the base of the food web.

The regional variation in stable carbon and nitrogen isotopes at the base of the food web occurs due to several factors, such as the predominant types of primary producers, nutrient and light levels, the intensity of the upwelling that results in the consequent transport of inorganic carbon and nitrogen, and the magnitude of fluvial, atmospheric or anthropogenic carbon and nitrogen inputs [34, 35, 41]. In the north-eastern Pacific Ocean, both $\delta^{15}N$ and $\delta^{13}C$ values at the base of the food web negatively correlate with latitude [30, 31, 41–43]. Moreover, the $\delta^{15}N$ values of residual nitrate tend to be enriched in ^{15}N near the tropics due to extensive denitrification regions in minimum oxygen zones (MOZs) [41, 44, 45]. Values of $\delta^{13}C$ at the base of the food web are generally depleted in ^{13}C at higher latitudes due to the lower sea surface temperature than that found at lower latitudes [35, 46], combined with light-limited low photosynthetic rates [24, 29].

Carbon and nitrogen stable isotope ratios have been used extensively to characterize feeding areas and movement patterns, and to track migration, residency, and food web structure for a variety of marine mammals. In the north-eastern Pacific Ocean, SIA has been successfully used to assess the foraging locations and preferences, trophic relationships and feeding grounds of several pinniped species, such as the harbor seal, the Steller sea lion (*Eumetopias jubatus*) [31], the northern fur seal (*Callorhinus ursinus*), the California sea lion (*Zalophus californianus*), and the northern elephant seal (*Mirounga angustirostris*) [26, 30, 42, 47].

Trophic niche width and foraging preferences have been characterized by the variance in the stable isotope ratios of populations of various taxa [48, 49]. The concept of ecological niche is defined as a hypervolume in n-dimensional space, in which an organism's feeding behavior can be represented on environmental and bionomic axes [50]. Therefore, the isotopic niche provides a quantitative metric for representing the trophic niche [51–54].

Tissue selection for the analysis must take into account the period over which the isotopic composition of the tissue has been integrated [26]. The isotopic enrichment between marine mammal mothers and their pups is similar to that observed in a prey-predator relationship. Isotope analyses of tissue taken from pups have served as good proxies for maternal foraging habits [23, 30, 33], because fur grows in utero approximately from the seventh month onwards and continues to grow until the last stage of gestation [55]. The harbor seal sheds its lanugo in utero prior to birth [56], with its fur molting one year post-birth [57–59]; therefore, harbor seal pup fur reflects the feeding habits of its mother (*i.e.* an adult female with offspring) over a time frame equivalent to winter (December to March), the last months of gestation (~four months before birth). As pups are easier to capture and restrain than adults, they offer the opportunity to obtain a sufficiently large sample size to enable robust statistical testing.

The aim of the present study was to characterize the trophic ecology of adult *P. v. richardii* females by analyzing the stable isotopes of pup fur at five sites representative of its distribution in Mexican waters. We defined potential feeding grounds (benthic vs. pelagic; coastal vs. oceanic) during the pupping season, as well as the trophic niche width, position and overlap, and the degree of specialization in the studied colonies.

Materials and methods

Ethics statement

All Pacific harbor seal fur samples used in this study were collected and processed under special permits issued by the following: Dirección General de Vida Silvestre (General Wildlife Directorate), Secretaría de Medio Ambiente y Recursos Naturales (Ministry for the Environment and Natural Resources), Nos. SGPA/DGVS/12269/13 and SGPA/DGVS/08370/14; *Secretaría de Gobernación* (Ministry of the Interior), Nos. UG/211/0087/2014 and UG/211/01022/ 2014; and *Reserva de la Biosfera El Vizcaíno* (*El Vizcaíno* Biosphere Reserve) No. F00. DRPBCPN.-000027. All tissue samples were collected using non-invasive sampling techniques.

Sample collection and processing

Our sampling sites were located along the west coast of the Baja California Peninsula, in a north to south direction: Todos Santos (TSI), San Jerónimo (SJI), Natividad (NI); and, San Roque (SRI) islands. TSI and SRI are separated by 4° latitude (Fig 1). These islands form part of the Baja California Peninsula Pacific Islands Biosphere Reserve [60]. The Pacific harbor seal pups were sampled at these four islands and at the Punta Banda Estuary (PBE) (Fig 1).

From February to April 2015, we collected 138 fur samples from suckling harbor seal pups aged two to four weeks and of both sexes (TSI, n = 39; PBE, n = 12; SJI, n = 29; NI, n = 27; SRI, n = 31). Pup counts in 2009 for the islands were 56 for TSI, 99 for SJI, 124 for NI, and 181 for SRI [9], while 34 pups were estimated at PBE in 2011 [12]. We applied a minimally-invasive fur-collection method that involved shaving a 10x10 cm patch from the dorsal region of the pups using a stainless-steel razor, with the samples registering a ~3–5 g wet weight. Care was taken to avoid removing the fur follicle. Fur samples were washed with acetone and double-distilled water in the laboratory to remove salt and sand residue, and dried at 60°C for 12 h to eliminate excess moisture. The fur samples were then homogenized in an agate mortar until a fine powder was obtained, which was then put in sealed tin capsules (5x9 mm) to achieve a target 0.5±0.05 mg dry weight.

Sample analyses

The nitrogen and carbon isotope ratios in the fur were determined using a continuous flow mass spectrometer (Delta plus Advantage, Thermo Fisher Scientific, Bremen, Germany), coupled to an elemental combustion analyzer (model 4010 ECS, Costech Analytical Technologies, California, USA) at the Stable Isotope Laboratory, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE, or the Ensenada Center for Scientific Research and Higher Education, in Baja California, Mexico). The stable isotope values were expressed as δ (‰), in line with international standards, with Vienna Pee Dee Belemnite (VPDB) used for carbon, and atmospheric nitrogen (N$_2$) used for nitrogen, in accordance with the equation:

$$\delta X = \left[\frac{R_{sample}}{R_{standard}} \right] \cdot 1000 \tag{1}$$

Where X is ^{13}C or ^{15}N and R$_{sample}$ and R$_{standard}$ are the molar quotients of the heavy isotopes over the light isotopes (^{13}C/^{12}C or ^{15}N/^{14}N) for the sample and laboratory standard, respectively. Secondary laboratory standards, including glutamic acid (δ^{13}C: -26.39‰ and δ^{15}N: -4.52‰), acetanilide (δ^{13}C: -30.37‰ and δ^{15}N: -0.83), seal pup lanugo (δ^{13}C: -16.04‰ and δ^{15}N: 15.32‰), and PF01 (δ^{13}C: -15.94‰ and δ^{15}N: 14.64‰) were routinely measured during sample analysis, with a high level of precision for all standards and both elements (<0.2 ‰
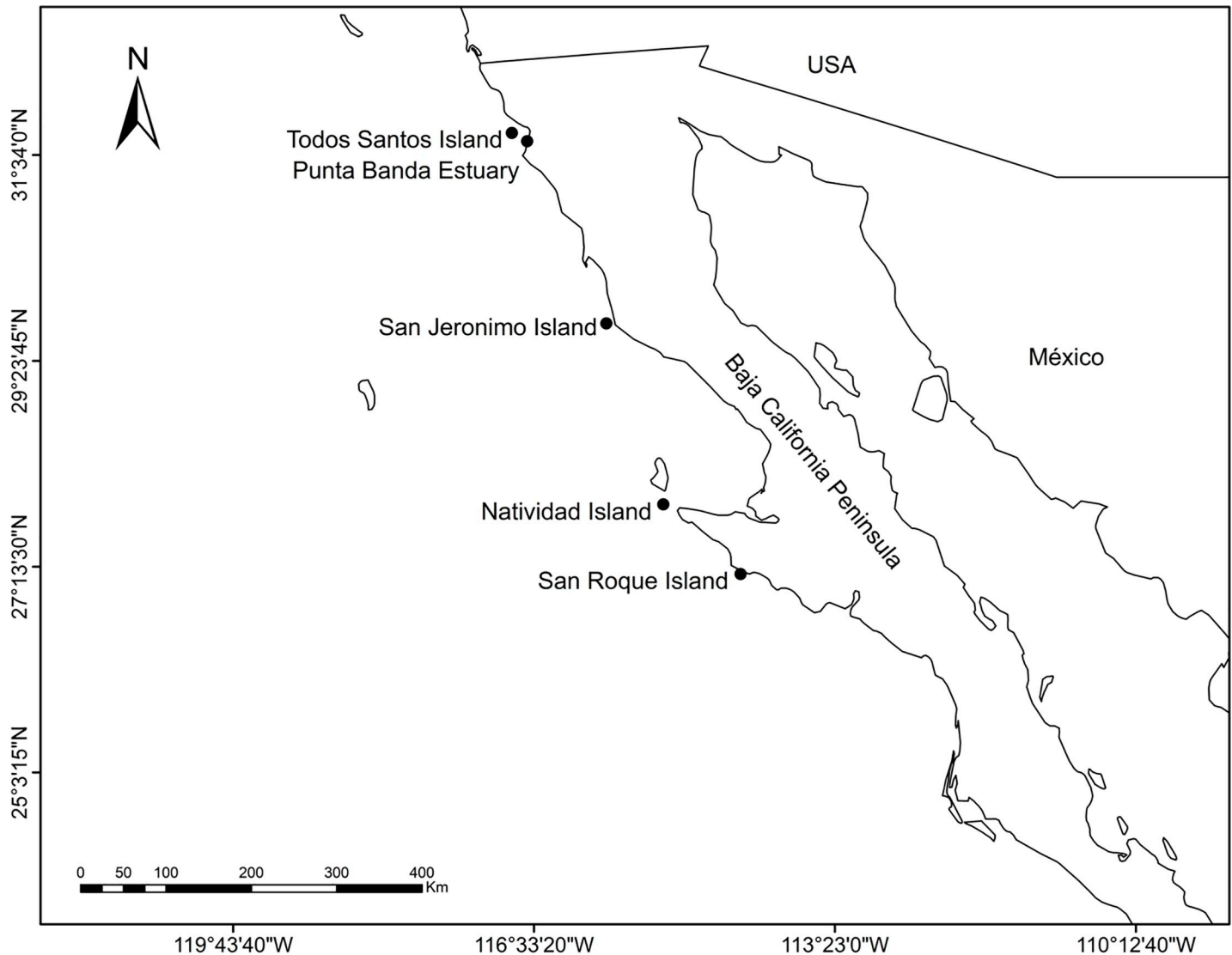
Fig 1. Location of sampling sites along the west coast of the Baja California Peninsula, Mexico, where fur sample were collected from Pacific harbor seal pups (n = 138) for stable isotope analysis.

