NRC Research Press

ARTICLE

Harbour seals target juvenile salmon of conservation concern

Austen C. Thomas, Benjamin W. Nelson, Monique M. Lance, Bruce E. Deagle, and Andrew W. Trites

Abstract: Knowing the species and life stages of prey that predators consume is important for understanding the impacts that predation may have on prey populations, but traditional methods for determining diets often cannot provide sufficient detail. We combined data from two methods of scat analysis (DNA metabarcoding and morphological prey ID) to quantify the species and life stages of salmon (*Oncorhynchus* spp.) consumed by harbour seals (*Phoca vitulina*) in the Strait of Georgia, Canada, where juvenile Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon survival is poor. Harbour seals primarily consumed adult salmon of lesser conservation concern in the fall (August–November): chum (*Oncorhynchus keta*: 18.4%), pink (*Oncorhynchus gorbuscha*: 12.6%), sockeye (*Oncorhynchus nerka*: 7.4%), Chinook (7.1%), and coho (1.8%). However, the opposite species trend occurred during the spring when seals preferred juvenile salmon of greater conservation concern (April–July): coho (2.9%), Chinook (2.9%), sockeye (2.5%), pink (1.4%), and chum (0.8%) — percentages that can equate to many individuals consumed. Our data suggest that harbour seals select juveniles of salmon species that out-migrate at ages >1 year and provide evidence of a potential causal relationship between harbour seal predation and juvenile salmon survival trends.

Résumé : Il importe de connaître l'espèce et l'étape du cycle biologique des proies que les prédateurs consomment afin de comprendre les impacts que la prédation peut avoir sur les populations de proies. Dans bien des cas, les méthodes traditionnelles de détermination du régime alimentaire ne peuvent toutefois fournir assez de détails. Nous avons combiné des données de deux méthodes d'analyse d'excréments (méta-codes-barres d'ADN et identification morphologique des proies) pour quantifier l'espèce et l'étape du cycle biologique de saumons (*Oncorhynchus* spp.) consommés par des phoques communs (*Phoca vitulina*) dans le détroit de Georgia (Canada), où la survie des saumons quinnats (*Oncorhynchus tshawytscha*) et cohos (*Oncorhynchus kisutch*) juvéniles est faible. Les données indiquent que, à l'automne (d'août à novembre), les phoques communs consomment principalement des saumons adultes dont la conservation est moins préoccupante, à savoir des saumons kétas (*Oncorhynchus keta* : 18,4 %), roses (*Oncorhynchus gorbuscha* : 12,6 %), rouges (*Oncorhynchus nerka* : 7,4 %), quinnats (7,1 %) et cohos (1,8 %). Cependant, cette tendance est inversée au printemps (d'avril à juillet), alors que les phoques privilégient des saumons juvéniles dont la conservation est plus préoccupante, à savoir des saumons cohos (2,9 %), quinnats (2,9 %), rouges (2,5 %), roses (1,4 %) et kétas (0,8 %), ces pourcentages pouvant équivaloir à de nombreux individus consommés. Nos données laissent croire que les phoques communs choisissent des juvéniles d'espèces de saumon qui migrent vers la mer à des âges de plus d'un an et indiqueraient une possible relation de causalité entre la prédation par les phoques communs et les tendances de survie des saumons juvéniles. [Traduit par la Rédaction]

Introduction

Predators can have different net effects on ecological communities depending on the life stage of prey they consume (Hastings 1983; 1988; Werner and Gilliam 1984). This is because prey species often fill different ontogenetic niches as they grow and mature and use different habitats or food resources as juveniles compared with their adult life stage (Werner and Gilliam 1984). Ecologists have therefore long recognized the need to account for age-specific predation on prey species when modeling predator–prey interactions or ecosystem dynamics (McCauley et al. 1993; Walters and Martell 2004).

The need for age-specific predation data is particularly apparent when attempting to calculate the number of individual prey consumed by a predator population. For example, a single harbour seal (*Phoca vitulina*) consuming an average of ~2 kg of fish per day would need to consume fewer than one individual adult coho salmon (*Oncorhynchus kisutch*) per day to meet its bioenergetic needs. However, if that same seal was consuming juvenile coho salmon shortly after ocean entry (~20 g smolts), it would need to eat ~100 individual coho salmon per day to meet its energy needs (assuming similar energy density). Thus, a seal diet percentage of 50% coho salmon would add up to a profoundly different number of individual coho consumed by seals depending on whether predation was focused on juveniles or adults.

To facilitate ecological modeling efforts, the ideal technique to determine predator diets would provide detailed information about the prey consumed, including species identification, prey life stage, and the relative proportions of prey in the overall predator diet (Bowen and Iverson 2013; Tollit et al. 2010). Using that information, ecologists could estimate life-stage-specific numbers of individual prey eaten by predator populations when diet data are combined with predator bioenergetic and demographic studies (Howard et al. 2013; Olesiuk 1993; Winship and Trites 2003).

Unfortunately many of the methods currently used to determine diets are unable to provide high taxonomic resolution of prey in addition to providing the life stage and relative propor-

Received 6 December 2015. Accepted 30 August 2016.

A.C. Thomas* and B.W. Nelson. Department of Zoology and Marine Mammal Research Unit, Institute for the Oceans and Fisheries, The University of British Columbia, Vancouver, B.C., Canada.
M.M. Lance. Wildlife Science Program, Washington Department of Fish and Wildlife, Lakewood, Washington, USA.
B.E. Deagle. Australian Antarctic Division, Channel Highway, Kingston, Tasmania, Australia.
A.W. Trites. Marine Mammal Research Unit, Institute for the Oceans and Fisheries, The University of British Columbia, Vancouver, B.C., Canada.
Corresponding author: Austen C. Thomas (emails: a.thomas@oceans.ubc.ca, athomas@smith-root.com).
*Present address: Smith-Root, Inc., 16603 NE 50th Avenue, Vancouver, WA 98686, USA.

tions of prey consumed (Tollit et al. 2006). Diets of seals and sea lions, for example, are commonly described from morphological identification of hard prey remains recovered in faecal samples (scats) (Bowen and Iverson 2013). Morphological ID is effective for estimating the sizes of prey consumed by the pinnipeds, but often cannot distinguish between closely related prey species (e.g., salmonids) or determine the relative proportions of prey (Laake et al. 2002; Lance et al. 2001; Phillips and Harvey 2009). Small prey with delicate bone structures may also be completely digested (i.e., no hard parts survive the digestive process), causing them to be highly underestimated. An alternative diet analysis method is therefore needed to generate all of the necessary information required to understand the impacts of pinniped predators on prey populations.

DNA metabarcoding diet analysis is an alternative to traditional morphological prey ID that offers high taxonomic resolution and increasingly quantitative information about the proportions of species consumed by pinnipeds and other animals (Kelly et al. 2014; Leray et al. 2015; Pompanon et al. 2012; Taberlet et al. 2012; Thomas et al. 2014). DNA metabarcoding is the process of characterizing species assemblages using diagnostic genetic markers (i.e., DNA barcodes) isolated from samples containing the DNA of multiple organisms, generally followed by high-throughput DNA amplicon sequencing. For the purpose of diet analysis, DNA metabarcoding is applied to scat samples or the stomach contents of individual animals, and DNA sequence percentages are used as a semiquantitative measure of the relative mass of species consumed (Deagle et al. 2010; Ford et al. 2016; Jarman et al. 2013; Pompanon et al. 2012).

We propose that a combined scat analysis method employing both DNA metabarcoding and morphological prey ID can be used to quantify the species and life stages of salmon consumed by pinnipeds. This is based on the sizes of prey bones in scats providing the life stage of salmon consumed (Buzzell et al. 2014; Lance et al. 2012) and DNA metabarcoding providing the salmon species ID in addition to the relative proportion of salmon in the overall seal diet. The approach is consistent with other recent studies that have highlighted the benefits of combining multiple diet analysis techniques to create enhanced data products (Chiaradia et al. 2014; Geiger et al. 2013; Méheust et al. 2014).

In the Pacific inland waters of British Columbia, Canada (Strait of Georgia), Chinook (*Oncorhynchus tshawytscha*) and coho salmon have experienced poor smolt-to-adult survival in recent decades, suggesting a high level of juvenile salmon mortality in the early marine phase of life (Neville et al. 2015; Welch et al. 2011; Zimmerman et al. 2015). Among the potential causes of increased juvenile marine mortality, Pacific harbour seals have been identified as a likely contributor due to their exponential population increase during the period of declining Chinook and coho survival (Olesiuk 2009; Riddell et al. 2009). To assess whether a potential causal relationship exists between harbour seal predation and juvenile salmon survival in the Strait of Georgia, proportional seal diet information is needed that can provide the species and life stages of salmon consumed by harbour seals in the region.

Our study therefore had two major objectives: (*i*) establish a new scatological analysis method that can be used to estimate the life stage, species, and relative proportions of salmon in pinniped diets and (*i*) apply our combined diet analysis method to harbour seal scats collected from the Strait of Georgia, British Columbia, where detailed seal diet information is needed to facilitate regional predator–prey modeling efforts. To accomplish this, we collected large numbers of harbour seal scat samples in the Strait of Georgia over 2 years and merged the resulting data sets from morphological prey ID and DNA metabarcoding diet analysis.

Materials and methods

Scat collection

Scat samples were collected from four locations used by Pacific harbour seals in the Strait of Georgia, British Columbia, Canada (Fig. 1). Previous research in the region indicated that salmon predation by seals is most intensive near river mouths (Olesiuk 1993; Olesiuk et al. 1990). Our study therefore focused primarily on the estuaries of major salmon-bearing rivers. Estuarine harbour seal haulout sites included Cowichan Bay, Fraser River, and Comox Bay (Fig. 1). For comparative purposes, we also collected scat samples from a rocky reef haulout site (Belle Chain) because the majority of seals in the Strait occupy such haulouts (Olesiuk 2009).

Sampling was stratified by collection site, year (2012, 2013), and season (spring: April–July; fall: August–November), targeting a total of 70 seal scat samples per stratum (Trites and Joy 2005). The seasons roughly corresponded to the temporal windows when juvenile salmon primarily out-migrate (spring) and when adult salmon return (fall) (Melnychuk et al. 2010; Quinn 2005). We attempted to attain an even sampling distribution within each stratum by collecting samples either monthly or biweekly from each site.

At the haulout sites, each individual scat sample was collected using a disposable wooden tongue depressor and placed in a 500 mL Histoplex jar lined with a 126 μm nylon mesh paint strainer (Orr et al. 2003). Samples were either preserved immediately in the field by adding 300 mL 95% ethanol to the collection jar or were taken to the lab and frozen at –20 °C within 6 h of collection (King et al. 2008). Later, samples were thawed and filled with ethanol prior to being manually homogenized with a disposable depressor inside the paint strainer to separate the scat matrix material from hard prey remains (e.g., bones, cephalopod beaks). The paint strainer containing prey hard parts was then removed from the jar, leaving behind the ethanol preserved scat matrix for genetic analysis (Thomas et al. 2014).

