

# Stable isotope mixing models elucidate sex and size effects on the diet of a generalist marine predator

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**ABSTRACT:** We applied a 2-step clustering algorithm and Bayesian stable isotope mixing model to examine intraspecific differences in the contribution of prey sources to the diet and foraging habitat of harbor seals *Phoca vitulina* in the Salish Sea, USA. We analyzed stable isotopes of carbon and nitrogen collected from 32 seals and 248 prey samples representing 18 of 25 of the most common seal prey items identified in seal scat. Stable isotope analyses identified significant harbor seal sex- and size-based differences in diet and foraging habitat use. In comparison to males, female harbor seals had a higher contribution of prey items that were more <sup>13</sup>C-enriched. This result may indicate that females derived more of their  $\delta^{13}\text{C}$  value from nearshore versus offshore food webs, an explanation supported by movement data on this population. However, large seals of both sexes displayed a greater offshore signal in their diet, indicating that seal mass effects on foraging habitat use were somewhat independent of sex. Our work contributes to understanding trophic linkages between these generalist consumers and their prey. The foraging differences that we detected between male and female harbor seals present complex challenges for fisheries management and for the design of marine reserves. Many marine reserves in the Pacific Northwest are located in close proximity to seal haul-out sites. By lowering the energetic costs of foraging of females, these reserves may ultimately have the unintended effect of increasing individual fitness, population growth rate, and influencing future predator-induced mortality on endangered species.

**KEY WORDS:** Stable isotopes · Bayesian mixing model · Harbor seal · *Phoca vitulina* · Pinniped · Salish Sea

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## INTRODUCTION

Intraspecific competition for habitat and food resources has been demonstrated in a variety of species, including sea snakes (Shine et al. 2003), crabs (Hines et al. 1987), cervids (Clutton-Brock et al. 1982), and birds (Parrish & Sherry 1994). Factors influencing

intraspecific habitat segregation are driven by the abundance and distribution of habitats and food resources and by age, sex, size, and behavioral differences within populations or species. In pinnipeds and other marine mammals, differing energy requirements and physiological limits may create spatial and temporal structuring of habitat/resource use by size,

gender, and reproductive status (Field et al. 2005, Wolf et al. 2005, Breed et al. 2006). These differences may in turn influence the spatial distribution and duration of foraging trips, as well as the types and quantity of prey consumed (Jeglinski et al. 2012, Leung et al. 2012, Hassrick et al. 2013). Because pinniped diet often includes forage fish or other commercially valuable fish species, a better understanding of diet and habitat use can be informative in managing pinniped–fisheries interactions (Spitz et al. 2010). For example, the effectiveness of marine reserves or harvest restrictions (e.g. time or area closures) to protect or rebuild depleted species or species of conservation concern can potentially be compromised by pinnipeds, depending on pinniped space use and their diet relative to the restricted area and season (Lance et al. 2012, Ward et al. 2012). After being depleted by hunting and other removals for more than a century, pinniped species in North America were protected in the 1970s and have largely recovered to historic levels (e.g. Jeffries et al. 2003). As their numbers have increased, so too has the recognition of their potential impact on fisheries and their role as upper-level marine predators in the nearshore environment (Lance et al. 2012, Peterson et al. 2012, Ward et al. 2012).

Despite the availability of a variety of tools and approaches to investigate the trophic linkages and dynamics of top predators such as cetaceans and pinnipeds, obtaining reliable data on diet or consumption from top predators remains challenging (Tucker et al. 2013). Most dietary reconstructions are based on indirect methods, each with associated caveats and limitations. In the early 1900s, marine mammal diets were assessed by harvesting individuals and sampling their stomachs (Scheffer & Sperry 1931); however, because of their protected species status, stomach collection now is limited to dead or stranded individuals. A commonly used alternative is the collection of scat samples from haul-out sites, but these methods may be biased toward prey species with identifiable digestion-resistant parts (Gales & Cheal 1992, Orr et al. 2004). Stomach samples and scat samples both integrate diet information over relatively short time scales, being limited to the recovery of the most recently consumed items (Phillips & Harvey 2009). Two approaches that integrate information over longer time scales are fatty acid (FA) and stable isotope (SI) signature analyses. The 2 methods can be used to evaluate support for coarse, large-scale hypotheses, such as evaluating support for migration between disparate habitats (Marra et al. 1998). More recently, both analyses have been used to estimate

the relative contribution of different prey items to a predator's tissues with the assumption that this reflects the predator's diet. The analysis of both FA and SI data requires the inclusion of correction coefficients or trophic discrimination factors to account for species-specific metabolic processes, which are rarely known *a priori* and are only available from laboratory studies. Because more SI than FA correction factors have been published, and their dimensionality is typically smaller (2–3 SIs versus 20–30 FAs), SI mixing models currently provide a coarser but more tractable option for diet estimation than FA models.

The isotopes most widely used to estimate the diet of predators are isotopes of carbon ( $^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}$ ). Gradients in ratios of carbon isotopes have been used to interpret sources of primary production to a consumer diet (Phillips 2012), and those of nitrogen have been used to estimate trophic positions in food webs (Post 2002). In the nearshore environment, both carbon and nitrogen have been used to differentiate resource use originating from the terrestrial versus the marine environment (Burton & Koch 1999). In the Northeast (NE) Pacific food web, for example, pinnipeds such as harbor seals *Phoca vitulina*, California sea lions *Zalophus californianus*, northern fur seals *Callorhinus ursinus*, and northern elephant seals *Mirounga angustirostris* may have depleted levels of  $^{15}\text{N}$  if they forage at a lower trophic level and depleted levels of  $^{13}\text{C}$  relative to individuals foraging offshore (Burton & Koch 1999). Tucker et al. (2013) suggested that the trends in  $^{13}\text{C}$  may be driven by differences in rates and magnitudes of phytoplankton production as well as the  $\delta^{13}\text{C}$  value of inorganic carbon available for photosynthesis. While these previous studies have described isotopic gradients between coastal and open water food webs as well as latitudinal variations, they have not directly incorporated these gradients into quantitative estimates of diet or consumption.

