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Feeding ecology of harbor seals (*Phoca vitulina*) in the outer Oslofjord quantified by DNA metabarcoding of scat soft-matrix and morphological analysis of hard prey remains

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Cover image: A harbor seal at Magdalenefjorden in Svalbard.
Photo by Andrew Weith.

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Master of Science in Biology – Marine Ecology and Resource Biology
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Abstract

The feeding ecology of harbor seals in the outer Oslofjord was investigated during late summer and autumn of 2019 using DNA metabarcoding and morphological hard-parts identification. To evaluate potential competition between seals and fisheries, the annual consumption of fish by harbor seals was estimated and compared to the fisheries landings in the outer Oslofjord. Fish species from 16 and 18 families were identified among the feces samples included in the DNA metabarcoding (n = 44) and hard-parts analysis (n = 77), respectively. In total, fish from 25 families were identified as prey. In addition to bony fishes, the DNA metabarcoding revealed that birds and skates may also be components of harbor seal diet in the region. The hard-parts analysis indicated cephalopods were prey as well. The results from molecular and morphological analyses were similar in regard to important prey species, but finer taxonomic resolution of important prey groups was achieved using DNA metabarcoding compared to the more traditional morphological analysis. Additionally, individual scats tended to contain a greater diversity of prey when analyzed by the DNA metabarcoding. In both methods of analysis, gadoid fishes comprised the largest part of the diet. In the hard-parts analysis, the most important prey in terms of relative diet contribution were fish in the unresolved cod/pollack/saithe group (13.0%), followed by *Trisopterus* spp. (Norway pout/poor cod/bib; 11.2%), Atlantic cod (8.7%), and haddock (8.5%). Similarly, through DNA metabarcoding the most important prey were found to be Atlantic cod (25.5%), haddock, (14.4%) and *Trisopterus* spp. (12.6%). Thus, the combined approach gives us reason to believe that much of the prey identified as cod/pollack/saithe in the hard-parts analysis may in fact be Atlantic cod. Pleuronectid flatfishes were also common prey in both methods of analysis. The total annual prey consumption by ca. 620 harbor seals in the outer Oslofjord (Færder and Hvaler sub-areas) was estimated as 1009 tons. Our data indicates increased predation on 0-group cod in a year when juvenile cod were exceptionally plentiful. Though prey size estimates showed that seals predate primarily on small fish below minimum allowed landing size for most commercial species, we speculate that harbor seals may expose preferred prey, such as coastal cod, to a "predator pit" phenomenon, and thus question whether seal predation is constraining the recovery of coastal cod in the outer Oslofjord and adjacent areas. Further investigation of diet across multiple seasons in subsequent years is needed to understand how harbor seals regulate prey populations.

Keywords: harbor seal, *Phoca vitulina,* DNA metabarcoding, feeding ecology, diet composition

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1 Introduction

Coastal ecosystems, such as fjords and coastal waters, are often very productive areas utilized by a great diversity of species. The complex topography of these coastal areas offers a multitude of habitats that support many essential ecological functions, in part by providing spawning, nursery, and feeding grounds. Knowledge of the structural and functional characteristics of an ecosystem is crucial for understanding its dynamics and responses to natural and anthropogenic perturbations. Coastal systems in southern Norway have experienced major changes in structure during the last few decades, including a major decline in coastal cod populations (Hagström et al. 1990, Fromentin et al. 1998, Svedäng 2003, Svedäng and Bardon 2003, Mieszkowska et al. 2009). Factors contributing to the collapse of coastal cod populations may include overfishing, predatory interactions, and climate change. Predation is a pervasive feature of coastal ecosystems, and in some ecosystems, the predation mortality constitutes a major fraction of the total mortality (Pedersen et al. 2008). For example, the fact that most coastal cod populations have stabilized at low densities may suggest that cod is trapped in a "predator pit" (e.g., Bakun 2006), implying that cod escapes predation at very low densities but suffers destructive predation above a certain density threshold sufficient to constitute a profitable prey resource for predators in the region. Analyzing the ecological role of important predators such as harbor seals in coastal communities is important to inform ecosystem-based management and resolve potential conflicts with fisheries, particularly in southern Norway where fishing-induced mortality has also been high (Julliard et al. 2001, Kleiven et al. 2016).

Assessing predator-prey interactions in marine ecosystems is difficult. Historically, predator diets have been assessed through examination of the stomach contents of animals found dead, killed incidentally (such as through bycatch), or killed deliberately, but such invasive methods are unfavorable because they yield biased estimates or necessitate killing of animals (Prime and Hammond 1990). For pinnipeds, identification of hard prey remains within feces is the most common and noninvasive method of diet quantification (Murie and Lavigne 1986, Prime and Hammond 1990, Pierce and Boyle 1991). This morphological method usually involves using fish otoliths and cephalopod beaks for taxonomic identification, though other hard remains such as eye lenses, scales, vertebrae, and miscellaneous skeletal structures may be used as well (Pierce and Boyle 1991, Olesiuk 1993, Cottrell et al. 1996, Brown and Pierce 1998, Browne et al. 2002, Tollit et al. 2003, Tollit et al. 2004).

Other noninvasive methods of pinniped diet assessment have become available during the last three decades, including stable isotope analysis (Burns et al. 1998, Post 2002, Cherel and Hobson 2005, Wild et al. 2020), fatty acid analysis (Iverson et al. 1997, Andersen et al. 2004, Iverson et al. 2004), and DNA-based techniques (Symondson 2002, Deagle et al. 2005, Dunshea 2009, Valentini et al. 2009, Pompanon et al. 2012, De Barba et al. 2014). Molecular analysis can be particularly useful for identifying prey species that lack otoliths, have small and fragile otoliths, or are partially ingested (e.g., through "belly-biting") without consumption of otoliths (Pierce and Boyle 1991, Moore 2003, Chouinard et al. 2005). Additionally, by providing higher taxonomic resolution (Matejusová et al. 2008, Voelker et al. 2020) recent DNA-based molecular methods may help to overcome major difficulties in the species-level identification of severely eroded fish otoliths, which are common with morphological analysis of scat contents (Nilssen et al. 2019). Because dietary metabarcoding data – like that from stable isotope and fatty acid analyses – is currently only semi-quantitative (Pompanon et al. 2012, Thomas et al. 2014), supplementing molecular techniques with morphological techniques may be the best approach (Matejusová et al. 2008, Bowen and Iverson 2013, Méheust et al. 2015).

The harbor seal is a well-studied, widely-distributed coastal pinniped species that is abundant in the north Atlantic (Teilmann and Galatius 2018). Previous studies of harbor seal diet show that they are generalist predators that typically feed on a great diversity of prey species (e.g., Härkönen and M.-P. Heide-Jørgensen 1991, Tollit and Thompson 1996, Pierce and Santos 2003, Andersen et al. 2004, Scharff-Olsen et al. 2019), though in some areas diet appears less diverse (Berg et al. 2002). Harbor seal diet composition may vary according to geographic location and season, generally reflecting the most spatially or temporally abundant prey species (e.g., Härkönen 1987, Olsen and Bjørge 1995, Tollit and Thompson 1996, Brown and Pierce 1998, Lance et al. 2012). In many regions, there is concern over potential conflicts between harbor seals and fisheries, either via operational interactions (e.g., seals causing damage to fishing gear and fisheries catches, seals killed in fishing gear or becoming entangled in fishing nets) or through competition for the same commercially important fish species (Beverton 1981, Beddington et al. 1986, Harwood and Croxall 1988, Olesiuk 1993, Bjørge 2002). Given their abundance and opportunistic, piscivorous foraging behavior, harbor seals have the potential to greatly influence ecosystem dynamics (Hansson et al. 2018, Voelker et al. 2020) and may impact fish mortality and stock recovery (Trzcinski et al. 2006, Lance et al. 2012). Alternatively, diversified generalist feeding behaviors in which harbor seals target the most abundant or most profitable prey may stabilize predator-prey dynamics and promote

coexistence (Rosenzweig and MacArthur 1963, Brown 2000). Accurate assessment of harbor seal diet is essential for improved understanding of pinniped impacts on food web dynamics and prey populations.

As in other parts of the world, harbor seals around southern Norway have also shown an opportunistic feeding strategy that involves preying on a variety of prey species and adjusting diet composition based on seasonal availability (Härkönen 1987, Härkönen and M.-P. Heide-Jørgensen 1991, Olsen and Bjørge 1995, Scharff-Olsen et al. 2019, Sørlie et al. 2020). Following population depletion by hunting in the early 20th century, the number of harbor seals in the Norwegian Skagerrak (which includes the outer Oslofjord) has increased, despite notable interruptions in the population recovery due to outbreaks of phocine distemper virus (PDV) in 1988 and 2002 that caused mass mortalities (Dietz et al. 1989, Markussen 1992, Härkönen et al. 2006, Reijnders et al. 2010, Nilssen et al. 2020). The most recent estimates of harbor seal population size in this region were 292 seals at Færder in western outer Oslofjord (Vestfold county) in 2014 and 325 seals at Hvaler in eastern outer Oslofjord (Østfold county) in 2016 (Nilssen et al. 2020). Increases in harbor seal numbers have coincided with or followed the collapse of several important fish stocks in the outer Oslofjord, motivating further investigation into harbor seal feeding ecology and improved assessment of the potential impacts of seal predation in the ecosystem (Hansen and Harding 2006, HELCOM 2018, Moland et al. 2020).

The aims of this study were: (1) to assess the diet composition of harbor seals in two sub-areas (Færder and Hvaler) in the outer Oslofjord using a combination of molecular scat analysis (DNA metabarcoding) and morphological identification of hard prey remains and (2) to compare the results from the two methodologies. Additionally, the annual prey consumption was estimated to evaluate the potential competition between harbor seals and commercial fisheries and to investigate the possible impacts of harbor seals on hampered coastal cod fish stocks.

2 Materials and Methods

2.1 Study areas

A total of 77 harbor seal scat samples (45 collected at Hvaler during August 2019 and 32 collected at Færder during November 2019) were analyzed (Figure 1). The study areas are separated by approximately 30 km; both consist of groups of small islands and rocks located in shallow near-shore waters where tidal amplitude is less than 50 cm. The collection sites were small, rocky skerries where harbor seals often congregate out of the water. Scat samples were taken from six collection sites at Hvaler (Båene, Flatekollen, Kuskjær, Lilleribba, Rokrasfluene, and Store Gråbein) and two collection sites at Færder (Flatskjær and Selskjær) (Figure 1).

