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DNA analysis of scats reveals spatial and temporal structure in the diversity of harbour seal diet from local haulouts to oceanographic bioregions

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ABSTRACT: Predation shapes ecosystems, and quantifying the impacts of predation on the distribution and abundance of prey requires substantial effort at appropriate spatial and temporal scales for diet estimation of predators. Here, we present diet estimates of a marine predator (harbour seal *Phoca vitulina richardsi*) from scat collections (n = 3420) sampled at multiple haulout sites (n = 64) in the Strait of Georgia and other coastal regions around Vancouver Island, British Columbia, Canada, between 2015 and 2019. DNA metabarcoding and hard part analysis were used to identify the proportion and sizes of prey species consumed, respectively. We found that harbour seals consumed 62 primary prey species. Diversity in the diet was highly affected by the number of samples collected and varied at small spatial scales (haulout sites) as well as among broad bioregions. Three to 5 species dominated the diet depending on location, season, and year, including Pacific hake *Merluccius productus*, Pacific herring *Clupea pallasii*, and walleye pollock *Gadus chalcogrammus*. Within the Strait of Georgia, both male and female harbour seals consumed more hake and walleye pollock in areas and seasons in which they were more abundant. Harbour seals consumed a wide size range of prey that also varied by species, season, and region. These results indicate that harbour seal foraging is influenced by both the local abundance and composition of prey. Attempts to model the impact of predation on species of concern risk bias in their estimates and underrepresenting uncertainty if spatial and temporal variation in the diet is not accounted for.

KEY WORDS: Harbor seal · DNA Metabarcoding diet analysis · Diet diversity · Species co-occurrence

1. INTRODUCTION

Diet is a critical link between an individual's energetic and nutritional fitness requirements, and species' impacts on ecosystems (i.e. response and effect traits; Loreau et al. 2001, Grime 2006, Schmitz et al. 2015). Some traits are constrained by phylogenetic history, while others, such as behavioural response traits, benefit from flexibility that allows individuals to adapt to a changing environment. Predators typically have a broad diet and respond to prey availability, diversity, and abundance through a multi-species functional response (Koen-Alonso & Yodzis 2005, Smith & Smith 2020). Foraging has wide-ranging impacts on the ecosystem, including direct, indirect, and non-consumptive effects on nutrient cycling and predator and prey abundance, distribution, and demography (Estes et al. 2016, Hammerschlag et al. 2019, Sinclair et al. 2019). For example, leeward waters of islands with pinniped and/or bird colonies are enriched and more productive, and the open ocean is fertilized by whales redistributing iron, phosphorus, nitrogen, and other micronutrients through the release of fecal material (Wing et al. 2014, Devred et al. 2021). Predators affect

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nutrient dynamics on global scales (Doughty et al. 2016), and the recovery of marine mammals and the rewilding of the ocean and terrestrial landscapes has been proposed as a powerful way to mitigate climate change (Schmitz et al. 2023).

Pinnipeds, like most generalist predators, forage over a wide area (e.g. Boyd et al. 2002, Breed et al. 2009, Vance et al. 2021) and have a broad diet (e.g. Trites & Joy 2005, Hui et al. 2017), which can vary greatly over different spatial and temporal scales (Beltran et al. 2021). Although the diets of many pinnipeds are generally dominated by a few species (e.g. Perez & Bigg 1986, Olesiuk 1993, Tollit et al. 1997), which is typical among the world's megafauna (Hutchinson et al. 2022), the ocean and nearshore areas where pinnipeds forage are highly variable environments subject to large seasonal or climate-related changes in ocean temperatures and currents. These environmental fluctuations affect primary productivity in nearshore areas across trophic levels (Allen & Wolfe 2013, Tanner et al. 2019, D'Alelio et al. 2020, Dai et al. 2023), impacting the abundance and distribution of prey available to pinnipeds (Chassot et al. 2010, Capuzzo et al. 2018). In addition to a high degree of spatial and temporal variation in pinniped diets within a species (Iverson et al. 1997, Labansen et al. 2007), diets also tend to vary among size- and age classes and between sexes (Field et al. 2007, Labansen et al. 2007, Tucker et al. 2007, 2009a).

In addition to environmental variation, many pinnipeds have undergone extreme historical fluctuations in population sizes. For example, on the west coast of North America, elephant seals *Mirounga angustirostris* (Lowry et al. 2014), California sea lions *Zalophus californianus* (Laake et al. 2018), Steller sea lions *Eumetopias jubatus* (Muto et al. 2020, DFO 2021), and harbour seals *Phoca vitulina richardii* (Olesiuk 2010, DFO 2022) have all increased from low abundance and either reached or are approaching pre-colonial abundances, a trend for many marine mammals around the globe (Duarte et al. 2020). In some cases, pinniped population increases have coincided with declines in commercial fish populations and/or a lack of recovery of commercial fish stocks following fishery closures (e.g. Trzcinski et al. 2006, Swain & Chouinard 2008). These observations have led some scientists to claim that pinnipeds are the cause of the declines and/or for the lack of recovery of commercially valuable fish such as Atlantic cod and Pacific salmon (Chasco et al. 2017b, Thomas et al. 2017, Nelson et al. 2019, Neuenhoff et al. 2019), which in turn has led to renewed calls to action including proposals for large culls with the intended effect of stimulating fish population recovery (Trzcinski 2020, Nelson et al. 2023).

Diet is a key first step to gauging the impact of pinnipeds on fish stocks (Mohn & Bowen 1996, Cook et al. 2015). While the best data available are used in modelling efforts to estimate impact, diet data are typically restricted spatially and temporally relative to a species' range and environmental variability (Cook et al. 2015, Chasco et al. 2017a,b), potentially introducing a high degree of bias and uncertainty. Furthermore, estimated impacts are typically made in a single-species context, ignoring the multivariate complexity of dietary space.

A critical aspect of any study of a species' diet is spatial–temporal sampling (Trites & Joy 2005, Bowen & Iverson 2013). Sampling pinnipeds in the marine environment is particularly difficult and is often opportunistic and uneven in design. Hutchinson et al. (2022) found that after consumer type (i.e. herbivore, carnivore), the largest effects on estimates of dietary generalization were sampling method, intra-annual extent, sampling size, and inter-annual extent, which highlights the importance of accounting for sampling method and spatial–temporal variables in the study design and analysis of diet.

The harbour seal *P. vitulina* Linnaeus, 1758 is the most widely distributed pinniped species globally (Andersen & Olsen 2010, Blanchet et al. 2021). Harbour seals forage throughout coastal and estuarine waters of British Columbia, Canada (Olesiuk 2010, DFO 2022), and typically exhibit a high degree of site fidelity with restricted foraging ranges (Härkönen & Harding 2001, Lowry et al. 2001, Steingass et al. 2019). The distribution and behaviour of harbour seals appear to be linked to prey availability (Harvey 1987, Thomas et al. 2011), predation pressures from killer whales *Orcinus orca* and shore-based predators (Nordstrom 2002, London et al. 2012) as well as human disturbance (Jansen et al. 2015). Harbour seals on the west coast of Canada have been heavily hunted and culled since European colonization, with the population decreasing to a low of ~10 000 individuals in the 1960s. Recovery occurred through the 1970s and 1980s, and by the 1990s, the subpopulation in the Strait of Georgia (SOG) had reached a stable abundance of between 35 000 and 45 000 seals (Olesiuk 2010, DFO 2022). The trends outside the SOG are less certain, but most subpopulations appear to be stable or decreasing slightly, except for the West Coast of Vancouver Island (WCVI), where they continue to increase (DFO 2022).

Previous work on harbour seal diets in the Pacific Northwest has employed a variety of methods, with

older studies using hard parts from scats (e.g. Olesiuk 1993) and newer studies applying fatty acid analysis (Bromaghin et al. 2013) and genetic techniques (e.g. Tollit et al. 2009, Nelson et al. 2019, Thomas et al. 2022). The methodologies have different inherent weaknesses and biases (e.g. taxonomic precision, retention times, erosion rates, quantification issues), making it difficult to directly compare diets derived from different approaches. Comparisons of diet are thus generally restricted to a few locales, seasons, and years. Extrapolating diet to space and time without data potentially creates a miss-alignment in our understanding of ecological effects and has the potential to incorrectly predict the consumption of a species when it is not even found in that area or during that season. Despite considerable differences in diet estimates depending on the method, the overall conclusion that harbour seal diets are dominated by a few (3–5) species is robust, assuming the same general locale is being compared. Both Olesiuk (1993) and Thomas et al. (2022) found that Pacific hake, herring, and salmon (identified as chum salmon by Thomas et al. 2022) tend to form the bulk of the diet. The proportion and size of prey in the diet can vary greatly among years, habitats, sexes, and seasons. Although not consistent across its range, male and female harbour seals in the northeast Pacific appear to have different foraging patterns, with females performing longer and deeper foraging dives and consuming more benthic prey (Wilson et al. 2014, Schwarz et al. 2018). Seals foraging at estuarine sites typically have a more diverse diet, which is composed of a different suite of species than in non-estuarine sites (Olesiuk 1993, Voelker et al. 2020), and respond to seasonal influx and concentrations in prey such as herring and salmon (Thomas et al. 2011, Allegue et al. 2020). The large spatial variation in diet is likely a reflection of both restricted foraging in the vicinity of preferred haulout sites and changes in prey community structure at both local and regional scales (Thomas et al. 2011, Vance et al. 2021, van Neer et al. 2023). In the Salish Sea (SOG and Puget Sound), foraging generally occurs within 10–25 km from preferred haulout sites; however, the spatial scale of harbour seal foraging can vary greatly among studies in different ecoregions, between sexes, and between mature and immature animals (Lowry et al. 2001, Thomas et al. 2011, Vance et al. 2021), all of which affects diet and spatial variation in diet estimates (Voelker et al. 2020).

Difficulties in accurately estimating diets of predators, and pinnipeds in particular, arise due to the feasibility of collecting observations and/or samples and uneven spatial–temporal sampling. Traditional methods of diet estimation include observations of predation events, examining stomach contents, and the analysis of hard parts in fecal samples (Bowen & Iverson 2013). The application of DNA metabarcoding to fecal matter or stomach contents now provides a high degree of species resolution, which was not possible using previous methods (Tollit et al. 2009, Bowen & Iverson 2013). Additionally, genetic material from the predator can be extracted from scat samples, and sex can be determined (Schwarz et al. 2018). As with any method of diet estimation, we recognize that there are limitations to the DNA metabarcoding methods (Deagle et al. 2019, Lamb et al. 2019, van der Loos & Nijland 2021). DNA metabarcoding is widely applied to dietary studies (Thomas et al. 2017, Brassea-Pérez et al. 2019), and although there are inherent biases in the method (de Sousa et al. 2019) which can affect the accuracy of estimates of proportion in the diet, results are robust in terms of species composition. While the absolute proportion of any given prey species in the diet may be uncertain, the relative contrasts of proportions of prey in the diet are valid, particularly within a study using consistent collection and data-processing methods.

Diet studies rarely attain a completely balanced sampling design, given the logistical difficulties of obtaining samples, but that does not preclude the need to capture spatial and temporal variation when generalizing results within ecosystems. In our study of harbour seal diets along the coast of Vancouver Island and the SOG, British Columbia, we collected scats from a wide array of locations (n = 64), seasons (n = 3), and years (n = 4) to provide a more synoptic picture of what is consumed. Sampling locations covered a range of habitats and spanned 3 out of the 4 marine ecoregions of British Columbia (Zacharias et al. 1998, Rubidge et al. 2016). The overall goal of our study was to quantify the diversity of harbour seal diet and how the diet varied over different regions, subregions, years, seasons, and between sexes. More specifically, we sought to determine (1) how many samples are required at a given location to quantify diet breadth and mean diet for a local population of harbour seals; (2) what are the principal prey species of harbour seals, and how do the diets of females and males differ; and (3) how variable is the diet in space and time, and are there prey species that are often found together? Addressing these questions is a key step in better quantifying the trophic position and impact of harbour seals on the British Columbia coastal ecosystem.

2. MATERIALS AND METHODS

2.1. Sampling summary

Potential collection sites were surveyed in 2015 at rocky-reef haulouts predominately used by harbour seals. Subsequently, fresh scats were collected at 64 sites from 2016 until 2019 (Fig. 1). These include maintaining collections at previously sampled sites of Cowichan Bay, Snake Island, and Belle Chain Islets. Sampling was undertaken monthly between April and November, attempting to target a total of 70 seal scat samples per site (Trites & Joy 2005). DNA analysis (described in Sections 2.2 & 2.3) of the host was used to ensure only harbour seal scats were used in our subsequent analyses. The number of samples collected by year–month–subregion are given in Table S1 in the Supplement at www.int-res.com/articles/suppl/m743p113_supp.pdf.