In this analysis, we apply a novel source (prey) grouping technique with a Bayesian mixing model (Moore & Semmens 2008) that incorporates individual covariates to SI data collected from a wide-ranging generalist species in the NE Pacific Ocean, viz. the harbor seal. Our study includes animals from haul-out sites in the Salish Sea, specifically the San Juan Islands and the southern Gulf Islands (Washington State, USA, and British Columbia, Canada). Given their rapid population increase in the latter half of the 20<sup>th</sup> century, the diet of these predators is of interest to fisheries managers because harbor seals are thought to consume non-negligible amounts of threatened and endangered salmon (*Oncorhynchus* spp.)

and rockfish (*Sebastes* spp.). Thus, better estimates of pinniped diet and understanding of resource partitioning between pinniped sex/age classes has implications for both conservation and fisheries management (see Könignson et al. 2013).

## MATERIALS AND METHODS

### SI analysis

Bromaghin et al. (2013) described the capture and collection of tissue and blubber samples from harbor seals between April 2007 and March 2008 from 4 sites in the San Juan Islands (Fig. 1) and putative prey species (June to December 2008). Samples of whole blood drawn from seals were centrifuged and frozen in liquid nitrogen at  $-80^{\circ}\text{C}$  until analysis. Prey types (whole homogenates) were freeze-dried. Seal and prey samples were sent to the Stable Isotope Core Laboratory of Washington State University (Pullman, WA) for SI analysis. There, consumer and putative prey samples were combusted to  $\text{N}_2$  and  $\text{CO}_2$  using a Costech Analytical ECS 4010 elemental analyzer; the gases were separated by a 3 m gas chromatography (GC) column and analyzed with a Thermo Finnigan Delta PlusXP continuous flow isotope ratio mass spectrometer. Isotope composition (parts per thousand and expressed in ‰ or  $\delta$  notation), represent the proportional deviation in the isotope ratio in the sample relative to a standard (Peterson & Fry 1987, Hobson et al. 1997). For C ( $\delta^{13}\text{C}$ ), the standard is Vienna PeeDee Belemnite and for N ( $\delta^{15}\text{N}$ ), the standard is relative to air. Samples were normalized using 2 internal running standards (acetanilide and keratin). Running standards were previously calibrated to NBS 19, RM8542, and IAEA-CO-9 for carbon and USGS 32, USGS 25, and USGS 26 for nitrogen. Blind reference materials (B2155 casein, Elemental Microanalysis) were interspersed with samples as a check of the normalization.

For each seal sampled, we also collected information on gender, size, and reproductive condition (pregnant or not). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were calculated for 32 individuals (14 females, 18 males). SI values

were analyzed for 248 samples of the 18 most common prey species identified by Lance et al. (2012) in their investigation of the seasonal and spatial variability in harbor seal diets. Lance et al. (2012) identified the most common seal prey as Pacific herring *Clupea pallasii*, Pacific sand lance *Ammodytes hexapterus*, and adult salmon *Oncorhynchus* spp. Our data included isotopic values for juveniles/mid-sized individuals of several species, generating a total of 25 unique prey items (Table 1). Because lipid-containing tissues are  $\delta^{13}\text{C}$  depleted relative to proteins and carbohydrates, variation in lipid content is a potential source of bias in SI analyses (Post et al. 2007). Lipids were not extracted from prey samples (or seals) prior to SI analysis. Instead, we employed a correction factor that uses the carbon:nitrogen ratio of the sampled material (Post et al. 2007). Specifically, the  $\delta^{13}\text{C}$  values obtained were corrected by  $\delta^{13}\text{C} = -3.32 + 0.99 \times \text{C:N}$  (Post et al. 2007).

### Statistical analysis

To estimate the relative contribution of different prey items, given the identified prey groupings and covariates, our statistical analysis was divided into 3 parts: (1) determining ecologically important prey groups, (2) identifying significant covariates, and

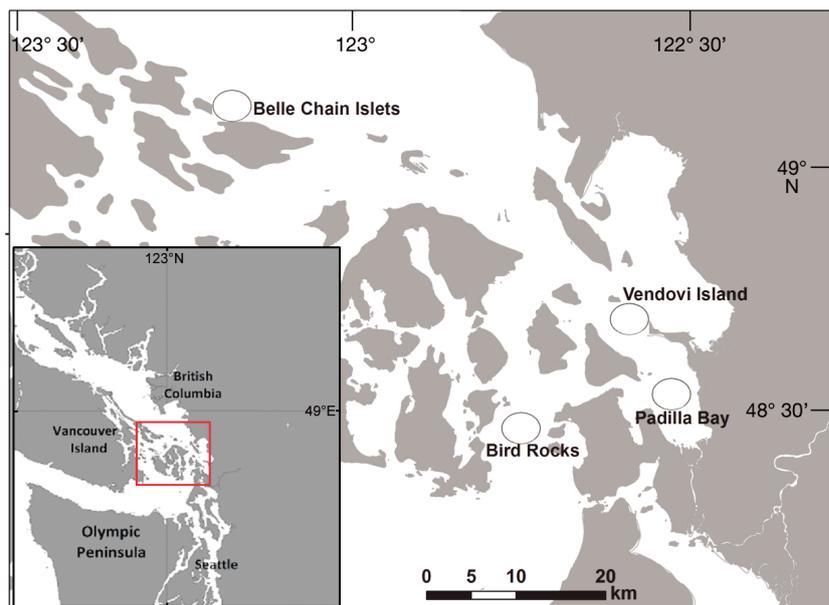


Fig. 1. Puget Sound and Straits of Juan de Fuca, Washington, USA. Harbor seals *Phoca vitulina* were sampled at 4 haul-out sites. Seal prey items from Padilla Bay and Bird Rocks were collected from seafood processors and sites within this area. For details see Lance et al. (2012), Bromaghin et al. (2013)

Table 1. Means, SD, and coefficient of variation (SD) of carbon and nitrogen isotopic values for harbor seal *Phoca vitulina* prey species groups. Twenty-five unique prey species–age class groups were combined to family level for major components. Minor contributors (kelp greenling, starry flounder, and spiny dogfish) were combined into an ‘other’ category (see Lance et al. 2012). In total, 10 groups were entered into the clustering (partitioning around medoids [PAM]) algorithm. PAM group indicates initial group assessment from clustering algorithm. Ad.: adult; juv.: juvenile