Prey hard parts analysis

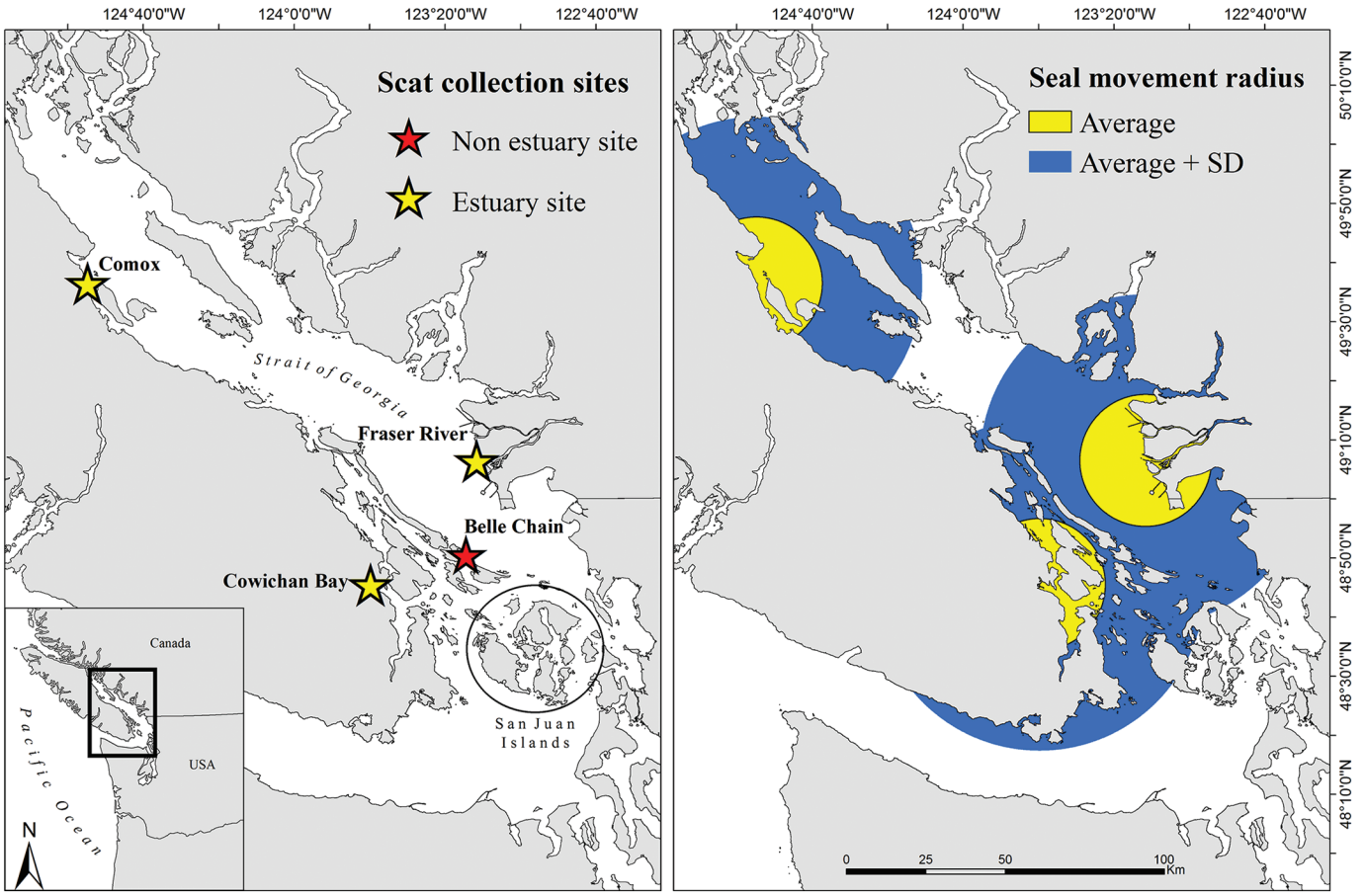
To remain consistent with the way previous harbour seal diet work in the region has been conducted using hard prey remains (i.e., hard parts), we used the "all structures" approach to identify harbour seal prey contained in individual scat samples (Olesiuk et al. 1990). Prey hard parts retained in paint strainers were cleaned of debris using either a washing machine or nested sieves. All diagnostic prey hard parts were identified to the lowest possible taxon using a dissecting microscope and reference fish bones from Washington and British Columbia, in addition to published keys for fish bones and cephalopod beaks (Clarke 1986; Harvey et al. 2000; Kashiwada et al. 1979; Morrow 1979; Wolff 1982). Samples containing prey hard parts identifiable only to the family level (e.g., Clupeidae) and bones identifiable to the species level of the same family (e.g., Pacific herring, *Clupea pallasii*) were both tallied (Lance et al. 2001).

For comparison with DNA metabarcoding diet percentage, prey hard parts species occurrences in samples were converted into population-level diet percentages using the Split Sample Frequency of Occurrence model (SSFO):

$$\mathrm{SSFO}_i = \frac{\sum_{k=1}^{s}\left[\frac{I_{i,k}}{\sum_{i=1}^{\omega} I_{i,k}}\right]}{s}$$

where ω is the number of prey categories, s is the number of samples, and I is the indicator function equal to 1 if the ith prey category is present in the kth sample and 0 if it is absent (Olesiuk et al. 1990; Tollit et al. 2010). Simply speaking, this model divides

Fig. 1. (Left) Harbour seal haulouts in the Strait of Georgia, British Columbia, Canada, where scats were collected. The sites include three estuary haulouts (Fraser River, Cowichan Bay, and Comox) and one non-estuary haulout (Belle Chain). (Right) Potential spatial area represented by "estuary" seal diet data based on satellite-tagged harbour seal movements in the region (Peterson et al. 2012). Seal movement radius buffers were generated around estuary collection sites using the average (20.7 km) and standard deviation (SD) (31.4 km) of the "median over-water distances between satellite locations and the capture site for all (tagged) seals" (Peterson et al. 2012). [Colour online.]



each species occurrence in a scat by the total number of occurrences in the scat (thereby converting to a proportion) and then calculates a population mean for each prey species across all scats in a collection. Alternative models for prey biomass reconstruction from hard parts are now known to be superior to SSFO (Phillips and Harvey 2009); however, this method was chosen so data could be directly comparable to published data from the 1980s (Olesiuk et al. 1990).

Salmon vertebrae diameters were measured to demonstrate the clear size differential between juvenile and adult salmon bones in seal scats, which is visually evident to taxonomic experts. Two representative salmon vertebrae classified as "juvenile" and two classified as "adult" were randomly (i.e., haphazardly) selected and measured from samples collected in each month and in both years. Not all months contained samples with salmon vertebrae in both age classes, resulting in 49 total measured salmon vertebrae (25 juvenile, 24 adult; see online supplementary material, Fig. S1[1]).

Fish otoliths in seal scats were also measured using an ocular micrometer and graded based on the observed level of digestion erosion (Tollit et al. 2004). Grade-specific length correction factors for salmon were applied to any salmon otoliths that were graded "good" (no or minimal erosion) or "fair" (small amount of erosion) (Phillips and Harvey 2009). Corrected otolith lengths were used to estimate the fork lengths of juvenile salmon consumed by seals using a published linear equation of the relationship between otolith length and fish length for Chinook salmon smolts (Neilson and Geen 1982).

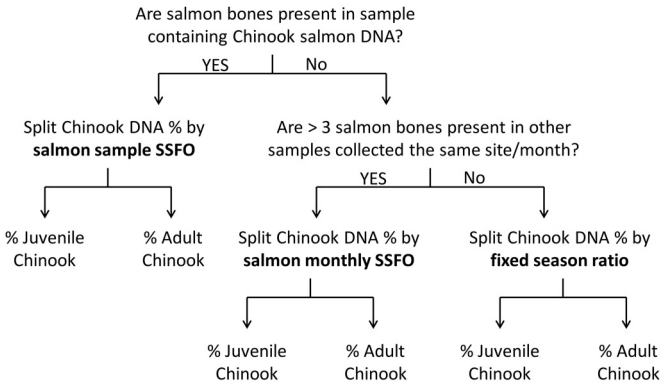
DNA metabarcoding diet analysis

The DNA diet analysis protocol we used is described in detail in Thomas et al. (2016). In brief, a multiplex PCR reaction was done with the extracted DNA from each individual scat sample using 16S primer sets designed to amplify both fish and cephalopod DNA. Samples were individually labeled with index sequences, and the pooled amplicons were sequenced on multiple Illumina MiSeq runs (v2-300 cycle SE). DNA sequences were compared with a custom BLAST reference database composed of 16S sequences of species known to occur in the geographic region. To remove potential DNA contaminants, species sequences that comprised <1% of a single sample were removed prior to calculating sample diet percentages. Prey species taxonomic assignments were finally renormalized to generate proportional DNA summaries for each individual scat sample.

Although Thomas et al. (2016) evaluated the application of relative correction factors (RCFs) to harbour seal scat samples to account for prey species-specific biases, RCFs were not applied in this study. Given that our objective was to characterize harbour seal population diet from a numerical aggregate of many scat

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2015-0558.

Fig. 2. A schematic diagram depicting the decision tree approach we developed to estimate salmon species and life stage in harbour seal diet. This example demonstrates how Chinook salmon DNA sequences in an individual seal scat sample can be assigned to juvenile or adult Chinook salmon based on the co-occurrence of salmon bones. If salmon bones are present in the sample containing Chinook DNA, the salmon sample split sample frequency of occurrence (SSFO) is used to split DNA percentage into adult and juvenile Chinook percentage. If no salmon bones are present in the sample, and more than three samples in the same monthly collection contain salmon bones, the salmon monthly SSFO is used. In the rare case when neither criterion is met, the species DNA percentage is split according to the fixed season ratio (see Materials and methods section for details).



samples, prey species-specific biases to DNA sequence counts are likely less influential in this application of DNA metabarcoding (Thomas et al. 2016). A statistical simulation study is currently needed to determine when RCFs are necessary to achieve study objectives.

For samples collected in 2012, a secondary metabarcoding marker was used to quantify the salmon portion of seal diet, because the primary 16S marker is unable to differentiate between coho (*O. kisutch*) and steelhead (*Oncorhynchus mykiss*) DNA sequences (Table S1[1]). This marker was a cytochrome oxidase I (COI) "minibarcode" specifically for salmonids within the standard COI barcoding region: Sal_COI_F (CTCTATTTAGTATTTGGTGCCTGAG), Sal_COI_R (GAG TCAGAAGCTTATGTTRTTTATTCG). The COI amplicons were sequenced alongside 16S such that the overall salmonid fraction of the diet was quantified by 16S, and the salmon species proportions within that fraction were quantified by COI. The salmon-specific marker was not used with 2013 samples because the steelhead diet component was determined to be quite small in 2012 and did not justify the additional expense for subsequent samples. However, future efforts should likely prioritize identification of steelhead in seal diet samples due to increased concern about possible interactions between seals and steelhead populations in the region (Berejikian et al. 2016; Moore et al. 2015).

Estimating salmon life stages

We created a novel decision tree approach to assign the recovered salmon DNA to either adult or juvenile by combining DNA and hard parts data from the same collection of scat samples (Fig. 2). For a given salmon species, we split the DNA percentage according to the ratio of adult to juvenile salmon and calculated the ratio in one of three ways depending on the available data ((*i*) salmon sample SSFO, (*ii*) salmon monthly SSFO, (*iii*) fixed season ratio).

The adult/juvenile ratio we applied depended on the available information for each specific scat sample. We calculated the salmon sample SSFO by dividing salmon hard parts occurrences in a sample (specified to life stage) by the total number of salmon occurrences within that sample. For example, if a sample contained hard parts from an adult salmon and a juvenile salmon, the ratio was 0.5:0.5 (adult:juvenile). However, the ratio was 1:0 if a sample contained only adult salmon bones, and the ratio was 0:1 if it contained only juvenile salmon bones. We calculated salmon monthly SSFO by averaging the salmon sample SSFO values for a particular month and collection site, similar to the equation detailed in "Prey hard parts analysis". Lastly, the fixed season ratio assumed that all salmon consumed in the spring season were juveniles (0:1, adult:juvenile ratio) and that all salmon consumed in the fall season are adults (1:0, adult:juvenile ratio) (see Results and Discussion for evaluation of this assumption).

A sample containing salmon species DNA as well as salmon bones resulted in the salmon species DNA percentage being split according to the salmon sample SSFO ratio. However, if no salmon bones were identified in the sample and greater than three samples contained salmon bones in the collection site and month, the species DNA percentage was split according to the salmon monthly SSFO ratio. If no salmon bones were present in the sample and fewer than three samples contained salmon bones in the collection site–month, the DNA percentage was split by the fixed season ratio (Fig. 2). This method of partitioning salmon between juvenile and adult life stages works on the assumption that the probable life stage of salmon species occurring in any individual scat can be inferred based on the co-occurrences of salmon bones in scats collected in the same location and month. Furthermore, this method prioritizes the best level of information available to partition the salmon species into life stages, rather than simply making assumptions based on regional fish life history information.

Seal diet confidence intervals

Means, standard deviations, and confidence intervals associated with the estimated salmon diet proportions were calculated using nonparametric bootstrapping techniques (Efron and Tibshirani 1993) implemented in the R Programming Environment (R Core Team 2014). We generated 1000 bootstrap replicates to calculate estimates. Confidence intervals (95%) were estimated using the "first percentile method" (Roff 2006), ranking bootstrap replicates from lowest to highest and identifying the 2.5th and 97.5th percentiles as the lower and upper limits of the confidence interval, respectively.

