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Using Bayesian stable isotope mixing models and generalized additive models to resolve diet changes for fish-eating killer whales *Orcinus orca*

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ABSTRACT: Understanding diet composition is fundamental to making conservation and management decisions about depleted species, particularly when nutritional stress is a potential threat hindering recovery. Diet in free-ranging marine mammals is challenging to study, but stable isotope mixing models are a powerful means of estimating the contribution of prey species to diet and can improve precision by leveraging information from multiple data sources. We evaluated diet composition of a fish-eating killer whale population (Southern Resident killer whales, *Orcinus orca*) using 2 approaches. First, we fit generalized additive models to evaluate seasonal and interannual patterns in isotopic values across age, sex, and pod, which revealed seasonal carbon enrichment for certain pods and a recent increased nitrogen enrichment that could suggest increased Chinook salmon consumption, changing isotopic values of prey, or nutritional stress. Second, we developed a Bayesian stable isotope mixing model that accounts for the different integration times represented by bulk stable isotopes and fecal samples. Results showed that estimated prey contributions are similar between prey data sources, though the precision of estimates from periods with smaller sample sizes was improved by using an informative prior to account for the different consumption windows of the data. This study illustrates the importance of improving our understanding of how killer whale diets vary over time (both seasonally and across years) and uses a novel approach to resolve 2 sources of diet information (stable isotope, fecal samples) with different consumption windows.

KEY WORDS: Southern Resident killer whale · Stable isotope · Mixing model · Diet estimation · Generalized additive models · Chinook salmon · Salish Sea

1. INTRODUCTION

Understanding the foraging ecology and energetic needs of top predators is critical to informing management and conservation strategies designed to recover depleted or endangered populations. Because precisely estimating the diet of free-ranging animals can be costly or even impossible, several approaches have been developed to make inference about diet. These include direct observation of feeding events (Redpath et al. 2001), analysis of stomach contents (Cortes 1997), examining fecal samples (Trites & Joy

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2005, Ford et al. 2016), and estimating diet through tissue chemical tracers such as fatty acids, stable isotopes (SIs), or persistent organic pollutants (Ben-David et al. 1997, Iverson et al. 2004, Herman et al. 2005, Krahn et al. 2007). Each of these methods has advantages, challenges, known biases, and different 'consumption windows' or integration times represented by the diet sample which might range from hours to months to years. It can therefore be beneficial to compare results from different diet data or even combine disparate sources for a more comprehensive understanding of population-level diet. In this study, we improved our understanding of the diet of a small population of killer whales (Southern Resident killer whales, SRKWs; *Orcinus orca*) using generalized additive models (GAMs) and a SI mixing model with fecal samples from whales (2006–2011) and SI data from both whales (2006–2016) and fish prey (2000–2010).

SRKWs are a fish-eating population composed of 3 social groups (J, K, and L pods) inhabiting coastal waters off British Columbia, Canada, and the west coast of the USA. SRKWs are of conservation concern with only 72 individuals as of July 2019 (Center for Whale Research 2019) and are listed under both the US Endangered Species Act and Canadian Species at Risk Act. Lack of prey or decreased prey quality has been highlighted as one of the main factors thought to be contributing to the population's decline from 98 whales in 1995 (NMFS 2008). Researchers have worked to elucidate foraging habits of SRKWs, though there is limited information about the spatio-temporal dynamics of prey availability and seasonal foraging distribution, with K/L pods thought to inhabit outer coastal waters as far south as California from winter through spring and both pods inhabiting inland waters to feed on Chinook salmon in the summer (Hauser et al. 2007).

Existing knowledge about SRKW diet comes primarily from direct observations and the genetic analysis of fecal samples and prey remains, and suggests Chinook salmon *Oncorhynchus tshawytscha* comprise the majority (>85%) of the summer (May–Sept) diet, and that coho salmon *O. kisutch* contribute up to 50% of diet in late summer (Sept) (Ford et al. 2010, 2016, Hanson et al. 2010). However, this evidence represents discrete seasonal snapshots and can only lend insight into diet during the summer, which may be different than other seasons and may also exhibit interannual variability. Skin tissue samples have also been collected from this population for over a decade, providing an opportunity to examine diet through SI analysis of the tissue over a longer time period than has been done to date. Bulk SI data may be useful, as indicators of dietary change throughout the year (particularly in non-summer months, when other data sources are more sparsely collected) or for integrating with other data, such as fecal samples, to improve the precision of existing diet estimates.