Prey group (no. of samples)	Composition	$\delta^{13}\text{C}$ Mean (SD)	%C Mean	$\delta^{15}\text{N}$ Mean (SD)	%N Mean	PAM group
Rockfish (36)	Black <i>Sebastes melanops</i> ; copper <i>S. caurinus</i> ; Puget Sound <i>S. emphaeus</i> ; yellowtail <i>S. flavidus</i> rockfish	–17.15 (1.23)	44.68	13.84 (0.46)	11.15	1
Juv. salmon (56)	<i>Oncorhynchus</i> spp.	–19.37 (0.87)	46.68	12.61 (0.86)	13.04	2
Northern anchovy (11)	<i>Engraulis mordax</i>	–20.81 (0.43)	53.55	11.19 (0.38)	8.07	2
Other (22)	Dogfish <i>Squalus acanthias</i> ; kelp greenling <i>Hexagrammos decagrammus</i> ; starry flounder <i>Platichthys stellatus</i>	–14.88 (2.95)	45.38	13.23 (0.89)	10.83	1
Pacific herring ad. and juv. (24)	<i>Clupea pallasii</i>	–20.59 (0.66)	50.88	11.86 (0.68)	10.24	2
Ad. salmon (50)	Chinook <i>O. tshawytscha</i> ; chum <i>O. keta</i> ; coho <i>O. kitsuch</i> ; pink <i>O. gorbuscha</i> ; sockeye <i>O. nerka</i>	–21.14 (2.04)	55.54	12.80 (1.89)	11.20	2
Sand lance (12)	<i>Ammodytes hexapterus</i>	–20.33 (0.49)	46.29	11.53 (0.38)	10.75	2
Shiner surfperch (12)	<i>Cymatogaster aggregata</i>	–16.08 (1.69)	49.22	13.27 (0.48)	8.95	1
Staghorn sculpin (12)	<i>Leptocottus armatus</i>	–12.78 (1.87)	44.08	13.33 (0.68)	12.36	1
Walleye pollock juv. (13)	<i>Theragra chalcogramma</i>	–18.26 (0.43)	43.23	12.04 (0.67)	11.93	2

(3) estimating parameters using a Bayesian mixing model with fixed and continuous predictors.

### Constructing prey groups

One of the biggest challenges in using SI mixing models is that if the number of prey (source) items is large, the relative contributions of prey that overlap in isotopic space cannot be estimated precisely. Several approaches for grouping prey have been proposed, including grouping by ecologically similar species (Phillips et al. 2005) or grouping based entirely on isotopic values (Ward et al. 2011). We used a hybrid approach for grouping prey items in the harbor seal diet, using cluster analysis to identify distinct groups based on dissimilarity measures in SI values and taxonomy. Cluster analysis provides an analytical basis on which to partition and evaluate potential groupings and patterns in the data (Fraleigh & Raftery 1998). We grouped the 25 prey items into 10 prey groups *a priori*, based on taxonomy (family level) and age class (Table 1). Beginning our analysis at the family level is similar to the approach used by Lance et al. (2012). We separated salmonid prey into 2 categories (adults and juveniles) because juvenile salmonid feeding ecology and diet are distinct from adult conspecifics (Quinn 2011). Walleye pollock *Theragra*

*chalcogramma* was the only gadid in our analysis and all specimens were juvenile. All rockfishes were placed in a ‘rockfish’ category, and very minor diet constituents (spiny dogfish, starry flounder, kelp greenling) into an ‘other’ category (Table 1).

Our dataset contained multiple samples of isotopic values for each prey type. While clustering on some measure of central tendency (e.g. mean or median) is an option, such an approach would ignore the within-group variability and process error in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, losing an important dimension (variability) in the data set. To incorporate the uncertainty surrounding these SI estimates, we employed the 2-step clustering approach of Cope & Punt (2009). An advantage of the 2-step approach is that the best data-supported number of clusters may be different in each of the 100 iterations, which tends to minimize overfitting when the final cluster assignment is made. In this approach, we first resampled the original estimates of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  assuming a normal distribution and employed the mean and standard deviation calculated from the simulated replicates. One hundred randomly drawn datasets of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of each prey group were then obtained, and clustering analysis was performed on each data set separately.

A partitioning analysis (*k*-medoids) was used to cluster prey items. This approach was chosen over hierarchical clustering methods because our goal

was to identify specific clusters rather than investigating their hierarchy or relationship to each other. A medoid represents the object in a cluster whose average dissimilarity to all the objects in the cluster is minimal.  $k$ -medoid is a common partitioning technique that clusters the data set of  $n$  objects into  $k$  clusters around the medoids, where  $k$  is specified *a priori*. This method minimizes the dissimilarity (rather than Euclidean distance) within clusters (Kaufman & Rousseeuw 1990). Because it is based on the most centrally located object in a cluster, it is also less sensitive to outliers in comparison with other clustering algorithms such as  $k$ -means clustering (Park & Jun 2009).

For the  $n = 10$  prey groups, we used cluster-validity diagnostics to evaluate the  $k$ -medoids method over all possible  $k$  clusters (for  $k = 2$  to  $n - 1$ ) to find the number of clustered prey groups best supported by that particular dataset. Once the best supported clusters were found, the cluster assignment of each prey group was retained for each dataset, yielding a matrix of 100 assignment values for each prey group. Clustering was then applied to these final sets of nominal cluster assignments of the prey to produce the final estimate of prey group clusters.

All analyses were conducted in R.2.15.1 (R Development Core Team 2012). We conducted the  $k$ -medoids analyses using the `pam()` function in R (partitioning around medoids, PAM). We assessed cluster validity using 2 measures: average silhouette coefficient (`sil`; Kaufman & Rousseeuw 1990) and Hubert's  $\Gamma$  (`Hg`; Halkidi et al. 2001). Although both measures consistently perform well, they offer contrasts in their tendencies to lump (`Hg`) or split (`sil`) (Cope & Punt 2009). We based our cluster assignments on the `Hg` statistic. The nominal clustering of assignments used the `daisy()` function to calculate pairwise dissimilarities. Silhouette plots were created to display the final cluster assignments. Silhouette values range from 1 (exact cluster match) to  $-1$  (no relationship to members of the cluster). Average silhouette width values  $\geq 0.5$  are considered indicative of significant cluster groupings, and values between 0.25 and 0.5 suggest some, albeit weaker, group structuring (Kaufman & Rousseeuw 1990). Values  $< 0.25$  do not support a group structure.