At the haulout sites, each individual scat sample was collected using a disposable wooden spoon and placed in a Ziplock® bag. Samples 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 water before being manually homogenized with a disposable wooden depressor inside a paint strainer to separate the scat matrix material from hard prey remains (e.g. bones, cephalopod beaks). Using a clean disposable transfer pipette, the scat sediment was transferred to 20 ml glass scintillation vials in duplicate, and the vials were topped off with 95% EtOH (ethanol). The paint strainer containing prey hard parts was rinsed and non-scat items (rocks, shells, seaweed, etc.) were removed. Hard parts were then air-dried on a coffee filter and stored in a vial for subsequent parallel morphological prey identification.

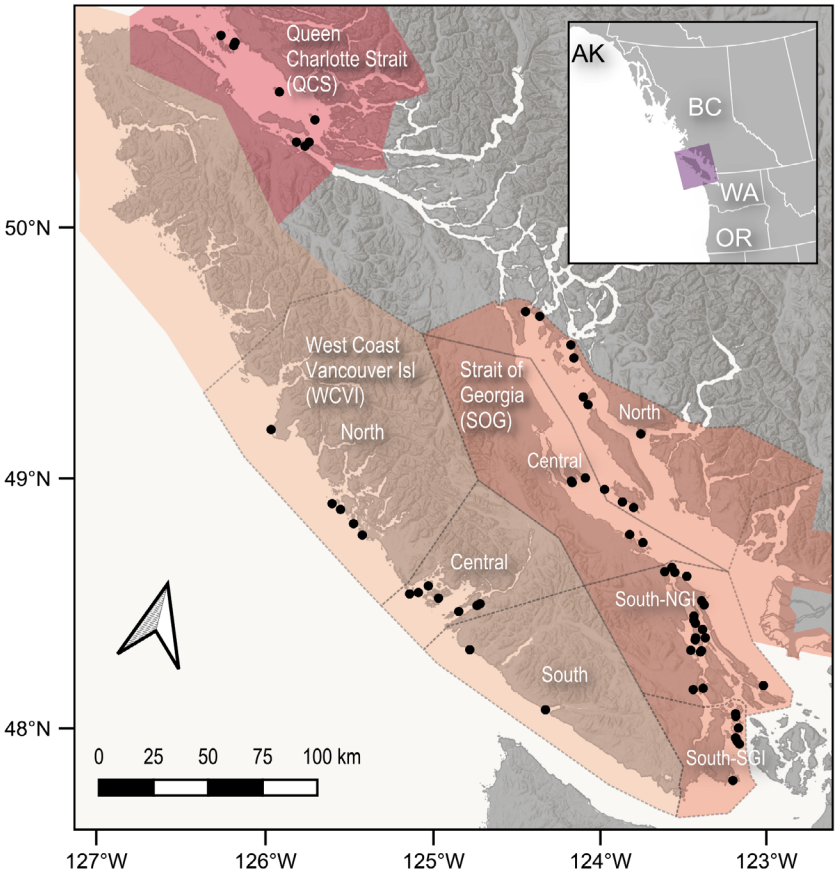

Fig. 1. Vancouver Island (southwestern British Columbia, Canada) showing regional divisions (coloured polygons), labelled subregions (dashed polygons), and sampling locations (black dots). Sampling location names can be found in Fig. S1. Subregion codes — QCS: Queen Charlotte Strait; SOG: Strait of Georgia (SOG North, SOG Central); SOG South–NGI: SOG South–Northern Gulf Islands; SOG South–SGI: SOG South–Southern Gulf Islands; WCVI: West Coast Vancouver Island (WCVI North, WCVI Central, WCVI South). Inset map shows Vancouver Island in relation to extents of British Columbia (BC), Alaska (AK), Washington (WA), and Oregon (OR)

A total of 3420 harbour seal scats were collected at 64 locations between 2016 and 2019 along the shores of Vancouver Island, including the western coast, the SOG, and Queen Charlotte Strait (QCS) (Fig. 1, Fig. S1). The number of scats collected per year and season varied greatly, and none were collected in the winter due to difficult weather conditions and higher water levels making sampling difficult by increased flushing of haulouts. In 2016, 1275 scats were collected, and in 2017, 2018, and 2019, 228, 1084, and 833 scats were collected, respectively, from 64 locations. Not all locations were sampled every year.

The coastal areas along Vancouver Island are highly variable in ocean conditions, primary production, and community structure (Boldt et al. 2022). Three bioregions are identified in this area which broadly represent different oceanographic and biological conditions (Zacharias et al. 1998, Rubidge et al. 2016). These include the SOG component of the Salish Sea, the Southern Shelf (off West Vancouver Island including the Strait of Juan de Fuca; WCVI), and the Northern Shelf Zone (QCS). These regions also demonstrate variation in harbour seal abundance and density

from surveys conducted between 2015 and 2019, where densities are highest within the SOG at 10.5 seals km^{-1}, lowest in QCS at 1.2 seals km^{-1}, and intermediate in WCVI at 2 seals km^{-1} (DFO 2022). As a primary goal of this study was to quantify spatial variation in harbour seal diet, given that sampling occurred at multiple sites within the SOG and WCVI, we further subdivided these areas into subregions. These similar-sized subregions conform to assessment subarea divisions (DFO 2022) and broadly reflect varying habitat and conditions within each area (i.e. Juan de Fuca Strait, Barkley Sound, and southern WCVI areas for west Vancouver Island) and indeed, depending on the metric, can reflect different sub-ecoregions in and of themselves (Orsi et al. 2007, Marchese et al. 2022). We did not subdivide the QCS region given its smaller size but included it in subsequent subregion contrasts. Therefore, the 8 subregions as defined here are QCS, SOG North, SOG Central, SOG South–Northern Gulf Islands (SOG South–NGI), SOG South–Southern Gulf Islands (SOG South–SGI), WCVI North, WCVI Central, and WCVI South. The number of samples collected varied greatly among years within each of these regions. Only 3 subregions (SOG North, SOG South–SGI, and SOG South–NGI) were sampled in all 4 years between 2016 and 2019, but not in all 3 seasons. In 2016 and 2017, all but a few samples were collected in the SOG (2016, n = 1262; 2017, n = 228), and in 2018 and 2019, scats were collected from all subregions except SOG Central (2018, n = 1084; 2019, n = 833).

2.2. DNA metabarcoding

The DNA diet analysis protocol we used is described in detail in Thomas et al. (2017). Scat extractions and subsampling were performed in a UV PCR hood, and the equipment and work surface were bleached and exposed to ultra-violet light prior to use. DNA was extracted from scats using the DNeasy PowerSoil HTP 96 kit (Qiagen) or the DNeasy 96 PowerSoil Pro kit (Qiagen) following the manufacturer's protocols. Approximately 450 µl of resuspended scat was added to the bead plate, and before processing, excess EtOH was decanted off following centrifugation at 4000 rpm (~2500 × *g*) for 3 min. Proteinase K and incubation at 60°C were added in both protocols prior to the addition of solution C2 in the inhibitor removal step. Samples were eluted with 100 µl of 10 mM tris(hydroxymethyl)aminomethane (solution C6).

Three amplicon types for the characterization of different taxonomic groups were applied to the extracted DNA samples. Amplicons used included a ~270 bp segment of the chordate (16SChord) and the cephalopod 16S rRNA gene (16SCeph) (Deagle et al. 2009), and a ~260 bp segment of the cytochrome oxidase I (COI) gene amplified with primers designed primarily for the amplification of salmonids (Thomas et al. 2017). A predator blocking primer (ATG GAG CTT TAA TTA ACT AAC TCA ACA GAR CAA) with a modified non-extendable primer (3′ C3 Spacer) was included in the 16S PCR reactions (Vestheim & Jarman 2008). The blocking primer overlaps the 3′ end of the 16S F primers, reducing but not eliminating the amplification of the harbour seal DNA. The blocking primer was included in the reactions at 10 times the PCR primer concentration.

The 16SChord and 16SCeph amplicons were multiplexed in one PCR reaction and the COI amplification was performed in a separate reaction. All PCR amplifications were performed in 20 µl volumes using the Multiplex PCR Kit (Qiagen). Reactions contained 10 µl (2×) master mix, 0.25 µM of each primer, and 2 µl template DNA. Thermal cycling conditions were as follows: 95°C for 15 min followed by 34 cycles of 94°C for 30 s, 57°C for 90 s, and 72°C for 60 s, and a final extension at 72°C for 600 s. Along with extraction and PCR blanks, a positive control for each of the 3 amplicons was added to the amplification tray. These consisted of ~1.0×10^{-5} diluted, synthesized Gene Fragments (Integrated DNA Technologies) designed to unique sequences that were encompassed within the matching amplicon primers.

Amplified 16SChord, 16SCeph, and COI amplicon samples were barcoded with unique, matching 10 bp forward and reverse tags with an edit distance of 5 and pooled by amplicon into single libraries. These were cleaned and concentrated using DNA Clean and Concentrate-5 columns (Zymo Research) prior to library preparation and indexing using the KAPA Low Throughput Library Preparation kit (KAPA Biosystems) for Illumina platforms as per the manufacturer's instructions. The quantity of the indexed library pools was assessed using the Qubit dsDNA High Sensitivity kit (Invitrogen) and quality- and size-assessed using the DNA 1000 Bioanalyzer chip (Agilent Technologies). Finally, the pools were combined into a single library of 66–33% ratio and processed on a 301 bp single end MiSeq V2 chip (Illumina) with a 5% phiX spike-in. The data were de-multiplexed by library pool (index) on the sequencer and produced as fastq files.

2.3. DNA bioinformatics and diet calculation

Sequences matching the full forward and reverse primers and the 10 bp primer tag (allowing up to 2 mismatches) were mapped to the scat sample of origin and enumerated using the macQIIME v.1.9.1 software package. Sequences were subsequently clustered using the incorporated National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST v.2.3.0) with a minimum usearch size of 3 and a similarity threshold of 0.99 before being assigned a taxonomy from a custom reference database listing 293 potential prey specific to the NE Pacific, including 29 variants of local salmonids (Thomas et al. 2022). A subset of sequences from each plate (25%) was uploaded to the online NCBI BLAST portal (https://blast.ncbi.nlm.nih.gov/Blast.cgi) at the time of processing to manually review taxonomies assigned using the most current version of the comprehensive nucleotide database. This additional step was taken to screen for additional species not already included in the local database and to supplement existing entries with newly added voucher samples or haplotypes. Three putative prey items were iteratively added to the reference library over the course of the study, and all data were re-processed with the most inclusive version prior to the analysis.

Sequences assigned to species were tabulated for each scat sample using scripts written in the R software, version 4.2.3 (R Core Team 2022). Despite the use of the blocker, a notable number of predator sequences remained in the 16S data and were used to confirm that samples were indeed from harbour seals. Rarely, scats were determined to be other than seal (typically sea lion scats or seabird droppings from a mixed-use haulout), and these samples were omitted from the study. Harbour seal sequences were removed and the remaining putative prey sequences (median: 2255 total prey reads per sample) were summed by species and their relative proportion was calculated.

Samples can be susceptible to spurious species detections due to minute contamination during collection, processing, or the amplification process. To balance the removal of potential contaminants with the loss of true prey species occurring in low amounts (e.g. Littleford-Colquhoun et al. 2022), we developed a 3-stage filtering process. First, we removed samples with <20 total prey sequences due to poor overall read depth of the sample. Second, we applied a species-specific minimum and removed species with 3 or fewer sequences but allowed for a stricter cutoff if required. We calculated the median number of positive control sequences that were amplified in non-control (prey) samples as a measure of technical error or 'bleed' between sample wells for each run. This enabled us to incorporate a variable minimum threshold for each MiSeq run and enforce it in the rare instances where the median bleed was >3 sequences. Third, we employed a proportional cutoff wherein species that comprised <1% of a single sample were removed before calculating sample diet percentages. An exception was made for 16S sequences categorized as flatfish, rockfish, or salmon (at <1%), as select species in those groups are less well resolved at the 16S marker (Thomas et al. 2017, 2022). For those categories, we calculated the group proportion and used the following decision tree: (1) flatfish, rockfish, and salmon species that remained at <1% even in aggregate were removed; (2) flatfish or rockfish species at <1% but which surpassed the 1% cutoff in aggregate were combined and relabeled as 'grouped flatfish' (9 cases, mean: 1.29%), or 'grouped rockfish' (30 cases, mean: 1.43%); (3) salmon occurring at <1% but >1% when grouped were retained as-is for further evaluation using the COI amplicon. Following all filtering, total sequence counts were renormalized and proportional species estimates (diet percentages), otherwise referred to as relative read abundances in metabarcoding studies (Deagle et al. 2019), were calculated for each individual scat sample. Finally, the 16S and COI information was combined such that the overall salmonid proportion in any sample was delimited by 16S and the specific salmon species percentages within that segment were quantified by COI. These per-sample DNA diet estimates were used in subsequent analyses.