Diet can be assessed through SI mixing models that incorporate isotopic information from potential prey sources to estimate the relative importance of multiple prey in a predator's diet. Advances in Bayesian methods over the last decade have supported the development of SI mixing models for analyzing diet composition (Bearhop et al. 2002, Moore & Semmens 2008, Semmens et al. 2009, Parnell et al. 2010, 2013), including applications to large whales (Ryan et al. 2014) and extensions to include multivariate analyses (Hopkins & Ferguson 2012) and the incorporation of multiple sources of uncertainty (Ward et al. 2010, Bond & Diamond 2011). The advantage of a Bayesian approach is that disparate data sources (stomach contents, feeding events, or fecal samples) can be combined with SI data via an informative prior distribution, potentially reducing biases that would arise when analyzing any single data source alone. However, 2 complicating issues in these SI mixing models have largely gone unaddressed thus far. The first is not accounting for the fact that sources of diet information represent different temporal consumption windows (e.g. minutes for feeding observations, hours to days for fecal samples, and months for SI data). For example, combining 2 sources of data with equal sample sizes but different consumption windows would inflate the importance of the data source with the shorter consumption window. A commonly employed strategy for dealing with data sources with different effective sample sizes is post hoc weighting of each likelihood according to its relative contribution (Francis 2011, Yeakel et al. 2011). However, in this study, we illustrate how these data can instead be combined via informative priors. The second complicating issue that has not been addressed in SI mixing models thus far is that variability in trophic enrichment factors (TEFs) across species, age, nutritional status, tissue type, and even environmental variables such as water temperature (Hobson et al. 1996, Newsome et al. 2009, Busquets-Vass et al. 2017) can strongly bias estimated diet source proportions (Bond & Diamond 2011). We attempted to address uncertainty in TEFs by conducting a sensitivity analysis, examining model results and convergence across a range of TEF values estimated in previous studies of cetacean species.

The objectives of this study were 2-fold. First, we examined interannual and seasonal variability of carbon and nitrogen SIs ($\delta^{13}C$ and $\delta^{15}N$) across pods, age groups, and sex to ascertain whether bulk SI data over the study period suggest changes in trophic position or foraging patterns that could be used to inform hypotheses about the population's continued decline. These bulk isotope data had not been previously analyzed. Second, we developed an integrated SI mixing model that accounts for different tissue turnover times and consumption windows and evaluates whether diet estimates using information recovered from fecal samples are comparable to those derived from SI samples. As part of this mixing model effort, we also conducted a sensitivity analysis of the impact of different TEFs on model results. This work makes a valuable contribution to ongoing conservation and management planning for a population subject to multiple anthropogenic stressors and climate-related ecosystem changes that likely impact the abundance, availability, and quality of prey.

2. MATERIALS AND METHODS

2.1. Sample collection

SRKW skin biopsy samples have been opportunistically collected by the Northwest Fisheries Science Center since 2006, with nearly every individual in the population having been biopsied at least once (Table S1 in the Supplement at www.int-res.com/articles/suppl/m649p189_supp.pdf). These samples are temporally concentrated in summer and fall months (Fig. S1; approximately 70% of samples), when the whales typically spend more time in the Salish Sea versus coastal waters (Hauser et al. 2007). A total of 109 samples from 90 individuals were collected from 2006–2016. Sampled individuals ranged from 1–80+ yr old and comprised 54% females. Approximately one-third (32%) were from J pod (K and L pods were grouped for the purposes of this study due to similar foraging ecology). Individuals were previously classified as calves (0–2 yr), juveniles (3–9 yr), adult females (10–42 yr), young adult males (10–21 yr), senescent females (43+ yr), and older males (22+ yr) (Ward et al. 2013). For the purposes of our analyses, we ultimately grouped animals as calves versus non-calves.

In this study, we used SI data derived from whole fish representing known SRKW prey species collected from fisheries in the marine and estuarine waters of the Salish Sea (O'Neill et al. 2014). These included maturing Chinook (n = 105 collected in Aug–Oct 2000, 2004, and 2009), coho (n = 40, Aug–Nov of 2000 and 2003), chum *Oncorhynchus keta* (n = 30, Nov 2003), and sockeye salmon *O. nerka* (n = 30, Jul–Aug 2004). Full details and data are provided in O'Neill et al. (2014). The SI data were collected from fish samples by grinding whole fish and collecting tissue samples that were stored at –20°C for subsequent SI analyses based on individual Chinook salmon and composites (representing 5–6 individuals from the same location). The stock origins of Chinook salmon collected in non-terminal fisheries were inferred from genetic analyses using a coast-wide set of genotypes at 13 microsatellite loci developed by a consortium of laboratories (Seeb et al. 2007).

SI values of fish prey used in the mixing model included the SI data for the aforementioned whole fish samples of Chinook, coho, chum, and sockeye salmon samples collected for this study and additional SI data from Chinook salmon muscle samples reported for the Lower Fraser, Harrison stock (n = 6; Cullon et al. 2009), and steelhead trout *O. mykiss* muscle samples (n = 45; Quinn et al. 2012). The SI values of the Chinook salmon varied by stock, associated with the regional differences in their marine distribution, so the SI values used in the mixing model were derived by adjusting the measured values for each stock based on their relative abundance from 2005–2009 in the Salish Sea (Ward et al. 2013). SI analyses of whole fish and killer whale skin samples were conducted on lipid-extracted tissues as described previously (Herman et al. 2005). Collectively, the SI data used in the model showed Chinook and coho salmon to be the most enriched of the prey species, with killer whale samples adjusted for trophic enrichment (Caut et al. 2011, Marcoux et al. 2012, Ryan et al. 2014) falling between those and the other 3 prey species (Fig. 1).

Fecal samples have also been collected opportunistically during encounters with the SRKWs since 2006 (n = 244) and were therefore also taken primarily in May–Sept in the Salish Sea. Given the spatially and temporally unbalanced sample collection of fecal and skin biopsy samples (Fig. S1), the SI values were grouped to match previously established seasonal periods for fecal samples (Ford et al. 2016), thus facilitating diet comparisons between early summer (May–July) and late summer (Sept) periods. Data and a description of fecal sampling methods are available in Ford et al. (2016).