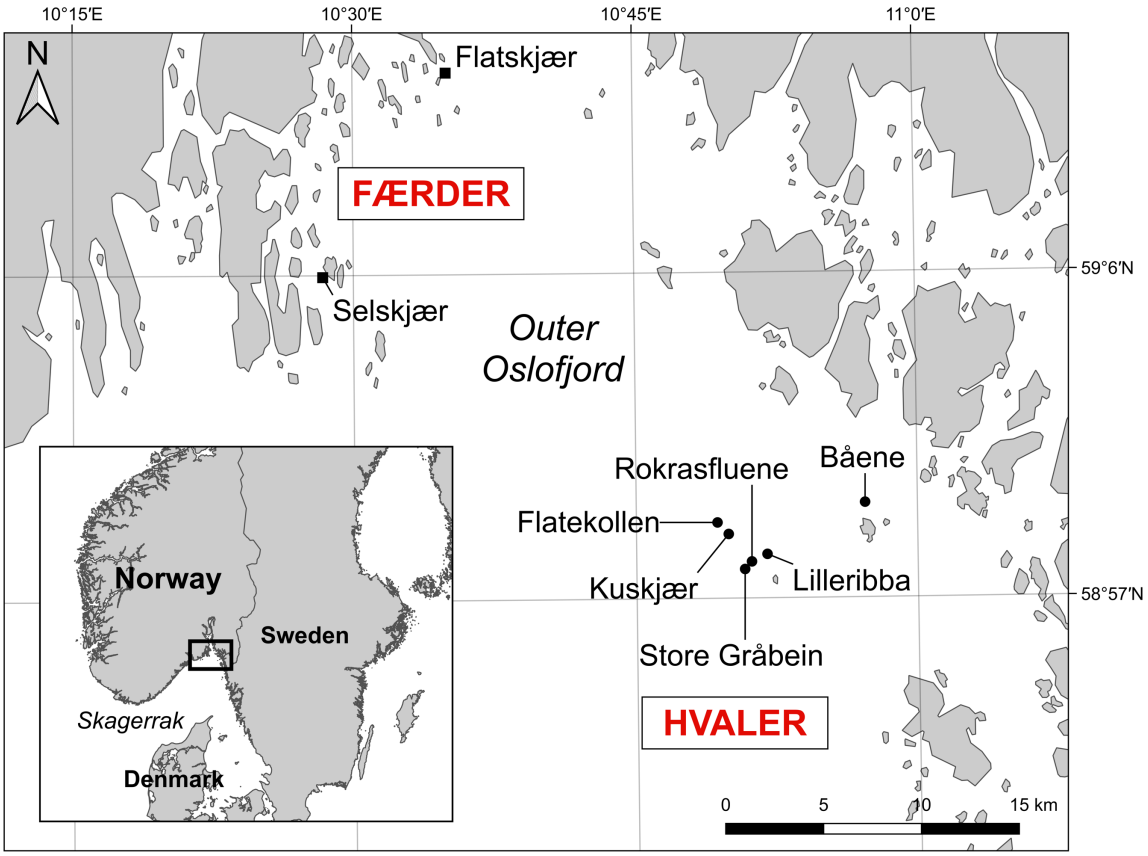

Figure 1. Haul-out sites in the outer Oslofjord where harbor seal scats were collected. The two study areas, Færder and Hvaler are indicated in red capitalized text. Collection locations are marked by black squares for sites in Færder and by black dots for sites in Hvaler. Coordinates for each site are as follows: Flatskjær: 59° 11’ 34.3428” N, 10° 35’ 0.3876” E; Selskjær: 59° 5’ 57.012” N, 10° 28’ 21.81” E; Flatekollen: 58° 59’ 5.6112” N, 10° 49’ 19.6536” E; Lilleribba: 58° 58’ 12.63” N, 10° 51’ 58.3632” E; Båene: 58° 59’ 37.0356” N, 10° 57’ 12.6864” E; Rokrasfluene: 58° 58’ 0.4548” N, 10° 51’ 8.3844” E; Store Gråbein: 58° 57’ 48.0132” N, 10° 50’ 46.248” E; and Kuskjær: 58° 58’ 46.326” N, 10° 49’ 55.1568” E.

All scats were collected in plastic bags and transferred to a freezer (-18 ˚C) for approximately one year until further processing.

2.2 DNA metabarcoding

DNA was extracted from the scat samples using the QIAamp Fast DNA Stool Mini Kit (QIAGEN). Following the manufacturer's protocol, the scat samples were kept frozen until lysing buffer was added. To preserve the structure of fragile otoliths and other hard parts required for subsequent morphological analysis, the frozen scats were kept intact and not blended. Because DNA from different prey species is not distributed evenly within a single scat (Deagle et al. 2005), fecal starting material was taken from multiple distinct fecal lobes. Approximately 1 g of feces was used as starting material for DNA extraction. The remaining DNA extraction process was conducted following the manufacturer's "Isolation of DNA from Stool for Human DNA Analysis" protocol beginning with the "Isolation of DNA from Larger Volumes of Stool" protocol in order to maximize the likelihood of purifying degraded prey DNA from the nonhomogeneous scat samples (QIAGEN Februrary 2020). The following modifications were made: (1) DNA was eluted in a 100 µl volume (rather than 200 µl) to increase DNA concentration in the eluate; and (2) after the first elution, a second elution centrifugation was conducted with the same solution to capture DNA remaining on the column filter and maximize yield. The concentration of DNA in the extract from each sample was measured by spectrophotometry (using the NanoDrop One Microvolume UV-Vis spectrophotometer from ThermoFisher) and a subset of 49 scat samples with relatively high concentrations were selected for further processing (DNA extraction was attempted unsuccessfully for a higher number of samples; e.g., desiccated "old" scats that may have contained degraded DNA and/or PCR hindering substances, as previously reported by Tollit et al. (2009)).

Standard polymerase chain reactions (PCRs) were conducted using universal primers described by Granquist et al. (2018) to amplify a barcoding fragment (~270 base pairs) of the mitochondrial 16s rDNA. These primers – 16sPreyF (5′-CGTGCRAAGGTAGCG-3′) and 16sPreyR (5′-CCTYGGGCGCCCCAAC-3′) – were designed to amplify sequences of jawed fish and other vertebrates (including birds) while avoiding over-amplification of the predator DNA through a mismatch between the phocid seal 16s sequence and the nucleotide at the 3' end of the forward primer (Granquist et al. 2018). To allow for multiplexing of individual samples in DNA libraries and subsequent demultiplexing of sequence data, the PCR amplicons

were labeled with one of nine variations of the forward primer, each with a unique six-nucleotide "barcode" sequence at the 5' end. This method was adapted from the method used by Granquist et al. (2018), which was originally suggested by Binladen et al. (2007).

PCR reactions were carried out in 70 µl reaction volumes, including 35 µl AmpliTaq Gold 360 Master mix (Applied Biosystems; catalog no. 4398881), 2.8 µl of each primer at 10 µM concentration, 8.4 µl of DNA extract solution, and 21 µl of nuclease-free PCR grade water. Thermal cycling conditions were as follows: initial denaturation at 95 °C for 10 min; 38 cycles of 94 °C for 30 s, 54 °C for 30 s, 68 °C for 30 s; then a final extension step of 72 °C for 7 min. Negative template controls using an additional 8.4 µl of nuclease-free PCR grade water in place of the template DNA extract solution were included in each PCR amplification run for all combinations of PCR primers. The PCR products of all negative template controls were assessed for presence of DNA through spectrophotometry (using the NanoDrop One Microvolume UV-Vis spectrophotometer from ThermoFisher), and all controls showed no evidence of PCR product formation. These negative controls were not processed further.

The PCR products for reactions with template DNA were then purified with the PureLink PCR Purification Kit (Invitrogen, catalog no. K3100-01), following the manufacturer's protocol. Out of the 49 scat samples subjected to PCR, 45 samples ultimately contained a suitable concentration of DNA for sequencing (91.8% PCR amplification success).

PCR products were pooled in sets of up to 8, with each sample in a pool corresponding to a different variation of the forward primer. The pools were normalized to contain approximately equimolar amounts of DNA from each sample by adding purified PCR products to pools in volumes calculated according to their DNA concentrations (Appendix A). Normalizing the pools ensures equal coverage of each sample in the resulting sequences, providing the best possibility of identifying representative prey sequences from each scat sample, including samples with lower DNA concentration after PCR. PCR products from a total of 45 samples were pooled into 6 groups that were used to prepare 6 different DNA libraries.

Library preparation and sequencing was done at the Norwegian Sequencing Centre, Oslo. Amplicon libraries were prepared using the low-input SMARTer ThruPLEX DNA-Seq Kit (Takara, catalog no. R400676) with indexed PCR primers carrying the "IDT for Illumina UD" index sequences from the SMARTer DNA Unique Dual Index Kits (Takara, catalog no. R400665- R400666). The amplicon libraries were pooled together and sequenced using Miseq

v2 (2 x 250 cycles). The sequencing center ran bcl2fastq v2.20.0.422 to demultiplex the data by index sequences; one mismatch was allowed. Reads that failed to match any known index sequence were discarded. The paired-end output was received in FastQ format. No further work was outsourced.

DNA metabarcoding bioinformatics were performed with QIIME 2 2021.4 (Bolyen et al. 2019) (see Appendix B.1 for complete QIIME 2 code). Raw, multiplexed sequence data with barcodes in the paired-end sequence reads were demultiplexed and trimmed to remove the indexing barcodes and PCR primers on the 5' ends using the Cutadapt tool (Martin 2011) (via the q2-cutadapt plugin); all sequences that lacked either the barcodes or the primers were discarded. Data were then denoised, joined, and dereplicated with DADA2 (Callahan et al. 2016) (via the q2-dada2 plugin). To correct for decreasing read quality, the 3' ends of forward read sequences were truncated to retain 185 nucleotides, while the 3' ends of reverse reads were truncated to retain 135 nucleotides. Reads with more than 3 expected errors were discarded. The RESCRIPt (REference Sequence annotation and CuRatIon Pipeline) plugin was used to compile a custom reference sequence database and taxonomy information from the NCBI GenBank sequences for teleost fishes, cartilaginous fishes (Chondrichthyes), birds (Aves), and true seals (family Phocidae) (Robeson et al. 2020). Species identification was carried out by assigning taxonomy to a representative set of amplicon sequence variants with BLAST+ (via the q2-feature-classifier plugin) (Camacho et al. 2009) (see Appendix B.2 for further details).

Samples that contained fewer than 100 assigned prey sequences were discarded. 44 of the 45 scat samples analyzed for prey DNA produced a sufficient number of matched prey sequences and could be used for further analysis.

2.3 Hard-parts analysis

Extraction and preparation of prey hard parts was completed after DNA extraction. Scat samples were first thawed, then rinsed and stirred with water through a set of nested sieves with 2.0, 1.0, and 0.5 mm mesh, following the method described by Orr et al. (2003). The remaining slurry of hard parts and particles from each sieve was transferred to a water basin and swilled and "panned" to separate the otoliths, which sink to the bottom, as explained by Treacy and Crawford (1981). Otoliths and other hard remains, including crustacean exoskeletons and cephalopod beaks, were collected and stored in 96% ethanol. Subsequently, the sagittal otoliths were removed from the ethanol, dried, and measured for length and/or width to the nearest 0.05

mm using the internal reticle scale of a microscope, which had been calibrated for accurate measurement. Each otolith was registered as belonging to the left or right side. Then, each sagittal otolith was identified to the lowest possible taxonomic group based on the 1986 guide from Härkönen (Härkönen 1986) with additional support from the Otoliths of North Sea Fish web-resource (Leopold et al. 2001).