We also use this approach to test for significant differences in diet percentages between prey species. The differences in diet percentages between two species in question was calculated for each sample in the data set, then bootstrapped (1000 replicates) to calculate 95% confidence intervals. If the 95% confidence intervals for the mean difference in diet percentage overlapped with zero, we did not reject the null hypothesis (concluding no difference). However, if the confidence intervals did not overlap zero, we rejected the null hypothesis and concluded there is a statistically significant difference between the two species in the diet.

Results

We collected a total of 1258 scat samples from all four sites combined during the study period. Of these, 18 samples were identified as belonging to California sea lions (*Zalophus californianus*) based on a high percentage of sea lion DNA present in the samples. Of the remaining 1240 harbour seal scat samples, 1166 (94.0%) produced sufficient prey DNA sequences to be analyzed, and 1168 (94.2%) contained identifiable prey hard parts. Illumina MiSeq sequencing of scat DNA produced on average 1227 prey DNA sequences per sample for those samples that passed filtering, and morphological analysis of scats identified on average 5.2 prey hard parts per sample.

Bootstrapped confidence intervals for the salmon component of harbour seal diet are given for each combination of sampling site and season (Table 1) and monthly for all estuary samples combined in each year (Table 2). Although not the focus of this salmon-centric

Table 1. Data aggregated by site, year, and season, including means, standard deviations (SD), and 95% confidence intervals from 1000 bootstrap replicates of the salmon components of harbour seal diet (%) for each collection location, year, and season.

	Spring										Fall									
	2012					2013					2012					2013				
Collection site	*n*	Mean	SD	2.5%	97.5%	*n*	Mean	SD	2.5%	97.5%	*n*	Mean	SD	2.5%	97.5%	*n*	Mean	SD	2.5%	97.5%
Fraser River																				
Juvenile																				
Chinook	70	2.27	1.21	0.48	4.87	88	2.74	1.43	0.53	5.79	83	0.53	0.51	0.00	1.58	70	0.01	0.01	0.00	0.02
Coho	70	0.61	0.39	0.13	1.47	88	0.94	0.64	0.06	2.36	83	0.00	0.00	0.00	0.00	70	0.00	0.00	0.00	0.01
Sockeye	70	0.12	0.08	0.00	0.30	88	2.86	1.42	0.50	6.06	83	0.01	0.01	0.00	0.03	70	0.00	0.00	0.00	0.00
Pink	70	0.28	0.17	0.03	0.66	88	2.15	1.36	0.11	5.14	83	0.12	0.10	0.00	0.33	70	0.03	0.02	0.00	0.09
Chum	70	0.16	0.09	0.02	0.38	88	0.38	0.24	0.02	0.92	83	0.20	0.09	0.04	0.39	70	1.29	1.22	0.00	3.84
Steelhead	70	0.49	0.44	0.00	1.35	—	—	—	—	—	83	0.00	0.00	0.00	0.00	—	—	—	—	—
Adult																				
Chinook	70	4.54	1.40	2.08	7.32	88	6.49	2.55	2.07	11.72	83	22.59	4.21	14.38	30.95	70	3.87	1.74	1.20	7.81
Coho	70	0.43	0.21	0.14	0.94	88	0.01	0.01	0.00	0.04	83	1.99	1.30	0.02	4.82	70	2.49	1.11	0.68	4.95
Sockeye	70	35.61	5.27	25.63	46.14	88	1.55	1.08	0.10	3.93	83	22.93	4.29	14.94	31.22	70	18.45	4.03	10.90	26.76
Pink	70	0.62	0.21	0.25	1.08	88	0.14	0.08	0.01	0.33	83	1.36	0.86	0.08	3.30	70	45.35	5.01	35.55	55.50
Chum	70	0.15	0.06	0.04	0.29	88	0.29	0.26	0.00	0.82	83	38.37	4.98	28.67	48.59	70	20.89	4.30	12.90	29.38
Steelhead	70	1.65	1.03	0.03	4.17	—	—	—	—	—	83	0.00	0.00	0.00	0.00	—	—	—	—	—
Comox																				
Juvenile																				
Chinook	85	4.55	1.66	1.80	8.14	98	0.84	0.35	0.27	1.61	111	0.14	0.07	0.03	0.28	73	0.27	0.15	0.05	0.60
Coho	85	4.97	1.87	1.67	8.69	98	4.37	1.77	1.31	8.10	111	0.12	0.10	0.01	0.33	73	2.56	1.72	0.03	6.39
Sockeye	85	1.33	0.38	0.61	2.15	98	4.40	1.66	1.48	8.07	111	0.27	0.11	0.09	0.50	73	1.36	0.66	0.29	2.82
Pink	85	1.82	0.86	0.56	3.67	98	0.47	0.21	0.11	0.92	111	0.98	0.60	0.04	2.31	73	2.19	0.86	0.80	4.01
Chum	85	0.95	0.47	0.16	1.99	98	2.16	1.20	0.14	4.79	111	0.62	0.46	0.05	1.68	73	0.10	0.07	0.00	0.27
Steelhead	85	1.28	1.17	0.07	3.75	—	—	—	—	—	111	0.00	0.00	0.00	0.01	—	—	—	—	—
Adult																				
Chinook	85	2.35	1.31	0.23	5.27	98	1.03	1.02	0.00	3.05	111	2.30	1.22	0.41	5.03	73	2.49	1.46	0.34	5.87
Coho	85	0.05	0.02	0.01	0.10	98	0.02	0.02	0.00	0.05	111	0.81	0.43	0.13	1.77	73	1.41	0.78	0.13	3.12
Sockeye	85	0.63	0.43	0.10	1.55	98	0.25	0.19	0.01	0.67	111	0.96	0.33	0.43	1.66	73	2.77	1.11	0.94	5.19
Pink	85	0.28	0.21	0.03	0.78	98	0.04	0.04	0.00	0.12	111	3.41	1.31	1.06	6.11	73	26.77	4.71	17.68	36.41
Chum	85	0.00	0.00	0.00	0.00	98	0.02	0.02	0.00	0.05	111	24.27	3.82	16.92	32.05	73	4.46	1.95	1.01	8.72
Steelhead	85	0.01	0.00	0.00	0.02	—	—	—	—	—	111	0.00	0.00	0.00	0.00	—	—	—	—	—
Cowichan Bay																				
Juvenile																				
Chinook	56	6.15	2.06	2.71	10.71	76	2.27	0.79	0.87	3.99	83	2.28	0.82	0.87	4.06	91	1.75	1.08	0.18	4.26
Coho	56	2.91	1.22	0.98	5.39	76	3.23	1.60	0.55	6.61	83	0.62	0.31	0.10	1.31	91	0.20	0.16	0.01	0.55
Sockeye	56	2.82	0.71	1.51	4.29	76	2.75	1.23	0.77	5.53	83	1.32	0.68	0.28	2.85	91	0.92	0.39	0.27	1.82
Pink	56	0.80	0.26	0.36	1.33	76	2.54	1.53	0.25	5.99	83	1.22	0.66	0.20	2.68	91	0.87	0.31	0.36	1.54
Chum	56	0.00	0.00	0.00	0.01	76	0.47	0.33	0.02	1.19	83	0.52	0.35	0.04	1.29	91	1.02	0.68	0.05	2.61
Steelhead	56	0.08	0.07	0.00	0.23	—	—	—	—	—	83	0.15	0.14	0.00	0.44	—	—	—	—	—
Adult																				
Chinook	56	0.00	0.00	0.00	0.00	76	0.72	0.73	0.00	2.28	83	8.99	2.74	4.08	14.46	91	3.45	1.65	0.62	7.21
Coho	56	0.00	0.00	0.00	0.00	76	0.30	0.30	0.00	0.92	83	0.47	0.34	0.05	1.26	91	2.58	1.33	0.45	5.56
Sockeye	56	0.00	0.00	0.00	0.00	76	0.00	0.00	0.00	0.00	83	3.25	1.38	1.02	6.27	91	2.30	1.07	0.56	4.60
Pink	56	0.00	0.00	0.00	0.00	76	0.02	0.02	0.00	0.06	83	2.14	1.17	0.41	4.91	91	5.84	1.63	3.06	9.57
Chum	56	0.00	0.00	0.00	0.00	76	0.00	0.00	0.00	0.00	83	11.77	3.43	5.46	18.77	91	9.46	2.45	5.00	14.54
Steelhead	56	0.00	0.00	0.00	0.00	—	—	—	—	—	83	0.25	0.26	0.00	0.98	—	—	—	—	—
Belle Chain																				
Juvenile																				
Chinook											85	7.49	2.38	3.45	12.72	77	0.05	0.02	0.01	0.10
Coho											85	0.60	0.23	0.19	1.08	77	0.84	0.70	0.02	2.32
Sockeye											85	5.39	1.45	2.74	8.49	77	0.03	0.02	0.00	0.07
Pink											85	0.55	0.21	0.19	1.04	77	0.35	0.17	0.09	0.73
Chum											85	0.42	0.24	0.05	0.92	77	1.29	0.55	0.46	2.52
Steelhead											85	0.14	0.11	0.01	0.38	—	—	—	—	—
Adult																				
Chinook											85	4.07	1.96	0.71	8.44	77	2.26	1.30	0.51	5.10
Coho											85	0.37	0.25	0.00	0.93	77	0.47	0.16	0.18	0.82
Sockeye											85	2.84	1.14	1.00	5.32	77	4.36	1.76	1.28	8.15
Pink											85	0.49	0.23	0.12	0.98	77	35.85	4.86	26.52	45.47
Chum											85	0.04	0.02	0.00	0.09	77	7.63	1.80	4.56	11.53
Steelhead											85	0.06	0.06	0.00	0.18	—	—	—	—	—

Note: Only a small number of samples were obtained from the non-estuary site (Belle Chain) in the spring season; therefore, data are not shown.

Table 2. Data aggregated by month and year (estuaries only), including means, standard deviations (SD), and 95% confidence intervals from 1000 bootstrap replicates of the salmon components of harbour seal diet (%) for each collection month and year.

	2012					2013				
	n	Mean	SD	2.5%	97.5%	*n*	Mean	SD	2.5%	97.5%
April										
Juvenile										
Chinook						72	1.20	0.61	0.26	2.54
Coho						72	4.79	2.11	1.20	9.27
Sockeye						72	3.11	1.58	0.63	6.77
Pink						72	0.26	0.19	0.00	0.70
Chum						72	0.24	0.13	0.03	0.54
Steelhead						—	—	—	—	—
Adult										
Chinook						72	2.71	1.92	0.00	6.87
Coho						72	0.00	0.00	0.00	0.00
Sockeye						72	0.00	0.00	0.00	0.00
Pink						72	0.00	0.00	0.00	0.00
Chum						72	0.00	0.00	0.00	0.00
Steelhead						—	—	—	—	—
May										
Juvenile										
Chinook	40	0.37	0.24	0.00	0.90	85	2.41	1.25	0.53	5.27
Coho	40	0.14	0.10	0.00	0.37	85	0.74	0.65	0.00	2.10
Sockeye	40	1.29	0.63	0.20	2.72	85	2.54	1.32	0.37	5.42
Pink	40	0.44	0.30	0.00	1.16	85	1.81	1.25	0.00	4.45
Chum	40	0.32	0.31	0.00	1.02	85	0.80	0.59	0.01	2.39
Steelhead	40	2.50	2.32	0.00	7.35	—	—	—	—	—
Adult										
Chinook	40	2.78	1.67	0.21	6.65	85	0.67	0.68	0.00	2.04
Coho	40	0.20	0.11	0.02	0.44	85	0.28	0.27	0.00	0.82
Sockeye	40	0.14	0.15	0.00	0.46	85	0.00	0.00	0.00	0.00
Pink	40	0.12	0.11	0.00	0.34	85	0.02	0.02	0.00	0.05
Chum	40	0.07	0.07	0.00	0.21	85	0.00	0.00	0.00	0.00
Steelhead	40	0.97	0.95	0.00	3.06	—	—	—	—	—
June										
Juvenile										
Chinook	70	5.27	1.89	2.01	9.28	45	1.85	0.71	0.65	3.41
Coho	70	4.22	1.47	1.68	7.40	45	1.08	0.62	0.10	2.50
Sockeye	70	1.04	0.41	0.32	1.95	45	6.35	3.15	0.80	13.19
Pink	70	2.01	1.00	0.52	4.22	45	0.68	0.31	0.15	1.34
Chum	70	1.13	0.53	0.26	2.29	45	1.64	1.17	0.00	4.42
Steelhead	70	0.58	0.42	0.07	1.52	—	—	—	—	—
Adult										
Chinook	70	0.55	0.25	0.15	1.12	45	2.21	2.16	0.00	6.60
Coho	70	0.31	0.20	0.06	0.75	45	0.00	0.00	0.00	0.00
Sockeye	70	0.07	0.04	0.00	0.16	45	0.00	0.00	0.00	0.00
Pink	70	0.14	0.08	0.02	0.32	45	0.00	0.00	0.00	0.00
Chum	70	0.08	0.05	0.01	0.18	45	0.00	0.00	0.00	0.00
Steelhead	70	1.07	0.85	0.00	3.11	—	—	—	—	—
July										
Juvenile										
Chinook	101	4.95	1.52	2.31	8.19	60	2.02	1.39	0.32	5.20
Coho	101	3.16	1.41	0.85	6.22	60	4.98	2.39	0.81	10.12
Sockeye	101	1.52	0.36	0.89	2.26	60	2.82	1.30	0.91	5.76
Pink	101	0.55	0.17	0.25	0.92	60	3.98	2.31	0.47	8.91
Chum	101	0.00	0.00	0.00	0.01	60	1.94	1.71	0.00	5.67
Steelhead	101	0.06	0.04	0.00	0.16	—	—	—	—	—
Adult										
Chinook	101	3.68	1.36	1.26	6.51	60	6.39	2.98	1.46	12.46
Coho	101	0.04	0.02	0.01	0.08	60	0.05	0.03	0.00	0.12
Sockeye	101	25.14	4.09	17.05	33.14	60	2.72	1.61	0.42	6.38
Pink	101	0.53	0.22	0.16	1.02	60	0.27	0.14	0.06	0.58
Chum	101	0.02	0.02	0.00	0.05	60	0.45	0.36	0.00	1.23
Steelhead	101	0.02	0.01	0.00	0.04	—	—	—	—	—

Table 2 (*concluded*).