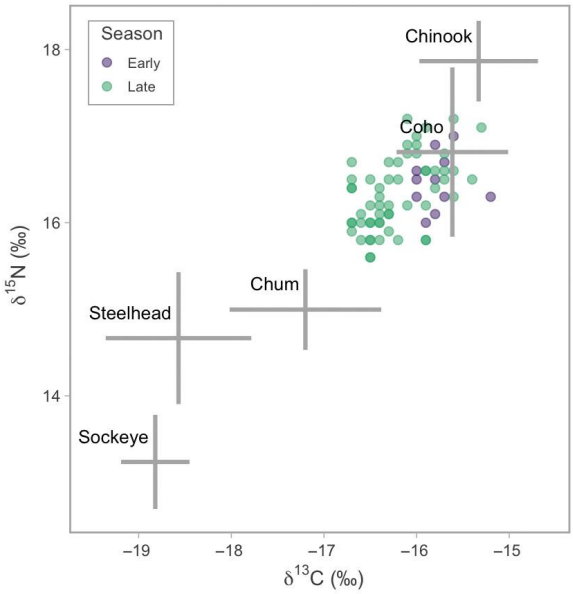

Fig. 1. Stable isotope values for fish prey (± 2 SD) and stable isotope values for Southern Resident killer whale skin samples in early versus late summer periods adjusted using average trophic enrichment factor values from the literature ($\delta^{13}C$ = 2.0; $\delta^{15}N$ = 2.85)

2.2. Spatio-temporal sampling caveats

Studying diet composition can be complicated by variation in both prey and predator SI signatures at both spatial and temporal scales. Previous studies have documented a pronounced difference in SI signatures among adults of some salmon species, with more enriched values of carbon and nitrogen in Chinook and coho salmon compared to other species (Johnson & Schindler 2009), but regional differences within a species have also been observed (Johnson & Schindler 2012, S. M. O'Neill unpubl. data), revealing the importance of using prey collected from the study area of interest and including the relative abundance of stocks throughout the region in the calculation of salmon SI signatures. Prey samples in this study largely represent the geographic distribution of SRKWs in inland waters, and are therefore less informative for inferring diet during winter months or when whales are distributed along the outer coast.

In addition to spatial variability, prey SI signatures may also vary at annual and seasonal temporal scales. Although sampling years for salmon differed from that of the SRKWs, we assumed that this would not notably affect our results. Annual variation in salmon SI signatures collected in Alaska over a 40 yr period that included large climate shifts were demonstrated to be far less than regional differences within a species (Johnson & Schindler 2012). Though SI values of adult salmon may vary seasonally throughout the Pacific Northwest, this variability has not yet been studied, and due to this knowledge gap, we made the necessary simplifying assumption that the SI signatures of fish prey are not highly variable over seasons. However, this assumption may be particularly problematic, as SI signatures for adult salmon in terminal areas may become enriched if and when individuals stop consuming prey.

2.3. Statistical analyses

2.3.1. GAMs

To evaluate evidence of non-linear interannual and seasonal changes in killer whale SI signatures from 2006–2016, we fit a series of GAMs (Hastie & Tibshirani 1990) with bulk $\delta^{13}C$ and $\delta^{15}N$ values from skin biopsies (n = 109) as the dependent variables. Several model structures were considered that included age, sex, and pod as linear predictors and smoothed effects for sampling month and year (see Table 1). A thin plate spline was used to estimate the effect of year and a cyclic cubic spline with 6 knots to estimate the monthly effect. The number of knots for the monthly effect was set rather than estimated to achieve a greater degree of smoothness suitable for the available data given the unbalanced sample design (Fig. S1, Table S1) and the longer consumption window. Additional models that included the complementary SI value as a fixed effect were also explored (i.e. $\delta^{13}C$ used as a covariate in the model for $\delta^{15}N$ and vice versa), though ultimately not included, with the 2 variables having a correlation of 0.49. SI signatures were very similar between sexes, and this was therefore eliminated as a potential covariate.

Parametric and smooth terms were estimated using the 'mgcv' package (Wood 2011) in R v.3.6.1 (R Core Team 2019) using restricted maximum likelihood. The contribution of fixed effects predictors to model fit was evaluated by comparing Akaike's information criterion (AIC) values of the global and null models, though pod group (J vs. K/L) and age (calf versus non-calf) were retained in all subsequent models to examine effect sizes, even if small, due to the pertinence of these variables to our research questions. Once fixed effects predictors were determined for both $\delta^{13}C$ and $\delta^{15}N$, the best model among different GAM structures was selected according to AIC values and the amount of deviance explained. Likelihood ratio tests were not used due to the challenge of

determining degrees of freedom given varying smoothness and number of penalties (Wood et al. 2016, Wood 2017). Model residual plots were examined for the effects of month and year (Figs. S2 & S3).