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standard deviation). The laboratory protocol used is available at https://dx.doi.org/10.17504/protocols.io.2m3gc8n and in the Supplementary Information (S1 Protocol) for this study. The $\delta^{13}C$ and $\delta^{15}N$ values for each pup fur sample are also available in the Supplementary Information (S1 Dataset) for this study and at the Figshare repository, DOI: https://doi.org/10.6084/m9.figshare.8156273.

Statistical analyses

The statistical tests were applied using the *Statistica* software (version 10.0, 2015), while the pups were grouped by sampling site. The $\delta^{13}C$ and $\delta^{15}N$ values were tested for normality using the Shapiro–Wilks test and for homoscedasticity, using the Levene test. Differences in $\delta^{13}C$ values among the five sampling sites were assessed using one-way analysis of variance (ANOVA). Kruskal-Wallis analysis was used for $\delta^{15}N$ values, because the nitrogen isotope

ratios did not meet the assumptions of parametric statistics. When significant differences in $\delta^{13}C$ were found among sampling sites, the Tukey's test was applied, while, for $\delta^{15}N$, multiple comparisons of mean ranks were used as a non-parametric *a posteriori* test, in which a p-value < 0.05 was considered significant.

Based on isotopic variance, the dietary niche width is one of the metrics applied to measure the degree of specialization. To determine the isotopic niche width for the individuals from all the colonies studied here (four islands and one estuary), we first calculated the convex hull area (TA) of the $\delta^{13}C$-$\delta^{15}N$ biplot for each colony [53]. The polygon was drawn around the outermost points of stable isotope values and is a direct measure of the area of isotopic niche space encompassed by a given colony. However, as the convex hull area can be sensitive to sample size [61], for comparative purposes, we also calculated isotopic ellipses using the Bayesian method SIBER (Stable Isotope Bayesian Ellipses in R) [53, 61], which was developed for the R package (R Development Core Team, 2008) and updated in February 2019 [62]. This method provides a robust estimate for comparing isotopic niche width, and is based on the calculation of standard ellipse areas (SEA) which are not as sensitive to limited sample sizes, as is the case for the convex hull model [61]. SIBER is frequently used in ecological studies with sample sizes n<50 [61]. Standard ellipse areas were corrected according to sample size (SEA$_c$) and were calculated to nullify potential bias due to differences in sample size between colonies, with SEA$_c$ the equivalent to the standard deviation in univariate cases, and less influenced by the extreme values of standard ellipses or convex hulls [61], containing approximately 40% of the data, SEA$_c$ has a 95% credible interval, while the SEA$_c$ differences among colonies were tested for statistical differences by comparing the SEA$_c$ probability distributions for the colonies generated, as the outcome of 10^4 resampling runs [61]. We calculated the overlap between two colonies by comparing their SEA$_c$ in ‰ ^{2}, in accordance with the following equation [61, 62]:

$$Overlap = \frac{overlap(A, B)}{total\ area\ A} \tag{2}$$

To test the above for geographical differences in isotopic values, we correlated the distance to the coast as well as the latitude and longitude (*i.e.*, the east-to-west gradient) of each colony with the mean $\delta^{13}C$ and $\delta^{15}N$ values for each colony. The distance or proximity of each colony to the coast was calculated using the Haversine equation:

$$a = sin^2\left(\frac{\Delta\varphi}{2}\right) + cos\varphi_1 \cdot cos\varphi_2 \cdot sin^2\left(\frac{\Delta\lambda}{2}\right) \tag{3}$$

$$c = 2 \cdot atan2\left(\sqrt{a}, \sqrt{(1-a)}\right)$$

$$d = R \cdot c$$

Where:
φ = Latitude
λ = Longitude
R = Radius of the Earth, assumed to be 6,371 km
d = Distance between two points

Relationship with diet

Stable isotopic values reflect the composition of the assimilated diet [63], but not necessarily the ingested diet [22]. Therefore, information relating to the ingested diet is not necessarily

recorded, while isotope niche widths can lead to the underestimation of the dietary niche. Conversely, species richness may not reflect the actual consumption of individual prey.

The trophic spectrum of the harbor seal's diet was correlated based on species richness estimates derived from the identification of the hard parts of fish and cephalopods found in scat [22], which were cross-referenced with our estimated isotopic niche width (*i.e.*, number of prey species for each colony *vs.* the SEA_c and the TA). Species richness data recorded during the pupping season were considered for TSI, SJI, NI, and SRI [22], while the data for PBE were excluded because scat was collected during the molting season, and the seals' diet tends to vary from season-to-season or, even, month-to-month [21]. The prey species identified from hard parts were also plotted in terms of their trophic level, Index of Prey Importance (IIMPi) [22] and their habitat type (demersal, bathydemersal, benthic, benthopelagic, or pelagic) [64].

Results

Inter-individual variation

The $\delta^{15}N$ values from individual Pacific harbor seal fur samples ranged between 16.8 and 21.5‰ (mean ± SD: 19.0 ± 0.7‰; Table 1, Fig 2). The SRI sampling site recorded the highest mean $\delta^{15}N$ value, while TSI recorded the lowest. The $\delta^{13}C$ values ranged between -18.3 and -13.6‰ (mean ± SD: -15.9 ± 0.8‰). The broadest range of $\delta^{13}C$ values was exhibited by the pups sampled at TSI (-17.1 to -13.6‰), while the broadest $\delta^{15}N$ values were found at the SRI colony (19.0 to 21.6‰). Individuals sampled at PBE showed the most limited range of isotopic values for both elements. Those individuals sampled at NI showed the most depleted carbon isotope ratios, in contrast to PBE, where the most enriched carbon isotope values were found.

Overall, significant differences were found in the $\delta^{13}C$ values for the pup fur samples collected at five colonies along the western coast of the Baja California peninsula (ANOVA, $F_{4, 133}$ = 9.321, p<0.001; Fig 2). For of the $\delta^{13}C$ values, NI was statistically different from all other sites, while SRI was statistically different from PBE and NI (Tukey´s test, p <0.05). There were also significant differences in $\delta^{15}N$ values among the colonies (KW, $H_{4, 138}$ = 68,795, p <0.001; Fig 2), while statistical differences were found between TSI in comparison with SJI, NI, and SRI. SRI was significantly different from all other study sites (non-parametric *a posteriori* test, Table 2).

Table 1. $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD) values for the pup fur of Pacific harbor seals sampled at five sites, given with their geographical location and distances to shore along the western coast of the Baja California Peninsula, Mexico.