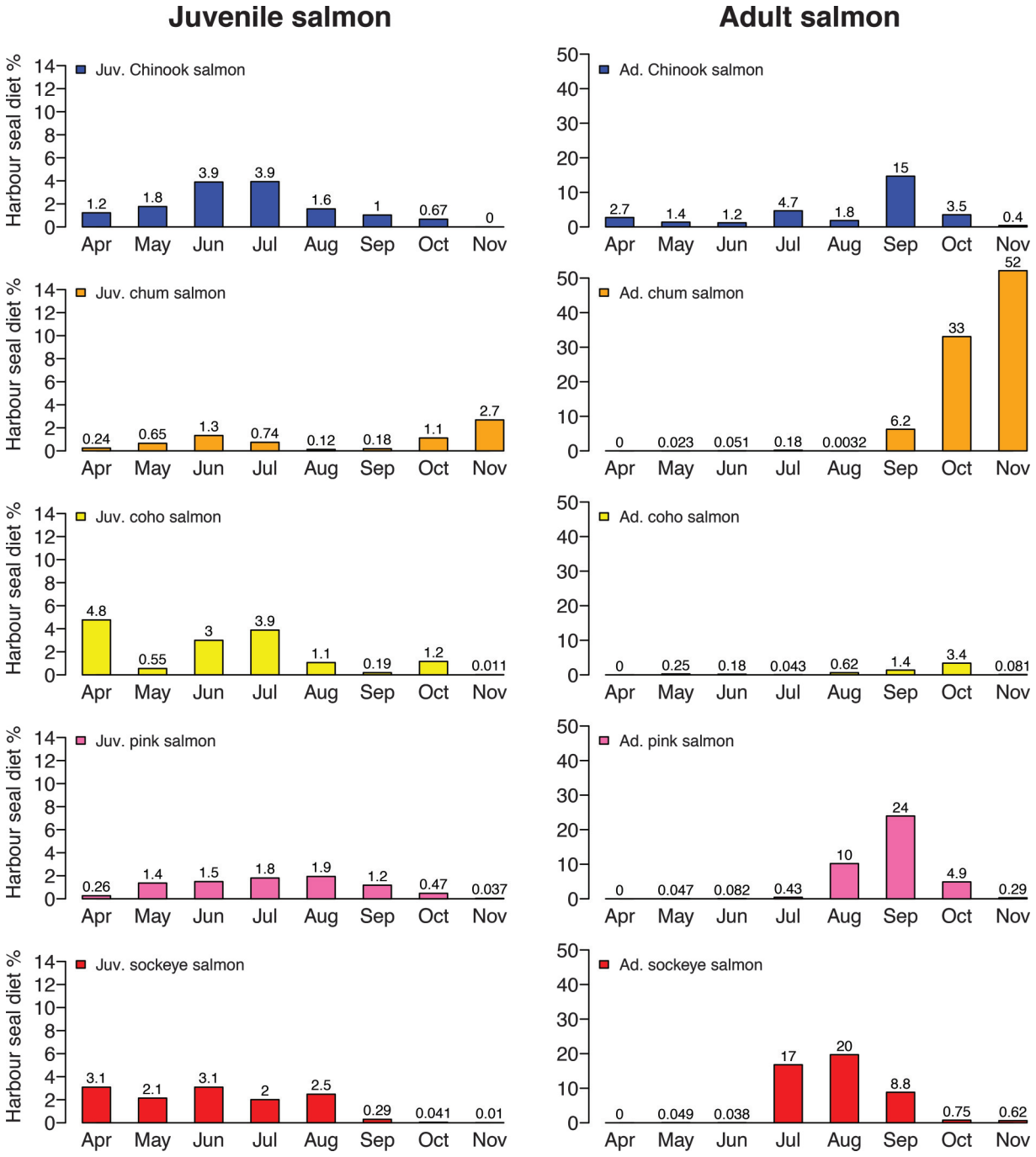
	2012					2013				
	n	Mean	SD	2.5%	97.5%	*n*	Mean	SD	2.5%	97.5%
August										
Juvenile										
Chinook	51	0.17	0.07	0.06	0.31	55	2.84	1.73	0.27	6.64
Coho	51	0.03	0.02	0.00	0.08	55	2.04	1.74	0.00	6.93
Sockeye	51	2.50	1.07	0.73	4.92	55	2.50	0.99	0.84	4.67
Pink	51	2.04	1.25	0.17	4.64	55	1.84	0.93	0.47	4.05
Chum	51	0.01	0.00	0.00	0.02	55	0.23	0.24	0.00	0.69
Steelhead	51	0.01	0.00	0.00	0.02	—	—	—	—	—
Adult										
Chinook	51	3.08	1.96	0.37	7.47	55	0.66	0.36	0.12	1.45
Coho	51	0.09	0.06	0.00	0.22	55	1.16	1.00	0.00	3.21
Sockeye	51	23.51	5.16	13.87	34.05	55	15.93	4.32	8.29	25.22
Pink	51	6.85	2.69	2.35	12.69	55	13.37	3.92	6.13	21.36
Chum	51	0.01	0.00	0.00	0.02	55	0.00	0.00	0.00	0.00
Steelhead	51	0.01	0.01	0.00	0.02	—	—	—	—	—
September										
Juvenile										
Chinook	103	1.81	0.71	0.57	3.35	97	0.21	0.12	0.03	0.49
Coho	103	0.30	0.20	0.01	0.78	97	0.07	0.04	0.00	0.18
Sockeye	103	0.13	0.07	0.02	0.29	97	0.48	0.28	0.09	1.15
Pink	103	1.06	0.55	0.16	2.28	97	1.35	0.49	0.49	2.39
Chum	103	0.34	0.12	0.12	0.61	97	0.02	0.01	0.00	0.05
Steelhead	103	0.12	0.11	0.00	0.35	—	—	—	—	—
Adult										
Chinook	103	23.16	3.83	16.07	30.64	97	5.77	1.94	2.34	9.75
Coho	103	1.89	1.08	0.22	4.27	97	0.88	0.41	0.27	1.84
Sockeye	103	9.11	2.51	4.63	14.30	97	8.68	2.18	4.53	13.11
Pink	103	2.22	1.11	0.46	4.72	97	47.03	4.50	38.18	55.66
Chum	103	11.95	2.94	6.59	17.94	97	0.17	0.13	0.00	0.46
Steelhead	103	0.20	0.19	0.00	0.59	—	—	—	—	—
October										
Juvenile										
Chinook	119	1.02	0.50	0.19	2.14	66	0.05	0.04	0.00	0.13
Coho	119	1.09	0.57	0.22	2.39	66	1.34	1.38	0.00	4.11
Sockeye	119	0.06	0.05	0.00	0.17	66	0.00	0.00	0.00	0.00
Pink	119	0.64	0.37	0.09	1.49	66	0.17	0.10	0.03	0.39
Chum	119	0.90	0.40	0.25	1.75	66	1.52	1.26	0.05	4.18
Steelhead	119	0.00	0.00	0.00	0.00	—	—	—	—	—
Adult										
Chinook	119	4.09	1.42	1.65	7.06	66	2.55	1.41	0.62	5.59
Coho	119	2.30	0.93	0.67	4.26	66	5.33	2.11	1.79	9.87
Sockeye	119	1.16	0.84	0.05	3.02	66	0.00	0.00	0.00	0.00
Pink	119	4.45	1.44	1.84	7.43	66	5.60	1.68	2.72	9.21
Chum	119	32.26	3.96	24.88	40.34	66	34.13	4.79	25.44	43.56
Steelhead	119	0.00	0.00	0.00	0.00	—	—	—	—	—
November										
Juvenile										
Chinook	33	0.00	0.00	0.00	0.00	16	0.00	0.00	0.00	0.00
Coho	33	0.00	0.00	0.00	0.00	16	0.04	0.03	0.00	0.10
Sockeye	33	0.01	0.01	0.00	0.05	16	0.00	0.00	0.00	0.00
Pink	33	0.00	0.00	0.00	0.01	16	0.10	0.10	0.00	0.31
Chum	33	1.53	1.48	0.00	4.72	16	5.11	3.61	0.11	13.18
Steelhead	33	0.00	0.00	0.00	0.00	—	—	—	—	—
Adult										
Chinook	33	0.57	0.56	0.00	1.77	16	0.00	0.00	0.00	0.00
Coho	33	0.00	0.00	0.00	0.00	16	0.25	0.16	0.00	0.63
Sockeye	33	0.89	0.61	0.04	2.24	16	0.00	0.00	0.00	0.00
Pink	33	0.23	0.12	0.00	0.52	16	0.44	0.41	0.00	1.25
Chum	33	66.86	7.01	52.56	79.61	16	21.90	9.12	5.85	40.67
Steelhead	33	0.00	0.00	0.00	0.00	—	—	—	—	—

Note: In April 2012, insufficient samples were collected to generate diet averages; therefore, data are not shown.

study, global diet summaries are also shown for each sampling stratum calculated using both prey hard parts SSFO percentage and prey DNA sequences percentage (Table S2[1]). Not all samples collected in each stratum produced sufficient prey DNA or hard parts information, so tabulated sample sizes indicate the number of samples that contributed to diet summary calculations.

When assigning salmon species DNA percentages to either adult or juvenile life stage, it was important to note which source

Fig. 3. Monthly amounts (%) of juvenile (left) and adult (right) salmon species present in harbour seal scats collected at haulouts in estuaries (2012–2013). Species and percentages were determined using DNA sequencing, and life stages were determined from a salmon hard parts decision tree analysis. Data represent means for all estuary sites and both years combined.