2.3.2. SI mixing model

To understand similarities or differences between published estimates of killer whale diet from fecal samples and bulk SI data collected from skin biopsies and account for seasonal variation in diet, we developed a Bayesian SI mixing model based on fish prey sources that comprise the majority of SRKW diet. To avoid introducing biases, we restricted the mixing model to a subset of the SRKW SI data that overlapped seasonally with the fecal samples. The subset of SI data contained samples taken during May through September and excluded calves (0–2 yr old) because they are primarily nursing rather than foraging (and thus may have higher $\delta^{15}N$ signatures), resulting in a subset of 69 samples from 62 individuals, 26% of which were juveniles, 30% from J pod, and 36% male. Though whale diet may change month to month (or seasonally) and the tissue turnover time of wild killer whales is uncertain and may be greater than 2 mo, we binned whale SI data into 'early summer' (May–July; n = 12) and 'late summer' (Sept; n = 57) periods to match the seasons considered in fecal sample analysis (no skin samples exist for the mid-summer August period).

To estimate the relative diet contribution of fish prey from the SI data alone, we first fit a 2 isotope, 5 source mixing model (Parnell et al. 2010) using the 'MixSIAR' package (Stock & Semmens 2016, Stock et al. 2018) in R. Because we were ultimately interested in exploring the effects of using the fecal samples as informative priors, we analyzed the data from early and late summer separately rather than treating season as a factor variable in the model. For the estimated contribution of the 5 fish prey species, we used Dirichlet priors that were uninformative on the simplex ($\alpha = 1$). Because of potential sensitivity to the choice of TEF values (Bond & Diamond 2011), we ran the above model with 3 alternative TEF values from the literature to evaluate which was most consistent with the killer whale and prey SI data: (1) muscle and skin samples from beluga whales *Delphinapterus leucas* ($\delta^{15}N = 2.57 \pm 0.52$, $\delta^{13}C = 2.29 \pm 0.59$; Marcoux et al. 2012), (2) skin samples from fin whales *Balaenoptera physalus* ($\delta^{15}N = 1.28 \pm 0.38$, $\delta^{13}C = 2.82 \pm 0.3$; Ryan et al. 2014, Borrell et al. 2012), and (3) skin samples from captive killer whales ($\delta^{15}N = 3.18 \pm 0.4$, $\delta^{13}C = 2.43 \pm 0.4$; Caut et al. 2011).

Because of different consumption windows, prior studies of killer whale diet from SI data and fecal samples may not be directly comparable. By using a Bayesian approach in this study, we were able to incorporate both sources of data into a single framework, with diet proportions estimated from the fecal samples, to construct informative prior distributions. Like other prior distributions, the uninformative Dirichlet distribution ($\alpha = 1$) can be adjusted for a varied prior sample size (Gelman & Rubin 1992, Gelman et al. 2004). The hyperparameters of the Dirichlet control the location and scale, $E[y_i] = \alpha_i / \sum \alpha_i$, but the sum of hyperparameters $\sum \alpha_i$ can also be interpreted as the effective sample size of the prior (Morita et al. 2008). We constructed informative priors for each season (early summer, late summer) using data from Ford et al. (2016) that included the number of fecal samples collected, relative contribution from each of the 5 prey species, and tissue turnover time. As an example of how these priors were constructed, suppose 50 fecal samples were collected and proportionally assigned to prey species as 35.5 Chinook, 9.5 coho, 4.25 chum, 0.50 sockeye, 0.25 steelhead. Because these fecal samples represent a smaller consumption window (1–2 d) compared to SI data, the prior effective sample size needs to be adjusted. Assuming a hypothetical turnover time of killer whale skin to be 30 d, the weighted prior can be calculated by multiplying the proportional assignments by (2/30), resulting in a weighted vector (2.33 Chinook, 0.67 coho, 0.28 chum, 0.03 sockeye, 0.02 steelhead) with a total effective sample size of 3.33 (= 50 × 2 / 30) instead of 50. The final set of hyperparameters is derived by adding the initial count from the uninformative prior ($\alpha_i = 1$) to each, yielding (3.33, 1.67, 1.28, 1.03, 1.02). Given uncertainties in the turnover time of skin tissue in wild killer whales, we ran a sensitivity analysis with 2 models for each informative prior, using skin turnover times of 30 and 60 d, in addition to the models with an uninformed prior as described above. Combinations of the informed TEF priors (n = 3), skin turnover times (n = 3), and season (n = 2) resulted in a total of 18 models. For each model, we ran 3 parallel Markov chain Monte Carlo simulations with 'MixSIAR' and 'JAGS' (Plummer 2003) and generated 50 000 samples from each, with the first 25 000 iterations discarded as a burn-in. Convergence was assessed using the 'CODA' package (Plummer et al. 2006) using $\hat{R}$ values < 1.1 (Gelman & Rubin 1992, Gelman et al. 2004). Code to replicate our analyses is included in Text S1 in the Supplement.