A total of 125 species were retained in our scat samples. While there are no definitive standards for exclusion, we opted to drop any species with a global mean of <0.1% of the diet from the summary analysis. This resulted in 62 species that were assumed to be primary prey in harbour seal diets (no species groupings), which formed 99.6% of the diet among all samples at all locations. Our statistical analyses of harbour seal diet are divided into 4 sections: (1) diversity in harbour seal diets, (2) analysis of mean diet, (3) analyses of size frequency of prey in the diet, and (4) quantifying spatial–temporal patterns in diet using unconstrained multivariate analyses.

2.4. Diversity in harbour seal diets

It is difficult to know exactly what a scat represents, as different species and their hard parts have different erosion rates and retention times, and this has un-

known effects on hard part analysis and genetic techniques, but a scat is typically thought to represent a meal or two (Bowen & Iverson 2013). Hence, any given scat sample is typically dominated by 1 or 2 species. Consequently, many scat samples are required to quantify the mean and variance in the diet. To understand the effect of sample size on estimating the diversity of species in the diet among years and seasons at a location, we calculated species accumulation curves for all locations where 30 or more scats were collected (n = 16 locations). A 'location' was defined as a haulout site where scat can be collected and which can range in size from a few meters to ~10s of meters. The distance between haulout sites varied greatly but was typically >10 km. The closest haulout locations in our study were Norris and Heron in the SOG, which were 1.7 km apart, and it is likely that some of the same seals were hauling out at both of these locations. It may be argued that these samples represent the same local seal population and should be combined. However, as the point of the present study was to analyse spatial and temporal variation at multiple scales, they were left distinct. In all our analyses of diet, scats collected at different haulout locations were treated as independent. In our analysis of diet richness, richness was estimated at 51 locations by year and season where 5 or more scats were collected (n = 131), and the effects of sample size (number of scats), year, season, and subregion were tested using a generalized linear model assuming a Tweedie error distribution. All models were parameterized in R using the package 'glmmTMB' (v.1.1.7; Brooks et al. 2017).

2.5. Analysis of mean diet

Other studies of harbour seal diet have shown that a few species are common and form the majority of the diet, while many other species are rare and comprise relatively small proportions (e.g. Wilson & Hammond 2019, Sørlie et al. 2020, Thomas et al. 2022). We filtered the data using 2 criteria. The first has already been mentioned: the mean percent in the diet had to be ⩾0.1% across all our scat samples (n = 3420) for it to be considered a primary species, resulting in 62 species. To compare diet differences among subregions, we analyzed the top 12 prey species based on rank ordering in the diets (representing 82.6% of diet across all subregions) and rolled the remaining 50 species into respective taxonomic or functional groups: cephalopods, flatfish, forage fish, gadids, hexagrammids, rockfish, salmonids, and a catch-all category 'other'. We calculated the mean diet for all locations within a subregion by year and season if a location had 5 or more scat samples collected (51 locations). Out of a total of 96 subregion by year by season combinations, 43 met this criterion. The lower and upper confidence intervals of the mean diet were calculated by bootstrapping the data using the package 'boot' (v.1.3-28.1) in R.

The most intensive sampling occurred at 9 locations within the SOG, which allowed us to take a finer look at spatial and temporal variation in the diet in this region. A different set of the top 12 species consumed was defined for the SOG, and the remaining species rolled up into the 8 functional groups as above. We calculated the mean diet for each location by year and season if n ⩾ 5 scat samples.

2.6. DNA sex identification

We estimated the sex of a harbour seal from scat for a subset of the data (1617 out of 3420 scats; 47%), of which 84% (n = 1364) could be positively attributed to a sex. We then estimated the mean proportion of prey in the diet by sex in the SOG, as this region was the most intensively sampled (n = 1136).

Quantitative polymerase chain reaction (qPCR) was used to determine the sex of the individual that deposited each scat using a modified version of the seal-specific assay developed by Matejusová et al. (2013). Briefly, we performed 3 TaqMan qPCR reactions that targeted the paralogous zinc finger X (ZFX) and zinc finger Y (ZFY) genes, respectively, along with the sex-determining region Y gene (SRY) to determine seal sex. ZFX acted as a positive control, as all scat samples should contain the ZFX gene, while the presence or absence of ZFY and SRY would determine the sex. All 3 probes were custom synthesized by Applied Biosciences and were diluted to 10× concentration. We used 2× TaqMan Gene Expression Master Mix from Applied Biosciences. Master mixes were made with 6.0 µl of 2× TaqMan Gene Expression Master Mix for every 0.22 µl of 50 µM F/R primer pair and 0.3 µl of 10 µM ZFX, ZFY, or SRY TaqMan probe. The optimized qPCR reaction comprised 11 µl ZFX, ZFY, or SRY Master Mix with 1 µl of gDNA, APC, or PCR water. The thermocycler protocol was as follows: one holding cycle (50°C for 2 min, 95°C for 10 min) followed by 60 cycles of denaturation and annealing/extension (95°C for 15 s, 60°C for 1 min). We ran 2 ZFX, ZFY, and SRY replicates for each sample. Each qPCR reaction profile was manually inspected for the presence of an amplification curve.

We decided against applying a maximum threshold cycle (C_T) value because we did not attempt to quantify the template DNA but were instead scoring the presence and absence of amplification for each marker. We assigned a score for no $C_T = 0$, $C_T < 40 = 1$ and $C_T > 40 = 2$. Therefore, each sample was assigned a score by assay or replicate (ZFX_1/ZFX_2/ZFY_1/ZFY_2/SRY_1/SRY_2). Within a typical sample, the SRY assay C_T was more sensitive and typically expressed ~4 C_T lower than the ZFX and ZFY assays. In classifying the samples as male or female, we utilized both the scoring pattern and the C_T ratios of the 3 assays. If none of the 3 assays amplified (0/0/0/0/0/0), we classified them as no result (3.4%), and if the assay score pattern and/or C_T ratios between the assays were not typical, we classified the sample as inconclusive (17.3%). If one or both of the ZFX duplicates amplified and none of the ZFY or SRY replicates amplified, the sample was classified as female (19.8%), while if 1 or 2 of the ZFY and SRY replicates showed amplification, the sample was classified as male (59.5%).

Five scat samples from known male harbour seals housed at the Vancouver Aquarium (British Columbia, Canada) and 5 scat samples from known females at the Point Defiance Zoo and Aquarium (Tacoma, Washington, USA) served as positive biological controls. Two of these controls were analyzed in duplicate for each assay per run. All of the females were identified as female (1/1/0/0/0/0), and all of the males were identified as male (1/1/1/1/1/1).

Artificial positive controls (IDT DNA) were synthesized for each assay and included as a 9-point, 10-fold dilution series in each run. All assays displayed efficiencies within 90–110% and $R^2 > 0.97$% over the 384 well tray runs. A limit of detection of 10 copies for each assay was determined 75% of the time.

Multiple negative controls (n = 8–11) were run in duplicate on every 384 well tray for a total of ~250 negative controls for each assay. The false positive rate was 0.4% for the ZFX and ZFY assay and 0% for the SRY assay. As a side note, the C_T ratios for the 3 sex marker assays were fairly equivalent when amplifying the APCs (synthesized constructs diluted to represent copy number). However, when amplifying known biological samples and a majority of unknown biological samples, this same trend was not observed. Instead, the C_T value for the SRY assay was ~4 C_T lower (more sensitive) than the ZFX and ZFY assays, which may point to the SRY gene existing as a multicopy gene in harbour seals as it does in dogs (7 copies) (Li et al. 2013).

2.7. Size frequency of prey derived from hard parts

We estimated the size of prey consumed from hard parts in the scat for 7 species (hake, herring, pollock, shiner perch, lingcod, English sole, starry flounder) and 2 species groups (salmon and rockfish; n = 3314 scats). Extraction and identification of hard structures for family or species ID and size-class categorization from harbour seal scats was conducted by the independent contracting company Pacific ID, using the 'all structures' approach, including measurements, amount of erosion, and size keys. That is, all diagnostic prey hard parts were considered in identifying structures to the lowest possible taxon using a dissecting microscope in conjunction with reference fish skeletons from the Eastern North Pacific. 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. Prey size was estimated using different prey size bins for each taxon. If a prey item could not be attributed to a non-overlapping size bin, the data were removed from the analysis.

The number of hard parts attributed to each size bin has the potential to repeatedly count a prey item in the diet. To partially address this issue and to test for differences in prey size among regions and seasons, the frequency of scats which were determined to have a prey species within a given size bin was calculated. The midpoint of a size bin was used in species-specific models of prey size consumed where the midpoint was weighted by the frequency of scats found to have that prey species and size. In most models, we assumed a Tweedie distribution with a parameter for zero-inflation, but we found that a negative binomial distribution fit the prey size data better for herring. All models were performed using the package 'glmmTMB' (v.1.1.7; Brooks et al. 2017).

2.8. Quantifying spatial–temporal patterns in diet

To explore the spatial and temporal variation in harbour seal diet and potential prey associations, the mean proportion in the diet was calculated for 12 species and 8 species groups by subregion and season (as in Section 2.5) and analyzed using correspondence analysis (CA). The mean diet was calculated for 43 subregion by year by season combinations that had 5 or more scat samples collected. The data were Hellinger-transformed and submitted to a CA, where the means were weighted by sample size (Bor-

card et al. 2018). A similar analysis was performed on the data from the 32 locations within the SOG using a SOG-specific suite of species (as above). The package 'FactoMineR' (v.2.8) was used for correspondence analysis and 'adonis2' in the 'vegan' package (v.2.6-4) was used for variance partitioning with 999 permutations.

3. RESULTS

3.1. Diversity in harbour seal diets

A total of 125 species were detected in the DNA analysis of harbour seal scats collected in our study between 2016 and 2019; 62 species comprised >0.1% of all estimated diets, which collectively represented 99.6% of the diet (species list and taxonomy in Table S2). The mean number of species in a scat was 1.9, the median was 2, and the range was between 1 and 13 species detected. The number of species detected in the diet at a given location by year by season combination increased as more scat samples were collected (Fig. 2). The rate at which new species were added to the diet varied among subregions and locations within subregions and slowed as more scat samples were collected but did not reach a plateau. At our most sampled and species-diverse site, Cowichan estuary (SOG South–NGI), 5 scats yielded about 5.1 prey species in the diet on average, 10 scats yielded 8.5 species, 15 scats yielded 11, and 30 scats yielded 16 species. At the Cowichan site, <0.2 prey species were being added for every scat collected after 50 scats, indicating significant diminishing returns. Across years and seasons, the mean diversity of harbour seal diets was highest on the WCVI (mean ± SE: 16.5 ± 1.4 species), followed by QCS (11.4 ± 1.1 species) and the SOG (10.7 ± 0.4 species).

An analysis of diversity in the diet at 131 location by year by season combinations showed that prey diversity was strongly affected by sample size (linear effect) and by subregion but only marginally by year (with the diversity being least in 2017) and not by season (Table 1). When model predictions were standardized to a sample size of 30 scats, the lowest diversity in the diet was found in SOG North (7.0 species, 95% CI: 5.3–8.7 species) and SOG Central (8.9 species, 95% CI: 5.7–12.1 species), and the highest in WCVI South (21.2 species, 95% CI: 16.8–25.6 species) and WCVI North (20.6 species, 95% CI: 13.9–27.3 species). The diversity in the mean diet of the WCVI North seals was significantly higher than the diet diversity in QCS and in SOG North and SOG Central, but not significantly different from the other regions, given the high variation within subregions (Table 1, Fig. 3). The highest diversity at an individual site was observed at Cowichan (SOG South–NGI) in the spring of 2018, with 33 species detected from 70 scats. Belle Chain (SOG South–SGI), Perez (WCVI North), and Wizard (WCVI Central) all had 25 or more species detected in the diet.