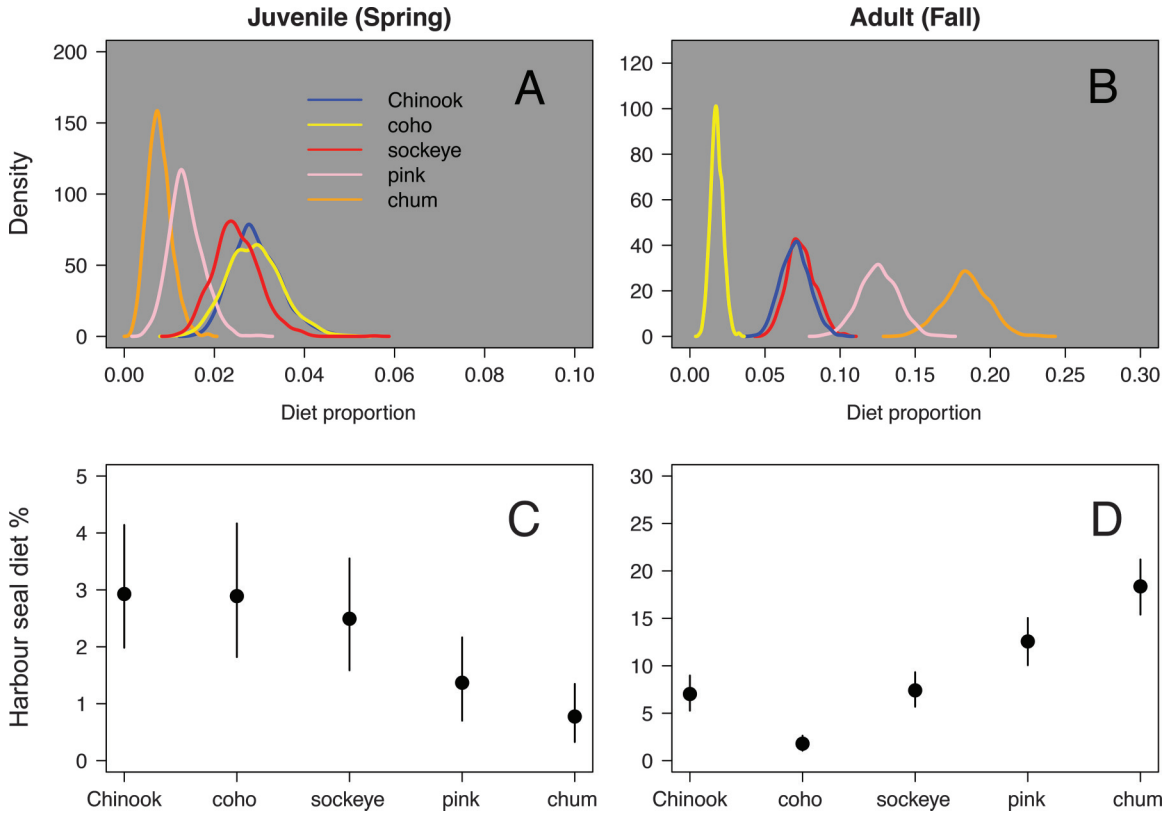


of information was used to generate the juvenile/adult salmon ratio. Of the 756 samples that produced salmon DNA sequences, 419 (55.4%) were assigned to a life stage using the salmon sample SSFO ratio (i.e., the same scat samples contained identified salmon hard parts). Another 285 (37.7%) samples did not contain salmon hard parts, but were assigned to life stage based on the salmon monthly SSFO ratio because greater than three samples from the same location–month contained salmon hard parts. Only 52 (6.9%) samples were assigned to salmon life stage based on the fixed season ratio. Salmon species assignment to life stage was therefore informed by salmon hard parts data for 93% of samples containing salmon DNA.

Life-stage-specific harbour seal diet percentages for salmon species resulted in clear seasonal trends in harbour seal salmon predation, pooling samples across years and all three estuary sites (Fig. 3). For clarity, references to salmon "species" in this manuscript indicate the subpopulations of those species that return to Strait of Georgia rivers to spawn. Only a small number of samples were obtained from the non-estuary site (Belle Chain) in the spring season — therefore, graphical data representations shown are aggregates of only samples collected from the three estuary sampling sties (Figs. 3, 4, and 5).

Adult salmon predation by harbour seals was primarily focused on chum (*Oncorhynchus keta*), pink (*Oncorhynchus gorbuscha*), and

Fig. 4. (A, B) Kernel density plots of the mean harbour seal diet proportion from bootstrap replicates for Chinook (blue), coho (yellow), sockeye (red), pink (pink), and chum (orange) salmon. (C, D) Mean diet percentage (circles) with 95% confidence intervals generated from bootstrapping methods. The first column (panels A and C) displays juvenile salmon consumed by seals in the spring (April–July), while the second column (panels B and D) indicates adult salmon eaten in the fall (August–November). Data represent means for all estuary sites and both years combined. [Colour online.]



sockeye (*Oncorhynchus nerka*) salmon, with different species peaking in different months and roughly matching the adult return timing of the different species (Fig. 3). With the exception of low-level consumption of Chinook in the spring, predation on adults initiated with sockeye in July, which peaked in August (20%) and diminished in September. Sockeye consumption was followed by pink salmon predation, which peaked in September (24%) and diminished in October. Chum salmon was the last and most important salmon species in harbour seal diet, beginning in September and peaking in November (52%). Although far less pronounced, two peaks were observed in adult Chinook predation, with a small peak in July (4.7%) and a larger peak in September (15%). Adult coho salmon was a surprisingly small component of the overall seal diet, peaking at 3.4% in October.

Seasonal trends in juvenile salmon predation by seals were less defined, but the importance of salmon juveniles in harbour seal diet varied largely between salmon species. In contrast with adult salmon predation, juvenile coho comprised a relatively large component of harbour seal diet in the spring, with peaks at 4.8% of seal diet biomass in April and 3.9% in July (Figs. 3 and 4). Juvenile Chinook salmon was also an important diet species, with a combined peak in June and July at 3.9%. Sockeye and pink juvenile salmon predation was consistent throughout the spring, with no clearly defined peaks in predation at the aggregate scale. Also in contrast with adult salmon predation, chum salmon was the least important juvenile salmon species in seal diets (Fig. 4).

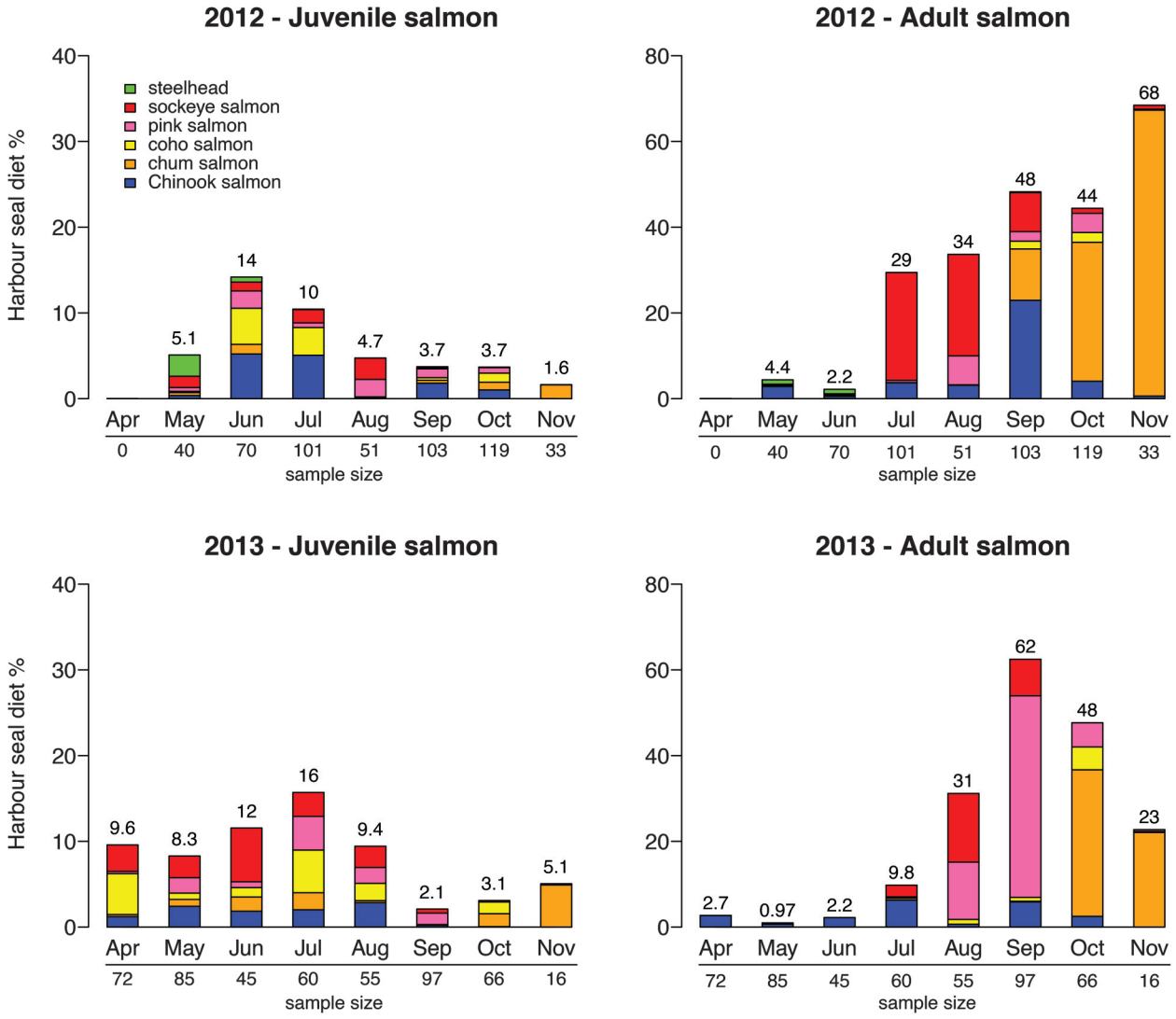
Significance tests via bootstrapping methods also indicate that harbour seals consumed significantly greater quantities of juvenile Chinook, coho, and sockeye salmon in the spring season than they did pink or chum salmon (Fig. 4; Table 3). The percentages of juvenile Chinook, coho, and sockeye in harbour seal diet did not differ significantly from one another at seasonal aggregate level, nor did the percentages of pink and chum salmon differ. In the fall season, the percentages of adult salmon in harbour seal diet differed significantly between species in all comparisons, with the exception of Chinook and sockeye, which did not significantly differ when both years were combined (Table 3).

Table 3. Statistical comparisons (S = significant, NS = nonsignificant) by bootstrapping of seal diet percentages composed of (*i*) juvenile salmon in the spring and (*ii*) adult salmon in the fall between salmon species.

	Chinook	Coho	Sockeye	Chum	Pink
Juvenile salmon % (spring)					
Chinook	—				
Coho	NS	—			
Sockeye	NS	NS	—		
Chum	S	S	S	—	
Pink	S	S	S	NS	—
Adult salmon % (fall)					
Chinook	—				
Coho	S	—			
Sockeye	NS	S	—		
Chum	S	S	S	—	
Pink	S	S	S	S	—

In addition to seasonal variability, we observed marked interannual variability in harbour seal salmon predation between 2012 and 2013 (Fig. 5). In 2012 for example, adult sockeye salmon was more important in seal diets (July = 25%, August = 24%) than it was in 2013 when adult sockeye peaked at 16%. Additionally, the per-

Fig. 5. Percentages of salmon (steelhead, sockeye, pink, coho, chum, and Chinook) by life stage (juvenile or adult) in the diets of harbour seals using estuary haulouts in 2012 and 2013. Diets were determined by month, and sample size indicates the number of scats collected each month. Differences in salmon species consumed between years reflect differences in year class strengths and life histories of the different salmonid species. Note that steelhead were only detectable in 2012 when the secondary (cytochrome oxidase I) salmon-specific DNA marker was used. [Colour online.]



centage of adult pink salmon in seal diet was far greater in 2013 (September = 47%) than it was in 2012 (September = 2.2%) and appeared to be inversely related to the percentage of adult Chinook salmon in the seal diet (i.e., the year with high pink salmon predation had low Chinook predation). Juvenile salmon predation by seals varied between years as well — coho and Chinook predation, for example, both peaked in June and July of 2012, but they did not exhibit the same unimodal pattern in 2013. Large differences were also detected in the percentage of juvenile sockeye consumed between years (Fig. 5).