Table 1. Null and candidate models estimating parametric and smooth effects of explanatory variables for Southern Resident killer whale $\delta^{13}C$ and $\delta^{15}N$ isotope values with corresponding Akaike's information criterion (AIC), ΔAIC, model weight, and deviance explained, with the best model indicated in **bold**. Null and linear models are not comparable to generalized additive models and therefore differences in model fit are not included (–)

Model	AIC	ΔAIC	Weight	Dev. expl. (%)
$\delta^{15}N_i \sim 1$	150.70	–	–	–
$\delta^{15}N_i \sim age_i + pod_i + sex_i + month_i + year_i$	123.51	–	–	–
$\delta^{15}N_i \sim age_i + pod_i + s(month_i) + s(1 \mid year_i)$	77.90	3.13	0.12	59
$\delta^{15}N_i \sim age_i + pod_i + s(month_i) + s(year_i)$	74.77	0.00	0.56	58
$\delta^{15}N_i \sim age_i + pod_i + s(month_i) + s(year_i, pod_i)$	81.43	6.65	0.02	59
$\delta^{15}N_i \sim age_i + pod_i + s(year_i, pod_i) + s(month_i, pod_i)$	**76.46**	**1.69**	**0.24**	**60**
$\delta^{15}N_i \sim age_i + pod_i + s(month_i, pod_i) + s(year_i, pod_i)$	79.16	4.38	0.06	61
$\delta^{13}C_i \sim 1$	116.18	–	–	–
$\delta^{13}C_i \sim age_i + pod_i + sex_i + month_i + year_i$	102.46	–	–	–
$\delta^{13}C_i \sim age_i + pod_i + s(month_i) + s(1 \mid year_i)$	42.25	2.71	0.16	60
$\delta^{13}C_i \sim age_i + pod_i + s(month_i) + s(year_i)$	41.53	1.99	0.23	60
$\delta^{13}C_i \sim age_i + pod_i + s(month_i) + s(year_i, pod_i)$	58.64	19.10	0.00	52
$\delta^{13}C_i \sim age_i + pod_i + s(year_i, pod_i) + s(month_i, pod_i)$	**39.54**	**0.00**	**0.61**	**62**
$\delta^{13}C_i \sim age_i + pod_i + s(month_i, pod_i) + s(year_i, pod_i)$	57.06	17.52	0.00	55

3. RESULTS

3.1. GAMs

The best models for $\delta^{13}C$ and $\delta^{15}N$ were selected based on the combination of AIC value, deviance explained, and our *a priori* interest in examining differences across pods (Table 1). The selected best model for both $\delta^{13}C$ and $\delta^{15}N$ included the fixed effects of pod and age group, a smooth term for year, and a pod-specific smooth term for month. Including predictor covariates notably improved model fit (Table 1).

As noted above, the best model for $\delta^{13}C$ values included non-significant linear predictors for age and pod, a global smooth term for year (effective degrees of freedom [edf] = 6.1, $p < 0.001$), and a significant smooth effect of month for J pod (edf = 1.9, $p < 0.01$) and K/L pods (edf = 3.3, $p < 0.001$) (Table 2), though there was also support for a global smooth effect of month (ΔAIC = 1.99). The shape of the smooth term for the effect of month on $\delta^{13}C$ was different between pods, with a peak in $\delta^{13}C$ in March and April for K/L pods, but a smaller, later peak in June and July for J pod (Fig. 2). The change in $\delta^{13}C$ over the study period as indicated by the smooth term for the effect of year indicated greater enrichment in 2010 and 2015 (Fig. 2).

For $\delta^{15}N$ values, the best model estimated lower SI signatures for non-calves ($\hat{b} = -0.68$, $p < 0.001$), no difference between pods, a global smooth effect for year (edf = 6.4, $p < 0.001$), and non-significant, pod-specific smooth effects of month, with a deviance explained of 60% (Tables 1 & 2). The pod-specific smooth effect of month was much smaller than that of $\delta^{13}C$ and showed a slight increase in $\delta^{15}N$ in May for K/L pods, but a later increase in July and August for J pod (Fig. 2). The smooth term for interannual variability indicated that values were notably more enriched in 2010, dropped, and then began increasing again in 2013 (Fig. 2). The interannual variability for $\delta^{15}N$ signatures was higher in magnitude than for $\delta^{13}C$ (edf = 6.07, $p < 0.001$).

3.2. SI mixing model

Of the 18 SI mixing models used in our analysis, all but 2 (models for late summer using TEFs from Caut et al. 2011 and Marcoux et al. 2012 with zero and 60 d skin turnover times respectively) successfully converged (Table 3). The estimated relative contribution of the 5 prey species was similar across models, but because all the models using the TEF prior from fin whales (Ryan et al. 2014) converged, we focused the

Table 2. Estimated parametric and smooth effects, effective degrees of freedom (edf), standard error, test statistic critical values (*t*-value/*F*), and p-values for best generalized additive models of Southern Resident killer whale $\delta^{13}C$ and $\delta^{15}N$ isotope values. ***$p < 0.001$; **$p < 0.01$

Term	Estimate/edf	SE	*t*/*F*	p
$\delta^{15}N_i \sim age_i + pod_i + s(year_i) + s(month_i, pod_i)$				
(Intercept)	16.97	0.15	116.81	***
age: non-calf	–0.68	0.15	–4.55	***
pod: K/L	0.02	0.07	0.28	
s(month, J pod)	1.11	4.00	0.52	
s(month, K/L pods)	1.31	4.00	0.67	
s(year)	6.40	7.38	10.99	***
$\delta^{13}C_i \sim age_i + pod_i + s(year_i) + s(month_i, pod_i)$				
(Intercept)	–16.18	0.12	–131.90	***
age: non-calf	–0.03	0.12	–0.28	
pod: K/L	0.04	0.06	0.68	
s(month, J pod)	1.91	4.00	2.66	**
s(month, K/L pods)	3.34	4.00	10.84	***
s(year)	6.07	7.09	9.11	***