3.2. Mean diet proportions

In total, 12 species across all study areas accounted for 80.1% of the diet (Table 2). Across all samples collected (regardless of space and time differences), the top 4 species in the diet collectively comprised 67.8% of the diet. Hake (36.6%, 95% CI: 35.3–38.3%) was the largest mean proportion of any species in the diet, followed by herring (22.5%, 95% CI: 21.4–23.9%), walleye pollock (5.0%, 95% CI: 4.4–5.6%), and chum salmon (4.5%, 95% CI: 3.8–5.1%). The percent diet of the remaining 8 species collectively comprised 11.5% of the diet and consisted of 3.4% plainfin midshipman (95% CI: 2.9–4.0%), 2.1% shiner surfperch (95% CI: 1.7–2.5%), 1.9% English sole (95% CI: 1.5–2.3%), 1.5% black rockfish (95% CI: 1.1–1.8%), 1.3% lingcod (95% CI: 1.0–1.6%), 1.3% sockeye (95% CI: 1.0–1.7%), 1.2% blue rockfish (95% CI: 0.9–1.5%), and 1.2% Chinook salmon (95% CI: 0.9–1.5%). The 8 species groups collectively comprised 19.8% of the diet (Table 2). The 'other' category comprised 40 species; each species averaged <1.1% of the diet across the entire data set and on average collectively comprised 4.4% (95% CI: 3.9–5%) of a harbour seal's diet. The mean diet of these 12 species and 8 functional groups by subregion are shown in Table S3.

There were noticeable differences in mean harbour seal diet among subregions, seasons, and years (Fig. 4). Herring comprised a large proportion of the diet in the QCS (34.4%, 95% CI: 29.7–38.7%) and the Northern and Southern Gulf Islands (SOG South–NGI, 34.8%, 95% CI: 32.7–37.4% and SOG South–SGI, 39.4%, 95% CI: 35.2–43.7%), and was consumed in roughly equal proportions in the spring, summer, and fall. Chum was a small proportion of the overall diet (4.5%, 95% CI: 3.8–5.1%) and was highest in fall in the SOG South–NGI (15.1%, 95% CI: 12.5–17.9%). Sockeye was consumed mostly at locations in the Southern Gulf Islands (Belle Chain), specifically in the fall of 2018 (5.0%, 95% CI: 3.6–6.5%). The 'other' species category in the diet was highest in SOG South–SGI at 9.2% (95% CI: 6.9–12.1%) and in WCVI South at 7.2% (95% CI: 5.2–9.8%).

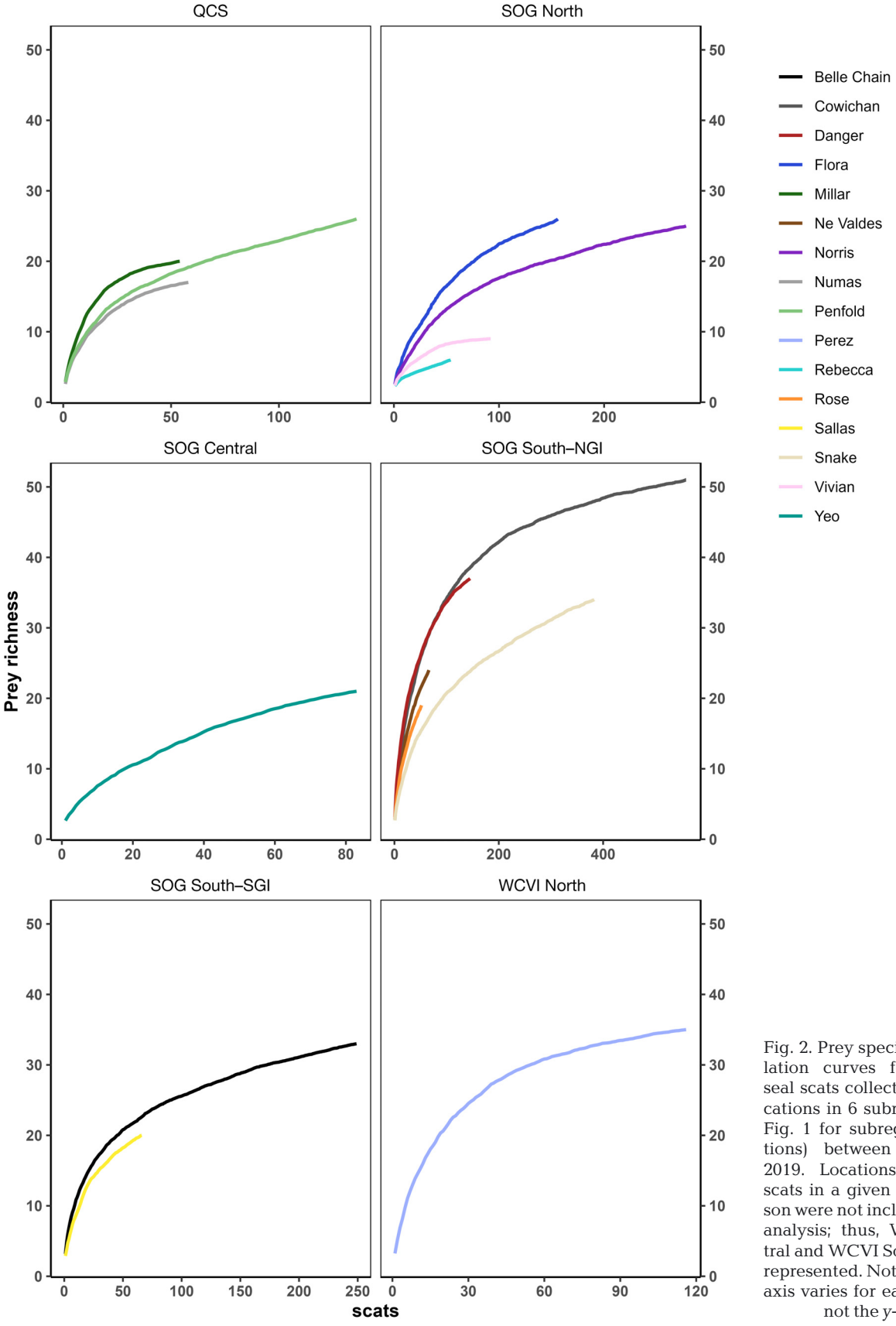

Fig. 2. Prey species accumulation curves for harbour seal scats collected at 16 locations in 6 subregions (see Fig. 1 for subregion definitions) between 2016 and 2019. Locations with <30 scats in a given year × season were not included in this analysis; thus, WCVI Central and WCVI South are not represented. Note that the *x*-axis varies for each plot but not the *y*-axis

Table 1. Generalized linear model results of harbour seal diet richness in 8 subregions over 4 years (2016–2019) and 3 seasons for 12 species and 8 prey groups (exclusive of species identifications) including a catch-all 'other' category. The model assumed a Tweedie distribution with a log link. Statistically significant results ($p \leq 0.05$) in **bold**. CI: 95% confidence interval

Parameter	Coefficient	CI low	CI high	*z*	p
Intercept	1.955	1.671	2.238	13.512	**<0.001**
n	0.014	0.011	0.017	9.418	**<0.001**
Year 2017	−0.284	−0.634	0.066	−1.588	0.112
Year 2018	0.109	−0.077	0.296	1.147	0.251
Year 2019	0.053	−0.140	0.246	0.538	0.591
Season Spring	0.124	−0.116	0.365	1.014	0.311
Season Summer	0.088	−0.062	0.237	1.153	0.249
SubRegion SOG Central	−0.374	−0.763	0.015	−1.883	0.060
SubRegion SOG North	−0.608	−0.900	−0.315	−4.075	**<0.001**
SubRegion SOG South–NGI	0.052	−0.191	0.294	0.418	0.676
SubRegion SOG South–SGI	0.116	−0.188	0.420	0.750	0.453
SubRegion WCVI Central	0.359	−0.071	0.788	1.635	0.102
SubRegion WCVI North	0.463	0.113	0.813	2.595	**0.009**
SubRegion WCVI South	0.479	0.223	0.736	3.667	**<0.001**

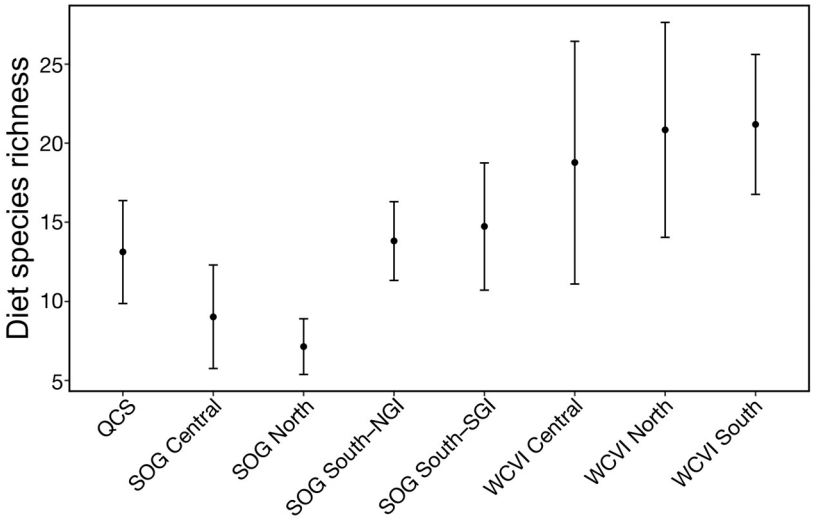

Fig. 3. Model predicted estimates (±95% CI) of harbour seal diet species richness for 8 subregions assuming a sample size of 30 scats. See Table 2 for model formulation and estimates. See Fig. 1 for subregion code definitions

Restricting the analysis to a finer scale of 9 locations within the SOG which had the most intensive sampling, there was considerable variation in mean harbour seal diet among locations, years, and seasons (Fig. 5). Hake and herring remained the most consistently consumed prey and made up the largest proportion of the diet, chum and sockeye were only consumed in the fall, and sole was only found at Danger, Miami, and Rose (SOG South–NGI).

For the subset of scats for which we determined the sex of the harbour seal, we had more male (n = 840) than female (n = 296) samples at 38 locations within the SOG. It is important to be aware of the sample size difference when interpreting the data. Males and females show some differences in the species composition and mean percent of the prey species in their diet, but subregion explained more variation and had a larger effect on diet (Fig. 6, Table 3). Males consumed more hake than females in SOG Central (males: 81.8%, 95% CI: 76.6–86.9%; females: 59.4%, 95% CI: 40.7–78.1%) and in the Northern Gulf Islands (males: 40.3%, 95% CI: 35.9–44.9%; females: 27.5%, 95% CI: 21.0–34.1%). Females consumed more shiner surfperch in the Northern Gulf Island (SOG South–NGI: females: 13.3%, 95% CI: 8.6–18.1%; males: 1.5%, 95% CI: 0.4–2.6%), and males consumed more chum in both the Northern and Southern Gulf Islands (SOG South–NGI: males: 14.1%, 95% CI: 10.6–17.5%, females: 1.5%, 95% CI: 0–3.3%; SOG South–SGI: males: 12.4%, 95% CI: 7.8–17.0%, females: zero chum).

Diet varied more strongly among subregions, with some smaller differences between sexes. Females consumed 3.4 times (76.4%, 95% CI: 65.9–87.0%) more hake and males consumed 7.4 times (78.6%, 95% CI: 72.6–84.5%) more hake in the SOG North than in the SOG South–SGI (females: 23.0%, 95% CI: 12.4–33.7%; males: 10.5%, 95% CI: 6.6–14.5%). Males consumed 10.2 times more walleye pollock in the southern SOG (SOG South–SGI: 13.3%, 95% CI: 8.4–18.2%, SOG South–NGI: 1.3%, 95% CI: 0–2.6%), and females consumed 21.0 times more pollock in the southern SOG (SOG South–SGI: 10.7%, 95% CI: 2.7–18.6%, SOG South–NGI: 0.5%, 95% CI: 0–1.5%). Females consumed more herring in the Northern Gulf Islands (SOG South–NGI: 26.9%, 95% CI: 20.7–33.0%; SOG North: 14.8%, 95% CI: 5.7–23.9%). Males showed a strong decline in the percent of herring in the diet from the south to the SOG Central (SOG South–SGI: 32.7%, 95% CI: 25.9–39.6%; SOG Central: 5.1%, 95% CI: 2.3–7.9%) (Fig. 7).