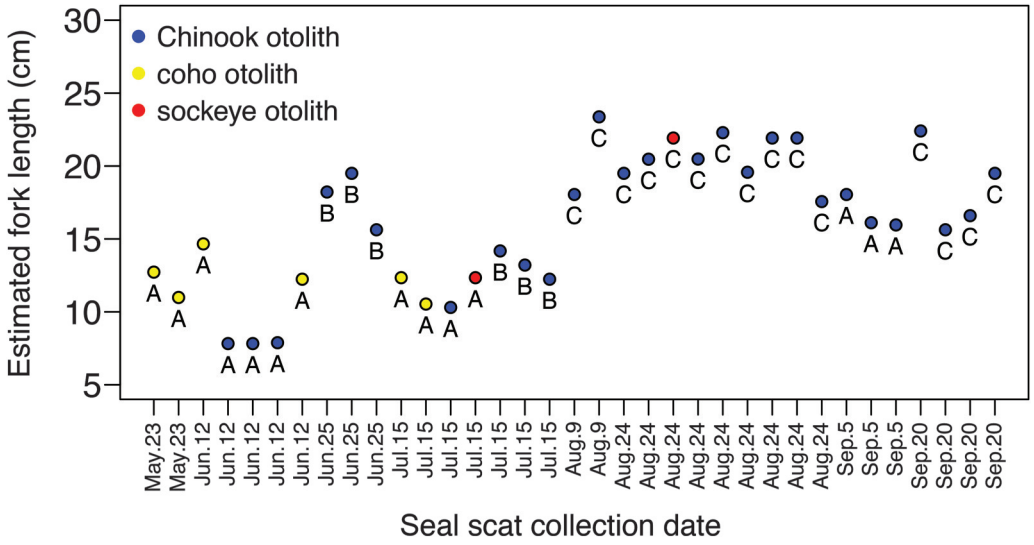
Of the 433 salmon otoliths recovered from harbour seal scats, 363 (84%) were graded as "poor" due to digestion erosion and could not be used to estimate fish lengths. The remaining salmon otoliths were paired to represent a minimum number of individual fish, and fork lengths of juveniles were estimated for 35 salmon otoliths identified to a species (Fig. 6). As stated, many juvenile salmon otoliths were too eroded to measure, and many more were likely completely digested. However, assuming these 35 otoliths are an unbiased representation of the juvenile salmon consumed, our results indicate that harbour seals consumed salmon juveniles between 7.8 and 23.4 cm (fork length). During spring (April–July), harbour seals primarily consumed juvenile salmon between 10 and 15 cm, and in the fall (August–November) seals targeted juveniles between 15 and 25 cm. The majority of identifiable otoliths ($N = 27$) were Chinook salmon, but coho and sockeye otoliths were also identified.

The non-salmonid portion of harbour seal diets was composed primarily of herring and gadoid species, consistent with previous harbour seal diet studies in the region (Table S2[1]). Several other prey species are worth noting because they contributed substantially (>2%) to the overall harbour seal diet in one of the two seasons (all estuary sites and years combined). Three-spined stickleback (*Gasterosteus aculeatus*) comprised 7.5% of overall seal diet in the spring season, and eulachon (*Thaleichthys pacificus*) comprised 5.5% of the spring diet as well. In the fall season, shiner perch (*Cymatogaster aggregata*) made up 5.3% of overall seal diet, and Pacific staghorn sculpin (*Leptocottus armatus*) contributed 3.5% (Table S2[1]).

Discussion

We applied combined DNA metabarcoding diet analysis and morphological prey hard parts analysis to 1240 harbour seal scat

Fig. 6. Estimated fork lengths of juvenile salmon (Chinook, coho, and sockeye) derived from otoliths recovered in seal scats that were not too eroded to measure. Colors indicate the species of salmon based on morphological ID, and the letters identify where the seal scat was collected (A = Comox, B = Cowichan Bay, C = Belle Chain). [Colour online.]



samples collected from haulout sites in the Strait of Georgia, Canada. A novel decision tree method was used to estimate the species and life stages of salmon consumed by seals, creating a data set useful for age-specific predator–prey modeling. The vast majority of samples containing salmon (93.1%) were assigned to a life stage (juvenile or adult) based on the co-occurrence of salmon bones in the sample or collection month. The combined data set indicates that harbour seals primarily consume adult salmon of lesser conservation concern species in the fall season (i.e., chum and pink salmon). However, seals appeared to target larger-bodied juvenile salmon that are of greater conservation concern (i.e., coho, Chinook, and sockeye) in the spring, despite an exceeding abundance of juvenile chum salmon in the region (Beamish et al. 2012*a*).

Combining DNA and hard parts diet data

Our first objective in this study was to establish a new diet analysis method that could estimate the life stage, species, and relative proportions of salmon in pinniped diets. Pinniped diet analysis using DNA metabarcoding is a relatively new technique that offers several advantages over previous molecular diet analysis tools (Clare 2014; Deagle et al. 2009; Symondson and Harwood 2014). Prior studies have mostly relied on species-specific primer sets to identify pinniped prey — often limiting DNA identification to only a subset of prey taxa and (or) requiring a mathematical model to convert prey occurrences into diet percentages (Parsons et al. 2005; Purcell et al. 2004; Tollit et al. 2009). Conversely, DNA metabarcoding diet analysis employs universal primers to simultaneously amplify many (if not all) prey species, relying on high-throughput DNA amplicon sequencing to identify and quantify prey. Furthermore, large numbers of individual samples can now be sequenced simultaneously and differentiated using bioinformatic techniques, dramatically reducing the per sample cost of DNA diet analysis.

Proportional estimates of predator global diet are important for calculating estimates of prey consumption, such as the numbers of individual fish eaten by a pinniped population (Howard et al. 2013; Olesiuk 1993; Winship and Trites 2003). In our study, prey DNA sequence percentages were averaged from large numbers of individual seal scat samples to calculate population-level diet percentages. This approach generally assumes a quantitative relationship between DNA sequence read proportions from seal scat samples and the overall biomass proportions of prey consumed by the seal population. Captive feeding studies with pinnipeds and other marine predators have indicated that the relationship between prey DNA sequence percentage and prey biomass is not linear, but most studies have ultimately concluded that DNA metabarcoding can be treated as a semiquantitative tool (Deagle et al. 2010; Pompanon et al. 2012; Thomas et al. 2014). In addition, studies such as ours that aim to characterize the diets of consumer populations appear to be less influenced by quantification biases than studies focused on the diets of individual animals (Thomas et al. 2016). The accuracy of our harbour seal DNA diet estimates could likely be improved in the future by creating a complete harbour seal prey library of tissue mix standards and applying species-specific correction factors (Thomas et al. 2016).

Merging harbour seal DNA diet data with prey hard parts information enabled us to estimate the proportion of seal diet contributed by adult and juvenile salmon, in addition to identifying the salmon species consumed. To our knowledge, these are the first such estimates generated from pinniped scat samples. The method we created to assign salmon species to life stage relies initially on the co-occurrence of salmon bones in the individual scat, then on occurrences of salmon bones in scats collected from the same site and month. As a last resort, assignments are made based on a fixed seasonal ratio of adults to juvenile salmon. Although this design prioritizes the best available information to assign salmon life stage and represents a major methodological advancement, the method also likely produces certain data artifacts. For example, juvenile pink salmon are an unlikely diet item in July and August of 2013 because the number of pink salmon that spawn in even-numbered years is very low. Similarly, it is not probable that seals consume appreciable numbers of juvenile chum salmon in November when the adult chum salmon are spawning. These occurrences are likely an incorrect assignment of salmon DNA to the juvenile life stage as a result of juvenile salmon bones co-occurring in seal scats that contained adult salmon DNA.

However, three pieces of evidence support the appropriateness of our method for assigning a life stage to salmon species in harbour seal diets. First, 93% of samples containing salmon DNA were assigned based on salmon bone occurrences in the same scat sample or in scat samples collected in the same location and month. Only 7% of salmon samples relied on the fixed season ratio to assign salmon life stage. Second, the fixed season ratio (assuming juvenile salmon consumption in spring and adult salmon con-

sumption in fall) is generally supported by the occurrences of adult and juvenile salmon bones in those seasons (Fig. S2[1]). The only major exception to this assumption was the occurrence of adult sockeye salmon in July in the Fraser River estuary; adults of all other species were primarily consumed in what we defined as the fall season. The final piece of evidence supporting our salmon life stage assignment protocol is the fact that the resulting estimates of harbour seal diet demonstrate a clear functional response by seals to the seasonal abundances of salmon species, which corresponds well with the movements of adult and juvenile salmonids in the Strait of Georgia (Quinn 2005).

Salmon in harbour seal diet

The second objective of our study was to apply our combined diet analysis method in a system where knowledge of the salmon species and life stages consumed by seals may have important management implications. Salmon stocks in the Strait of Georgia have substantial economic, cultural, and recreational value, and the sustainable management of salmon resources in British Columbia is a high priority (Cohen 2012). Unfortunately, Chinook and coho salmon populations in the Strait of Georgia have experienced a long-term decline in smolt-to-adult survival — a pattern that has been linked to high mortality rates in the first 4 months after smolt ocean entry (Beamish et al. 2010; Neville et al. 2015). The survival patterns of these populations also appear to be moderately independent of the coast-wide species trends, suggesting that local factors specific to the basin scale are likely driving variability in Chinook and coho survival (Zimmerman et al. 2015). In contrast, chum and pink salmon populations in the Strait of Georgia have increased over the same period, indicating that these regional forces are not impacting all salmon species in the same way (Irvine et al. 2014). The search for a causal mechanism(s) explaining the marine survival patterns of Chinook and coho salmon in the Strait of Georgia (Salish Sea) is the focus of a major transboundary research effort (Riddell et al. 2009).

Based on retrospective analysis, the factors likely to be driving regional survival patterns of Chinook and coho salmon should have the following characteristics: (1) have changed substantially during the period of declining Chinook and coho survival, (2) have a greater influence in the Strait of Georgia than in other nearby coastal areas, (3) cause mortality in the first 4 months after smolt ocean entry, and (4) disproportionately impact Chinook and coho compared with other salmon species such as chum and pink salmon (Beamish et al. 2010; Neville et al. 2015; Zimmerman et al. 2015). While it is known that harbour seals increased exponentially in the Strait of Georgia during the period of declining Chinook and coho marine survival (meeting criteria 1 and 2) (Olesiuk 2009), methodological limitations have made it difficult to assess whether seals consume juvenile salmon in the first 4 months after ocean entry, or if they impact certain salmon species more than others.

Our data indicate that the adult salmon primarily consumed by harbour seals in the Strait of Georgia estuaries were not the species currently of greatest conservation concern (Irvine et al. 2009; Welch et al. 2011). Seals mostly targeted adult chum salmon in the fall, with pink and sockeye salmon also contributing substantially to seal diet in alternate years. Regional populations of pink and chum salmon appear to be thriving in spite of predation pressure on adults from harbour seals (Irvine et al. 2009; Irvine et al. 2014). Also interesting was the inverse interannual relationship in the percentage of adult Chinook salmon in seal diet relative to pink salmon in seal diet. Regional pink salmon runs are large in odd-numbered years and low in even-numbered years. It is possible there is a predation masking effect occurring, whereby the presence of many pink salmon in September reduces seal predation pressure on adult Chinook salmon (Evans 2008; Holling 1966). These results emphasize the importance of using diet techniques that can resolve prey to the species level when assessing potential impacts of predators on prey populations.

While the species composition of adult salmon eaten by harbour seals does not raise concern for salmon stocks, the composition of juvenile salmon species in seal diet displayed the opposite trend. Harbour seals consumed significantly higher percentages of juvenile coho, Chinook, and sockeye salmon in the Strait of Georgia compared with pink and chum salmon, even though regional trawl surveys indicate that juvenile chum salmon are available to seals in much higher abundance (Beamish et al. 2012*a*). This implies that harbour seals may be selecting juveniles of some salmon species over others (i.e., consuming disproportionately more fish than expected based on abundance; Manly et al. 1993). Positive selection often occurs when less abundant prey are for some reason more profitable (e.g., contain higher energy density or require less energy to capture) than the more abundant prey species (Stephens and Krebs 1986; Tollit et al. 1997).

Interestingly, all three of the juvenile salmon species consumed by seals in relatively high proportion (coho, Chinook, and sockeye) contain stocks that undergo seaward migration at ages >1 year. In contrast, the juvenile salmon species eaten by the seals in smaller proportions (pink and chum) all out-migrate at age <1 year (Quinn 2005; Randall et al. 1987). Harbour seals may be selecting for older, larger salmon smolts that are more profitable to pursue than chum and pink fry. In addition, a recent acoustic tagging study in nearby Puget Sound, Washington, found evidence of harbour seals targeting juvenile steelhead (a salmonid that also outmigrates at ages >1 year; Berejikian et al. 2016). These older juvenile salmonids may also better fit the prey search image of harbour seals in their physical appearance by being similar to the typical forage fish (Tollit et al. 1997). There is, however, some size overlap between stocks of juvenile Chinook and chum salmon in the Strait of Georgia during the summer months (Beamish et al. 2012*b*; Parker 1971); therefore, this apparent selectivity for juvenile coho and Chinook salmon may be driven by other factors such as fish energy density, distribution, or schooling behaviour that make them more favourable prey for harbour seals.