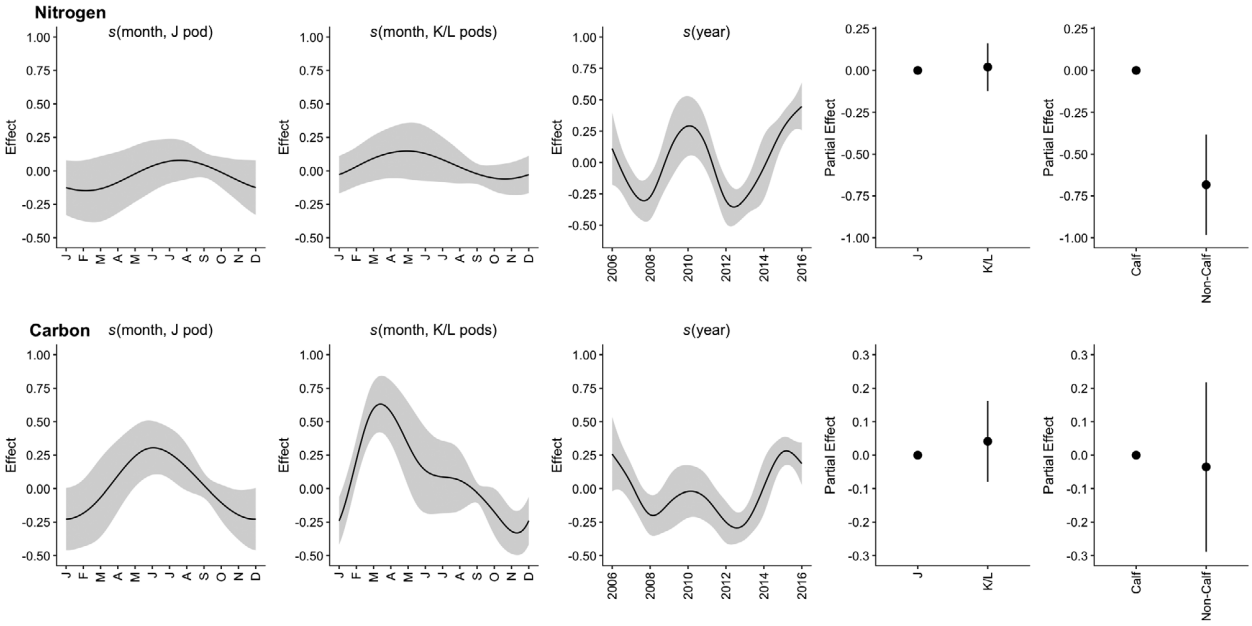

Fig. 2. Predicted stable isotope values based on best-fit generalized additive models showing Southern Resident killer whale pod-specific smooths for month and a global smooth for year in addition to fixed effects for pod and age group for $\delta^{15}N$ and $\delta^{13}C$, with 95% CIs represented by grey shading and black bars

following results on those. Because of the uncertain lag time of tissue assimilation, estimates from the SI mixing model may not be directly comparable to the fecal samples alone (Ford et al. 2016) but are summarized below.

As expected, using SI data to infer killer whale diet suggests that diet is dominated by Chinook salmon, where in both early and late summer periods, the contribution of Chinook was estimated to be more than 50% on average. Using the SI data alone (no prior information), the estimated consumption of Chinook was higher in samples taken in late summer (Table 3). However, when the informative prior was included, the contribution of Chinook decreased in samples from the late summer period (59 vs. 50%, and 54 vs. 51% for 30 and 60 d, respectively; Fig. 3), similar to what was suggested by the fecal data (Ford et al. 2016). The difference between these 2 results is likely due to the increased precision that is achieved when using an informative prior for the estimate in the early

Table 3. Summary of 18 stable isotope mixing models considered in the study, including trophic enrichment factors (TEFs), season (early or late summer), assumed skin turnover time in days (for integration with fecal samples, with 0 representing models with an uninformative prior), and the maximum value of $\hat{R}$ used to assess convergence (values <1.1 indicate convergence). The posterior summaries of the estimated diet proportion of Chinook salmon is also shown (mean, lower 95%, upper 95%)

TEFs (Reference)	Season	Skin turnover	Max. $\hat{R}$	Chinook diet proportion Lower 95%	Mean	Upper 95%
$\delta^{15}N = 1.28 \pm 0.38$, $\delta^{13}C = 2.82 \pm 0.30$ (Ryan et al. 2014)	Early	0	1.01	0.21	0.50	0.70
		30	1.03	0.40	0.59	0.73
		60	1.03	0.33	0.54	0.70
	Late	0	1.03	0.35	0.53	0.63
		30	1.05	0.32	0.50	0.61
		60	1.06	0.34	0.51	0.62
$\delta^{15}N = 3.18 \pm 0.4$, $\delta^{13}C = 2.43 \pm 0.4$ (Caut et al. 2011)	Early	0	1.02	0.19	0.49	0.69
		30	1.01	0.39	0.58	0.72
		60	1.01	0.34	0.55	0.72
	Late	0	1.13	0.34	0.54	0.63
		30	1.03	0.32	0.50	0.61
		60	1.06	0.31	0.51	0.62
$\delta^{15}N = 2.57 \pm 0.52$, $\delta^{13}C = 2.29 \pm 0.59$ (Marcoux et al. 2012)	Early	0	1.03	0.22	0.49	0.70
		30	1.04	0.39	0.59	0.72
		60	1.08	0.33	0.55	0.71
	Late	0	1.08	0.30	0.52	0.63
		30	1.08	0.31	0.49	0.60
		60	1.13	0.29	0.50	0.62