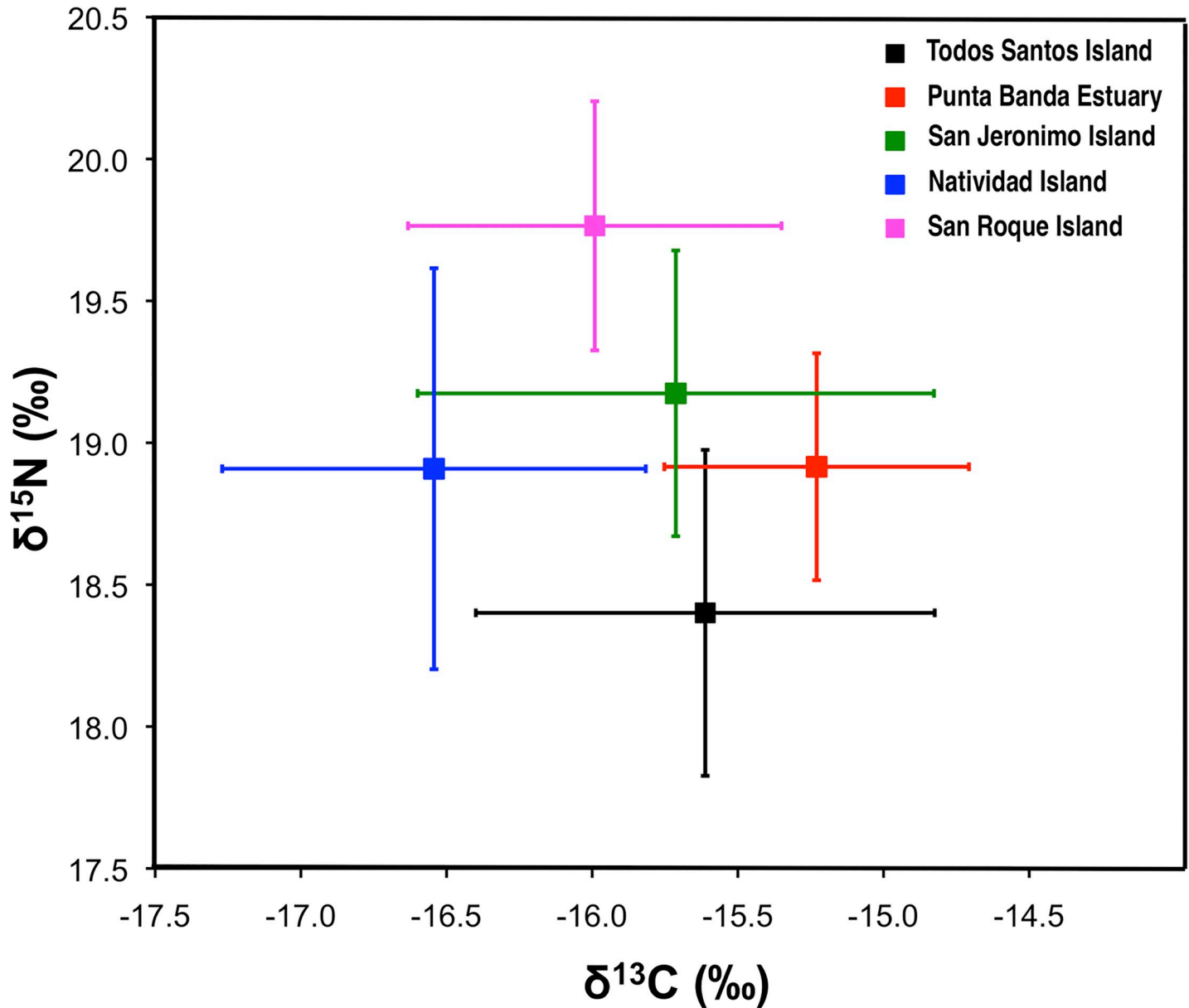| Sampling sites	Distance to shore (km)	n	$\delta^{13}C$ mean± SD (‰)	$\delta^{15}N$ mean ± SD (‰)
Todos Santos Island	16.46	39	-15.6± 0.8	18.40±0.6
(31˚47' 59" N, 116˚47' 20" W)				
Punta Banda Estuary	0.29	12	-15.23±0.5	18.92±0.4
(31˚ 42' 47" N, 116˚ 40' W)				
San Jerónimo Island	9.44	29	-15.71±0.9	19.17±0.5
(29˚47' 33" N, 115˚47'29" W)				
Natividad Island	9.80	27	-16.54±0.7	18.91±0.7
(27˚52' 55" N, 115˚11'33")				
San Roque Island	1.8	31	-15.99±0.6	19.76±0.4
(27˚8' 48 " N, 114˚22'40" W)				

Fig 2. $\delta^{15}N$ and $\delta^{13}C$ values (mean ± SD) of Pacific harbor seal pup samples at different sites along the west coast of the Baja California Peninsula (TSI = Todos Santos Island, PBE = Punta Banda Estuary, SJI = San Jerónimo Island, NI = Natividad Island, and SRI = San Roque Island).

Trophic niche

Variance in carbon and nitrogen stable isotope values within sampling sites was low (<1‰), both for $\delta^{13}C$ (TSI: ±0.6, PBE: ±0.3, SJI: ±0.8, NI: ±0.5, and SRI: ±0.4 in ‰) and for $\delta^{15}N$ (TSI: ±0.3, PBE: ±0.3, SJI: ±0.3, NI: ±0.5, and SRI: ±0.3‰). The convex hull total areas (TA) were largest for TSI and NI (5.1 and 5.9, respectively), while PBE presented the smallest TA (1) (Fig 3). The highest overlap in isotopic space was between SJI and NI (3.0), followed by NI and TSI (2.7); while the smallest overlap found was between SRI and PBE (0.1) (Table 3).

Based on the SEA$_c$ values, NI presented the largest trophic width (1.6) and PBE the smallest (0.6) (Fig 4, Table 4). A large overlap (0.5) was found between SJI and NI, followed by PBE and

Table 2. Results of the paired comparisons of the nitrogen isotope ratios of Pacific harbor seal pup fur among five sampling sites along the west coast of the Baja California Peninsula (TSI = Todos Santos Island, PBE = Punta Banda Estuary, SJI = San Jerónimo Island, NI = Natividad Island, and SRI = San Roque Island).

| | A posteriori test | | | |
| | p-value | | | |
	TSI	PBE	SJI	NI
PBE	0.54			
SJI	< .01	1.00		
NI	0.03	1.00	1.00	
SRI	<0.01	< .01	< .01	< .01

SJI (0.5), and TSI and PBE (0.3). The smallest trophic overlap found for this metric was between TSI and SRI.

Geographical variation

No correlation was found between the proximity to shore and the $\delta^{15}N$ and $\delta^{13}C$ pup fur values. However, we observed a high explained variance (r = 0.58, p = 0.081) for carbon isotope ratios, a positive and significant correlation between mean $\delta^{15}N$ values for both colonies and longitude (r = 0.78, p = 0.046), and an enrichment trend in ^{15}N towards the south, but which was not significant (r = 0.57, p < 0.087). In contrast, the correlations between mean $\delta^{13}C$ values and proximity to the shore, as well as latitude and longitude were not significant.

Relationship with diet

There were no significant correlations between SEA_c and TA estimates in our study with species richness identified by hard remains previously [22] (SEA_c: r = -0.40, p = 0.75; TA: r = -0.80, p = 0.33; Table 5). This absence of correlation is due to the fact that San Jerónimo and San Roque islands had the highest species richness (26 and 20 taxa, respectively, Fig 5B and 5D), which did not coincide with the largest TAs and SEA_c (Table 5). In addition, we did not find a significant correlation between the number of prey species shared between sites and their corresponding overlap areas (r = 0.21, p = 0.689, Fig 5, Table 6).

The absence of significant correlation between species richness and overlap areas between colonies reflects different prey preferences among the colonies (IIMPi). For example, demersal species, such as *Sebastes* spp., *Synodus lucioceps* and *Octopus* spp., appear with larger IIMPi in TSI, SJI and SRI than NI (Fig 5).

Discussion

The factors controlling isotopic incorporation into tissue have to be taken into account by trophic ecology studies inferred from stable isotopes. The present study used fur, a metabolically inert tissue that reflects the isotopic composition of the prey assimilated during biosynthesis [65, 66]. Dietary changes are recorded in fur as a function of the time required for isotope equilibration between the diet assimilated and formation of the fur [67–69]. As explained in the introduction of the present paper, harbor seal pup fur is a good proxy for the mother's feeding habits in a time-frame equivalent to winter (December to March), based on the last months of gestation in the region of study [12].

To date, only a few studies have analyzed stable isotope values in fur samples taken from harbor seal pups pertaining to Mexican colonies [23, 70, 71], with one of those studies [72] reporting higher mean $\delta^{13}C$ values for the NI colony ($\delta^{13}C$: -15.4±0.6‰) than those obtained

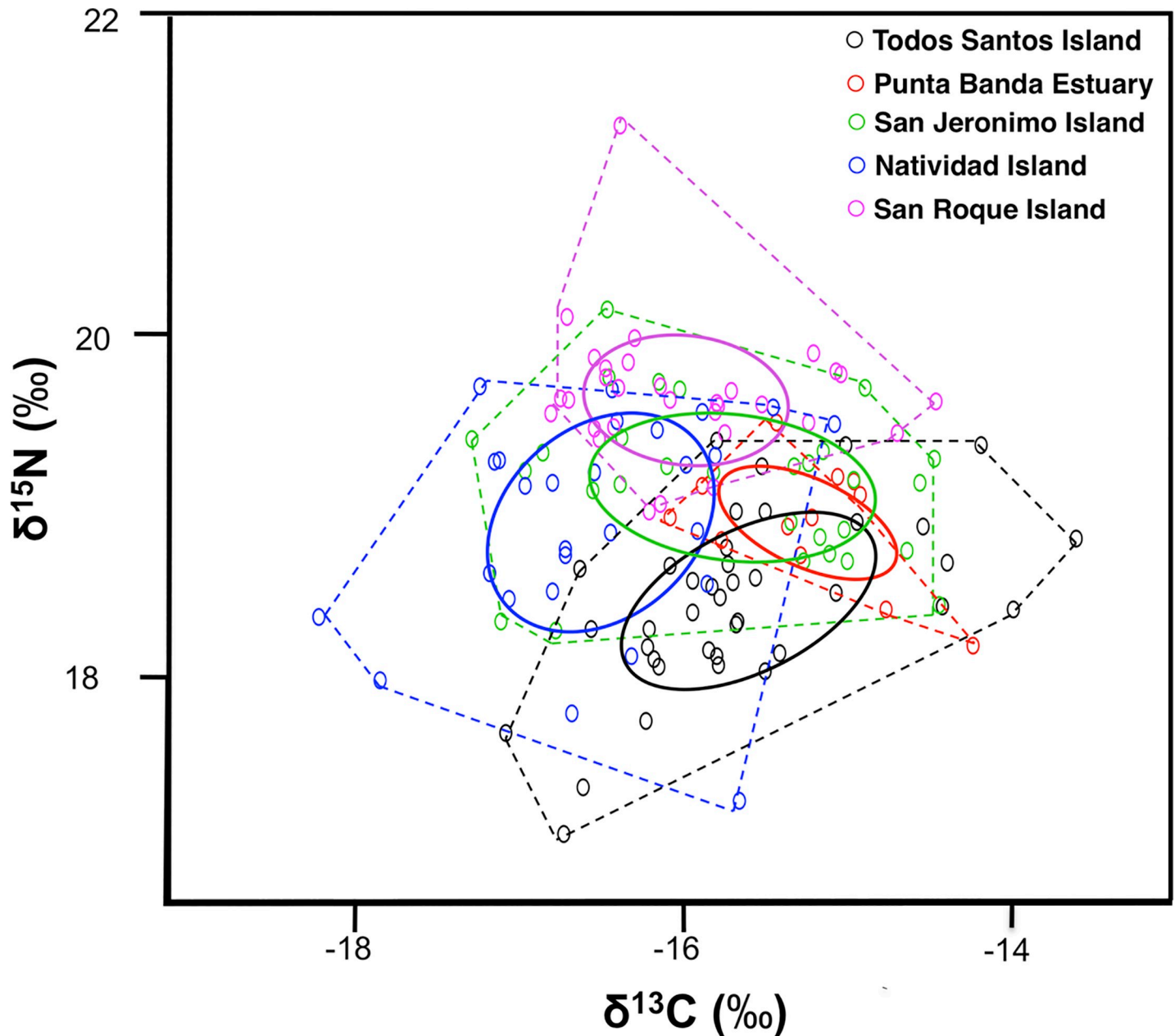**Fig 3. Individual δ¹⁵N and δ¹³C values of pup fur samples for pup fur samples from Pacific harbor seals at five sampling sites along the west coast of the Baja California Peninsula.** Solid lines represent the standard ellipses corrected for sample size (SEA$_c$) and dashed lines represent the convex hull areas.