Although the percentages of juvenile salmon species in harbour seal diets were relatively small (generally <5% per species), such percentages can be significant when converted to numbers of fish — particularly when a large number of predators consume many small-bodied prey species. Using our April 2013 data, for example, ~40 000 harbour seals (the most recent population estimate in the Strait of Georgia; Olesiuk 2009) consuming a mean of 2 kg per day (Howard et al. 2013) would consume ~5.7 million coho smolts in one month (95% CI = 1.4–11.1 million), assuming the average hatchery coho smolt weighs 20 g, and seal diet is 4.8% juvenile coho (95% CI = 1.2%–9.3%; Table 2). Considerably more smolts could be consumed if the smolts were smaller (e.g., wild coho smolts). This simple example highlights the importance of obtaining age-specific predation information when attempting to assess the consumptive impacts of a large predator population. In-depth modeling will be required to produce robust estimates of harbour seal consumption of juvenile and adult salmon species in the Strait of Georgia based on the dietary data we generated (B.W. Nelson, A.C. Thomas, A.W. Trites, M.K. McAllister, and C.J. Walters, unpublished data).

The calculation above raises the question of whether the seal diet data collected in this study are representative of the greater Strait of Georgia harbour seal population. Our collections were focused primarily on estuarine seal haulout sites where interactions between seals and salmon may be much higher than in non-estuary habitats (Olesiuk 1993; Olesiuk et al. 1990). However, a satellite telemetry study found that harbour seals in this region make regular movements >20 km from their haulout site, indicating that seals occupying estuary sites likely also forage in non-estuary areas (Peterson et al. 2012). After incorporating the standard deviation of the median over-water distances between satellite loca-

tions and the capture site for tagged seals, the three estuary sites we sampled could easily be representative of harbour seal foraging in the majority of the Strait of Georgia (Fig. 1).

Further evidence that harbour seal foraging on salmon smolts is not isolated to estuary haulouts comes from our Belle Chain sampling site (a non-estuary harbour seal haulout). The single highest percentage of juvenile Chinook salmon in harbour seal diet for any stratum in our study was at Belle Chain, which peaked in August — much later in the season than would be expected of seals feeding on out-migrating smolts in estuaries. In addition, the most comprehensive recent harbour seal diet study in the region found that the frequency of occurrence of juvenile salmon bones in seal scats was 11.6% in the nearby San Juan Islands (non-estuary haulouts) during July–September (Lance et al. 2012) (Fig. 1). Comparing this to our estuary-focused data set during the same temporal window, we find that estuary seals actually have a lower frequency of occurrence value for juvenile salmon bones (9.9%) than seals in the San Juan Islands. Combined, this evidence suggests that our data set is likely representative of a large portion of the Strait of Georgia and may actually offer a conservative estimate of harbour seal juvenile salmon predation by being biased toward estuary haulout sites.

Prior to our study, no data were available to assess whether harbour seals are a major source of mortality for juvenile Chinook and coho salmon in the first 4 months after ocean entry or whether seal-related mortality is preferentially focused on certain salmon species. Based on the current data set, we can conclude that harbour seals are major predators of juvenile Chinook and coho salmon in the summer months and that seals appear to select for these two species while ignoring large numbers of juvenile chum salmon in the system. Thus, harbour seals meet all four of the criteria outlined above for a factor that may be driving regional survival patterns of Chinook and coho salmon. It is worth noting, however, that the stock composition of juvenile salmon in Strait of Georgia appears to change between spring and fall seasons, and not all stocks within a species have experienced the same survival patterns (Beamish et al. 2016; Neville et al. 2015). Ideally, future consumption estimates derived from our data set would account for such seasonal changes when estimating harbour seal impacts on salmon stocks in the Strait of Georgia.

Although we found evidence that harbour seals may be impacting Chinook and coho salmon populations, we recognize that such predation could simply be a symptom of a larger underlying problem facing salmon populations. For instance, seals may be consuming larger numbers of juvenile salmon in recent years because smolts are physically compromised by pathogens, contaminants, or poor food supply, implying that seals are only the proximate cause of mortality (Beamish et al. 2010; Godwin et al. 2015; O'Neill and West 2009; Tucker et al. 2016). Likewise, there is evidence of a major change in the Salish Sea food web indicated by a shift from high-energy forage fish to low-energy prey such as sticklebacks and jellyfish in Puget Sound (Greene et al. 2015). Harbour seals in some areas could be utilizing salmon resources that are supplemented by hatchery programs because their preferred prey (forage fish) are no longer available in historical abundance. Caution should therefore be exercised when drawing conclusions from studies such as ours, because there are many additional factors at work that can influence the apparent interactions between species.

Data accessibility

Individual scat sample data (i.e., proportional prey summaries) from DNA and hard parts techniques are available upon request from the authors.

Acknowledgements

We are grateful to the many volunteers who assisted with seal scat collections and laboratory processing during the 2 years of study. We thank Ilai Karen for assistance in merging the seal diet data sets and Brian McConeghy for refining the molecular laboratory protocols. We also thank Marc Trudel, Carl Walters, and Anthony Sinclair for providing useful feedback on several early drafts of this manuscript and the reviewers for their constructive comments and suggestions. The harbour seal scats were collected under Fisheries and Oceans Canada Marine Mammal Research License (MML 2011-10) and a University of British Columbia Animal Care Permit (A11-0072). This is Publication Number 4 from the Salish Sea Marine Survival Project (marinesurvivalproject.com). Funding was provided by the Pacific Salmon Foundation, the Pacific Salmon Commission's Southern Endowment Fund via Long Live the Kings, by Washington State with equal in-kind contributions by those participating in the research.

References

Beamish, R.J., Sweeting, R.M., Lange, K.L., Noakes, D.J., Preikshot, D., and Neville, C.M. 2010. Early marine survival of coho salmon in the Strait of Georgia declines to very low levels. Mar. Coast. Fish. Dyn. Manage. Ecosyst. Sci. **2**: 424–439. doi:10.1577/C09-040.1.

Beamish, R., Neville, C., Sweeting, R., and Lange, K. 2012*a*. The synchronous failure of juvenile Pacific salmon and herring production in the Strait of Georgia in 2007 and the poor return of sockeye salmon to the Fraser River in 2009. Mar. Coast. Fish. **4**(1): 403–414. doi:10.1080/19425120.2012.676607.

Beamish, R.J., Sweeting, R.M., Neville, C.M., Lange, K.L., Beacham, T.D., and Preikshot, D. 2012*b*. Wild Chinook salmon survive better than hatchery salmon in a period of poor production. Environ. Biol. Fishes, **94**(1): 135–148. doi:10.1007/s10641-011-9783-5.

Beamish, R.J., Neville, C.M., Sweeting, R.M., Beacham, T.D., Wade, J., and Li, L. 2016. Early ocean life history of Harrison River sockeye salmon and their contribution to the biodiversity of sockeye salmon in the Fraser River, British Columbia, Canada. Trans. Am. Fish. Soc. **145**(2): 348–362. doi:10.1080/00028487.2015.1123182.

Berejikian, B.A., Moore, M.E., and Jeffries, S.J. 2016. Predator–prey interactions between harbor seals and migrating steelhead trout smolts revealed by acoustic telemetry. Mar. Ecol. Progr. Ser. **543**: 21–35. doi:10.3354/meps11579.

Bowen, W., and Iverson, S. 2013. Methods of estimating marine mammal diets: a review of validation experiments and sources of bias and uncertainty. Mar. Mammal Sci. **29**(4): 719–754. doi:10.1111/j.1748-7692.2012.00604.x.

Buzzell, B., Lance, M.M., and Acevedo-Gutiérrez, A. 2014. Spatial and temporal variation in river otter (*Lontra canadensis*) diet and predation on rockfish (Genus *Sebastes*) in the San Juan Islands, Washington. Aquat. Mamm. **40**(2): 150–161. doi:10.1578/AM.40.2.2014.150.

Chiaradia, A., Forero, M.G., McInnes, J.C., and Ramírez, F. 2014. Searching for the true diet of marine predators: incorporating Bayesian priors into stable isotope mixing models. PLoS ONE, **9**(3): e92665. doi:10.1371/journal.pone.0092665. PMID:24667296.

Clare, E.L. 2014. Molecular detection of trophic interactions: emerging trends, distinct advantages, significant considerations and conservation applications. Evol. Appl. **7**(9): 1144–1157. doi:10.1111/eva.12225. PMID:25553074.

Clarke, M.R. 1986. A handbook for the identification of cephalopods beaks. Oxford University Press.

Cohen, B. 2012. Cohen Commission of inquiry into the decline of Sockeye Salmon in the Fraser River — Final Report. The uncertain future of Fraser River sockeye 1.

Deagle, B.E., Kirkwood, R., and Jarman, S.N. 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. Mol. Ecol. **18**(9): 2022–2038. doi:10.1111/j.1365-294X.2009.04158.x. PMID:19317847.

Deagle, B.E., Chiaradia, A., McInnes, J., and Jarman, S.N. 2010. Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out? Conserv. Genet. **11**(5): 2039–2048. doi:10.1007/s10592-010-0096-6.

Efron, B., and Tibshirani, R. 1993. An introduction to the bootstrap. Monographs on Statistics and Applied Probability (EUA)(57).

Evans, E.W. 2008. Multitrophic interactions among plants, aphids, alternate prey and shared natural enemies — a review. Eur. J. Entomol. **105**(3): 369–380. doi:10.14411/eje.2008.047.

Ford, M.J., Hempelmann, J., Hanson, M.B., Ayres, K.L., Baird, R.W., Emmons, C.K., Lundin, J.I., Schorr, G.S., Wasser, S.K., and Park, L.K. 2016. Estimation of a Killer Whale (*Orcinus orca*) population's diet using sequencing analysis of DNA from feces. PLoS ONE, **11**(1): e0144956. doi:10.1371/journal.pone.0144956. PMID:26735849.

Geiger, G.L., Atkinson, S., Waite, J.N., Blundell, G.M., Carpenter, J.R., and Wynne, K. 2013. A new method to evaluate the nutritional composition of marine mammal diets from scats applied to harbor seals in the Gulf of Alaska. J. Exp. Mar. Biol. Ecol. **449**: 118–128. doi:10.1016/j.jembe.2013.09.005.

Godwin, S.C., Dill, L.M., Reynolds, J.D., and Krkošek, M. 2015. Sea lice, sockeye salmon, and foraging competition: lousy fish are lousy competitors. Can. J. Fish. Aquat. Sci. **72**(7): 1113–1120. doi:10.1139/cjfas-2014-0284.

Greene, C., Kuehne, L., Rice, C., Fresh, K., and Penttila, D. 2015. Forty years of change in forage fish and jellyfish abundance across greater Puget Sound,

Washington (USA): anthropogenic and climate associations. Mar. Ecol. Progr. Ser. **525**: 153–170. doi:10.3354/meps11251.

Harvey, J.T., Loughlin, T.R., Perez, M.A., and Oxman, D.S. 2000. Relationship between fish size and otolith length for 63 species of fishes from the eastern North Pacific Ocean. NOAA Technical Report, NMFS 150.

Hastings, A. 1983. Age-dependent predation is not a simple process. I. Continuous time models. Theor. Popul. Biol. **23**(3): 347–362. doi:10.1016/0040-5809(83)90023-0.

Hastings, A. 1988. Food web theory and stability. Ecology, **69**: 1665–1668. doi:10.2307/1941143.

Holling, C.S. 1966. The functional response of invertebrate predators to prey density. Mem. Entomol. Soc. Can. **98**(S48): 5–86. doi:10.4039/entm9848fv.

Howard, S., Lance, M.M., Jeffries, S.J., and Acevedo-Gutiérrez, A. 2013. Fish consumption by harbor seals (*Phoca vitulina*) in the San Juan Islands, Washington. Fish. Bull. **111**(1): 27.

Irvine, J.R., Fukuwaka, M.-a., Kaga, T., Park, J.-H., Seong, K.B., Kang, S., Karpenko, V., Klovach, N., Bartlett, H., and Volk, E. 2009. Pacific salmon status and abundance trends. North Pacific Anadromous Fish Commission Document 1199.

Irvine, J.R., Michielsens, C.J.G., O'Brien, M., White, B.A., and Folkes, M. 2014. Increasing dominance of odd-year returning pink salmon. Trans. Am. Fish. Soc. **143**(4): 939–956.