Fish length and weight were estimated using regression equations based on otolith length (or otolith width in select cases) from Härkönen (1986), Leopold (2001), and Silva et al. (2013) (Appendix C). The length distributions of prey identified to groups rather than species were estimated by using the mean size calculated from the regression equations of the species comprising the group, weighted by the prevalence of each species in the area. Species prevalence was calculated as frequency of their occurrence in trawls conducted in the outer Oslofjord and Skagerrak in February 2020 by the Norwegian Institute of Marine Research.

Otoliths, in contrast to cephalopod beaks, were identified to the lowest possible taxonomic unit; species that could not be reliably distinguished were pooled into groups (Appendix D). For visualization purposes, the resulting prey species and groups were sometimes combined into fewer, broader groups. Otoliths from each species/group were subjectively paired based on side (left and right) and length. Complementary left and right otoliths from a given species/prey group were paired by most similar length possible, as long as the difference in length between the two was no greater than that which could reasonably be expected to result from degradation of equivalently-sized otoliths in the harbor seal digestive tract, as approximated by digestion coefficients given by Wilson et al. (2017) and Grellier and Hammond (2006). Otoliths of unknown side were paired according to the general procedure described in Tollit et al. (2003). The minimum number of individuals (MNI) was counted from the side (left/right) with the greatest number of otoliths from each species/group after pairing (Ringrose 1993, Orr et al. 2003, Tollit et al. 2003).

Hard parts were first identified from the 45 samples for which DNA was sequenced followed by 32 additional samples in a random order. Thus, a total of 77 scat samples were analyzed morphologically; 32 of these samples had been collected from Færder and 45 has been collected from Hvaler (Table 1).

Table 1. Number of samples analyzed by DNA metabarcoding, number of samples analyzed by hard-parts analysis, number of samples with otoliths, number of otoliths, and number of prey fish identified per study location.

Study location	Samples analyzed by DNA metabarcoding*	Samples analyzed by hard-parts identification	Samples with otoliths	Otoliths	Prey fish (identified by otoliths)
Færder	24	32	30	2051	1141
Hvaler	20	45	43	1001	657
Total	**44**	**77**	**73**	**3052**	**1798**

*49 samples (25 from Færder and 24 from Hvaler) were initially subjected to PCR. PCR was successful for 45 of these samples (25 from Færder and 20 from Hvaler) and they were sent for sequencing. Ultimately, 44 of these 45 contained enough prey DNA to be used in the analysis (as displayed in the above table).

2.4 Feeding indices and statistical analysis

Predator diet studies employ feeding indices to estimate relative contribution or importance of prey species (Hyslop 1980). Because no singular measure provides a complete picture of diet composition, two feeding indices were used to assess importance of different prey species in the diet of harbor seals. The frequency of occurrence of each prey group, (FO_i) was calculated as:

$$FO_i = s_i/s_t \times 100 \quad (1)$$

where s_i is the number of scat samples containing prey group i, and s_t is the total number of scat samples examined. This index was calculated with data from both molecular and hard-parts analysis. FO_i calculations with the DNA metabarcoding data were based on prey taxa that comprised >1% of prey sequences for that sample, and prey taxa that were detected in sequence amounts below this threshold were considered as absent from the sample. The relative contribution of each prey group (RC_i) was calculated as:

$$RC_i = p_i/p_t \times 100 \quad (2)$$

where for molecular DNA data p_i is the number of sequences of prey group i in the samples, and p_t is the total number of sequences identified as prey, while for morphological hard-parts data p_i is the total biomass (g) of prey group i – calculated using regressions (Appendix D; e.g., Härkönen 1986, Leopold et al. 2001) – and p_t is the total biomass (g) of all prey. Thus, when applied to data from the DNA metabarcoding RC_i represents relative read abundance, and when applied to data from the hard-parts analysis RC_i represents relative biomass. When analyzing the molecular data, mean RC_i was used to ensure equal weight of all samples by avoiding overrepresentation of samples containing very high numbers of prey sequences.

To further assess the relative contribution of prey families, samples were categorized according to the dominant prey group, defined as the prey group represented by >70% of the sequences in the molecular data or by >70% of the biomass in the hard-parts data. This method was adapted from that used in a metabarcoding study by McInnes et al. (2017). A sample was classified as "mixed" when no single prey family dominated.

The scat samples analyzed with molecular analysis ($n = 44$) were also analyzed during the hard-parts analysis, which allowed for paired comparison between the methods. As stated above, additional samples were analyzed through morphological analysis alone, but these samples were excluded from the dataset whenever the techniques were being compared. Prey taxa were grouped prior to comparison when species-specific information was not consistently available across both techniques. A chi-square test was used to determine if there was a significant relationship between the frequency of occurrence of prey groups and the method of analysis. The results from the molecular analysis were further compared to the results from the morphological analysis with respect to the number of prey taxa identified per sample by Wilcoxon signed rank test.

Differences in the harbor seal diet between the two locations were investigated by chi-square and Fisher's exact tests. A constrained correspondence analysis (CCA) was conducted on prey biomass data to test if the factor of location explained a significant amount of the variation in relative importance of prey groups by biomass. The CCA was chosen because there was a unimodal relationship (gradient length > 4) between the diet matrix variables and the explanatory variable (location) (e.g., Legendre and Anderson 1999, Lepš and Šmilauer 2003). Prior to running the CCA, prey groups were consolidated into fewer categories and any prey occurring in less than 5% of the scat samples were excluded from the analysis in order to reduce the number of zeros present in the dataset. To normalize the data and reduce the effect of outliers, the biomass data were transformed prior to analysis using the Hellinger transformation (Roberts 2019). The CCA was performed in R (R Core Team 2021) using the *vegan* package (Oksanen et al. 2020) and plots were created using the *ggplot2* package (Wickham 2016).

2.5 Prey consumption

The annual consumption of prey by harbor seals was estimated as in a previous study by Sørlie et al. (2020) using 4 kg as the daily per capita food consumption for harbor seals (Bjørge 2002). This value represents the daily prey biomass needed to meet the energy requirements of seals

averaged across the different age and sex groups and was used since the scat samples could not be attributed to seals of specific age or sex. Assuming the relative contribution of prey groups in the diet of harbor seals determined from the available samples was representative for the diet throughout the year, total annual biomass consumption was calculated for the two locations (Færder and Hvaler) by multiplying 4 kg per capita per day by 365 days per year and then by the number of seals at the location. Prey species were assumed to have equal energy densities, and annual biomass consumption was distributed across the various prey species and groups using the percentage biomass estimates (RC_i) found in the present study. These calculations can be represented as:

$$AC_{i,L} = 4\ \mathrm{kg} \cdot \mathrm{day}^{-1} \times 365\ \mathrm{day} \cdot \mathrm{year}^{-1} \times\ N_L \times \frac{RC_{i,L}}{100} \qquad (3)$$

where $AC_{i,L}$ is the annual consumption of prey group i at location L, N_L is the number of harbor seals at location L, and $RC_{i,L}$ is the relative contribution of prey group i at location L (see equation 2).

Standard Monte Carlo resampling methods were used to include some uncertainty in the prey consumption estimates. 1000 simulations were run for each location with respect to relative diet composition ($RC_{i,L}$) and harbor seal population size (N_L). The uncertainty in relative diet composition was accounted for by bootstrapping the diet data 1000 times, whereas the population size was randomly drawn from the upper 50% of a normal distribution of simulated harbor seal abundance with mean equal to the most recent minimum counts ($N_{Færder}$ = 292, N_{Hvaler} = 325) (Nilssen and Bjørge 2019) and standard deviation ($SD_{Færder}$ = 43.8, SD_{Hvaler} = 48.75) calculated from the coefficient of variation (CV = population standard deviation/population mean) in harbor seal abundance counts, as estimated by Cunningham et al. (2010).

Estimates of annual prey consumption by harbor seals were compared with fisheries landings in the region (ICES division IIIa, subdivision 09, areas 16-22) using landings statistics from the 2018 and 2019 Norwegian catch statistics of the Directorate of Fisheries (see Appendix E for map of areas). Since fishing of cod was banned in Oslofjord in 2019, the 2018 fisheries statistics were used for cod; 2019 statistics were used for all other species.

3 Results

DNA was amplified in 45 samples, but 44 samples yielded >100 prey sequences and were utilized in further analyses. Of these samples, 24 were from scats collected at Færder and 20 were from scats collected at Hvaler (Table 1). Taxonomy was determined for a total of 1996221 sequences, attributed to 669 amplicon sequence variants. Though the primers were chosen to mismatch phocid seal sequences, a small amount of seal DNA was detected (<1% of all sequences to which taxonomy was assigned).

Of the 77 scat samples examined morphologically for hard-parts analysis, two samples from each location lacked otoliths, leaving a total of 73 (94.8%) samples that contained otoliths. There were therefore 30 samples with otoliths from Færder and 43 samples with otoliths from Hvaler (Table 1). The sample that did not yield >100 prey sequences was one of the samples from Færder that contained no otoliths, though the other three samples without otoliths successfully yielded prey DNA. A total of 3052 otoliths representing 1798 prey items were identified, 2051 otoliths (1141 prey items) in scats from Færder and 1001 otoliths (657 prey items) in scats from Hvaler (Table 1). Despite morphological examination of fewer scat samples from Færder, more otoliths were recovered and more prey individuals were identified in the scats from Færder than from Hvaler (Table 1). Four (5.2%) of the 77 scats contained cephalopod beaks; all scats with cephalopod beaks were from Hvaler, thus cephalopod prey occurred in 8.9% of Hvaler samples. Fourteen (18.2%) scats contained crustacean remains.

3.1 Diet composition

3.1.1 Detection of prey taxa

A total of 48 prey taxa were identified in the molecular analysis. Among these, 17 taxa occurred in concentrations too low (<1% of sequences in the sample) for groups to be considered present as prey in any samples. Therefore, 31 of the prey taxa identified molecularly were considered present in the samples. In all, bony fish prey taxa found to be present in the samples belong to 16 families and include 17 species, 5 genera-level groups, and 6 family-level groups. Bird prey taxa found to be present in the samples include the bird families Anatidae and Turdidae (genus *Turdus*). Skates identified as prey belonged to genus *Amblyraja*.

Through morphological analysis, a total of 32 fish species and 17 prey groups were identified. Cephalopods were not distinguished further and therefore comprise one prey group. The

remaining prey groups contain specimens that were identified either to family or to a selection of species (or a genus) within the same family whose otoliths are similar and sometimes made indistinguishable by degradation. Fish prey identified morphologically belonged to at least 18 families.