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by the present study (δ¹³C: -18.3±0.7‰). This discrepancy could be caused by the fact that the sampling for the studies was conducted in different sampling years (April 2013 vs. December 2014-February 2015), meaning that, therefore, there was either a distinct difference in isotopic composition at the base of the food web or distinct difference in prey availability. A warm water anomaly in the northeast Pacific, which started off the coast of Alaska and subsequently spread to southern Baja California [73], occurred from winter 2013–2014 to summer 2015 and caused a 1-4˚C increase in sea surface temperature. This event may have changed the composition and structure of the region's trophic webs by modifying the abundance and distribution

Table 3. Areas and overlap of convex hulls calculated for the carbon and nitrogen isotope ratios for Pacific harbor seal pup fur sampled at five sites along the west coast of the Baja California Peninsula (TSI = Todos Santos Island, PBE = Punta Banda Estuary, SJI = San Jerónimo Island, NI = Natividad Island, and SRI = San Roque Island).

	Convex hull areas (‰^{2})	Overlap (‰^{2})			
		TSI	PBE	SJI	NI
TSI	5.14				
PBE	1.00	0.98			
SJI	4.70	2.24	0.95		
NI	5.86	2.66	0.51	3.00	
SRI	3.21	0.23	0.14	1.67	0.87

of the organisms and also, therefore, the availability of harbor seal prey. The study discussed above [72] collected samples in April 2013, approximately ten months before the warm water anomaly began, while our samples were collected in January-February 2015, when the anomaly was already well established.

Isotopic niche width can be considered a measurement of a species' degree of specialization [74]. The low variance in carbon and nitrogen stable isotopes in pup fur ($<$1‰) suggests that female harbor seals adhere to specialized foraging habits [48, 75]. In fact, previous studies indicate that female harbor seals prefer a narrow range of prey species, with three to five dominant species documented in their diet [48]. The results of the present study suggest that the largest convex hull total area calculated for NI indicates more generalized foraging habits or a lower degree of specialization, compared to the other colonies studied here. In contrast, PBE presented the smallest convex hull area, low variance, and smaller ellipse areas, and, therefore, the highest degree of specialization. This finding may be due to the high IIMPi of three fish species found in the diet of the colony's members—the California lizardfish (*Synodus lucioceps*), Verrill's two-spotted octopus (*Octopus bimaculatus*) and the California market squid (*Dorytheutis opalescens*) [21]. However, up to 23 species of ichthyofauna have also been documented as prey for the individuals of this colony [76]. The PBE colony occupies a small geographical area [12], with a previous study finding that the prey species of its Pacific harbor seal population were significantly different from those of the TSI, which is located less than 20 km away from PBE [21], probably because the PBE harbor seal colony seems to feed only on species found in the estuary [77]. In addition, the central region of the Baja California Peninsula shows a lower number of fish habitats and species groups compared with the northern region [78], which may explain the smaller trophic niche of the PBE colony. This smaller isotopic niche means that the PBE colony is of ecological relevance, given that specialist species are more sensitive to changes in resource availability than generalists [77, 79, 80].

The differences in δ^{13}C values among Mexican Pacific harbor seals colonies seem to reflect different site-specific oceanographic conditions that have caused variations in the isotopic baseline, although a latitudinal pattern in the δ^{13}C values of harbor seal pup fur was not evident. The greatest discrepancy was found between NI and PBE. With PBE located inshore and NI near Punta Eugenia, the latter colony is more strongly influenced by the California Current and the North Equatorial Countercurrent than the former. This oceanic influence is consistent with the lower δ^{13}C values found at NI [72], which contrast with the higher δ^{13}C values found at PBE, that is a coastal lagoon, and result from the higher contribution of benthic carbon sources or benthic macrophytes, which are significantly enriched in ^{13}C in relation to phytoplankton [46, 81]. The δ^{13}C values were highest in the coastal colonies at TSI and PBE, which probably reflects the significant presence of benthic-demersal prey items [82].
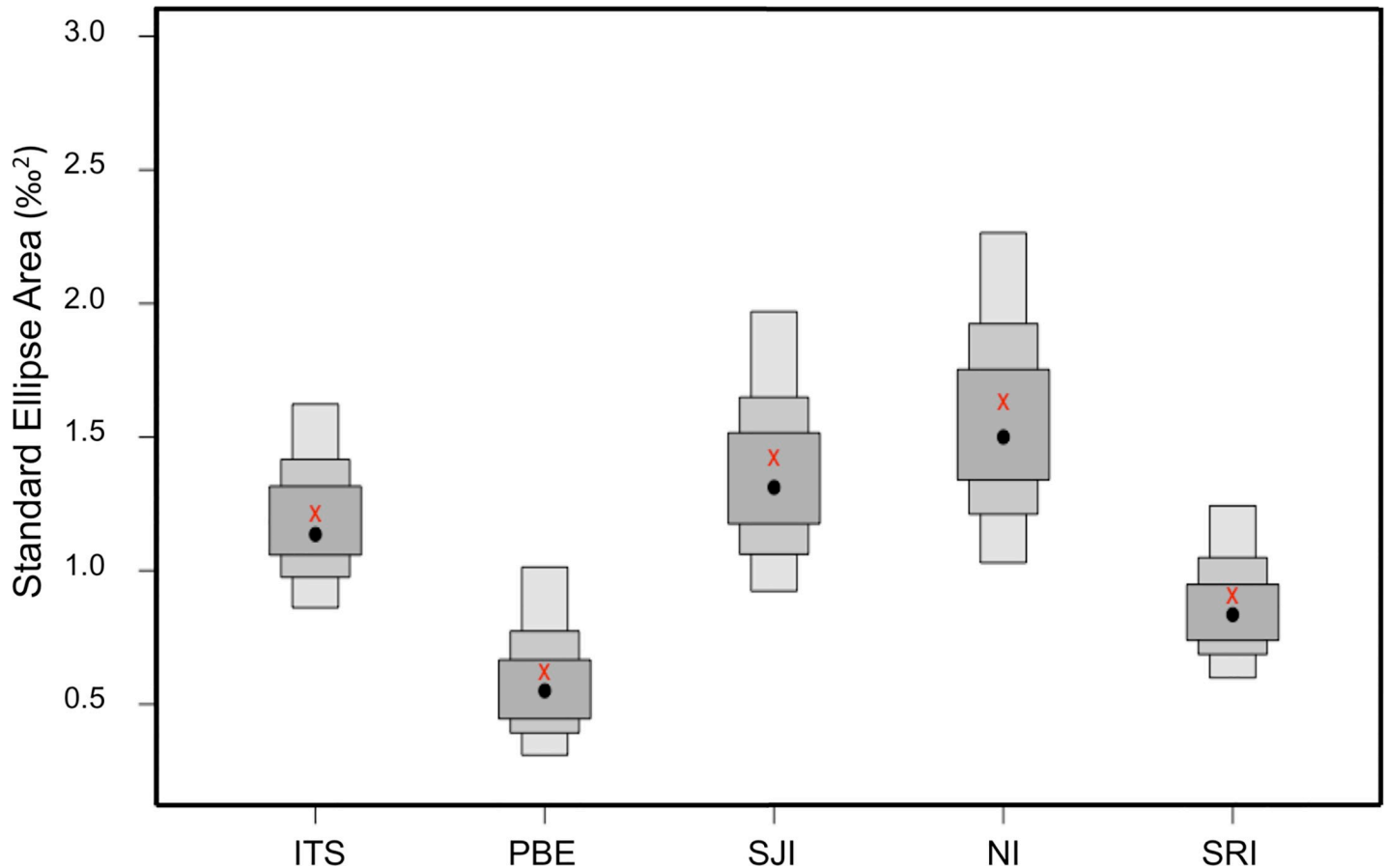
Fig 4. SIBER density plot, with credibility intervals (50% dark grey boxes, 75% mid grey boxes, 100% light grey boxes), for the Bayesian-generated ellipses (SEA—black dots) for the Pacific harbor seal pup isotope data overlaid with the sample size corrected for uncertainty in the estimates (SEA$_c$—red crosses). The distribution in the estimates of the standard ellipse area (‰) is based on 10^6 resampling runs.

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Isotopic differences in $\delta^{15}N$ values among all colonies were significant, with the exception of TSI and PBE, which are located in close proximity to each other. The higher $\delta^{15}N$ values found at SRI likely reflect a latitudinal ^{15}N enrichment of the baseline towards the south, caused by regional denitrification processes in the tropical equatorial Pacific and the subsurface transport of enriched nitrate to the north by the California Counter Current [83]. The high $\delta^{15}N$ pup fur values from SRI, the southernmost site in the present study, likely reflects

Table 4. Areas and overlap of Standard Ellipse Areas corrected for the sample size (SEA$_c$) and calculated for the carbon and nitrogen isotope ratios of Pacific harbor seal pup fur at five sampling sites along the west coast of the Baja California Peninsula (TSI = Todos Santos Island, PBE = Punta Banda Estuary, SJI = San Jerónimo Island, NI = Natividad Island, and SRI = San Roque Island).