### Identification of covariates

Harbor seals are largely viewed as generalist predators (Burns 2009). However, recent studies suggest individual foraging specialization within populations (Lance et al. 2012, Bromaghin et al. 2013, Wilson et

al. 2014). Hence, we considered a number of covariates that may help explain the trophic signature and inferred seal diets. Because of the transformations required when incorporating covariates in SI mixing models (e.g. Francis et al. 2011), it is more difficult to identify significant covariates within mixing models, compared to identifying covariates that describe variation in isotopic space. The predictors we considered included sex, reproductive status (pregnant, not pregnant), length, weight, body condition, and haul-out location (Bird Rocks, Vendovi Island, and Belle Chain Islets; see Table S1 in Supplement 1 at [www.int-res.com/articles/suppl/m526p213\\_supp.pdf](http://www.int-res.com/articles/suppl/m526p213_supp.pdf)). Because length and weight were highly correlated, they were not included as predictors in the same model (Supplement 1). We also calculated the body condition of each animal as a derived variable: the residuals of log-length versus log-weight regressions (see Schulte-Hostedde et al. 2005). For each seal, we used the 2-dimensional vector of isotope values as the response, and modeled the effects of covariates linearly,  $\mathbf{Y} = \mathbf{BX}$ , where  $\mathbf{Y}$  is the  $N \times 2$  matrix of isotope values;  $\mathbf{X}$  is a matrix representing predictors (sex, reproductive status, length, weight, body condition and haulout location); and  $\mathbf{B}$  is the vector of coefficients of these predictors. Because our sample size of seals was relatively small, the design was unbalanced; consequently, we utilized a leave-one-out cross validation (LOOCV) procedure in a multivariate regression framework over traditional approaches (e.g. multivariate ANOVA). We analyzed females and males separately, first calculating the LOOCV sum of squares for the null (intercept only) model. Variables were added in a stepwise manner (including interactions between variables) and compared to the results from the null model. Because LOOCV has the same properties as Akaike's information criterion, variables that perform better than the null model have good explanatory and predictive power (Stone 1977). All data needed to replicate this analysis are provided in Supplement 1.

### Bayesian mixing model

To examine how covariates affect diet, we constructed a Bayesian mixing model that included residual variation (Moore & Semmens 2008, Parnell et al. 2010). Source means and variances were calculated for the groups identified in the cluster analysis. As a cautionary note, our clustering algorithm may be useful in identifying isotopically similar species, but is not intended to correct poor source geometry,

which is one of the necessary requirements of mixing models. To address this requirement, our mixing model was extended to include a 2-level factor for sex, and a continuous covariate for weight. Instead of including covariates in the mixing model in transformed space (Francis et al. 2011), we parameterized the coefficients as derived parameters, and estimated the relative source contributions at the extremes of weight (45, 110 kg). In other words, we estimated the diet composition of the smallest individuals,  $p_{\text{small}}$ , and the largest individuals,  $p_{\text{large}}$ , and linearly interpolated between these values for weights between 45 and 110 kg. This procedure kept estimated proportions between 0 and 1, and did not require compositional transformations. We used the mean and variances calculated from each of the groups identified in the cluster analysis as source means and variances. Fractionation values were obtained from previously published feeding trials with phocid seals (Lesage et al. 2002):  $0.8 \pm 0.2\%$  for  $^{13}\text{C}$  and  $3.1 \pm 0.4\%$  for  $^{15}\text{N}$ . Our mixing model was constructed in JAGS (Plummer 2003) to implement Markov Chain Monte Carlo sampling. After a burn-in of 50 000, we ran 5 parallel chains for 100 000 iterations. A thinning rate of every 20<sup>th</sup> sample was used to ensure that the Gelman-Rubin statistic for each parameter was  $<1.02$  (Gelman et al. 2004). All data and code necessary to replicate our analysis are in the Supplements (available at [www.int-res.com/articles/suppl/m526/p213\\_supp.pdf](http://www.int-res.com/articles/suppl/m526/p213_supp.pdf)).

## RESULTS

### SI analysis

Mean  $\pm$  SD  $\delta^{13}\text{C}$  values for prey groups ranged from  $-21.14 \pm 2.04$  for adult salmon to  $-12.78 \pm 1.87$  for staghorn sculpin (Table 1). Estimates of mean  $\delta^{15}\text{N}$  for prey ranged from  $11.19 \pm 0.38$  for northern anchovy to  $13.84 \pm 0.46$  for rockfish. Mean  $\delta^{13}\text{C}$  corrected for fractionation and lipid content was  $-12.73 \pm 1.61$  for female harbor seals and  $-15.51 \pm 1.48$  for males. The mean  $\delta^{15}\text{N}$  for female seals was  $16.56 \pm 0.41$  and  $15.84 \pm 0.69$  for males.

### Constructing prey groups

The PAM algorithm returned a 2-cluster solution for the final assignment (Fig. 2), and the performance of the clustering algorithm was similar for both the sil and Hg cluster validity measures. Group 1 (rockfish,