There existed a difference in the capacity of the methods to identify prey other than bony fishes. The molecular analysis indicated presence of birds and skates (genus *Amblyraja*) in 31.8% and 2.3% of the samples, respectively, but was not able to detect invertebrate prey (such as cephalopods). In contrast, cephalopods were identified in 5.2% of samples in the morphological analysis – though not in any of the samples that had also been analyzed molecularly.

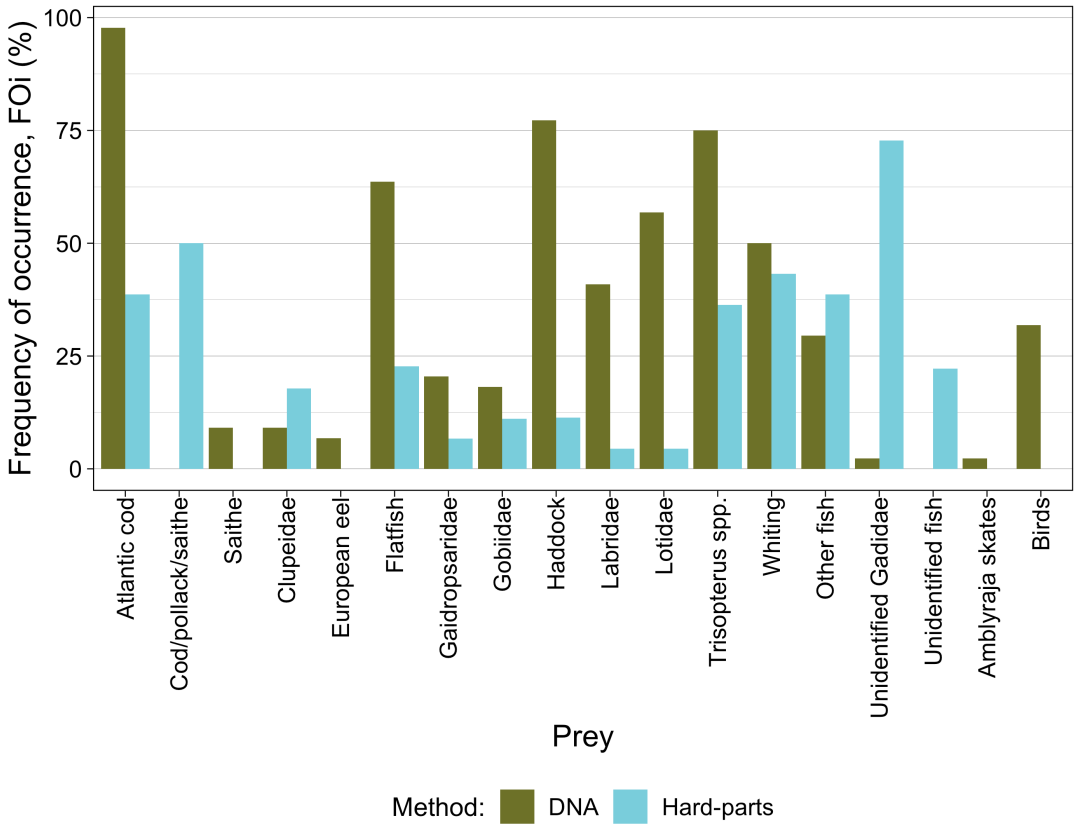

Figure 2. Frequency of occurrence (FO_i) of different prey taxa in the harbor seal diet using molecular DNA analysis in comparison with data from hard-parts analysis of the same samples (n = 44).

In the hard-parts analysis of all samples (n = 77), greater prey diversity was found at Hvaler: 34 prey species/groups were found in the samples from Hvaler, while only 20 prey species/groups were identified in the samples from Færder. The same trend remained in the hard-parts data after adjusting the groups for the subset of samples for which DNA was analyzed (n = 44), with 13 prey groups in samples from Færder and 22 prey groups in samples

from Hvaler. However, the DNA analysis of these same samples did not find much difference in prey diversity between the two locations; 19 prey groups occurred in the samples from Færder, and 20 prey groups occurred in the samples from Hvaler.

3.1.2 Frequency of occurrence

The DNA analysis revealed that Atlantic cod (*Gadus morhua,* FO_i = 97.7%) occurred most frequently in the scats, followed by haddock (*Melanogrammus aeglefinus,* 77.3%), *Trisopterus* spp. (*T. esmarkii/T. luscus/T. minutus*, 75%), and unidentified Lotidae and unidentified Pleuronectidae (both 56.8%) (Figure 2). When looking at the locations individually, these same prey are amongst the most frequently occurring in the DNA metabarcoding data at both Færder and Hvaler (Figure 3). The most frequently occurring prey at Færder were Atlantic cod (100%), haddock (79.2%), *Trisopterus* spp. (75%), unidentified Lotidae and whiting (*Merlangius merlangus*) (both 54.2%), and unidentified Pleuronectidae (50%) (Figure 3). The most frequently occurring prey at Hvaler were Atlantic cod (95%), haddock and *Trisopterus* spp. (both 75%), unidentified Pleuronectidae (65%), unidentified Lotidae (60%), and whiting (45%) (Figure 3). The DNA metabarcoding analysis did not find a significant relationship between the frequency of occurrence of prey groups and location ($\chi^2_{30,0.05} = 32.535$, $P = 0.3431$).

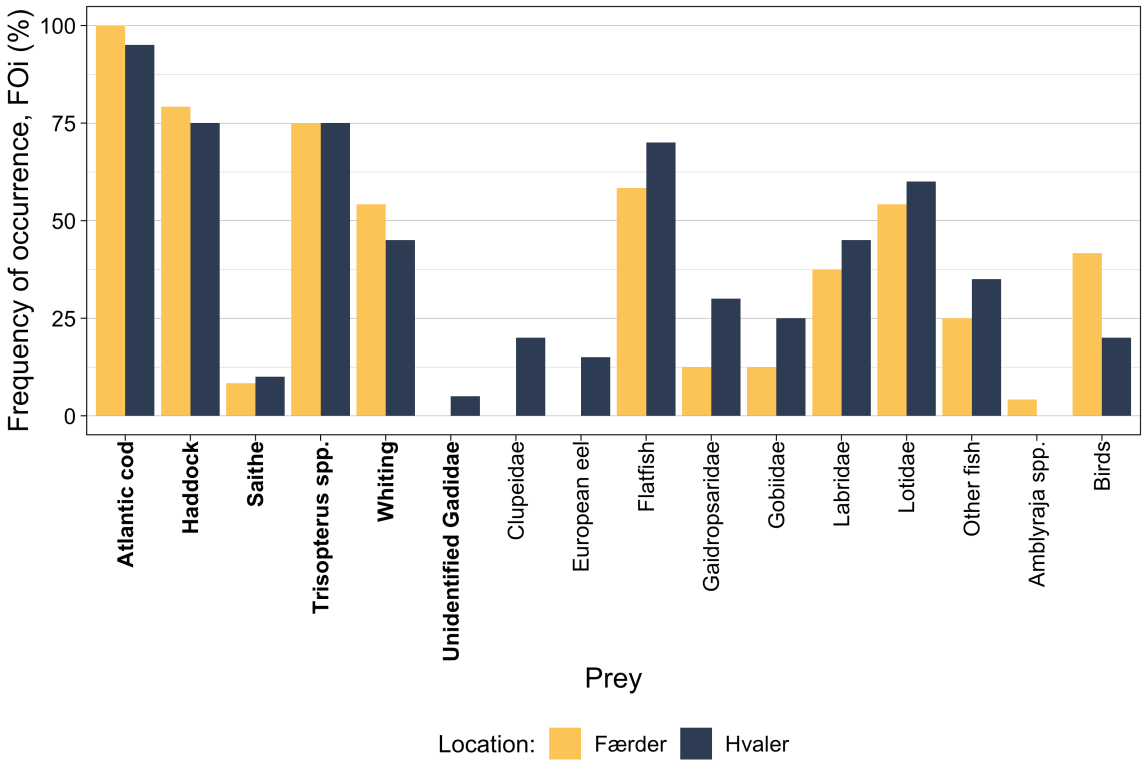

Figure 3. Frequency of occurrence (FO_i) of different prey taxa in the harbor seal diet, as identified through molecular DNA analysis of 44 scat samples. Data is presented for each of the two study locations. Prey belonging to the family Gadidae are indicated in bold.

Alternatively, hard-parts analysis of all 77 samples revealed that the prey occurring most frequently (FO_i) overall were unidentified Gadidae (61.0%), cod/pollack/saithe (*Gadus morhua/Pollachius pollachius/Pollachius virens*, 39.0%), *Trisopterus* spp. (36.4%), whiting (33.8%), and Atlantic cod (28.6%) (Figure 2; Table 2). The prey groups occurring most frequently at Færder were the same as overall: unidentified Gadidae (78.1%), whiting (59.4%), cod/pollack/saithe (50.0%), Atlantic cod (43.8%), and *Trisopterus* spp. (40.6%) (Figure 4; Table 2). The prey groups occurring most frequently at Hvaler were also unidentified Gadidae (40.0%), *Trisopterus* spp. (27.3%), and cod/pollack/saithe (25.5%), but unidentified Pleuronectidae (25.5%) was also common (Figure 4; Table 2). Unlike in the DNA metabarcoding analysis, the hard-parts analysis revealed significant difference in the frequency of occurrence of prey groups between the two locations ($\chi^2_{36,0.05} = 77.905$, $P < 0.0001$).