Table 2. Mean percent of prey species in the diet estimated from DNA in 3420 scats across years (2016–2019), regions (see Fig. 1), and seasons (spring, summer, fall) for the 12 species and 8 prey groups (exclusive of species identifications) including a catch-all 'other' category. lCI and uCI: lower and upper 95% confidence intervals estimated by bootstrapping the data. Estimates mean prey size (mean size) from hard parts collected from scats. n = total number of scats with hard parts of given species or group, lCI and uCI: lower and upper 95% confidence intervals of prey size estimated assuming a normal distribution

Common name	Family	Species	Prey species % in diet			Prey size			
			Mean	lCI	uCI	n	Mean	lCI	uCI
Pacific hake	Merlucciidae	*Merluccius productus*	36.6	35.3	38.3	1503	30.0	29.4	30.6
Pacific herring	Clupeidae	*Clupea pallasii*	22.5	21.4	23.9	1059	12.6	12.3	12.9
Walleye pollock	Gadidae	*Gadus chalcogrammus*	5.0	4.4	5.6	483	25.2	24.2	26.2
Chum salmon	Salmonidae	*Oncorhynchus keta*	4.5	3.8	5.1	–	–	–	–
Plainfin midshipman	Batrachoididae	*Porichthys notatus*	3.4	2.9	4.0	156	19.5	18.0	21.1
Black rockfish	Scorpaenidae	*Sebastes melanops*	1.5	1.1	1.8	–	–	–	–
Lingcod	Hexagrammidae	*Ophiodon elongatus*	1.3	1.0	1.7	36	41.6	34.1	49.1
Sockeye salmon	Salmonidae	*Oncorhynchus nerka*	1.3	1.0	1.7	–	–	–	–
Blue rockfish	Scorpaenidae	*Sebastes mystinus*	1.2	0.9	1.5	–	–	–	–
Giant Pacific octopus	Octopodidae	*Enteroctopus dofleini*	1.1	0.9	1.4	–	–	–	–
Starry flounder	Pleuronectidae	*Platichthys stellatus*	1.0	0.8	1.3	9	31.5	21.5	41.5
Rex sole	Pleuronectidae	*Glyptocephalus zachirus*	0.7	0.5	1.0	97	22.1	20.1	24.2
Prey group									
Forage fish	–	–	4.4	3.8	5.0	–	–	–	–
Other	–	–	4.4	3.9	5.0	–	–	–	–
Flatfish	Pleuronectidae	–	3.6	3.0	4.2	–	–	–	–
Salmonids	Salmonidae	–	2.9	2.4	3.4	233	37.9	36	39.8
Rockfish	Scorpaenidae	–	2.0	1.6	2.4	326	12.7	11.8	13.6
Cephalopods	–	–	1.1	0.8	1.4	–	–	–	–
Hexagrammids	Hexagrammidae	–	0.7	0.5	1.0	–	–	–	–
Gadids	Gadidae	–	0.7	0.5	0.9	–	–	–	–

3.3. Size frequency of prey

The analysis of hard parts in scat shows that harbour seals eat a wide size range of prey, from small shiner perch (5–15 cm) to large lingcod (16–99 cm; Fig. S2). While there is considerable variation in the size of prey, species-specific models show that the size consumed changed among regions and seasons depending on the species. For example, larger hake were consumed on the WCVI and smaller hake in the QCS, larger herring were consumed in the spring and the smallest herring consumed in the summer, the largest salmon were consumed in the fall, and the walleye pollock consumed were on average larger in the QCS (Fig. S3, Table S4). Models for the prey size of lingcod, shiner perch, English sole, rockfish species, and starry flounder were not significant ($p > 0.05$ for region and season effects).

3.4. Quantifying spatial–temporal patterns in diet

There were large differences in harbour seal diets among subregions and seasons. The first 2 axes of the correspondence analysis collectively explained 51% of the variation in mean diet among regions, subregions, years, and seasons (Fig. 8). The first axis explained 35.4% of the variation and is primarily related to the proportion of blue and black rockfish in the diet at one extreme and hake at the other. The second axis explained 15.6% of the variation and is primarily related to the proportion of pollock, sockeye, and chum salmon in the diet at one extreme and the proportion of blue rockfish, giant octopus, and lingcod at the other. There were large differences in the community composition in the diet among regions and subregions. Blue and black rockfish, rex sole, and lingcod were mostly consumed on the WCVI; gadids and sockeye in the QCS; pollock in the Southern Gulf Islands; and hake and midshipmen in the North and Central SOG. A large proportion of the variance in harbour seal diets was explained by subregion with less, but significant, variation explained by year and season (Table 4).

The SOG has a different prey community from the WCVI or QCS, and correspondence analysis of 32 locations in the SOG showed different patterns in the mean diet (Fig. 9). The first 2 axes collectively ex-

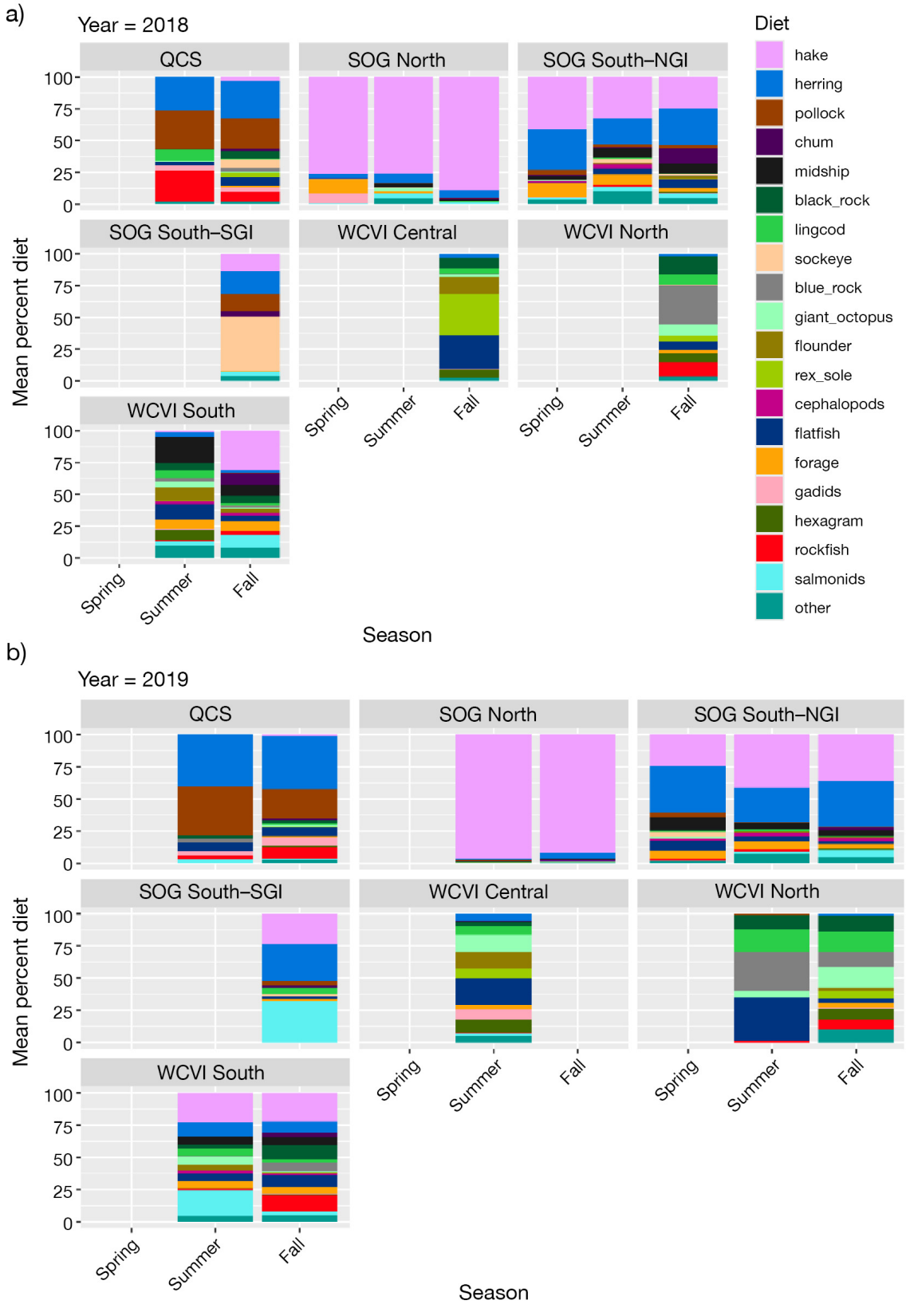

Fig. 4. Mean percent harbour seal diet for 12 species and 8 prey groups (exclusive of species identifications) including a catch-all 'other' category by subregion and season in (a) 2018 and (b) 2019. See Fig. 1 for subregion code definitions

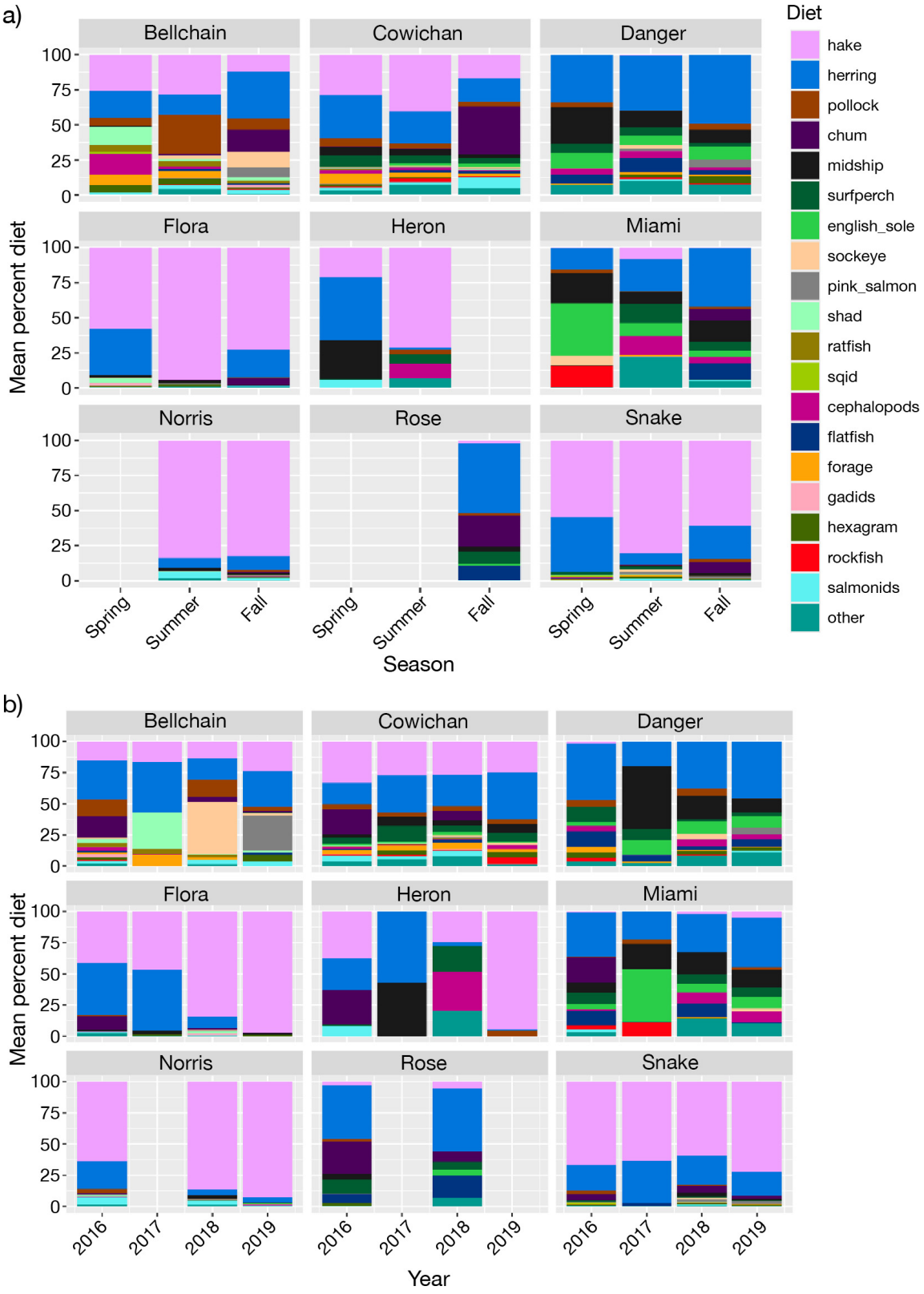

Fig. 5. Mean percent harbour seal diet for 12 species, 8 prey groups (exclusive of species identifications) including a catch-all 'other' category at 9 locations in the Strait of Georgia by (a) season and (b) year. These 9 locations were the most intensively sampled. See Fig. 1 for subregion code definitions