	SEA$_c$ (‰^{2})	Overlap (‰^{2})			
		TSI	PBE	SJI	NI
TSI	1.22				
PBE	0.62	0.29			
SJI	1.42	0.21	0.45		
NI	1.64	0.05	0	0.53	
SRI	0.91	<0.01	<0.01	0.29	0.12

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Table 5. Species richness (number of prey species) in four colonies with their corresponding Standard Ellipse Area (SEA$_c$) and Total Area (TA) calculated from pup fur isotope analysis.

Site	SEA$_c$ (‰^{2})	TA (‰^{2})	Species richness
Todos Santos Island	1.22	5.14	19
San Jerónimo Island	1.42	4.70	26
Natividad Island	1.64	5.86	15
San Roque Island	0.91	3.21	20

the greater contribution of heavy nitrate. The 1‰ increase in the mean isotope ratios of the fur taken at SRI and TSI, colonies separated by four degrees of latitude, is consistent with the equatorward ^{15}N enrichment shown in other studies [8, 39, 84].

Isotopic comparison among pinniped species

Comparing our isotopic data with fur samples taken from other pinniped species in the northeastern Pacific may be useful for understanding the trophic ecology of this subspecies at the southern end of its distribution [23, 31, 71, 85, 86]. The δ^{15}N values observed in the present study (19.0±0.7) were low compared to those previously reported for California sea lions (*Zalophus californianus*) in the Gulf of California (21.8 ±0.7‰) [39, 87], although they were higher than those reported for Galapagos sea lions (*Zalophus wollebaeki*, 12.94±0.43‰, Table 7).

The δ^{13}C values for the fur taken from Guadalupe fur seals at Guadalupe Island were approximately 2.6‰ lighter (-18.4±0.4, Table 4) than the values obtained for Mexican Pacific harbor seals. The δ^{15}N (17.6±1.3) and δ^{13}C (-17.2±0.8) values for the fur taken from Northern elephant seals (*Mirounga angustirostris*) at San Benito Islands were lower and more variable than our measurements for harbor seals (Table 4) [70].

The comparatively lower δ^{15}N values found by the present study on the west coast of the Baja California Peninsula revealed differences among trophic web baselines at other sites, such as the Gulf of California and the Galapagos islands [39, 87].

The differences in δ^{13}C values found among the fur samples taken from Guadalupe fur seals at Guadalupe Island and the values obtained from Mexican Pacific harbor seals most likely occur because Guadalupe Island is located at an oceanic location 250 km from the coast of the peninsula and the fact that Guadalupe fur seals forage on offshore squid [89]. The difference between the δ^{15}N values obtained for Northern elephant seals and those found in the samples taken in the present study may indicate a latitudinal gradient, as the elephant seal forages in northern waters (as far north as the Gulf of Alaska) and offshore areas on the west coast of North America [14, 75]. The differences observed in δ^{13}C values may result from the effect of both latitude and distinct diets, in that the Northern elephant seal feeds primarily on squid [14]. However, this possibility has to be considered with caution, as we did not analyze isotopic values at the food web baseline. In addition, unlike the elephant seal, the harbor seal does not undertake long-distance migrations and long feeding trips, instead foraging in nearshore habits, which most likely explains the relatively higher δ^{13}C values in the former than the latter. *P.v. richardii* shows a high degree of site fidelity [90, 91], which is especially the case for females during the breeding season, when they forage no more than 30 km from their haul-out site [91–93]. All the evidence discussed here suggests that the Pacific harbor seal follows local foraging habits in Mexican waters, which contrasts with other pinnipeds in the region, which forage in open waters further offshore.
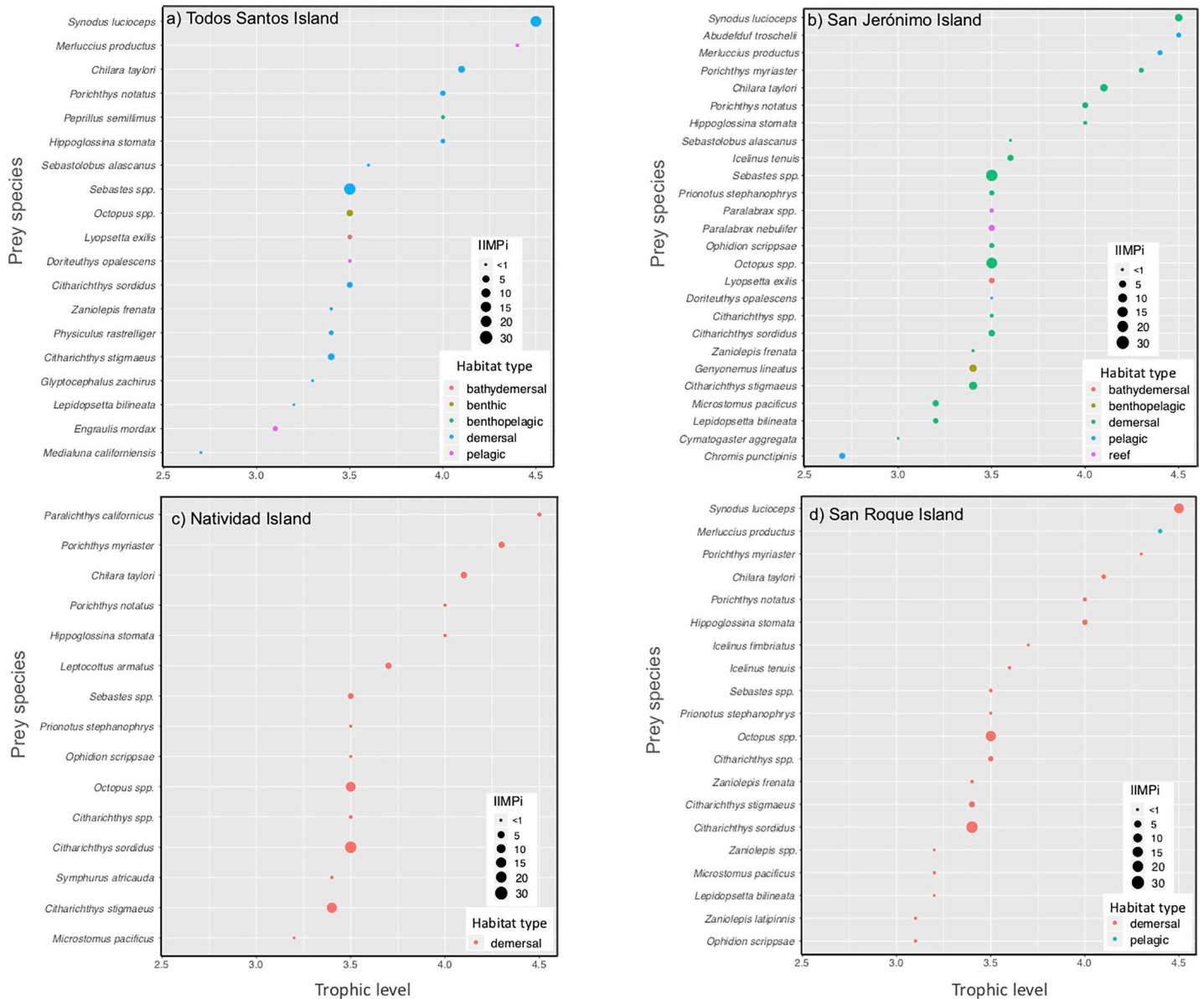
Fig 5. Harbor seal prey species identified by hard remain analysis of scat [22], with their corresponding estimated trophic level (x axis), Importance Index (IIMPi, which varies according to circle sizes), and predominant habitat type (colored circles).

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Table 6. Number of prey species shared between sites, with their corresponding overlap areas (‰²).

SITES	Number of shared prey species	Overlap areas (‰²)
ITS-ISJ	14	0.21
ITS-IN	6	0.05
ITS-ISR	11	0
ISJ-IN	10	0.53
ISJ-ISR	12	0.29
IN-ISR	11	0.12

https://doi.org/10.1371/journal.pone.0225889.t006

Table 7. Stable isotope values (mean ± SD) in fur taken from different pinniped species in the eastern Pacific Ocean and the Gulf of California, Mexico.

Common name	Species	Age	Sampling year	Locations	δ^{13}C	δ^{15}N	Reference
California sea lion	*Zalophus californianus*	Pups	2008	Gulf of California	-15.9±0.5	21.8±0.7	[39]
Galápagos sea lion	*Zalophus wollebaeki*			Galápagos Islands	-14.5±0.5	13.1±0.5	[39]
Pacific harbor seal	*Phoca vitulina richardii*	Pups (2mo)	2012	Natividad Island	-15.40±0.6	19.10±0.3	[72]
Galápagos sea lion	*Zalophus wollebaeki*	Pups (2mo)	2009	Galápagos Islands (avg.)	-16.2±0.3	12.94±0.4	[88]
				Cabo Douglas	-15.2±0.5	13.12±0.3	[88]
				Caamaño	-16.42±0.2	12.92±0.6	[88]
				Post Office	-16.59±0.3	12.79±0.4	[88]
				Malecon	-16.62±0.3	12.96±0.5	[88]
Pacific harbor seal	*Phoca vitulina richardii*	Pups (1–2 mo)	2013	Natividad Island	-15.4±0.6	19.1±0.3	[70]
Northern elephant seal	*Mirounga angustirostris*	Pups (1–2 mo)		San Benito Islands	-17.2±0.8	17.6±1.3	[70]
Guadalupe fur seal	*Arctocephalus philippii townsendi*	Pups (1mo)	July 2013	Guadalupe Island	-17.6± 0.3	18.4±0.4	[89]
				San Benito Islands	-17.1±0.4	18.8±0.4	[89]

https://doi.org/10.1371/journal.pone.0225889.t007

Relationship with diet

This study did not find a significant correlation between the trophic spectrum and isotopic niche width (SEA$_c$), which may be due to the fact that hard parts remaining in scat reflect the diet ingested over one or even several days prior to sampling, while the SEA$_c$ reflects the diet assimilated over the preceding months, during which period tissue synthesis occurred. The harbor seal seems to prefer three to five prey species within a wide potential trophic spectrum, with its prey's different habitats and trophic levels possibly increasing isotopic variability. For example, previous hard remain analysis [22] shows that the harbor seal prey species with the highest IIMPi for TSI were the Redfish (*Sebastes* spp.) and the California lizardfish (*Synodus lucioceps*), which adhere to demersal habits. Fur samples taken from the TSI colony yielded relatively high δ^{13}C values, which is consistent with feeding on prey that are at least partially dependent on benthic carbon sources [39, 70].