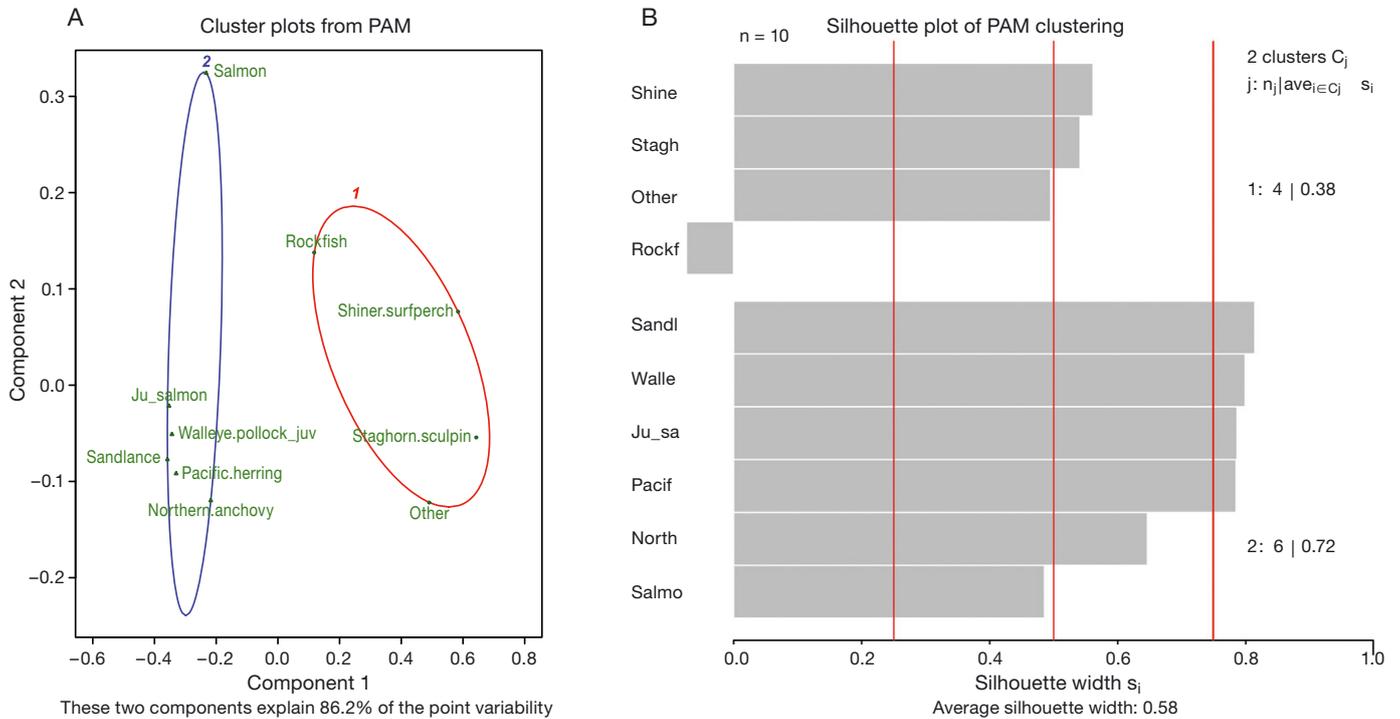
surfperch, staghorn, sculpin, and the 'other' taxa) was weakly clustered. The second group (anchovy, Pacific sand lance, Pacific herring, juvenile salmon, adult salmon, and walleye pollock) showed a stronger cluster structure. Given the large size of the second cluster, we repeated the iterative 2-step clustering process to further break out the large group (Fig. 3). Clustering on Group 2 alone produced 2 clusters: (a) juvenile salmon, walleye pollock, sand lance, Pacific anchovy and Pacific herring, and (b) adult salmon. Based on this clustering, we therefore assigned the prey items to the following groups for input into the mixing model (Table 2, Fig. 4): (1) adult salmonids; (2) small pelagics; juvenile salmon, juvenile pollock, sand lance, northern anchovy, Pacific herring; (3) a nearshore/estuarine bottom-feeding/semi-pelagic group; staghorn, sculpin, surfperch, and the 'other' category (sculpin, kelp greenling, dogfish, starry flounder); and (4) rockfish.

### Identification of covariates

A model including seal sex and mass as covariates performed the best with respect to predicting isotopic signatures, although a model using location and sex performed almost as well (Table 3). The importance of mass as a predictor of isotopic signatures was due largely to the negative correlation between  $\delta^{13}\text{C}$  enrichment and weight ( $p < 0.02$ ). We found no strong trend or differences in  $\delta^{15}\text{N}$  values with either increasing size or sex (Fig. 5).

### Dietary composition from Bayesian mixing model

Because size is a continuous covariate in our SI mixing model, we focused our comparison on smaller individual seals, where the estimated difference in diet between sexes is greatest. In general, the  $^{13}\text{C}$  values of female seals were more enriched compared to males, likely due to a greater consumption of prey items that are more enriched in  $^{13}\text{C}$  (e.g. staghorn sculpin, Table 4, Fig. 6). The estimated source contributions for the diets of small males and females revealed that differences in diet were most pronounced with respect to the consumption of pelagics (including salmon and pollock) versus rockfish and staghorn sculpins (Fig. 6). SI analysis indicated that salmon and small pelagics constituted the largest contributor of any prey group to small male harbor seals' diet (median  $p$ ,  $\tilde{p} = 0.80$ , Table 4) while this group contributed much less to the diet of small females ( $\tilde{p} =$



These two components explain 86.2% of the point variability

Fig. 2. Results of 2-step clustering of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of harbor seal *Phoca vitulina* prey using the partitioning around medoids (PAM) method. The 2-step clustering uses the CVs of the isotopic signatures as a starting point for the clustering algorithm, resampling these values for 100 iterations. Cluster results for each iteration are stored and the second clustering step is done on these nominal assignments. (A) The best solution from cluster diagnostics based on Hubert's  $\Gamma$  (Hg) was a 2-group cluster. (B) Red lines on the silhouette plot indicate levels of significant clustering ( $\geq 0.25, 0.5, 0.75$ ). Silhouette width  $s_i$  is average dissimilarity between  $i$  and all other points of the cluster to which  $i$  belongs,  $C_j$  is cluster  $j$ ,  $n_j$  is the number of elements or groups in cluster  $j$  and  $\text{ave}_{i \in C_j}$  is the average silhouette width of the cluster. Abbreviations for groups—Shine: shiner surfperch; Staghn: staghorn sculpin; Rockf: rockfish; Sandl: sand lance; Walle: walleye pollock; Ju\_sa: juvenile salmon; Pacif: Pacific herring; North: northern anchovy; Salmo: adult salmon

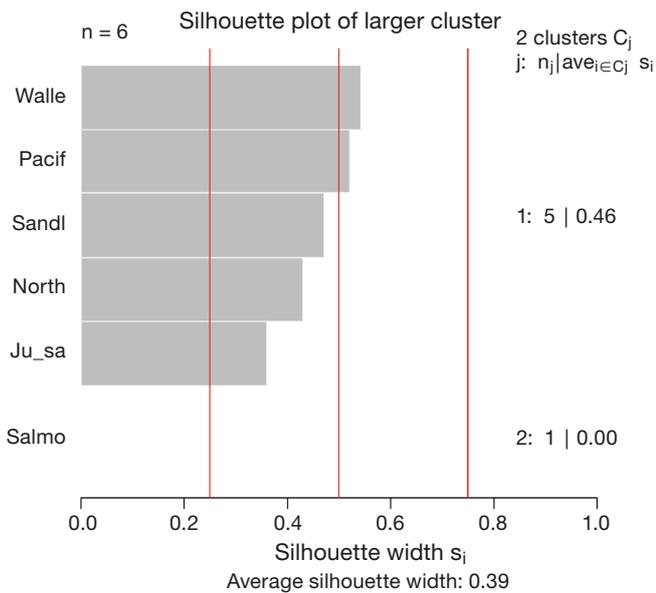


Fig. 3. Partitioning around medoids (PAM) clustering applied to the large cluster, Cluster 2, from Fig. 2. The 2-step clustering using the PAM algorithm was applied to further break out Groups. Cluster diagnostics based on Hubert's  $\Gamma$  (Hg). Abbreviations as in Fig. 2

0.25). SI results suggest that females supplemented their diet with more rockfish and staghorn sculpins/other prey items (instead of salmon and schooling pelagics) than their male counterparts (Fig. 6).