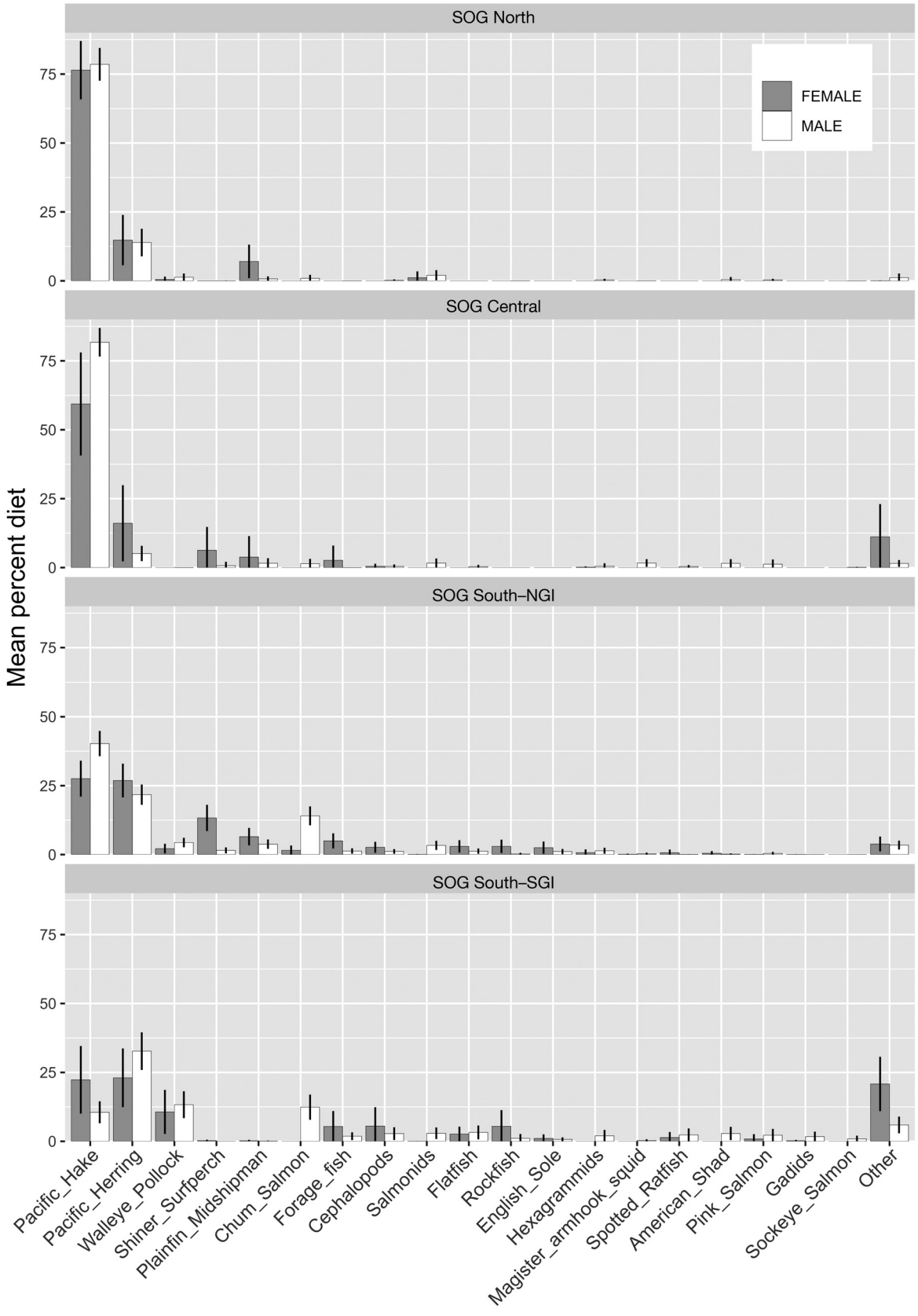

Fig. 6. Mean percent diet (±95% CI) for male and female harbour seals in the Strait of Georgia (SOG) for 12 species and 8 prey groups (exclusive of species identifications) including a catch-all 'other' category. We only tested for sex differences in diet in the SOG (i.e. not in QCS or WCVI), as it was the most intensively sampled. See Fig. 1 for subregion code definitions

Table 3. Variance partitioning (permutational multivariate analysis of variance) of female and male harbour seal diet in 4 subregions in the Strait of Georgia over 4 years (2016–2019) and 3 seasons for 12 species and 8 prey groups including a catch-all 'other' category (see Fig. 4 for species and prey groups). Season: spring, summer, fall. See Fig. S1 for subregions. The model was run for 999 permutations. Statistically significant results ($p \leqslant 0.05$) in **bold**

	df	SS	R^2	*F*	Pr(>*F*)
Year	3	1.16	0.10	3.220	**0.001**
Season	2	0.76	0.06	3.156	**0.001**
Subregion	3	4.05	0.33	11.227	**0.001**
Sex	1	0.55	0.05	4.549	**0.002**
Year:Season	4	0.91	0.07	1.888	**0.016**
Year:Subregion	5	1.56	0.13	2.601	**0.002**
Season:SubRegion	5	0.91	0.07	1.508	0.068
Year:Sex	3	0.68	0.06	1.874	**0.025**
Season:Sex	2	0.36	0.03	1.513	0.139
Residual	10	1.20	0.10	–	–
Total	38	12.14	1.00	–	–

plained 35% of the variation. Axis 1 explained 22% of the variation, in which Pacific hake loaded heavily at one extreme and English sole and surfperch at the other. The second axis had high loadings of gadids, ratfish, shad, and sockeye at one extreme and midshipmen and squid at the other, explaining 13% of the variation. There were large differences in the community composition in the diet among seasons and locations. In variance partition, a large proportion of variance in harbour seal diets was explained by locations within the SOG, with less, but significant, variation explained by year and season (Table 5).

4. DISCUSSION

We found that harbour seals are generalist predators with a broad and diverse diet that varies both at local and regional spatial scales as well as at seasonal and annual temporal scales. Our study builds upon previous studies (Olesiuk 1993, Thomas et al. 2022) and allows us to make broader inferences, largely because of an extensive spatial–temporal sampling effort. As far as we are aware, these are the first diet estimates for seals in areas outside of the SOG in British Columbia. In the areas along the coastal regions of Vancouver Island and the SOG, harbour seals consumed at least 62 different species, their diet was typically dominated by 3–5 species, and diet composition varied considerably over space and time. Collectively, harbour seal diets in the waters along the coast of Vancouver Island and the SOG are principally made up of hake, herring, rockfish, and pollock, but region and season greatly affect which of those species dominate the diet. Scat collected at nearby locations can produce different diet estimates, but within a given region, certain species groups can typically be found together in the diet. For example, the following species regularly co-occurred within the SOG: hake and squid; English sole and midshipmen; all 5 species of salmon, pollock, shad, and ratfish; this indicates that some consistent regional species assemblages are available to seals at some points within a season.

Harbour seal diets varied among the 3 main bioregions and were strikingly different at the spatial scale of our 8 study subregions. Hake and herring dominated the diet in the SOG, whereas rockfish, flatfish, lingcod, and octopus dominated the diet along the coast of WCVI Central and WCVI North, and hake and plainfin midshipmen in WCVI South. Other species like salmon pollock, surfperch, and sole form larger and smaller portions of the diet depending on location, region, season, and year. Hake comprised a large proportion of the diet in the SOG and WCVI South but was absent or at very low levels in QCS, WCVI North, and WCVI Central. Herring comprised a large proportion of the diet around the Gulf Islands (SOG South–NGI, SOG South–SGI) and QCS. The diet of harbour seals in the southern SOG was different than the rest of the SOG, with higher proportions of sockeye and pink salmon, depending on the year (2018 and 2019, respectively). These subregional differences likely reflect seasonal and spatial differences in the prey field, and harbour seals likely adjust their diet to temporal changes in abundance (e.g. herring and salmon influxes into the SOG; Schwarz et al. 2018, Allegue et al. 2020).

A harbour seal meal in the SOG consisted mostly of hake and herring, and the variation in the proportion of hake in the diet explains the largest proportion of the spatial–temporal variation in mean diet. When hake comprised a low proportion of the diet in the SOG, other demersal fish, namely sole, midshipmen, and surfperch typically comprised higher proportions of the diet. Other sites had higher proportions of chum salmon and shad in the diet or high proportions of squid and midshipman.

Our diet estimates are roughly similar to Olesiuk's (1993) study of harbour seal diets in the SOG, particularly for the dominant prey species, despite a ~30 yr time span between studies, a period which also covers large regime shifts in ocean productivity and community structure (Allen & Wolfe 2013, Schweigert et al.

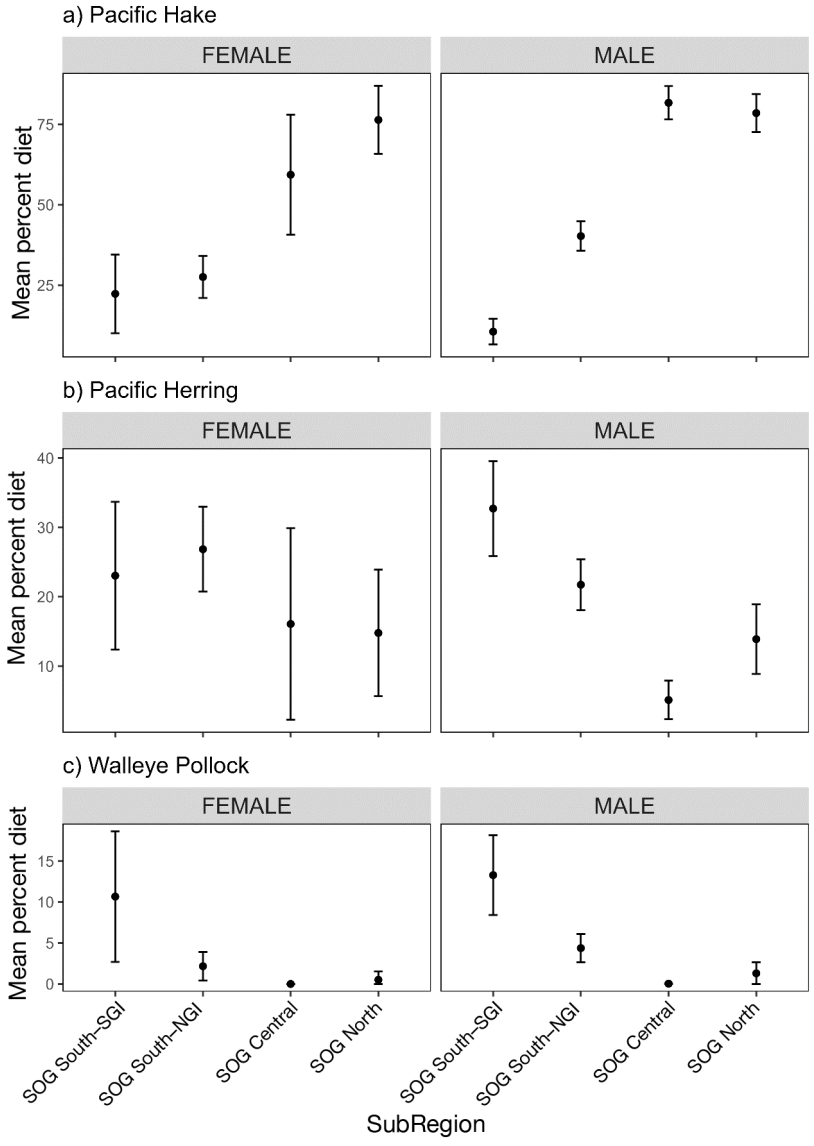

Fig. 7. Mean percent diet (±95% CI) for male and female harbour seals in the Strait of Georgia (SOG) for 3 main prey species: (a) Pacific hake, (b) Pacific herring, and (c) walleye pollock. See Table 2 for prey species taxonomic information and Fig. 1 for subregion code definitions

2013). In both studies, seasonal shift was a large driver of diet differences and probably reflects seasonal changes in prey local abundance. We did not categorize sites into estuary or non-estuary in this analysis but found similarly large spatial differences at both the broad spatial scales of subregion and among sites within the SOG, suggesting that there are other habitat features, such as hard, mixed, and soft substrate types, that may be driving the observed differences (Gregr et al. 2013).