Limitations

The more precise interpretation of the isotopic values within or among animal tissue requires information yielded by three sources of isotopic variation: 1) the isotopic composition of potential prey; 2) verification as to how isotopic fractionation occurs between sources and animal tissue; and, 3) the feeding period as reflected by specific tissue [26].

1) The present study attempted to correlate SIA results obtained with prey identified from hard remains [22], although no significant correlation was found. Future studies would be more successful if they were able to characterize the isotopic baseline and the isotopic composition of potential prey. A thorough characterization of an isotopic baseline requires extensive sampling that adequately captures spatial and temporal sources of variation or nutrient sources, as well as primary producers, and the prey. Although the intensity of the sampling effort needed and the remoteness of our sampling sites made it prohibitive to adequately characterize the baseline, specific compounds have recently been analyzed to obtain the baseline isotopic values in food webs. For example, nitrogen and carbon compound-specific isotopic analyses (CSIA) of some aminoacids have been shown to reflect the isotopic baseline [94].

2) In phocid seals, very different isotope fractionation has been reported among tissue types, including blood serum, red cells, and fur, ranging from 0.8 to 3.1 ‰ [68]. Little is known about isotope fractionation in the mother-to-foetus nutritional transference that occurs during gestation. While in many pinniped species, a pup's isotopic composition has been

reported as higher than its mother's [39, 87, 95], this has not been reported either for this subspecies or in this tissue (fur) sampled by the present study. Measuring mother-to-pup fractionation requires the handling of adult females and their pups in the wild, which has proven to be difficult.

3) In this study, we assumed that the harbor seal pup fur has an isotopic composition that reflects the last few months of gestation. Furthermore, we assumed that pup fur begins to be isotopically enriched while being biosynthesized *in-utero*, which begins during the seventh month of gestation. Therefore, the pups' isotopic values will correspond to the maternal foraging habits during the last three months of gestation, rather than the diet ingested over the entire 11–12 month gestation period.

As our results solely explain the trophic ecology of those adult females that were mothers at the time of sampling, no information was obtained on other females, males, and other age categories (juveniles or adults). Energy requirements may limit foraging behavior in other pinniped species, mostly during the breeding season [96].

Conclusions

The significant differences in both δ^{15}N and δ^{13}C revealed by this research among Mexican Pacific harbor seal colonies, coupled with previously established coastal-oceanic gradients for δ^{13}C values and a regional latitudinal gradient for δ^{15}N values, suggest specific local biogeochemical conditions that lead to the differences in isotopic baseline observed in the pups. Based on δ^{13}C values that are relatively high, we can conclude that the feeding habitat of adult female harbor seals is mainly benthic-demersal and nearshore in nature, with slight differences occurring due to type of prey consumed in each colony. The relatively higher δ^{15}N values recorded at the southernmost island (SRI) and the midpoint of SJI imply a latitudinal ^{15}N-enrichment at the base of the food web, which was, apparently, mostly influenced by denitrification and microbial processes. Thus, the variability observed in the δ^{15}N values obtained in the present study may involve taxonomic similarity among prey throughout the colonies, over a time-scale isotopically reflected in the fur. An alternative explanation may be that there is an isotopic similarity among different prey, because of their demersal habitat, which causes an underestimation of the degree of resource partitioning among colonies. The low isotopic variance observed in our results indicated that the Pacific harbor seal is a specialist consumer.

Supporting information

S1 Protocol. Laboratory protocol. Laboratory protocol for δ^{15}N and δ^{13}N stable isotope analysis in harbor seal pup fur samples.
(PDF)

S1 Dataset. Stable carbon and nitrogen values. δ^{13}C and δ^{15}N values for each pup fur sample to analyze trophic ecology of Pacific harbor seal females at five sites west off the Baja California Peninsula, Mexico.
(XLSX)

Acknowledgments

Author Contributions

Conceptualization: Maricela Juárez-Rodríguez, Gisela Heckel, Fernando R. Elorriaga-Verplancken, Sharon Z. Herzka.

Data curation: Maricela Juárez-Rodríguez.

Formal analysis: Maricela Juárez-Rodríguez, Juan Carlos Herguera-García.

Funding acquisition: Gisela Heckel, Yolanda Schramm.

Investigation: Maricela Juárez-Rodríguez.

Methodology: Gisela Heckel, Juan Carlos Herguera-García, Sharon Z. Herzka.

Project administration: Gisela Heckel, Yolanda Schramm.

Resources: Juan Carlos Herguera-García, Fernando R. Elorriaga-Verplancken, Sharon Z. Herzka, Yolanda Schramm.

Software: Fernando R. Elorriaga-Verplancken.

Supervision: Gisela Heckel.

Validation: Juan Carlos Herguera-García.

Visualization: Maricela Juárez-Rodríguez.

Writing – original draft: Maricela Juárez-Rodríguez.

Writing – review & editing: Gisela Heckel, Juan Carlos Herguera-García, Fernando R. Elorriaga-Verplancken, Sharon Z. Herzka, Yolanda Schramm.

References

1. Garvey JE, Whiles RM. Trophic Ecology. Florida: CRC Press; 2016.

2. Herreman JK, Blundell GM, Ben-David M. Evidence of bottom-up control of diet driven by top-down processes in a declining harbor seal *Phoca vitulina richardsi* population. Mar Ecol Prog Ser. 2009; 374: 287–300. https://doi.org/10.3354/meps07719

3. Bossart GD. Marine Mammals as Sentinel Species for Oceans and Human Health. Oceanography. 2006; 19(2): 134–7. https://doi.org/10.5670/oceanog.2006.77

4. Heithaus MR, Frid A, Wirsing AJ, Worm B. Predicting ecological consequences of marine top predator declines. Trends Ecol Evol. 2008; 23(4): 202–10. https://doi.org/10.1016/j.tree.2008.01.003 PMID: 18308421

5. Páez-Rosas D, Aurioles-Gamboa D. Alimentary niche partitioning in the Galapagos sea lion, *Zalophus wollebaeki*. Mar Biol. 2010; 157(12): 2769–81.

6. Páez-Rosas D, Rodríguez-Pérez M, Riofrío-Lazo M. Competition influence in the segregation of the trophic niche of otariids: a case study using isotopic bayesian mixing models in Galapagos pinnipeds. Rapid Commun Mass Spectrom. 2014; 28(23): 2550–8. https://doi.org/10.1002/rcm.7047 PMID: 25366402

7. Committee on Taxonomy. List of marine mammal species and subspecies 2019 [Accessed 19 November 2019]. Available from: www.marinemammalscience.org.

8. Kelly JF. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool. 2000; 78(1): 1–27.

9. Lubinsky-Jinich D, Schram Y, Heckel G. The Pacific harbor seal's (*Phoca vitulina richardii*) breeding colonies in Mexico: abundance and distribution. Aquat Mamm. 2017; 43(1): 73–81. https://doi.org/10.1578/AM.43.1.2017.73

10. Harvey J. *Phoca vitulina* ssp. *richardii*. The IUCN Red List of Threatened Species 2016: e. T17022A66991556. 2016. https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T17022A66991556.en

11. Temte JL, Bigg MA, Wiig Ø. Clines revisited: The timing of pupping in the harbour seal (*Phoca vitulina*). J Zool. 1991; 224(4): 617–32. https://doi.org/10.1111/j.1469-7998.1991.tb03790.x

12. Fernandez-Martin EM, Heckel G, Schramm Y, García-Aguilar MC. The timing of pupping and molting of the Pacific harbor seal, *Phoca vitulina richardii*, at Punta Banda Estuary, Baja California, Mexico. Cienc Mar. 2016; 42(3): 195–208. https://doi.org/10.7773/cm.v42i3.2615

13. Ruiz-Mar G. Determinación del periodo de lactancia y cuidado materno en la foca de puerto *Phoca vitulina richardii* y su relación con el disturbio humano en el estero de Punta Banda,Baja California, México. M.Sc. Thesis. Ensenada Center for Scientific Research and Higher Education. Ensenada, Baja California, México. 2016. Available from: https://biblioteca.cicese.mx/catalogo/tesis/ficha.php?id=24593

14. Le Boeuf BJ, Crocker DE, Costa DP, Blackwell SB, Webb PM, Houser DS. Foraging ecology of northern elephant seals. Ecological Monographs 2000; 70: 353–82.

15. Bjørge A, Thompson D, Hammond P, Fedak M, Bryant E, Aarefjord H, et al. Habitat use and diving behaviour of harbour seals in a coastal archipelago in Norway. In: Blix AS, Walløe L, Ulltang Ø, editors. Developments in Marine Biology. 4: Elsevier Science; 1995. pp. 211–23.