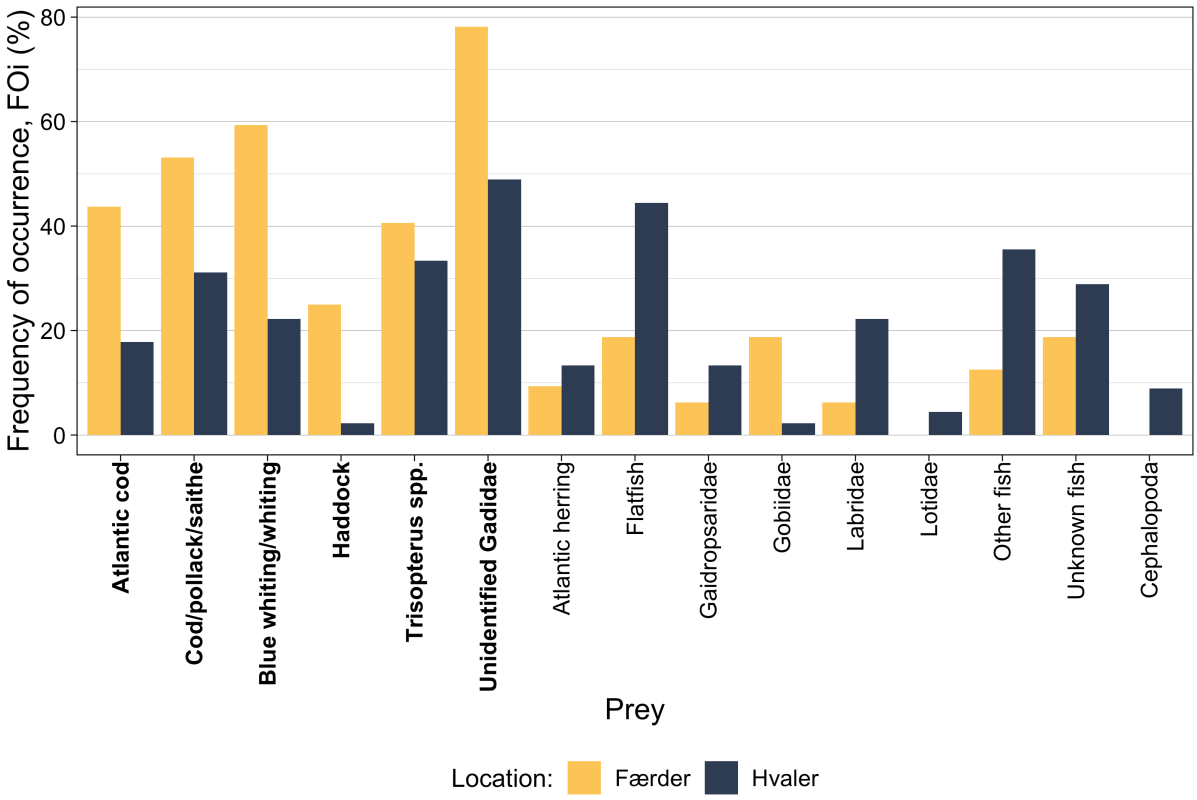

Figure 4. Frequency of occurrence (FO_i) of different prey taxa in the harbor seal diet, as identified through hard-parts analysis of 77 scat samples. Data is presented for each of the two study locations. Prey belonging to the family Gadidae are indicated in bold.

In both the molecular and morphological analyses, Atlantic cod, *Trisopterus* spp. and whiting were amongst the most frequently occurring prey overall. Nonetheless, there were significant differences in the occurrences of prey species between the molecular analysis and the hard-parts analysis of the same samples ($\chi^2_{30,0.05} = 140.95$, $P < 0.0001$) (see also Appendix F).

Table 2. Frequency of occurrence (FO_i) and relative diet contribution (RC_i) in terms of percentage biomass, for all prey species and prey groups identified through hard-parts analysis. Biomass could not be reconstructed for *Gaidropsarus vulgaris* or Cephalopoda.

Hard-parts Analysis						
Prey items	**Location**					
	Færder		**Hvaler**		**Both locations**	
	FO_i (%)	**RC_i (%)**	**FO_i (%)**	**RC_i (%)**	**FO_i (%)**	**RC_i (%)**
Anarhichadidae						
Anarhichas spp. (wolffishes)	0	0	1.82	0.63	1.30	0.32
Belonidae						
Belone belone (garfish)	0	0	1.82	3.47	1.30	1.77
Callionymidae						
Callionymus spp. (dragonets)	0	0	1.82	0.27	1.30	0.14
Clupeidae						
Clupea harengus (Atlantic herring)	9.38	0.19	10.91	3.13	11.69	1.68
Cottidae						
Myoxocephalus scorpius (fourhorn sculpin)	3.13	2.16	0	0	1.30	1.06
Myoxocephalus spp. (sculpins)	3.13	0.58	0	0	1.30	0.29
Gadidae						
Gadiculus thori (silvery pout)	6.25	0.35	3.64	0.01	5.20	0.17
Gadus morhua (Atlantic cod)	43.75	10.43	14.55	7.04	28.57	8.70
Gadus morhua/Pollachius pollachius/Pollachius virens (cod/pollack/saithe)	50.00	20.39	25.46	5.85	38.96	12.99
Melanogrammus aeglefinus (haddock)	25.00	16.93	1.82	0.36	11.69	8.50
Merlangius merlangus (whiting)	59.38	6.67	12.73	1.09	33.77	3.83
Micromesistius poutassou (blue whiting)	0	0	9.09	0.99	6.49	0.51
Micromesistius poutassou or *Merlangius merlangus* (blue whiting/whiting)	6.25	0.16	1.82	0.19	3.90	0.18
Pollachius pollachius or *Pollachius virens* (pollack or saithe)	6.25	2.33	7.27	5.61	7.79	4.00
Raniceps raninus (tadpole fish)	0	0	1.82	0.74	1.30	0.38
Trisopterus spp.	40.63	16.84	27.27	5.78	36.36	11.21
Unidentified Gadidae	78.13	13.35	40.00	3.32	61.04	8.25
Gaidropsaridae						
Enchelyopus cimbrius (fourbeard rockling)	6.25	0.28	10.91	0.96	10.39	0.62
Gaidropsarus vulgaris (three-bearded rockling)	0	0	3.64		2.60	
Unidentified Gaidropsaridae	0	0	1.82	0.12	1.30	0.06
Gobiidae (gobies)	18.75	0.13	1.82	0.00	9.09	0.07
Labridae (wrasses)	6.25	1.08	18.18	9.83	15.58	5.53
Lotidae						
Molva molva (ling)	0	0	3.64	2.44	2.60	1.24

Table 2 (*concluded*).

Prey items	Location					
	Færder		Hvaler		Both locations	
	FO_i (%)	RC_i (%)	FO_i (%)	RC_i (%)	FO_i (%)	RC_i (%)
Merlucciidae						
Merluccius merluccius (European hake)	3.13	0.34	7.27	0.50	6.49	0.42
Pholidae						
Pholis gunnellus (rock gunnel)	0	0	1.82	0.03	1.30	0.02
Pleuronectidae						
Hippoglossus hippoglossus (Atlantic halibut)	0	0	3.64	0.04	2.60	0.02
Hippoglossoides platessoides or *Glyptocephalus cynoglossus* (long rough dab/witch flounder)	9.38	0.30	14.55	3.11	14.29	1.73
Limanda limanda (common dab)	0	0	9.09	4.42	6.49	2.25
Platichthys flesus or *Pleuronectes platessa* (flounder/plaice)	12.50	3.09	18.18	11.49	18.18	7.37
Unidentified Pleuronectidae	12.50	3.98	25.46	10.13	23.38	7.11
Scombridae						
Scomber scombrus (Atlantic mackerel)	0	0	1.82	4.40	1.30	2.24
Scophthalmidae	0	0	9.09	1.86	6.49	0.95
Soleidae						
Solea solea (common sole)	0	0	3.64	2.49	2.60	1.27
Stichaeidae						
Lumpenus lampretaeformis (snakeblenny)	0	0	7.27	2.32	5.19	1.18
Zoarcidae (eelpouts)	0	0	7.27	0.99	5.19	0.50
Unidentifed fish prey	18.75	0.43	23.64	6.40	24.68	3.47
Cephalopoda	0	0	7.27		5.19	

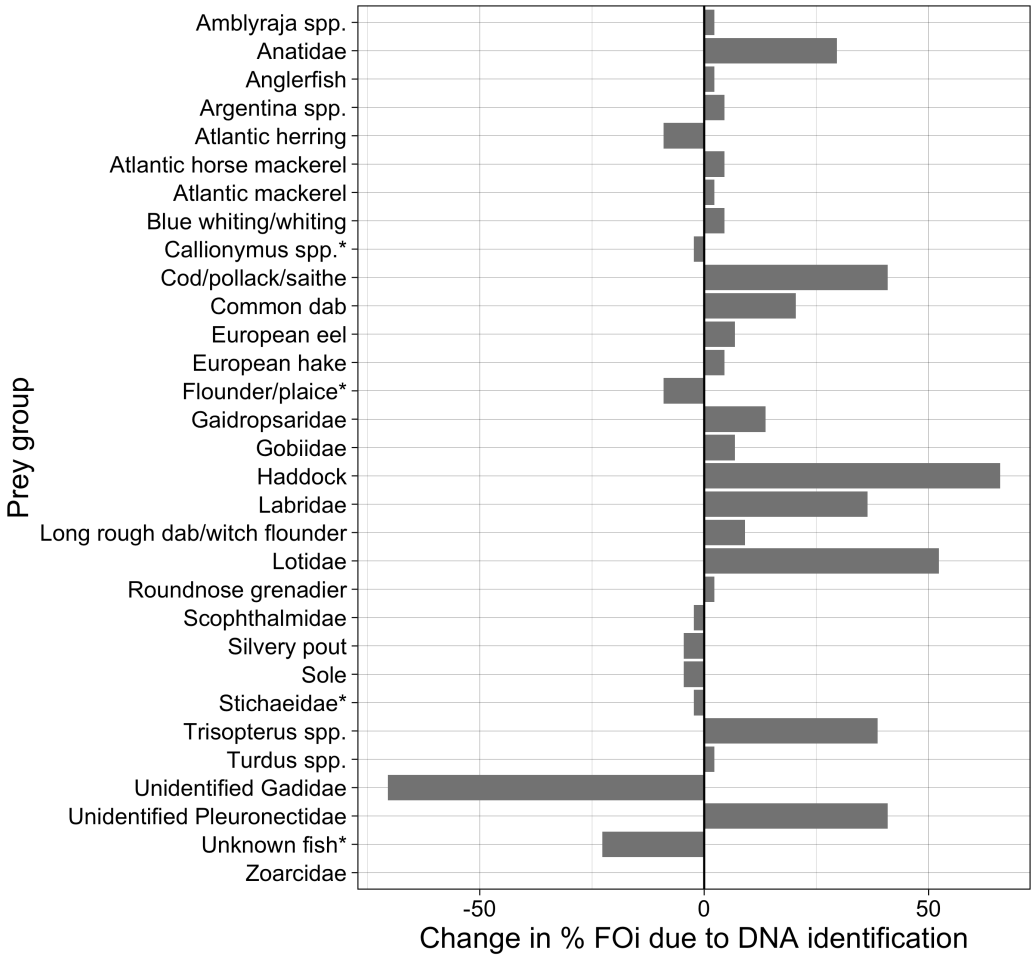

Figure 5. Change in percentage frequency of occurrence (FO_i) of prey groups due to DNA metabarcoding analysis. Negative values indicate that a prey taxon was identified more frequently in the hard-parts analysis. Only data from samples analyzed through both methods are shown. Prey groups marked by an asterisk (*) were detected in the DNA analysis but never at levels above the threshold (>1% of prey sequences for a sample) required to constitute an occurrence.

Most prey groups occurred significantly more frequently in the DNA metabarcoding compared with the hard-parts analysis (Figure 5). The DNA metabarcoding indicated reduced frequency of occurrence of unidentified gadoid fishes and found increased frequency of occurrence for the gadoid species haddock, Atlantic cod (shown in group cod/pollack/saithe), and *Trisopterus* spp., which could suggest that the DNA identification increased taxonomic resolution of these gadoids compared to the morphological hard-part identification. Additionally, the frequency of occurrence of prey in families Lotidae and Labridae was notably higher in the DNA metabarcoding analysis than in the hard-parts analysis (Figure 5). Conversely, sole (*Solea solea*) and silvery pout (*Gadiculus thori*) were only detected in the hard-parts analysis and were not identified by the DNA metabarcoding. While detected in the metabarcoding *Callionymus* spp., flounder/plaice (*Platichthys flesus/Pleuronectes platessa*), and Stichaeidae –

as well as unknown fish – occurred in concentrations too low (<1% of sequences in the sample) to be considered present in any samples. However, otoliths of these species were recovered and thus they were recorded as occurrences in the hard-parts data.