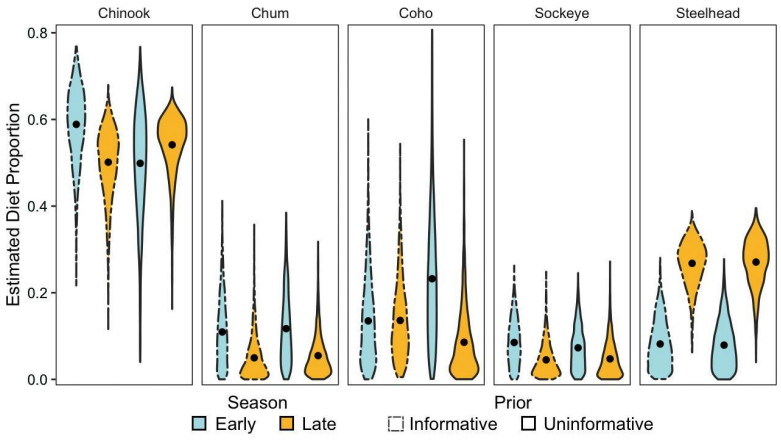

Fig. 3. Mean posterior estimates of prey contribution to Southern Resident killer whale diet in early versus late summer sampling periods using trophic enrichment factor values from Ryan et al. (2014) as the informative prior and assuming that skin tissue samples represent 30 d of diet information

summer period (Fig. 3) when sample sizes for SI data were smaller. The finding that the SI mixing model with both data sources generally supports that of the fecal data alone was expected and potentially provides useful information for conservation and management because fecal samples are more readily available and affordable to collect.

After Chinook, coho salmon comprised the next greatest contribution to overall diet, followed by the other 3 salmonid species, depending on the season. Similar to Chinook, the contribution of coho changes slightly depending on the season and inclusion of the informative prior. With an uninformative prior, consumption of coho was estimated to be approximately 23 and 9% from the early and late summer period samples, respectively (Fig. 3). Using the informative prior, however, the mean posterior contribution was approximately 13% in both periods (Fig. 3), which is closer to what is expected based on fecal data alone. The contribution of chum and sockeye salmon was estimated to be very small in samples from both seasons regardless of TEFs used, which again is in agreement with the fecal samples (Fig. 3). Similarly, the estimated proportion of steelhead was notably lower in samples from early summer compared to late summer (8 vs. 27%), using both informed and uninformed priors.

4. DISCUSSION

Through the application of GAMs and a Bayesian SI mixing model, this study examined the relative proportion of fish prey in SRKW diets throughout the summer and how the nitrogen signatures (commonly used as a proxy for trophic position) and/or carbon food web source may have changed over the study period for different pods. Results from the SI mixing model indicated that estimated prey contributions were relatively similar when incorporating the fecal data as an informative prior versus using the SI values alone with an uninformative prior. However, it is notable that precision was higher for estimated Chinook and coho salmon contributions using an informative prior from the fecal sample data, particularly when sample sizes were low (i.e. early summer). With large sample sizes (i.e. late summer), our informative prior was overwhelmed by the killer whale SI data and there was little difference between the prior and posterior estimates. While in general we recommend the use of informative priors for SI mixing models, we caution that discrepancies may arise between prior and posterior estimates when the tissue turnover times associated with SI data are on different timescales than the sampling of data used for generating the prior. As an extreme case, integrating SI data and fecal data sampled from the same individual on a single day attempts to reconcile short-term diet information (1–2 d) with diet over a much longer timescale.

The results from both the SI mixing model and GAMs support and validate previous SRKW diet estimates (e.g. the contribution of Chinook salmon decreases into the fall and winter; Ford et al. 2016) and what is known about seasonal whale distribution patterns (Olson et al. 2018). Prey isotopic signatures show that Chinook and coho salmon have more enriched $\delta^{15}N$ and $\delta^{13}C$ than other Pacific salmon, consistent with other evidence indicating that they feed at a higher trophic level and use coastal ecosystems more extensively than chum, sockeye, and steelhead (Quinn 2018). Because of tissue assimilation time, the skin tissue enriched in $\delta^{13}C$ in spring (Fig. 2) could be indicative of higher Chinook or coho consumption. Another potential reason for this $\delta^{13}C$ enrichment in killer whales is that $\delta^{13}C$ signatures for Chinook likely differ by run type (spring, summer, fall) and life history type (resident, ocean migrant). The pod-specific month effects predict peak $\delta^{13}C$ values for K/L pods in the spring, when these whales may be encountering Chinook enriched in $\delta^{13}C$ (or consuming non-salmon prey species; M. B. Hanson

unpubl. data). The seasonal pattern in the GAMs could also be mirroring the higher proportion of steelhead and lower proportion of Chinook estimated in the mixing model for the late summer period, though this connection is difficult to make given the uncertainty in the lag time between prey consumption and tissue assimilation.