16. Gibble CM, Harvey JT. Food habits of harbor seals (*Phoca vitulina richardii*) as an indicator of invasive species in San Francisco Bay, California. Mar Mammal Sci. 2015; 31(3): 1014–34. https://doi.org/10.1111/mms.12214

17. Luxa K, Acevedo-Gutiérrez A. Food habits of harbor seals (*Phoca vitulina*) in two estuaries in the central Salish Sea. Aquat Mamm. 2013; 39: 10–22. https://doi.org/10.1578/AM.39.1.2013.10

18. Pitcher KW. Stomach contents and feces as indicators of harbor seal (*Phoca vitulina*) foods in the Gulf of Alaska. Fish B-NOAA. 1980; 78: 797–8.

19. Tollit DJ, Steward MJ, Thompson PM, Pierce GJ, Santos MB, Hughes S. Species and size differences in the digestion of otoliths and beaks: implications for estimates of pinniped diet composition. Can J Fish Aquat Sci. 1997; 54(1): 105–19. https://doi.org/10.1139/f96-264

20. Bowen WD. Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Can J Fish Aquat Sci. 2000; 57: 898–905. https://doi.org/10.1139/f00-032

21. Alamán de Régules R. Hábitos alimentarios de la foca de puerto, *Phoca vitulina richardsi*, en la Bahía Todos Santos, Baja California, México. M.Sc. Thesis. Autonomous Universityof Baja California. Ensenada, Baja California, México. 2014.

22. Durazo-Rodríguez P. Variabilidad espacial y temporal de los hábitos alimentarios de la foca de puerto (*Phoca vitulina richardii*) en México. M.Sc. Thesis. Autonomous Universityof Baja California. Ensenada, Baja California, México. 2015.

23. Elorriaga-Verplancken FR, Morales-Luna L, Moreno-Sánchez XG, Mendoza-Salas I. 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. Aquat Mamm. 2013; 39(4): 415–21. https://doi.org/10.1578/AM.39.4.2013.415

24. Brassea-Pérez E, Schramm Y, Heckel G, Chong-Robles J, Lago-Lestón A. Metabarcoding analysis of the Pacific harbor seal diet in Mexico. Mar Biol. 2019; 166(8): 106.

25. Michener RH, Kaufman L. Stable Isotope Ratios as Tracers in Marine Food Webs: An Update. In: Lajtha H, Michener RH, editors. Stable Isotopes in Ecology and Environmental Science. Boston: Blackwell; 2008. pp. 238–82.

26. Newsome SD, Clementz MT, Koch PL. Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mammal Sci. 2010; 26(3): 509–72. https://doi.org/10.1111/j.1748-7692.2009.00354.x

27. Hobson KA, Clark RG. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor. 1992; 94: 181–8. https://doi.org/10.2307/1368807

28. Hobson KA, Clark RG. Assessing avian diets using stable isotopes. II. Factors influencing diet-tissue fractionation. Condor. 1992; 94: 189–97. https://doi.org/10.2307/1368808

29. Bearhop S, Waldron S, Votier SC, Furness RW. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol Biochem Zool. 2002; 75(5): 451–8. https://doi.org/10.1086/342800 PMID: 12529846

30. Aurioles-Gamboa D, Koch PL, Le Boeuf BJ. Differences in foraging location of Mexican and California elephant seals: evidence from stable isotopes in pups. Mar Mammal Sci. 2006; 22(2): 326–38. https://doi.org/10.1111/j.1748-7692.2006.00023.x

31. Hobson KA, Sease JL, Merrick RL, Piatt JF. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. Mar Mammal Sci. 1997; 13(1): 114–32. https://doi.org/10.1111/j.1748-7692.1997.tb00615.x

32. Kurle CM. 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. Can J Zool. 2002; 80 (5): 902–9. https://doi.org/10.1139/z02-069

33. Porras-Peters H, Aurioles-Gamboa D, Cruz-Escalona VH, Koch PL. Trophic level and overlap of sea lions (*Zalophus californianus*) in the Gulf of California, Mexico. Mar Mammal Sci. 2008; 24(3): 554–76. https://doi.org/10.1111/j.1748-7692.2008.00197.x

34. France RL. Carbon-13 enrichment in benthic compared to planktonic algae: Foodweb implications. Mar Ecol Prog Ser. 1995; 124: 307–12. https://doi.org/10.3354/meps124307

35. Graham BS, Koch PL, Newsome SD, McMahon KW, Aurioles D. Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In: West JB, Bowen GJ, Dawson TE, Tu KP, editors. Isoscapes. USA: Springer; 2010. pp. 299–318.

36. Minagawa M, Wada E. Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. Geochimica et Cosmochimica Acta. 1984; 48(5): 1135–40. https://doi.org/10.1016/0016-7037(84)90204-7

37. Hobson KA, Welch HE. Determination of trophic relationships within a high Arctic marine food web using δ^{13}C and δ^{15}N analysis. Mar Ecol Prog Ser. 1992; 84(1): 9–18. https://doi.org/10.3354/meps084009

38. Vanderklift A, Ponsard S. Sources of variation in consumer-diet δ^{15}N enrichments: a meta-analysis. Oecologia. 2003; 136: 169–82. https://doi.org/10.1007/s00442-003-1270-z PMID: 12802678

39. Aurioles-Gamboa D, Newsome SD, Salazar-Pico S, Koch PL. Stable isotope differences between sea lions (*Zalophus*) from the Gulf of California and Galapagos Islands. J Mammal. 2009; 90(6): 1410–20. https://doi.org/10.1644/08-MAMM-A-209R2.1

40. Gannes LZ, Martínez del Rio C, Koch P. Natural Abundance Variations in Stable Isotopes and their Potential Uses in Animal Physiological Ecology. Comp Biochem Physiol A Mol Integr Physiol. 1998; 119 (3): 725–37. https://doi.org/10.1016/s1095-6433(98)01016-2 PMID: 9683412

41. Altabet MA, Pilskaln C, Thunell R, Pride C, Sigman D, Chavez F, et al. The nitrogen isotope biogeochemistry of sinking particles from the margin of the Eastern North Pacific. Deep Sea Res Pt I. 1999; 46 (4): 655–79. https://doi.org/10.1016/S0967-0637(98)00084-3

42. Burton RK, Koch PL. Isotopic tracking of foraging and long-distance migration in northeastern Pacific pinnipeds. Oecologia. 1999; 119: 578–85. https://doi.org/10.1007/s004420050822 PMID: 28307717

43. Takai N, Onaka S, Ikeda Y, Yatsu A, Kidokoro H, Sakamoto W. Geographical variations of carbon and nitrogen stable isotopes ratios in squid. J Mar Biol Assoc UK. 2000; 80: 675–84.

44. McMahon KW, Hamady LL, Thorrold SR. A review of ecogeochemistry approaches to estimating movements of marine animals. Limnol Oceanogr. 2013; 58(2): 697–714. https://doi.org/10.4319/lo.2013.58.2.0697

45. Wada E, Hattori A. Nitrogen in the sea: forms, abundance, and rate processes. USA: CRC press; 1990.

46. Goericke R, Fry B. Variations of marine plankton δ^{13}C with latitude, temperature, and dissolved CO_2 in the world ocean. Global Biogeochemical Cycles. 1994; 8(1): 85–90. https://doi.org/10.1029/93GB03272

47. Smith RJ, Hobson KA, Koopman HN, Lavigne DM. Distinguishing between populations of fresh and saltwater harbour seals (*Phoca vitulina*) using stable isotope ratios and fatty acid profiles. Can J Fish Aquat Sci. 1996; 53(2): 272–9. https://doi.org/10.1139/f95-192

48. Bearhop S, Adams CE, Waldron S, Fuller RA, MacLeod H. Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol. 2004; 73(5): 1007–12. https://doi.org/10.1111/j.0021-8790.2004.00861.x

49. Newsome SD, Tinker MT, DH M, Oftedal OT, Ralls K, Staedler MM, et al. Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology. 2009; 90(4): 961–74. https://doi.org/10.1890/07-1812.1 PMID: 19449691

50. Hutchinson GE. An Introduction to Population Biology. USA: Yale University Press; 1978.

51. Cherel Y, Hobson KA, Guinet C, Vanpe C. Stable isotopes document seasonal changes in trophic niches and winter foraging individual specialization in diving predators from the Southern Ocean. J Anim Ecol. 2007; 76(4): 826–36. https://doi.org/10.1111/j.1365-2656.2007.01238.x PMID: 17584388

52. Das K, Beans C, Holsbeek L, Mauger G, Berrow SD, Rogan E, et al. Marine mammals from northeast Atlantic: relationship between their trophic status as determined by δ13C and δ15N measurements and their trace metal concentrations. Marine Environmental Research. 2003; 56(3): 349–65. https://doi.org/10.1016/S0141-1136(02)00308-2 PMID: 12738219

53. Layman CA, Arrington DA, Montaña CG, Post DM. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology. 2007; 88(1): 42–8. https://doi.org/10.1890/0012-9658 (2007)88[42:csirpf]2.0.co;2 PMID: 17489452

54. Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL. A niche for isotopic ecology. Front Ecol Environ. 2007; 5(8): 429–36. https://doi.org/10.1890/060150.1

55. Odell DK. Studies on the biology of the California sea lion and the northern elephant seal on San Nicolas Island. Los Angeles, USA: University of California; 1972.