### DISCUSSION

Assessing the diet and feeding ecology of upper-trophic level marine predators is important in understanding the structure and functioning of marine food webs. Our examination of the relationship between sex and size and harbor seal diets identified through SI Bayesian mixing modeling contributes new information and perspectives on resource partitioning in pinnipeds, and phocids in particular, including the first description of sex differences in the diet of harbor seals.

These predator sex- and size-based differences may arise from differing spatial foraging patterns and/or differences in the location from where the consumed prey derived their  $^{13}\text{C}$  (nearshore vs. off-

Table 2. Isotopic values (mean, SD) of final clusters of harbor seal prey groups produced by the PAM algorithm

Group	Cluster	Cluster size	$\delta^{13}\text{C}$ corrected		$\delta^{15}\text{N}$	
			Mean	SD	Mean	SD
1	Adult salmon	50	-19.46	2.13	12.80	1.89
2	Small pelagics	36	-16.49	0.82	13.84	0.46
3	Staghorn sculpins/ surfperch/other	116	-18.79	1.09	12.14	0.88
4	Rockfish	46	-13.66	2.10	13.27	0.74

shore). Gradients in  $\delta^{13}\text{C}$  have been used in previous marine environment SI studies to differentiate foraging behavior in the nearshore versus offshore environment (Hobson et al. 1994). Because the nearshore environment is more enriched as a result of terrestrial carbon inputs, the negative relationship identified between weight and  $\delta^{13}\text{C}$  may indicate that larger harbor seals of both sexes received more of their dietary carbon from the offshore (Fig. 5), or the greater consumption of more pelagic (as opposed to benthic) prey. Combined, the effects of size, sex, and behavior support the idea that small females have isotopic values closer to that of the nearshore environment, while large males had values closer to the offshore environment. However, size effects were found to be somewhat independent of sex, with larger animals of both sexes exhibiting a diet consisting of prey deriving their  $^{13}\text{C}$  from depleted sources (i.e. offshore). The fact that larger individuals of both

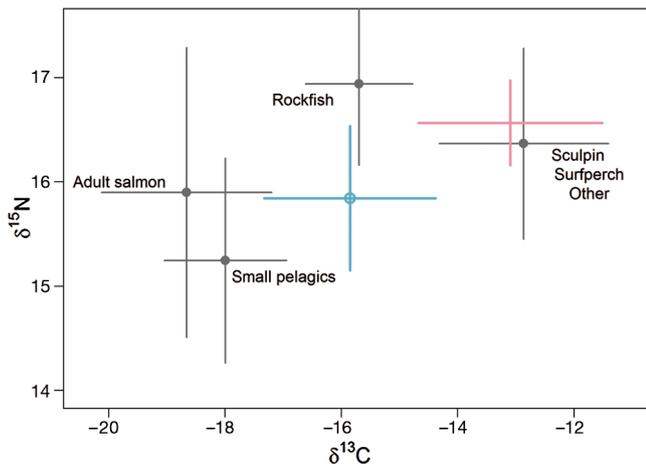


Fig. 4. Biplot of source and consumer isotopic signatures, using the source groups from our clustering algorithm. Points represent the means, and error bars represent standard deviations for each group. Harbor seal *Phoca vitulina* values displayed in pink (female) and blue (male) symbols. Source means and variances also include added effects of fractionation, and  $\delta^{13}\text{C}$  values for prey were corrected for lipids, following Post et al. (2007)

sexes may be foraging farther offshore to exploit offshore and pelagic food resources may be driven by resource partitioning resulting from intra-specific competition (Field et al. 2005).

As generalist predators, both male and female harbor seals likely capitalize on peaks of seasonal prey abundance, such as returning pink salmon (Ward et al. 2012). However, females may not be able to exploit these ephemeral changes in

prey abundance if those periods coincide with pupping and nursing seasons. The energetic costs for reproduction and lactation may constrain foraging to areas close to haul-out sites, which may be surrounded by shallower habitats. In contrast, male seals may leave the haul-out sites for longer periods. Small lactating females may need to supplement energy stores by more frequent feeding (Härkönen & Harding 2001). The requirements of more frequent nursing may limit these bouts to closer to the haul-out area than larger animals. Telemetry studies for this seal population offer some evidence for a sex-related spatial gradient. Females, especially those in estuarine areas and during the pupping season (spring and summer), displayed more localized movement, whereas other animals, mostly males, moved longer distances (e.g. traveling from the San Juan Islands to the outer Washington coast; Peterson et al. 2012). Sex-related differences in foraging trip duration and range have been observed in harbor seals in Scottish waters (Thompson et al. 1998) and in hooded seals *Cystophora cristata* and harp seals *Pagophilus groen-*

Table 3. Cross-validated sum of squares (SS) for multivariate regression analysis linking harbor seal *Phoca vitulina* covariates to isotopic signatures ( $n = 35$ ). Lower SS values are better; covariates that perform better than the null model (intercept only) are retained. The number of levels associated with the Location factor is included in parentheses

Covariate	Average cross-validated SS
Sex + ln(Weight)	2.73
Sex × Location	2.74
Sex + Location	2.76
Sex × ln(Weight)	2.81
Sex	2.82
Sex + ln (Length)	2.85
Location (3)	2.87
Sex × ln (Length)	2.99
Sex × Pregnant	3.01
ln (Weight)	4.2
ln(Length)	4.5
Null model	4.81
Body condition	5.04