Individual samples tended to be more diverse, containing a greater number of prey groups (once prey groups were adjusted to be consistent), in the molecular analysis than in than hard-parts analysis (Wilcoxon signed-rank test for paired samples, $P < 0.0001$). The mean number of prey groups identified per sample by DNA metabarcoding was 6.20 ± 2.92 SD, compared to 3.57 prey groups ± 2.20 SD in the morphological hard-parts analysis (Figure 6). DNA increased prey diversity in terms of number of prey groups identified in 79.5% (35 of 44) of the samples. Identical species composition between the two methods was never found.

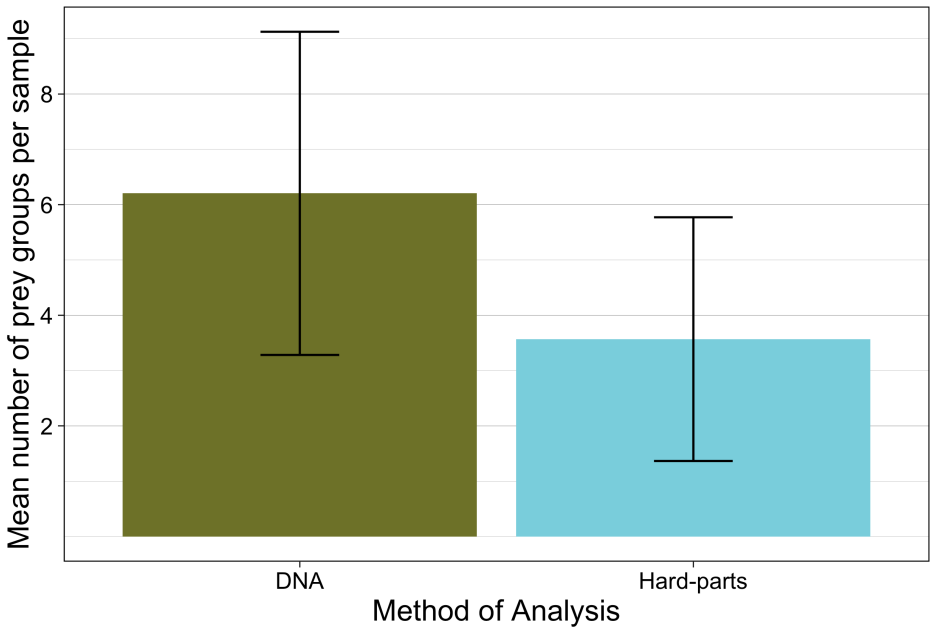

Figure 6. Mean number of prey groups identified in each scat sample by the two methods of analysis, DNA-metabarcoding (6.20 ± 2.92 SD) and morphological hard-parts identification (3.57 ± 2.20 SD). The error bars show standard deviation.

3.1.3 Relative prey importance

Overall, the most important prey in terms of relative diet contribution were found to be Atlantic cod, haddock, and *Trisopterus* spp. In the DNA metabarcoding analysis, the prey groups with the highest average RC_i overall, as determined by relative read abundance, were Atlantic cod (25.5%), haddock (14.4%), and *Trisopterus* spp. (12.6%), followed by unidentified Pleuronectidae (10.1%) and birds in the duck family Anatidae (7.3%) (Figure 7). However, it is important to note that the DNA analysis revealed considerable variation in the most important prey (according to RC_i) between scat samples (see data for individual samples in Appendix G).

In the hard-parts analysis, the prey groups with the highest RC_i overall, as determined by relative biomass, were cod/pollack/saithe (13.0%) and *Trisopterus* spp. (11.2%), followed by Atlantic cod (8.7%), haddock (8.5%), and unidentified Gadidae (8.3%) (Figure 8; Table 2).

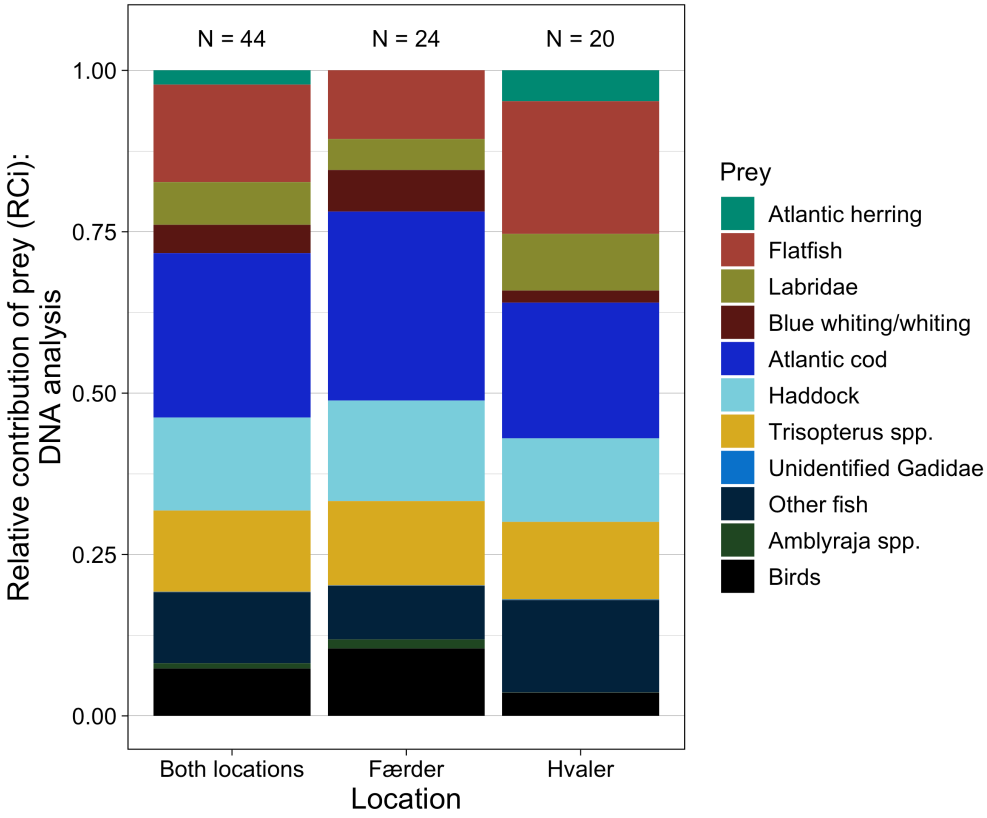

Figure 7. Relative contribution of prey (RC_i) given as the mean relative read abundance for all prey identified through DNA metabarcoding of harbor seal scat samples, by location. The first column represents all data across both locations. The tops of the bars are labelled with the number of scat samples analyzed from each location. The group 'flatfish' refers to prey from order Pleuronectiformes (including families Pleuronectidae and Scophthalmidae). 'Other fish' consists of *Anarhichas* spp., anglerfish (*Lophius piscatorius*), *Argentina* spp., Atlantic horse mackerel (*Trachurus trachurus*), Atlantic mackerel (*Scomber scombrus*), *Callionymus* spp., *Chelidonichthys* spp., European eel (*Anguilla anguilla*), European hake (*Merluccius merluccius*), European sprat (*Sprattus sprattus*), Gaidropsaridae, garfish (*Belone belone*), Gobiidae, Lotidae, lumpfish (*Cyclopterus lumpus*), roundnose grenadier (*Coryphaenoides rupestris*), saithe, *Salmo* spp., Stichaeidae, tadpole fish (*Raniceps raninus*), and Zoarcidae.

Some regional differences in the most important prey in terms of relative diet contribution were seen. At Færder, the three most important prey were determined to be Atlantic cod, haddock, and *Trisopterus* spp. In the DNA metabarcoding analysis of Færder samples, Atlantic cod (29.2%), haddock (15.6%), and *Trisopterus* spp. (13.1%) were the most important prey in terms of relative contribution of sequences (RC_i), followed by Anatidae ducks (10.4%) and whiting (6.4%) (Figure 7). Similarly, in the hard-parts analysis, the prey groups with the highest RC_i at Færder, as determined by relative biomass, were cod/pollack/saithe (20.4%), haddock (16.9%),

Trisopterus spp. (16.8%), unidentified Gadidae (13.4%), and Atlantic cod (10.4%) (Figure 8; Table 2).

At Hvaler, the prey groups with the highest RC_i in the DNA metabarcoding analysis, as determined by relative read abundance, were Atlantic cod (21.0%), unidentified Pleuronectidae (15.0%), haddock (12.9%), *Trisopterus* spp. (12.0%), and wrasses in genus *Symphodus*. (6.3%) (Figure 7). The prey groups with the highest RC_i at Hvaler in the hard-parts analysis, by relative biomass, were flounder/plaice (11.5%), unidentified Pleuronectidae (10.1%), and wrasses (family Labridae, 9.8%), followed by Atlantic cod (7.0%) (Figure 8; Table 2).