56. Cottrell PE, Jeffries S, Beck B, Ross PS. Growth and development in free-ranging harbor seal (*Phoca vitulina*) pups from southern British Columbia, Canada. Mar Mammal Sci. 2002; 18(3): 721–33. https://doi.org/10.1111/j.1748-7692.2002.tb01069.x

57. Oftedal OT, Bowen WD, Widdowson EM, Boness DJ. The prenatal molt and its ecological significance in hooded and harbor seals. Can J Zool. 1991; 69(9): 2489–93. https://doi.org/10.1139/z91-351

58. Stutz SS. Foetal and Postpartum Whitecoat Pelage in *Phoca vitulina*. J Fish Res Board Can. 1966; 23 (4): 607–9. https://doi.org/10.1139/f66-051

59. Ling JK, Thomas CDB. The skin and hair of the southern elephant seal, *Mirounga leonina* (Linn.). II. Pre-natal and early post-natal development and moulting. Aust J Zool. 1967; 15(349–365). https://doi.org/10.1071/ZO9670349

60. Rau GH, Sweeney RE, Kaplan IR. Plankton 13C: 12C ratio changes with latitude: differences between northern and southern oceans. Deep Sea Research Part A Oceanographic Research Papers. 1982; 29 (8): 1035–9. https://doi.org/10.1016/0198-0149(82)90026-7.

61. Jackson AL, Inger R, Parnell AC, Bearhop S. Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. J Anim Ecol. 2011; 80(3): 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x PMID: 21401589

62. Jackson AL. Introduction to SIBER 2019 [Accessed 22 April 2019]. Available from: https://cran.r-project.org/web/packages/SIBER/vignettes/Introduction-to-SIBER.html.

63. Phillips DL. Converting isotope values to diet composition: the use of mixing models. J Mammal. 2012; 93(2): 342–52. https://doi.org/10.1644/11-MAMM-S-158.1

64. Froese R, Pauly D. FishBase. World Wide Web electronic publication. www.fishbase.org, version (02/2019). 2019.

65. Hobson KA. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia. 1999; 120 314–26. https://doi.org/10.1007/s004420050865 PMID: 28308009

66. Nakamura K, Schoeller DA, Winkler FJ, Schmidt HL. Geographical variations in the carbon isotope composition of the diet and hair in contemporary man. Biol Mass Spectrom. 1982; 9(9): 390–4. https://doi.org/10.1002/bms.1200090906 PMID: 7139058

67. Hobson KA, Schell DM, Renouf D, Noseworthy E. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can J Fish Aquat Sci. 1996; 53(3): 528–33.

68. Lesage V, Hammill MO, Kovacs KM. Diet- tissue fractionation of stable carbon and nitrogen isotopes in phocids seals. Mar Mammal Sci. 2002; 18(1): 182–93. https://doi.org/10.1111/j.1748-7692.2002.tb01027.x

69. Yang D-Y, Chen Y-W, Gunn JM, Belzile N. Selenium and mercury in organisms: interactions and mechanisms. Environmental Reviews. 2008; 16(NA): 71–92.

70. Elorriaga-Verplancken F, Morales-Luna L, Heckel G, Schramm Y. Foraging ecology of harbour seals (*Phoca vitulina*) and Northern elephant seals (*Mirounga angustirostris*) from Baja California, Mexico: inferences from stable isotopes in pups. J Mar Biol Assoc UK. 2016; 96(4): 903–8. https://doi.org/10.1017/S0025315415002143

71. Pablo-Rodríguez N, Aurioles-Gamboa D, Montero-Muñoz JL. Niche overlap and habitat use at distinct temporal scales among the California sea lions (*Zalophus californianus*) and Guadalupe fur seals (*Arctocephalus philippii townsendi*). Mar Mammal Sci. 2015; 32(2): 466–89. https://doi.org/10.1111/mms.12274

72. Elorriaga-Verplancken FR, Morales-Luna L, Moreno-Sánchez XG, Mendoza-Salas I. 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. Aquat Mamm. 2013; 39(4): 415–21. https://doi.org/10.1578/AM.39.4.2013.415

73. Cavole LM, Demko AM, Diner RE, Giddings A, Koester I, Pagniello CM, et al. Biological impacts of the 2013–2015 warm-water anomaly in the Northeast Pacific: Winners, losers, and the future. Oceanography. 2016; 29(2): 273–85.

74. Martínez del Rio C, Sabat P, Anderson-Sprecher R, Gonzalez SP. Dietary and isotopic specialization: the isotopic niche of three Cinclodes ovenbirds. Oecologia. 2009; 161(1): 149–59. https://doi.org/10.1007/s00442-009-1357-2 PMID: 19424728

75. Thomson JA, Heithaus MR, Burkholder DA, Vaudo JJ, Wirsing AJ, Dill LM. Site specialists, diet general-ists? Isotopic variation, site fidelity, and foraging by loggerhead turtles in Shark Bay, Western Australia. 2012; 453: 213–26. https://doi.org/10.3354/meps09637 pub.1071170128.

76. Beltrán-Félix JL, Hammann MG, Chagoya-Guzmán A, Alvarez-Borrego S. Ichthyofauna of Estero de Punta Banda, Ensenada, Baja California, Mexico, before a major dredging operation. Cienc Mar. 1986; 12(1): 79–92. https://doi.org/10.7773/cm.v12i1.483

77. McPeek MA. Trade-offs, food web structure, and the coexistence of habitat specialists and generalists. The American Naturalist. 1996; 148: S124–S38.

78. Allen LG, Pondella DJ, Horn MH. The ecology of marine fishes: California and adjacent waters. 1st ed ed. USA: University of California Press; 2006.

79. Bolnick DI, Yang LH, Fordyce JA, Davis JM, Svanbäck R. Measuring individual-level resource speciali-zation. Ecology. 2002; 83(10): 2936–41.

80. Laidre KL, Stirling I, Lowry LF, Wiig Ø, Heide-Jørgensen MP, Ferguson SH. Quantifying the sensitivity of Arctic marine mammals to climate-induced habitat change. Ecological Applications. 2008; 18(sp2): S97–S125.

81. Fry B, Wainright SC. Diatom sources of 13C-rich carbon in marine food webs. Mar Ecol Prog Ser. 1991; 76: 149–57.

82. Payne PM, Selzer LA. The distribution, abundance and selected prey of the harbor seal, Phoca vitulina concolor, in southern New England. Mar Mammal Sci. 1989; 5(2): 173–92 https://doi.org/10.1111/j.1748-7692.1989.tb00331.x

83. Sigman DM, Granger J, DiFiore PJ, Lehmann MM, Ho R, Cane G, et al. Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin. Global Biogeochemical Cycles. 2005; 19(4).

84. McMahon KW, Hamady LL, Thorrold SR. A review of ecogeochemistry approaches to estimating move-ments of marine animals. Limnol Oceanogr. 2013; 58(2): 697–714.

85. Hirons AC, Schell DM, Finney BP. Temporal records of δ13C and δ15N in North Pacific pinnipeds: infer-ences regarding environmental change and diet. Oecologia. 2001; 129: 591–601. https://doi.org/10.1007/s004420100756 PMID: 24577700

86. Newsome SD, Koch PL, Etnier MA, Aurioles-Gamboa D. Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. Mar Mammal Sci. 2006; 22(3): 556–72. https://doi.org/10.1111/j.1748-7692.2006.00043.x

87. Elorriaga-Verplancken F, Aurioles-Gamboa D, Newsome SD, Martínez-Díaz S. δ15N and δ13C values in dental collagen as a proxy for age-and sex-related variation in foraging strategies of California sea lions. Mar Biol. 2013; 160(3): 641–52. https://doi.org/10.1007/s00227-012-2119-y

88. Páez-Rosas D, Aurioles-Gamboa D. Spatial variation in the foraging behaviour of the Galapagos sea lions (Zalophus wollebaeki) assessed using scat collections and stable isotope analysis. J Mar Biol Assoc UK. 2014; 94(6): 1099–107. https://doi.org/10.1017/S002531541300163X

89. Amador-Capitanachi M, Moreno-Sánchez X, Juárez-Ruiz A, Ferretto G, Elorriaga-Verplancken F. Tro-phic Variation Between the Two Existing Guadalupe Fur Seal Colonies on Guadalupe Island and the San Benito Archipelago, Mexico. Aquat Mamm. 2017; 43(1): 14–25. https://doi.org/10.1578/AM.43.1.2017.14

90. Härkönen T. Influence of Feeding on Haul-Out Patterns and Sizes of Sub-Populations in Harbor Seals. Netherlands Journal of Sea Research 1987; 21: 331–9.

91. Härkönen T, Harding KC. Spatial structure of harbour seal populations and the implications thereof. Can J Zool. 2001; 79(12): 2115–27. https://doi.org/10.1139/z01-172

92. Tollit DJ, Black AD, Thompson PM, Mackay A, Corpe HM, Wilson B, et al. Variations in harbour seal Phoca vitulina diet and dive-depths in relation to foraging habitat. J Zool. 1998; 244(02): 209–22. https://doi.org/10.1111/j.1469-7998.1998.tb00026.x

93. Womble JN, Gende SM. Post-breeding season migrations of a top predator, the harbor seal (Phoca vitulina richardii), from a marine protected area in Alaska. PLoS One. 2013; 8(2): 1–15. https://doi.org/10.1371/journal.pone.0055386 PMID: 23457468

94. McMahon KW, McCarthy MD. Embracing variability in amino acid δ15N fractionation: mechanisms, implications, and applications for trophic ecology. Ecosphere. 2016; 7(12).

95. Elorriaga-Verplancken FR, Hadrys LM-L, Moreno-Sánchez X, Mendoza-Salas I. 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. Aquatic Mammals. 2013; 39(4). https://doi.org/10.1578/AM.39.4.2013

96. Boyd I. Time and energy constraints in pinniped lactation. The American Naturalist. 1998; 152(5): 717–28. https://doi.org/10.1086/286202 PMID: 18811346