Jarman, S.N., McInnes, J.C., Faux, C., Polanowski, A.M., Marthick, J., Deagle, B.E., Southwell, C., and Emmerson, L. 2013. Adélie penguin population diet monitoring by analysis of food DNA in scats. PLoS ONE, **8**(12): e82227. doi:10.1371/journal.pone.0082227. PMID:24358158.

Kashiwada, J., Recksiek, C.W., and Karpov, K.A. 1979. Beaks of the market squid, *Loligo opalescens*, as tools for predator studies. Calif. Coop. Ocean. Fish. Investig. Rep. **20**: 65–69.

Kelly, R.P., Port, J.A., Yamahara, K.M., and Crowder, L.B. 2014. Using environmental DNA to census marine fishes in a large mesocosm. PLoS ONE, **9**(1): e86175. doi:10.1371/journal.pone.0086175. PMID:24454960.

King, R.A., Read, D.S., Traugott, M., and Symondson, W.O.C. 2008. INVITED REVIEW: Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. **17**(4): 947–963. doi:10.1111/j.1365-294X.2007.03613.x. PMID:18208490.

Laake, J., Browne, P., DeLong, R.L., and Huber, H.R. 2002. Pinniped diet composition: a comparison of estimation models. Fish. Bull. **100**: 434–447.

Lance, M.M., Orr, A.J., Riemer, S.D., Weise, M.J., and Laake, J.L. 2001. Pinniped food habits and prey identification techniques protocol AFSC Processed Report 2001-04. Alaska Fisheries Science Center, National Marine Fisheries Service, Seattle, Wash.

Lance, M.M., Chang, W.Y., Jeffries, S.J., Pearson, S.F., and Acevedo-Gutiérrez, A. 2012. Harbor seal diet in northern Puget Sound: implications for the recovery of depressed fish stocks. Mar. Ecol. Progr. Ser. **464**: 257–271. doi:10.3354/meps09880.

Leray, M., Meyer, C.P., and Mills, S.C. 2015. Metabarcoding dietary analysis of coral dwelling predatory fish demonstrates the minor contribution of coral mutualists to their highly partitioned, generalist diet. PeerJ, **3**: e1047. doi:10.7717/peerj.1047. PMID:26137428.

Manly, B.F., McDonald, L., and Thomas, D. 1993. Resource selection by animals: statistical design and analysis for field studies. Chapman & Hall, London.

McCauley, E., Wilson, W.G., and de Roos, A.M. 1993. Dynamics of age-structured and spatially structured predator–prey interactions: individual-based models and population-level formulations. Am. Nat. **142**: 412–442. doi:10.1086/285547. PMID:19425984.

Méheust, E., Alfonsi, E., Le Ménec, P., Hassani, S., and Jung, J.-L. 2014. DNA barcoding for the identification of soft remains of prey in the stomach contents of grey seals (*Halichoerus grypus*) and harbour porpoises (*Phocoena phocoena*). Mar. Biol. Res. **11**(4): 385–395. doi:10.1080/17451000.2014.943240.

Melnychuk, M.C., Welch, D.W., and Walters, C.J. 2010. Spatio-temporal migration patterns of Pacific salmon smolts in rivers and coastal marine waters. PLoS ONE, **5**(9): e12916. doi:10.1371/journal.pone.0012916. PMID:20886121.

Moore, M.E., Berejikian, B.A., Goetz, F.A., Berger, A.G., Hodgson, S.S., Connor, E.J., and Quinn, T.P. 2015. Multi-population analysis of Puget Sound steelhead survival and migration behavior. Mar. Ecol. Progr. Ser. **537**: 217–232. doi:10.3354/meps11460.

Morrow, J.E. 1979. Preliminary keys to otoliths of some adult fishes of the Gulf of Alaska, Bering Sea, and Beaufort Sea. NOAA Technical Report NMFS Circular **420**: 40.

Neilson, J.D., and Geen, G.H. 1982. Otoliths of Chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. Can. J. Fish. Aquat. Sci. **39**(10): 1340–1347. doi:10.1139/f82-180.

Neville, C.M., Beamish, R.J., and Chittenden, C.M. 2015. Poor survival of acoustically-tagged juvenile chinook salmon in the Strait of Georgia, British Columbia, Canada. Trans. Am. Fish. Soc. **144**(1): 25–33. doi:10.1080/00028487.2014.954053.

Olesiuk, P.F. 1993. Annual prey consumption by harbour seals (*Phoca vitulina*) in the Strait of Georgia, British Columbia. Fish. Bull. **91**(3): 491–515.

Olesiuk, P. 2009. An assessment of population trends and abundance of harbour seals (*Phoca vitulina*) in British Columbia. DFO Canadian Science Advisory Secretariat Research Document 105.

Olesiuk, P.F., Bigg, M.A., Ellis, G.M., Crockford, S.J., and Wigen, R.J. 1990. An assessment of the feeding habits of harbour seals (*Phoca vitulina*) in the Strait of Georgia, British Columbia, based on scat analysis. Can. Tech. Rep. Fish. Aquat. Sci. **1730**: 135.

O'Neill, S.M., and West, J.E. 2009. Marine distribution, life history traits, and the accumulation of polychlorinated biphenyls in Chinook salmon from Puget Sound, Washington. Trans. Am. Fish. Soc. **138**(3): 616–632. doi:10.1577/T08-003.1.

Orr, A.J., Laake, J.L., Dhruw, M.I., Banks, A.S., DeLong, R.L., and Huber, H.R. 2003. Comparison of processing pinniped scat samples using a washing machine and nested sieves. Wildl. Soc. Bull. **31**(1): 253–257.

Parker, R.R. 1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. J. Fish. Res. Board Can. **28**(10): 1503–1510. doi:10.1139/f71-231.

Parsons, K.M., Piertney, S.B., Middlemas, S.J., Hammond, P.S., and Armstrong, J.D. 2005. DNA-based identification of salmonid prey species in seal faeces. J. Zool. **266**(3): 275–281. doi:10.1017/S0952836905006904.

Peterson, S.H., Lance, M.M., Jeffries, S.J., and Acevedo-Gutiérrez, A. 2012. Long distance movements and disjunct spatial use of harbor seals (*Phoca vitulina*) in the inland waters of the Pacific Northwest. PLoS ONE, **7**(6): e39046. doi:10.1371/journal.pone.0039046. PMID:22723925.

Phillips, E.M., and Harvey, J.T. 2009. A captive feeding study with the Pacific harbor seal (*Phoca vitulina richardii*): implications for scat analysis. Mar. Mammal Sci. **25**(2): 373–391. doi:10.1111/j.1748-7692.2008.00265.x.

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., and Taberlet, P. 2012. Who is eating what: diet assessment using next generation sequencing. Mol. Ecol. **21**(8): 1931–1950. doi:10.1111/j.1365-294X.2011.05403.x. PMID:22171763.

Purcell, M., Mackey, G., LaHood, E., Huber, H., and Park, L. 2004. Molecular methods for the genetic identification of salmonid prey from Pacific harbor seal (*Phoca vitulina richardsi*) scat. Fish. Bull. **102**(1): 213–220.

Quinn, T.P. 2005. The behavior and ecology of Pacific salmon and trout. University of Washington Press.

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2012. ISBN 3-900051-07-0.

Randall, R.G., Healey, M.C., and Dempson, J.B. 1987. Variability in length of freshwater residence of salmon, trout, and char. Am. Fish. Soc. Symp. **1**: 27–41.

Riddell, B., Pearsall, I., Beamish, R., Devlin, B., Farrell, A., McFarlane, S., Miller-Saunders, K., Tautz, A., Trites, A., and Walters, C. 2009. Strait of Georgia Chinook and coho proposal, Vancouver, B.C., Canada.

Roff, D.A. 2006. Introduction to computer-intensive methods of data analysis in biology. Cambridge University Press.

Stephens, D.W., and Krebs, J.R. 1986. Foraging theory. Princeton University Press, Princeton, N.J.

Symondson, W.O.C., and Harwood, J.D. 2014. Special issue on molecular detection of trophic interactions: Unpicking the tangled bank. Mol. Ecol. **23**(15): 3601–3604. doi:10.1111/mec.12831. PMID:25051891.

Taberlet, P., Coissac, E., Hajibabaei, M., and Rieseberg, L.H. 2012. Environmental DNA. Mol. Ecol. **21**(8): 1789–1793. doi:10.1111/j.1365-294X.2012.05542.x. PMID:22486819.

Thomas, A.C., Jarman, S.N., Haman, K.H., Trites, A.W., and Deagle, B.E. 2014. Improving accuracy of DNA diet estimates using food tissue control materials and an evaluation of proxies for digestion bias. Mol. Ecol. **23**(15): 3706–3718. doi:10.1111/mec.12523. PMID:24102760.

Thomas, A.C., Deagle, B.E., Eveson, J.P., Harsch, C.H., and Trites, A.W. 2016. Quantitative DNA metabarcoding: improved estimates of species proportional biomass using correction factors derived from control material. Mol. Ecol. Resour. **16**(3): 714–726. doi:10.1111/1755-0998.12490. PMID:26602877.

Tollit, D., Greenstreet, S.P.R., and Thompson, P.M. 1997. Prey selection by harbour seals, *Phoca vitulina*, in relation to variations in prey abundance. Can. J. Zool. **75**(9): 1508–1518. doi:10.1139/z97-774.

Tollit, D., Heaslip, S., Zeppelin, T., Joy, R., Call, K., and Trites, A. 2004. A method to improve size estimates of walleye pollock (*Theragra chalcogramma*) and Atka mackerel (*Pleurogrammus monopterygius*) consumed by pinnipeds: digestion correction factors applied to bones and otoliths recovered in scats. Fish. Bull. **102**: 498–508.

Tollit, D., Heaslip, S., Deagle, B., Iverson, S., Joy, R., Rosen, D., and Trites, A. 2006. Estimating diet composition in sea lions: which technique to choose. Sea lions of the world. Alaska Sea Grant College Program, University of Alaska, Fairbanks, Alaska. pp. 293–308.

Tollit, D.J., Schulze, A.D., Trites, A.W., Olesiuk, P.F., Crockford, S.J., Gelatt, T.S., Ream, R.R., and Miller, K.M. 2009. Development and application of DNA techniques for validating and improving pinniped diet estimates. Ecol. Appl. **19**(4): 889–905. doi:10.1890/07-1701.1. PMID:19544732.

Tollit, D., Pierce, G., Hobson, K., Bowen, W.D., and Iverson, S. 2010. Diet. Marine Mammal Ecology and Conservation: A handbook of techniques. Oxford University Press, Oxford, UK. pp. 165–190.

Trites, A.W., and Joy, R. 2005. Dietary analysis from fecal samples: how many scats are enough? J. Mammal. **86**(4): 704–712. doi:10.1644/1545-1542(2005)086[0704:DAFFSH]2.0.CO;2.

Tucker, S., Hipfner, J.M., and Trudel, M. 2016. Size- and condition-dependent predation: a seabird disproportionately targets substandard individual juvenile salmon. Ecology, **97**(2): 461–471. PMID:27145620.

Walters, C.J., and Martell, S.J. 2004. Fisheries ecology and management. Princeton University Press.

Welch, D.W., Melnychuk, M.C., Payne, J.C., Rechisky, E.L., Porter, A.D., Jackson, G.D., Ward, B.R., Vincent, S.P., Wood, C.C., and Semmens, J. 2011. *In situ* measurement of coastal ocean movements and survival of juvenile Pacific salmon. Proc. Natl. Acad. Sci. U.S.A. **108**(21): 8708–8713. doi:10.1073/pnas.1014044108. PMID:21558442.

Werner, E.E., and Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size-structured populations. Annu. Rev. Ecol. Syst. **15**: 393–425. doi:10.1146/annurev.es.15.110184.002141.

Winship, A.J., and Trites, A.W. 2003. Prey consumption of Steller sea lions (*Eumetopias jubatus*) off Alaska: how much prey do they require? Fish. Bull. **101**: 147–167.

Wolff, G. 1982. A beak key for 8 eastern tropical pacific cephalopod species with relationships between their beak dimensions and size. Fish. Bull. **80**(2): 357–370.

Zimmerman, M.S., Irvine, J.R., O'Neill, M., Anderson, J.H., Greene, C.M., Weinheimer, J., Trudel, M., and Rawson, K. 2015. Spatial and temporal patterns in smolt survival of wild and hatchery coho salmon in the Salish Sea. Mar. Coast. Fish. **7**(1): 116–134. doi:10.1080/19425120.2015.1012246.