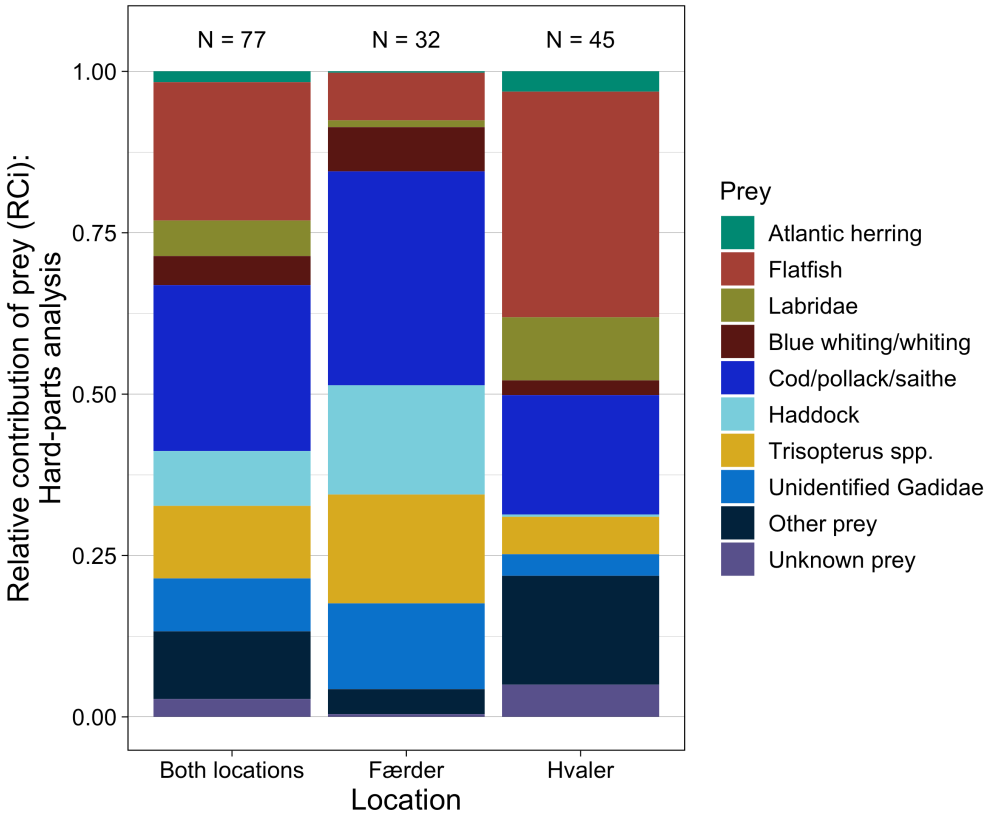

Figure 8. Relative contribution of prey (RC_i) given as the percentage biomass for all prey identified morphologically from hard prey remains in harbor seal scat samples, by location. The first column represents all data across both locations. The tops of the bars are labelled with the number of scat samples examined from each location. The group 'flatfish' refers to prey from order Pleuronectiformes (including families Pleuronectidae, Scophthalmidae, and Soleidae). 'Other prey' consists of *Anarhichas* spp., Atlantic mackerel, *Callionymus* spp., Cephalopoda, Cottidae, European hake, Gaidropsaridae, garfish, Gobiidae, Lotidae, rock gunnel (*Pholis gunnellus*), silvery pout, snakeblenny (*Lumpenus lampretaeformis*), tadpole fish, and Zoarcidae. 'Unknown prey' were fish remains not identifiable to family.

Overall, the importance of the different prey groups by biomass was significantly correlated to location (CCA, $F_1 = 2.5846$, $\chi^2 = 0.2156$, $P = 0.005$; plot in Appendix H). Although the difference in frequency of occurrence (FO_i) of pleuronectid prey between the two locations was

not significant in either the DNA ($\chi^2_{1,0.05} = 0.23653$, $P = 0.6267$) or hard-parts ($\chi^2_{1,0.05} = 3.0081$, $P = 0.0825$) analysis, pleuronectids were amongst the most important prey (by RC_i) at Hvaler, in contrast to Færder where all of the most important prey belonged to Gadidae. In addition to gadoids and pleuronectids, wrasses (family Labridae) were also amongst the most important prey at Hvaler.

To further assess the relative contribution of prey families, samples were categorized according to the dominant prey group. In the DNA analysis, samples fell into one of six categories when classified according to the dominant prey family (>70% of sequences): Gadidae, Pleuronectidae, Anatidae, Clupeidae, Labridae, or "mixed" (no one family dominant) (Figure 9a). In the hard-parts analysis, samples fell into one of 11 categories when classified by the dominant prey family by biomass (>70% biomass): Belonidae, Clupeidae, Gadidae, Labridae, Merlucciidae, Pleuronectidae, Scombridae, Scophthalmidae, Stichaeidae, Zoarcidae, or "mixed" (Figure 9b). The families Belonidae, Merlucciidae, Scombridae, Scophthalmidae, Stichaeidae, and Zoarcidae each dominated only one sample.

While there was no significant difference in the proportion of samples classified to each of the dominant prey families between the two locations based upon DNA sequences (Fisher's exact test, $P = 0.4336$), such a difference was evident in the morphological data (Fisher's exact test, $P = 0.0001$). This is consistent with the trends discussed previously, where frequency of occurrence of prey groups differed between the two locations only in the morphological analysis.

In both methods of analysis, the highest number of samples were dominated by gadoid prey (45.5% in the DNA and 45.8% in the hard-parts), but there were also many mixed samples (40.9% in the DNA and 30.6% in the hard-parts). However, differences in family dominance due to method of analysis were seen at both locations (Figure 9). At Færder – in addition to a sample dominated by Anatidae bird DNA (prey not detectable in the hard-parts) – samples were found to be dominated by Pleuronectidae in the DNA data while no Pleuronectidae dominated according to the hard-parts data. Indeed, there was also significantly higher frequency of occurrence of Pleuronectidae at Færder in the DNA analysis than in the hard-parts analysis ($\chi^2_{1,0.05} = 9.0234$, $P = 0.0027$) and Pleuronectidae were only found to be amongst the most frequently occurring prey at Færder in the DNA analysis (not in hard-parts data).

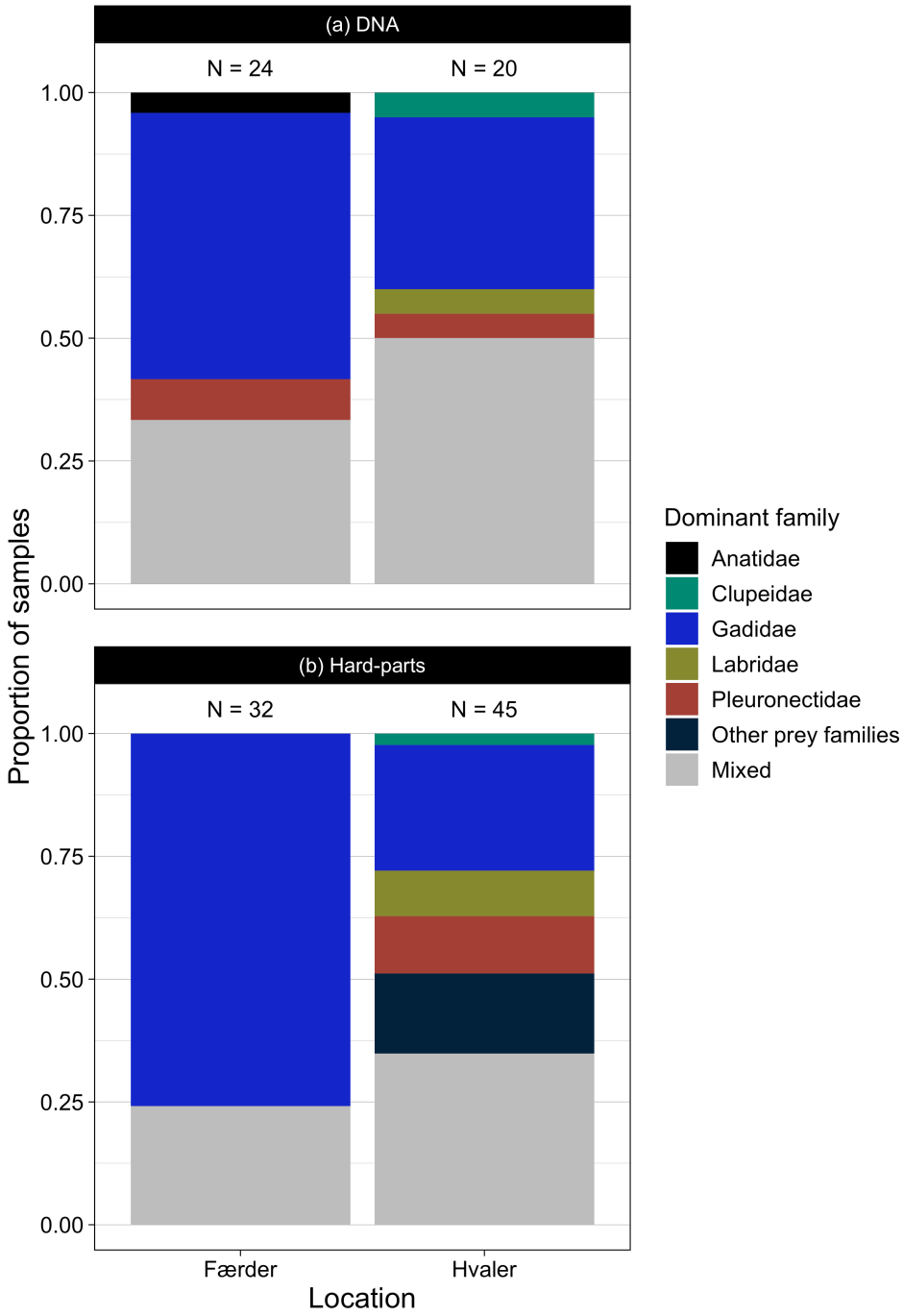

Figure 9. The proportion of harbor seal scat samples from the two study locations that were dominated by each prey family. Samples classified as 'mixed' were not dominated by any one group. (a) Prey family dominance according to the DNA metabarcoding analysis, where dominant prey family was defined as the family represented by >70% of the sequences in a sample. (b) Prey family dominance according to the hard-parts analysis, where dominant prey family was defined as the family represented by >70% of the total sample biomass. 'Other prey families' includes families that each dominated only one sample: Belonidae, Merlucciidae, Scombridae, Scophthalmidae, Stichaeidae, and Zoarcidae.