Our results indicate that harbour seal diet responded strongly to spatial differences in the prey abundance of hake and walleye pollock in the SOG. An acoustic survey targeting aggregations of hake, walleye pollock, and herring was conducted in the SOG in March 2016. The biomass of Pacific hake in the SOG was estimated to be ~50 000 mt, which was up from a low of ~12 000 mt in 2010 and close to the average of 9 surveys since 1981 (mean: ~54 000 mt; Guan et al. 2017). The biomass of walleye pollock was estimated at ~30 000 mt. Adult hake were more abundant in the SOG North and SOG Central, juvenile hake were most abundant in the SOG North, walleye pollock were most abundant in the SOG South–NGI, and herring were found throughout the Strait with higher abundances in nearshore areas. Female and male harbour seals consumed 3 and 7 times more hake in the northern SOG, respectively, 21 and 10 times more walleye pollock in the southern SOG, and significantly more herring in the SOG South–SGI and SOG South–NGI, with some difference in diet between the sexes depending on the prey species. We cannot estimate prey availability for most species, as most fish surveys are not undertaken at the nearshore scale of harbour seal foraging. If seals consume prey in proportion to their abundance, as they appear to for hake and walleye pollock in the SOG, then seal diets may be more informative than most fish surveys in our region in delineating trends in epipelagic species within a few km of the tidal zone adjacent to haulouts.

Although our spring diet sampling was limited by a lack of available samples, we found some evidence that harbour seals also respond to temporal changes in abundance with seasonal changes in their diet, but the response was generally weaker than spatial differences. Similar seasonal responses have been noted previously. Harbour seals switched from a diet of adult herring to juvenile herring during the spawning season just south of our study area in Washington, USA (Thomas et al. 2011). In the SOG, Allegue et al. (2020) found that a few tagged harbour seals (4 out of 17) changed their foraging patterns post release of

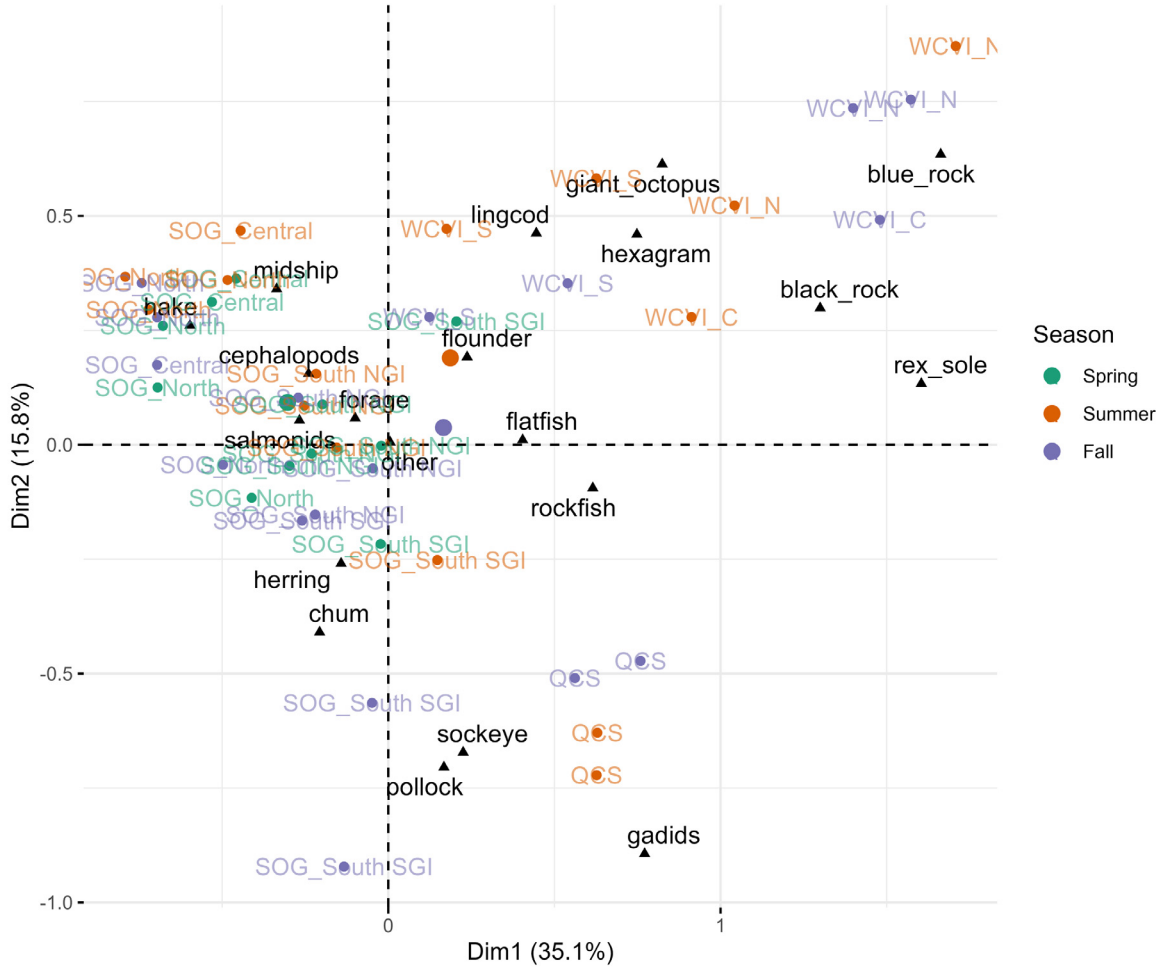

Fig. 8. Correspondence analysis of mean harbour seal diet in 8 subregions over 4 years (2016–2019) and 3 seasons for 12 species and 8 prey including a catch-all 'other' category (see Table 2 and Fig. 4 for prey species and groups, and Fig. S1 for subregion delineations). The mean percent of a species in the diet in a subregion by year × season combination was weighted by sample size. Results are colour-coded by season. Dim1 and Dim2: correspondence analysis axis 1 and 2, respectively, with percent variation explained in parentheses

Table 4. Variance partitioning (permutational multivariate analysis of variance) of harbour seal diet in 8 subregions over 4 years (2016–2019) and 3 seasons for 12 species and 8 prey groups including a catch-all 'other' category (see Fig. 4 for species and prey groups). Season: spring, summer, fall. See Fig. S1 for subregions. The model was run for 999 permutations. Statistically significant results ($p \leqslant 0.05$) in **bold**

	df	SS	R^2	*F*	Pr(>*F*)
Year	3	1.51	0.09	6.739	**0.001**
Season	2	0.85	0.05	5.707	**0.001**
Subregion	7	10.70	0.63	20.566	**0.001**
Year:Season	4	0.64	0.04	2.143	**0.041**
Year:SubRegion	12	1.54	0.09	1.713	0.064
Season:Subregion	9	1.29	0.08	1.912	**0.037**
Residual	5	0.37	0.02	—	—
Total	42	17.00	1.00	—	—

hatchery-reared coho and Chinook salmon smolts, indicating individual variation in diet such that some individuals will cue in on temporal changes in abundance and increase consumption while others of the same local population will not.

We found some diet differences between females and males in the SOG, but differences in the diet among subregions were larger. Males consumed more hake in the SOG Central and the SOG South–NGI, whereas males and females ate similar amounts of herring and pollock. Foraging behaviour and movement patterns differ between males and females in many sexually size-dimorphic phocid seals (Le Boeuf et al. 2000, Breed et al. 2009, Hindell et al. 2016, Anderson et al. 2013), and fairly large differences in diet potentially associated with differences in foraging patterns have also been noted (Lewis et al. 2006, Beck et al.

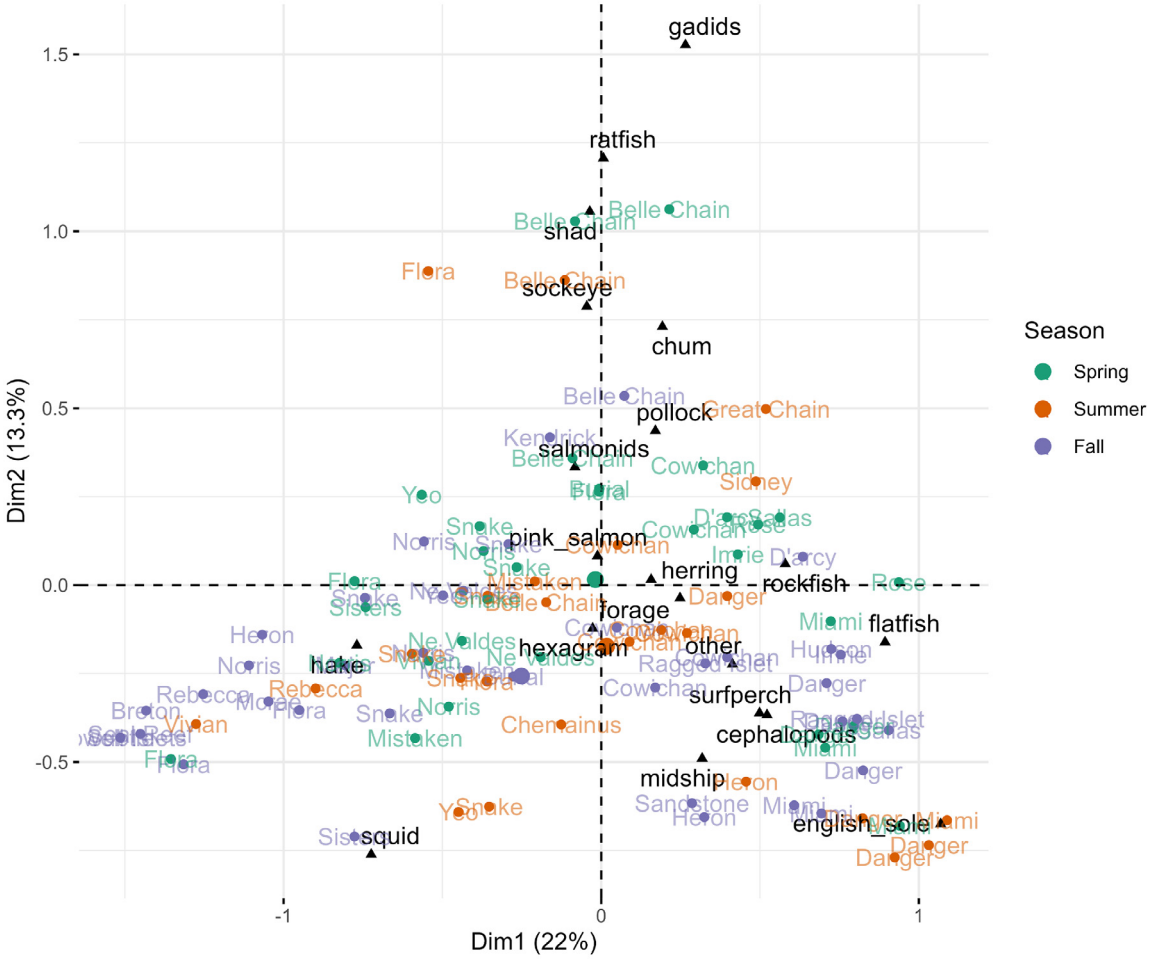

Fig. 9. Correspondence analysis of mean harbour seal diet estimated at 32 locations in the Strait of Georgia for 12 species and 8 prey groups including a catch-all 'other' category (see Table 2 and Fig. 5 for prey species and groups). The mean percent of a species in the diet in a subregion by year × season combination was weighted by sample size. Results are colour-coded by season. Dim1 and Dim2: correspondence analysis axis 1 and 2, respectively, with percent variation explained in parentheses

Table 5. Variance partitioning (permutational multivariate analysis of variance) of harbour seal diet in 32 locations in the Strait of Georgia over 4 years (2016–2019) and 3 seasons for 12 species and 8 prey groups including a catch-all 'other' category (see Fig. 5 for species and prey groups). The model was run for 999 permutations. Statistically significant results ($p \leq 0.05$) in **bold**

	df	SS	R^2	*F*	Pr(>*F*)
Year	3	1.78	0.04	4.810	**0.002**
Season	2	2.44	0.06	9.920	**0.001**
Location	31	24.76	0.62	6.485	**0.001**
Year:Season	4	0.84	0.02	1.707	0.097
Year:Location	25	5.40	0.14	1.752	**0.020**
Season:Location	19	3.20	0.08	1.367	0.136
Residual	10	1.23	0.03	–	–
Total	94	39.64	1.00	–	–

2007, Tucker et al. 2009a). Three mechanisms for producing sex differences in diet have been proposed: (1) sexual size dimorphism, (2) reduced intersexual competition for food resources, and (3) different reproductive costs (Bowen & Jonsen 2022). Harbour seals are not sexually size-dimorphic, yet sex differences in movement and diving behaviour have been noted (Coltman et al. 1997, Härkönen et al. 1999, Wilson et al. 2014); however, the differences in movement patterns are not consistent between studies (Frost et al. 2001, Russell et al. 2015). Regional differences in foraging behaviours are more likely related to variation in habitat types (Tollit et al. 1998, Hastings et al. 2004), as harbour seals are considered opportunistic predators with inter-individual specific behaviour, feeding on locally, seasonally, and annually abundant prey (Middlemas et al. 2006, this study).