In terms of evaluating changes in $\delta^{15}N$ over time, it is clear that there is interannual variability, but available data limits our ability to identify the likely cause of this variability. It may be that shifting isotopic signatures of prey are driving observed interannual variability in SRKW SI values (individual salmon stocks have variable distributions year to year, and by age; Quinn et al. 2014, Quinn 2018), though sockeye salmon stocks in Alaska exhibited relatively stable SI values across a range of oceanographic conditions and over a 40 yr period (Johnson & Schindler 2012). Year-to-year variability in SRKW $\delta^{15}N$ over time could be both an indicator of poor nutritional status in some years, and/or that killer whales consume more Chinook salmon in years when they are more abundant. Greater enrichment in isotopic values in 2010 and since 2013 could indicate greater Chinook consumption, which aligns with increasing coastwide salmon indices observed since 2008 (Pacific Salmon Commission 2018). Unfortunately, both prey and killer whale bulk SI data are limited for 2012 and 2013 when whale SI values were lowest, preventing a robust comparison to 2008 when enrichment was similarly low. Additionally, examining SRKW $\delta^{15}N$ values and indices of salmon abundance is complicated by the fact that the available indices of abundance may not reflect what is available to the whales. Future work formally examining correlations between $\delta^{15}N$ and indices of salmon abundance or other measures of SRKW health could help determine whether this trend is due to changes in salmon diet (and therefore SI signatures) based on ocean conditions (Brodeur et al. 2007) or SRKW catabolism of endogenous protein tissues due to nutritional stress (Kurle & Worthy 2001, Newsome et al. 2010, Horstmann-Dehn et al. 2012, Matthews et al. 2019).

While there is currently no direct evidence linking trophic position or diet composition with nutritional stress, ongoing efforts are aiming to link variability in prey abundance to changes in individual nutritional stress and body condition. Interannual variability estimated by GAMs indicated that there were no significant differences between the pod groups for either $\delta^{15}N$ and $\delta^{13}C$ when pod was included as a factor variable, and no sex-specific differences were found in SI signatures despite recent evidence suggesting greater foraging effort in males, particularly at depths corresponding to Chinook salmon habitat (Tennessen et al. 2019).

Making inferences about seasonal patterns in the diet of a top predator based on imperfect knowledge about tissue assimilation and unbalanced spatio-temporal sampling of consumer and prey is challenging. Disentangling the potential effects of diet shifts of predators and prey in addition to nutritional stress highlights the multiple uncertainties that underlie SRKW diet studies and potential future research directions. First, it is likely that K and L pods consume a small amount of other groundfish species (for which SI data are not available) on the coast during winter and spring (M. B. Hanson et al. unpubl. data), and though our mixing model includes the majority of prey items in killer whale diets, not accounting for all prey sources can lead to bias in SI mixing models (Phillips & Gregg 2001). We therefore cannot make inference about the contribution of non-salmon species to overall diet. Second, the wide range of tissue assimilation and TEF values estimated for cetaceans across species, tissue types, water temperatures, and geographic ranges (latitude) represent a large area of uncertainty, since TEFs and the consumption window are foundational to examining seasonality in SI data. Even within a species, considerable variation has been estimated depending on population and methodology (Wild et al. 2018). For SRKWs, it is possible that TEFs differ across age groups (due to different growth rates or reproductive status) and pods (due to differential metabolic demands in inland versus coastal environments). Third, there is a large degree of uncertainty surrounding salmon SI data, where isotopic signatures likely vary due to differences between specific stocks, seasonal runs, fish age, and size. It is also unknown to what extent killer whales consume resident Chinook salmon that do not migrate to the open ocean and therefore differ in their isotopic composition and spatio-temporal distribution. Data aggregated across these elements likely does not capture the specificity of foraging that may be needed in order to prioritize salmon recovery actions that could benefit SRKWs, though it is clear that Chinook salmon are a critical prey resource for SRKWs.

Despite these uncertainties, results from this analysis underscore that understanding timescales matters, particularly for diet studies of long-lived species. Timescales govern seasonal movements and foraging, and are coupled with salmon runs and physical ocean conditions. This intricacy is difficult to simplify and yet very important in developing management alternatives that address potential nutri-

tional stress. To that end, while uncertainty about SRKW diet remains, this study illustrates a method that further enhances the power of SI mixing models by accounting for different consumption windows across different diet data sources. Combining multiple data sources maximizes the utility of the unique information represented by each, and the chained hierarchical approach prevents one source from masking information in another due to varying sampling sizes. The mixing model with both fecal and SI data resulted in improved parameter estimation when sample sizes were low but also resulted in an estimated diet composition relatively similar to that of the fecal data alone, which will likely prove useful given the affordability and greater availability of fecal samples. This framework notably improves the precision of diet proportion estimates in certain circumstances and could be developed further through formal integration of not only different diet data but also information about tissue turnover rates, digestion, energetic requirements, and even age or reproductive status. Working to evaluate existing data in ways that can inform hypotheses about the continued decline of this population and the importance of Chinook salmon recovery will be critical to future conservation and management strategies.

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