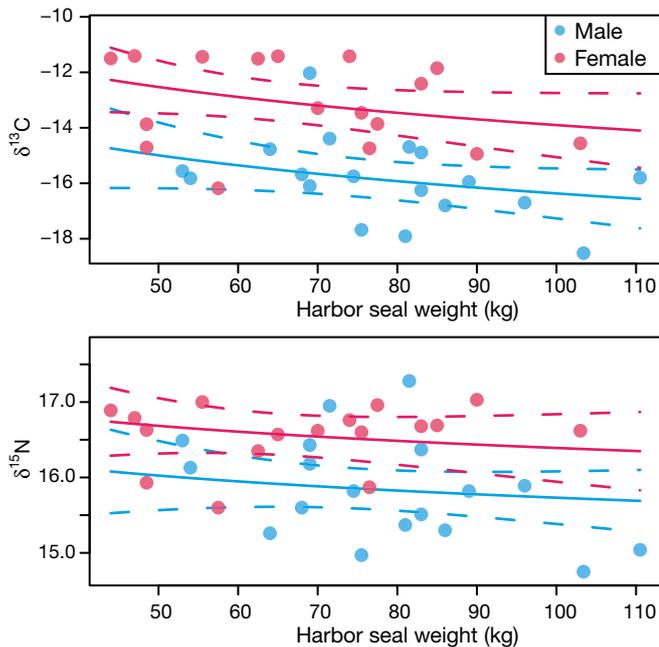


Fig. 5. Predicted and observed relationship between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in Puget Sound harbor seals *Phoca vitulina*, as a function of weight (kg) and sex. Lower carbon signatures may be associated with more offshore resource utilization or consumption of more pelagic resources, and higher nitrogen signatures are associated with higher trophic levels. Shown are the predicted mean relationships, with 95% credible intervals around the mean. Credible intervals for  $\delta^{15}\text{N}$  show a strong overlap for males and females

*landicus* (Tucker et al. 2009). Tucker et al. (2009) considered that these differences may be related to sex-specific costs of pregnancy, lactation, and reproduction and/or the costs of maintaining a large body size.

We found no evidence of the expected  $\delta^{15}\text{N}$  enrichment of seal tissue relative to that of their prey. The lack of an effect of sex or length on nitrogen values of harbor seals is consistent with findings of other studies (Kurlle 2002, Ruiz-Cooley et al. 2004, Drago et al. 2009). A number of factors may account for the lack of a trophic gradient. One possible explanation for this lack of enrichment is the movement of these

mobile taxa across spatial gradients in isotopic values or isoscapes (Popp et al. 2007). Other factors that may influence  $\delta^{15}\text{N}$  include seasonality, differences in elemental concentration of prey, isotopic routing, and dietary quality and quantity (Pearson et al. 2003, Karnovsky et al. 2012).

Karnovsky et al. (2012) compared seabird dietary estimation across direct sampling (lavage, pellets, and scat analysis), FA, and SI techniques. They noted that when applied in combination, the 3 techniques have the potential to reveal pathways of energy flux across marine ecosystems and to provide new insight into marine ecosystem dynamics. In a similar vein, estimating dietary source contributions from this population of harbor seal presents a unique opportunity to compare our results with those obtained from both scat (Lance et al. 2012) and FAs (Bromaghin et al. 2013) from the same population, some of the same individuals, and from the same temporal periods. Integrating the 3 sets of results provides an in-depth picture of the foraging landscape at varying temporal scales and breadths (19 prey types to 5 prey types); together they reveal the complexity of harbor seal diets as a function of sex, size, and season.

Hard parts recovered from scats provide unequivocal evidence of the presence of the species in the diet, but may not provide accurate assessment of the importance or proportion in the diet (Phillips & Harvey 2009). For example, scat samples from marine predators may under-represent small fishes or cephalopods. Scat analyses integrate information over a much shorter window (days) and may provide higher seasonal resolution. Scats from this same population of seals indicated that seals switched from a diet dominated by herring and sand lance in the winter and spring to a diet dominated by adult salmon in the summer/fall, coinciding with the increased availability of salmon (Lance et al. 2012).

SI provides information on the protein pathways, and SI analysis of whole blood provides resolution on time scales of months, while other blood components

integrate diet information on much shorter or longer time scales depending on the tissue (Hobson et al. 1996, Kurlle 2002). SI mixing models estimate the proportional contribution of prey isotopic signatures to that of the predator tissue assessed (Moore & Semmens 2008, Ward et al. 2010, Parnell et al. 2013). However, dietary items can be under- or over-represented in a given tissue because of diet-tissue discrimination or because SIs from various

Table 4. Stable isotope analysis of diets of female and male harbor seal *Phoca vitulina* in the Salish Sea. Diet composition estimated using a Bayesian mixing model. Values are estimated median proportions and the posterior 95 percentile credible range in parentheses. Estimates are shown for small seals (45 kg individuals)

Group	Prey item	Females	Males
1	Adult salmon	0.14 (0.007–0.357)	0.33 (0.015–0.803)
2	Small pelagics	0.11 (0.007–0.314)	0.47 (0.050–0.841)
3	Sculpin/surfperch/other	0.46 (0.141–0.741)	0.09 (0.003–0.286)
4	Rockfish	0.29 (0.027–0.666)	0.01 (0.003–0.318)