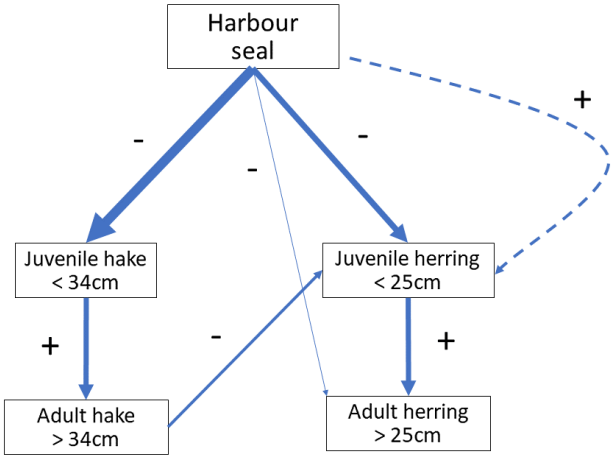

Fig. 10. Potential indirect positive effect of harbour seal predation on herring abundance in the Strait of Georgia. Solid lines: direct effects; dashed line: indirect effect resulting from the juvenile hake–adult hake–juvenile herring interaction pathway. Line width represents hypothesized interaction strength

We found a male skew in the sex ratio as estimated from our scat collections with a mean ratio of ~3:1, as also found in a previous study (Schwarz et al. 2018). We do not currently have data on the population age structure, but if males and females have different survival rates, then the sex ratio in the population will vary by age. There is potentially a behavioural component creating the male skew in the sample sex ratio, with large males probably more inclined to haul out higher up beyond the water's edge. As most scats are collected above the tideline, a behavioural difference in haulout use may affect the sex ratio of scat collections. The sex ratio becomes approximately equal (not significantly different from 1:1) in the summer during the pupping season, when females are likely motivated to be hauled out well above the tideline and males may start to establish underwater territories and haul out less frequently (Hayes et al. 2004, Boness et al. 2006). During 'beach rush' captures, which by default favour the capture of animals further from the water, there is a propensity to catch males in the late spring, while this generally shifts to pregnant females later in the season (C. A. Nordstrom unpubl. data).

Data on the sex ratio of the SOG harbour seal population has not been collected since the 1960s (Bigg 1969), when the population was near minimum following exploitation (Olesiuk 2010). Collections of all life stages (fetus, pups, maturing and mature) demonstrated a consistent 50:50 sex ratio, as would be expected from an expanding population. The harbour seal population in the SOG increased exponentially during the 1970s and 1980s, stabilizing in the mid-1990s to ~40 000 up to the last assessment in 2019 (DFO 2022), suggesting that the population has been at carrying capacity for ~30 yr and is likely resource-limited. For philopatric species with further dispersal of male offspring, as in harbour seals (Härkönen & Harding 2001), the resource competition model (Clark 1978, Silk 1983) for offspring sex-ratio adjustment in mammals predicts that if resources are limiting, females should produce a male-biased sex ratio to avoid direct competition with their offspring (Johnson 1988, Dittus 1998). Male and female northern elephant seals have distinct forage resources and have also been shown to adjust the sex ratio (Lee & Sydeman 2009). Therefore, it remains possible that our harbour seal scat samples are, to some degree, reflecting a real phenomenon in the population that requires further exploration.

Harbour seals eat prey in a wide range of species sizes, from small surfperch (5–15 cm) and herring (8–35 cm) to large salmon (15–90 cm) and lingcod (16–99 cm). The size range of individuals consumed within a prey species is huge, with the largest individuals consumed being 3–8.7 times bigger than the smallest prey items (Fig. S2). The size frequency distribution for each species shows some central tendency which shifts by season, with the smallest herring, surfperch, and pollock eaten in the summer or fall, the largest lingcod in the spring, and the largest salmon in the fall, indicating that harbour seals are likely responding to both the species and size of prey available. For example, lingcod spawning takes place over winter and males actively defend nests through spring (Hart 1973), likely making them more readily available to seals. Similarly, spawning of herring and pollock takes place in the spring and new recruits of these species would be available in summer, while adult chum salmon return to natal rivers in southern British Columbia in fall (Urawa et al. 2018).

Our results show that the impact of harbour seals on the coastal ecosystems of British Columbia is likely to vary widely because their diet depends upon the food web in which they are embedded and the size of the prey eaten. Focus is often given to the direct negative effect of predation on prey populations in tightly coupled predator–prey systems, but harbour seals are generalist predators and forage in a highly connected food web, increasing the potential for important indirect effects (Yodzis 2001). Herring in the SOG increased from 2006 to a record spawning biomass in 2019 during a period in which harbour seal abundance was high, suggesting that bottom-up (Schweigert et al. 2013, Godefroid et al. 2019) and possibly indirect effects are more important in determining

herring abundance than direct consumption by harbour seals. Hake is the primary species consumed by harbour seals in the SOG while also being the dominant fish predator (Preikshot et al. 2013). It is possible that the herring population benefits from seal predation on hake, particularly if we consider the size distribution of hake and herring eaten (mean hake: 30.0 cm, 95% CI: 29.4–30.6 cm; mean herring: 12.6 cm, 95% CI: 12.3–12.9 cm; Table 2, size distributions in Fig. S2). Harbour seal predation on juvenile hake could have an indirect positive effect on juvenile herring (<25 cm), as fewer hake reaching maturity (>34 cm) would result in reduced predation on juvenile herring (Fig. 10). This indirect positive effect of harbour seal predation on juvenile herring may also apply to other small pelagic fish, like Chinook and coho salmon smolts. These mechanisms are hard to demonstrate, but a hake fishery might have a similar impact.

There are at least 2 main sources of variation in seal diet aside from sampling error: (1) differences in prey availability in time and space and (2) variation in foraging behaviour among males and females (Kernaléguen et al. 2012, Wilson et al. 2014), juveniles and adults, and potentially among individuals (Kernaléguen et al. 2015, Schwarz et al. 2021). While the sex of an individual can be determined from DNA recovered in a scat, there is currently no reliable method to age an animal from DNA. Future studies of harbour seal diets in our study region using alternative means of diet estimation (stable isotopes or fatty acids) that are readily tied to individuals of known age and sex may be informative in discriminating diet differences among sexes and age classes (Beck et al. 2007, Tucker et al. 2009b, Bromaghin et al. 2013). These techniques typically integrate over larger temporal and spatial scales but have less taxonomic resolution.

Our sampling was as extensive as logistically possible, yet important gaps remained in our spatial–temporal coverage. As noted by previous studies, scats only represent a snapshot of the diet in time and space, and it may require 30 scats or more to accurately represent the diversity in the diet of a local population (Trites & Joy 2005, this study). Here, 63% of all scats collected between 2016 and 2019 were dominated by a single species (comprising 90% or more of the diet). Of the 12 major species on which we focused, one of these species comprised more than 90% of the diet in 58% of the scat samples. As such, a single scat is likely to have a low diversity of species detected in the diet (mean number of prey species in a scat was 1.9), while a large number of scats collected in a particular location and season will reveal a highly diverse diet. We used an arbitrary number of scats (5 scats) as a cutoff for our spatial–temporal correspondence analysis of harbour seal diet and weighted our multivariate analysis by sample size. A higher or lower sample size cutoff could lead to slightly different estimates, but sensitivity analyses showed that our conclusions about the principal species in the diet and the highly variable nature of harbour seal diet across space and time are robust. Given our current data, it is impossible to determine dietary 'preference', as that requires quantifying if a species is selected greater than its availability (e.g. Fig. 5 in Hutchinson et al. 2022), for which data are currently unavailable. However, our results support the notion that harbour seals are generalist predators that adapt to the local communities in which they live. Indeed, harbour seals are ubiquitous across the Northern Hemisphere and rely on disparate prey fields to achieve comparable growth and fitness endpoints. For example, in Mexico, 49 prey species were found in the diet of harbour seals, the diet varied widely by location, and the most frequently consumed were large-tooth flounder species *Citharichthys* spp. and California lizardfish *Synodus lucioceps* (Brassea-Pérez et al. 2019). Harbour seal diets varied widely among regions of Britain, where prey species richness in the diet varied from 10 to 46 species, with sandeels (Ammodytidae) and large gadids (Gadidae) dominating the diet (Wilson & Hammond 2019). In Japan, harbour seals consumed 46 prey species, and the diet was dominated by walleye pollock *Theragra chalcogramma*, sculpins (Cottidae), and snailfishes (Liparidae) (Hui et al. 2017).

Here, we used DNA sequence counts converted to percentages, generating proportional diet estimates to provide relative contrasts between harbour seal scats sampled from different locations over time. The method relies on the assumption that quantities of DNA detected from scats equate to the biomass proportions of food consumed (Thomas et al. 2017, 2022). The relationship between proportions of biological material in a sample and sequence reads recovered by high-throughput sequencing has been studied and generally confirmed in many experiments (summarized in Deagle et al. 2019, Lamb et al. 2019, van der Loos & Nijland 2021), and we are currently conducting our own controlled feeding trial (C. A. Nordstrom et al. unpubl. data). Biases can be biological in origin (mass-specific differences in target gene copy number between prey species and differential digestion of prey species) or introduced by the methodological protocols (PCR, primer tag, sequencing biases), which

can ultimately result in preferential or differential species-specific yields (Deagle et al. 2019). To mitigate known biases in the method (see Deagle et al. 2019, de Sousa et al. 2019), we implemented a number of checks during the initial processing of sequence reads, discarding rare sequences to avoid incorporation of background sequencing errors, as done by Quéméré et al. (2013) and Thomas et al. (2017). After this, sequences are assigned to a taxonomy, and a threshold number of reads is required for each taxon to be tallied as an occurrence to address sequencing depth variation. That being said, the approach is more accurate when the mean number of food taxa in samples is small (Deagle et al. 2019), which is the case here, with only a few prey items identified in each scat. Furthermore, captive feeding studies have explicitly examined quantitative prey DNA recovery on both captive sea lions and seals (Deagle & Tollit 2007, Bowles et al. 2011, Thomas et al. 2014) providing a high degree of confidence for the application of the method for relative contrasts. However, we emphasize the term relative and caution against taking diet proportions as absolute.

Lastly, we stress that the important regional differences in harbour seal diets need to be taken into account when estimating the ecological impact of harbour seal foraging. Estimates of mean diet from samples restricted in time and space and then applied to harbour seal foraging across the Salish Sea or all of British Columbian coastal waters and beyond are likely to be misleading, as they do not take into account the underlying spatial–temporal variation in harbour seal diets and the underlying uncertainty in such estimates (e.g. Chasco et al. 2017a,b). However, it must also be recognized that more meaningful estimates of diet bounded by space and time are limited by the difficulties of collecting samples and obtaining a large enough sample size. Species that form smaller proportions of the diet, such as Chinook, coho, and sockeye salmon, and for which harbour seals likely adapt to local abundances, will also have a high variance in the estimate of mean diet and are particularly prone to bias if spatial–temporal sampling is not considered. The combination of low proportion in the diet and high variance makes it all the more difficult to quantify the impact of harbour seal predation on the survival of fish of commercial interest (Trzcinski et al. 2006, Mohn 2009) because of high uncertainty. These uncertainties make it equally difficult to evaluate any proposed adaptive management actions to reduce harbour seal impact by altering their distribution and abundance, such as those explored by Nelson et al. (2023).

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