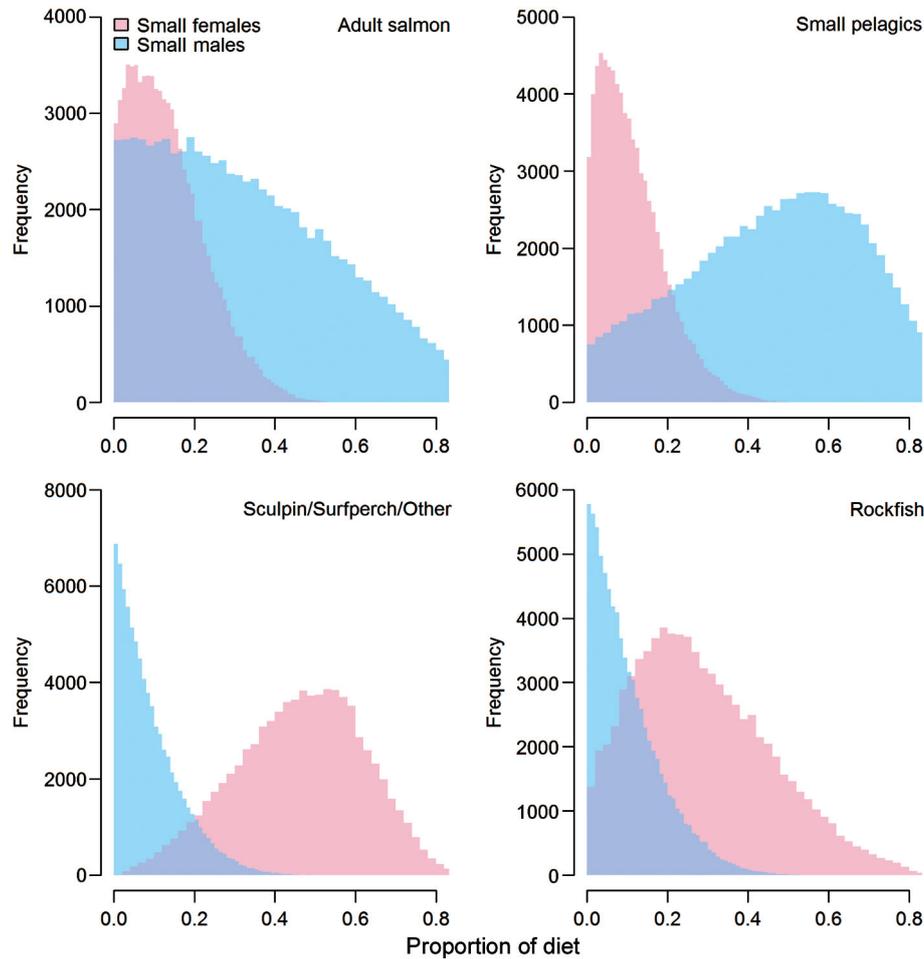


Fig. 6. Estimated harbor seal *Phoca vitulina* diet from the Bayesian stable isotope mixing model. Although the model was run using all seals, for simplicity, we show the estimated diet of small males along with the estimated diet of small females (45 kg individuals) to show the contrast between them

macronutrients in the diet may be differentially routed into tissues (i.e. carbon from dietary protein and carbohydrate into proteinaceous tissue; Robbins et al. 2005, Podlesak & McWilliams 2006). Isotopic routing may be less of an issue for seals than omnivorous species because all dietary items are relatively high in proteins and low in carbohydrates. We address this issue of diet-tissue discrimination by using experimentally derived fractionation factors for phocid seals (Lesage et al. 2002). In addition, observed changes in isotopic signatures that are the result of metabolic routing of dietary nutrients and discrimination tend to be relatively small compared to changes due to diet switches (Del Rio & Wolf 2005, Podlesak et al. 2005).

FA analysis compares FA signatures in predator adipose tissue to that of their potential prey (Iverson et al. 2004, Bromaghin et al. 2013). FA analysis, which integrates diet over several weeks, provides a

tool for analyzing sources of lipids (Karnovsky et al. 2012, Bromaghin et al. 2013). FAs also may be useful in analyzing prey at a finer resolution than SIs (19 prey species, Bromaghin et al. 2013). Applied to harbor seals, FA analyses, like SI approaches, also appear to show differences in consumption of salmon between males and females (Bromaghin et al. 2013). In interpreting the results of FA analyses, consideration should be given to the importance of a specific prey item in building fat tissues in the predator. Chinook salmon are a minor component of the seal diet sampled, yet they contribute disproportionately to building seal fat tissue because of their high fat content (Bromaghin et al. 2013).

Scat, FA, and SI analyses all indicate that commercially important species such salmon, rockfish, and Pacific herring are important contributors to harbor seal diets (Lance et al. 2012, Bromaghin et al. 2013, this study). Scat and FA analyses indicate that sal-

Table 5. Puget Sound harbor seal *Phoca vitulina* diets as reconstructed from 3 methods: quantitative fatty acid signature analysis (QFASA), stable isotope (SI), and fecal analysis (scat). No. prey groups: relative contribution to the diet, with 1 being the largest proportion. No sex-specific estimates were made for scat

Method:	QFASA	SI	Scat		
			11		
No. prey groups:	19	5	Spring	Summer/ fall	Winter
Dominant prey group-♂	Black and yellow rockfish	Small schooling pelagics (Pacific herring, northern anchovy, juvenile salmon and pollock, sand lance)	Clupeids	Adult salmon	Sand lance
	Chinook salmon (mature)		Gadids	Clupeids	Pollock
	Pacific herring (≥ 2 yr)		Others	Gadids	Anchovy
	Shiner surfperch		Sand lance	Others	Clupeids
	Spiny dogfish				
Dominant prey group-♀	Shiner surfperch	Staghorn sculpin/surfperch/other			
	Chinook (mature)	Rockfish			
	Black and yellow rockfish	Adult salmon			
	Herring (≥2 yr)				

mon and herring are among the top 2 sources depending on the season (Table 5). The FA and SI analyses both suggest sex differences in diet, with greater consumption of benthic/kelp forest/rocky bottom species by female seals, and greater consumption of pelagic/forage species by male seals. Fatty acid analyses indicated that males also ate more and larger herring and spiny dogfishes than females. Results from SI also indicate that males eat much more schooling pelagics than females. The results from the 3 methods diverged on several points. Scat analysis suggested that rockfish consumption was negligible compared to the other 2 methods. The SI analyses suggest that females consume more rockfish and surfperch, whereas FA analysis indicated that males eat more black and yellowtail rockfish.

The generalist diet of harbor seals appears to be a collection of individual specialists, something indirectly suggested by hard parts remaining in scat, FA and SI analyses, and even diving behavior (Lance et al. 2012, Bromaghin et al. 2013, Wilson et al. 2014). Although SI and FA analyses may indicate the importance of different food items to building specific tissues, the scat analysis identifies actual components of seal diet and is critical for parameterizing both the SI and FA approaches. Despite their individual strengths and weaknesses, together these 3 methods suggest that harbor seals have some degree of individual foraging specialization, and this specialization occurs on seasonal and perhaps longer time scales.

The foraging differences between male and female harbor seals present complex challenges for manage-

ment and for the design of marine reserves. Our findings also suggest a complex food web between harbor seals and their prey as exemplified by the following potential scenario. While female harbor seals appear to consume less salmon than males, they may have a secondary positive indirect effect on salmon because sculpins appear to be a more common diet item for female seals, and sculpins and other cottids are major predators of salmon eggs and fry (Mace 1983, Berejikian 1995, Foote & Brown 1998, Tabor et al. 1998). It is possible that female harbor seal consumption of sculpins may improve conditions for salmon, while male seals may have an opposite effect. In terms of designing reserves, many established marine reserves in the Pacific Northwest are located in close spatial proximity to seal haul-out sites. If smaller females forage closer to haul-out sites, then they may have a greater impact, either positive or negative, on prey populations in these areas. Given this possibility, future studies should assess the positive and negative impacts of this differential mortality on fisheries and on marine reserves.

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