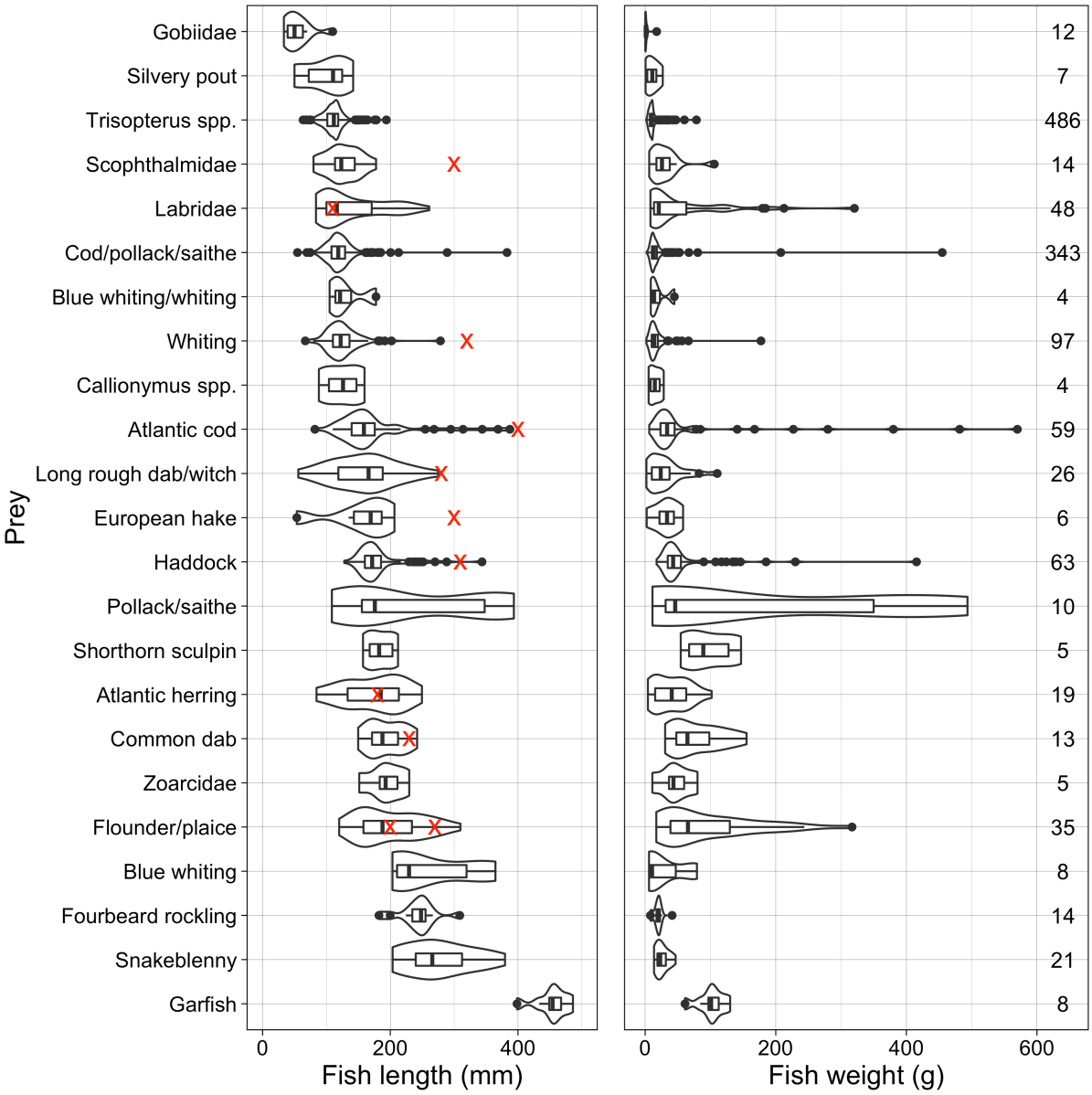

Figure 10. Length (mm) and weight (g) distribution for prey species/groups identified morphologically in harbor seal feces. The width of each violin indicates the distribution of observations. The maximum violin width is equal for all prey categories, regardless of number of observations. The box plots within the violins show the median value and 25% and 75% quartiles. The red x symbols indicate the minimum allowed landing size (Lovdata 2005). For the prey group Scophthalmidae, the minimum allowed landing size for brill is shown. For the prey group Labridae, the minimum allowed landing size for wrasses (110 mm) is shown, which excludes ballan wrasse (140 mm) and corkwing wrasse (120 mm). For the prey group flounder/plaice, the x at 200 mm marks the minimum allowed landing size for European flounder, while the x at 270 mm marks the minimum allowed landing size for European plaice. For the prey group long rough dab/witch flounder (shortened to 'long rough dab/witch' in the figure), the minimum allowed landing size for witch flounder is shown. The numbers on the right side of the figure indicate the number of prey fish; only prey groups for which more than three individuals were recovered are shown.

3.2 Prey size

Fish lengths and weights were estimated based on otolith measurements (Appendix C). Overall, prey fish ranged in size from 30 mm length and 0.1 g weight (Gobiidae) to 542 mm length and 1023.1 g weight (Atlantic mackerel, *Scomber scombrus*). 76.6% of the fish eaten by the harbor seals at both Færder and Hvaler were estimated to be less than 150 mm in length, while 98.0% were estimated to be less than 300 mm in length (Figure 10).

Of the most common prey, *Trisopterus* fishes were generally smaller, with a mean length of 111 mm and a mean weight of 10.6 g. Fishes from the cod/pollack/saithe group were also on the smaller end, 121 mm in length and 17.3 g in weight, on average. Prey classified to species as Atlantic cod tended to be larger, with a mean length of 173 mm and a mean weight of 67.5 g. Similarly, haddock prey fish averaged 182 mm in length and 61.7 g in weight. Pleuronectid prey had a mean length of 164 mm and a mean weight of 48.8, ranging from 159 mm and 30.4 g (for the group comprised of long rough dab, *Hippoglossoides platessoides*, and witch flounder, *Glyptocephalus cynoglossus*) to 199 mm and 96.2 g (for the flounder and plaice group) (Figure 10).

In both sampling periods (August and November 2019), seals appeared to be foraging primarily on juvenile cod (Figure 11). Atlantic cod were classified as juveniles (0-group) if fish length was less than 180 mm in late summer/autumn (Rogers et al. 2011). In order to assess which age class of cod the harbor seals in this study were preying upon, fish identified to the prey groups Atlantic cod and cod/pollack/saithe were considered (Figure 11). The mean size of prey fish identified as Atlantic cod or placed in the prey group cod/pollack/saithe was 129 mm in samples collected in August 2019 and 125 mm in samples collected in November 2019. In both periods, at least 60% of possible cod prey were smaller than 180 mm. In August, 64% of the cod foraged were juvenile, compared to 86% juvenile cod in November. Therefore, in both periods seals foraged on juvenile cod, but juvenile cod were targeted more heavily as prey in November.

Mean fish lengths were smaller than minimum allowed landing size in Norwegian waters (Lovdata 2005) for Atlantic cod, Atlantic herring (*Clupea harengus*), common dab (*Limanda limanda*), European hake (*Merluccius merluccius*), European plaice, haddock, scophthalmids (brill, *Scophthalmus rhombus*), whiting, and witch flounder (Figure 10). For European flounder and some wrasses (Labridae), mean fish length of the harbor seal prey was within the allowed landing size for fisheries. All of the Atlantic cod, European hake, whiting,

and witch flounder found in the scat samples were below the minimum landing size (Figure 10). 63% of Atlantic herring, 15% of common dab, and 1.6% of haddock were within allowed landing size. Of fish belonging to the flounder/plaice prey group, 43% were within allowed landing size for European flounder, while 11% were within allowed landing size for European plaice. Additionally, 40% of fish identified as Labridae were within allowed landing size for ballan wrasse (*Labrus bergylta*), 46% within allowed landing size of corkwing wrasse (*Symphodus melops*), and 54% within allowed landing size for other wrasses.

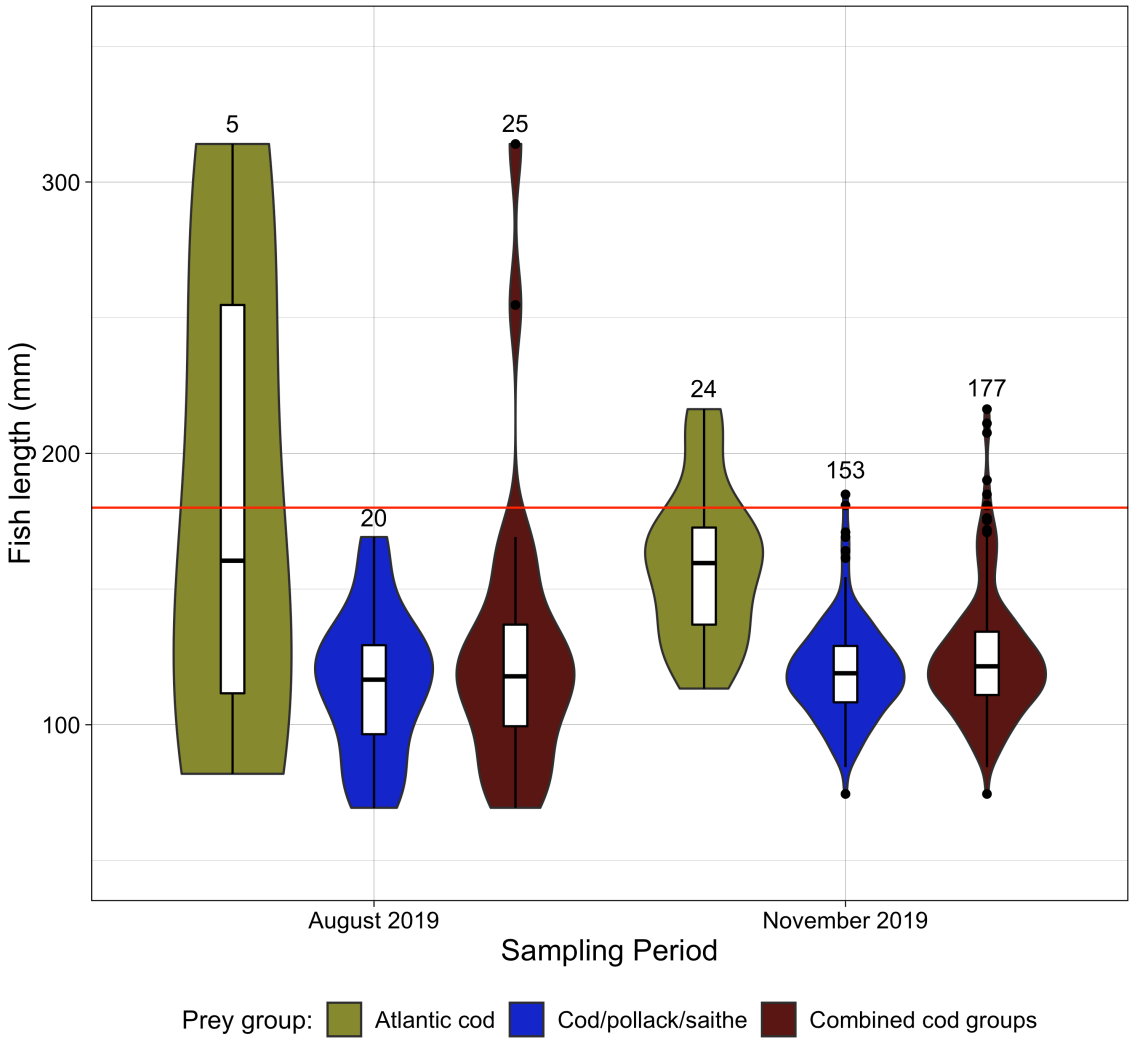

Figure 11. Length (mm) distribution for prey classified as either Atlantic cod or cod/pollack/saithe during the two sampling periods, August 2019 (Hvaler) and November 2019 (Færder). The dark red plots show the data for prey within the two aforementioned groups combined, representing most of the possible cod prey. The width of each violin indicates the distribution of observations with maximum width equal, regardless of total number of observations. The numbers at the top of the violins indicate the total number of cod and cod/pollack/saithe prey fish identified in each sampling period. The horizontal line (red) indicates the maximum size of juvenile cod, 180 mm.

3.3 Prey consumption

The estimated total annual consumption of all the harbor seals at Færder and Hvaler was 1009.3 tons of fish (477.1 tons by the ~292 seals at Færder and 532.2 tons by the ~325 seals at Hvaler). Gadoids (mean 604.7 tons), flatfishes (mean 166.3 tons), and wrasses (mean 87.2 tons) were estimated to be consumed in the largest quantities in 2019 (Table 3). The three most consumed prey groups on average were cod/pollack/saithe (189.3 tons), *Trisopterus* spp. (133.9 tons) and wrasses in family Labridae (87.2 tons). Additionally, 84.1 tons of unidentified gadoids were consumed (Table 3).

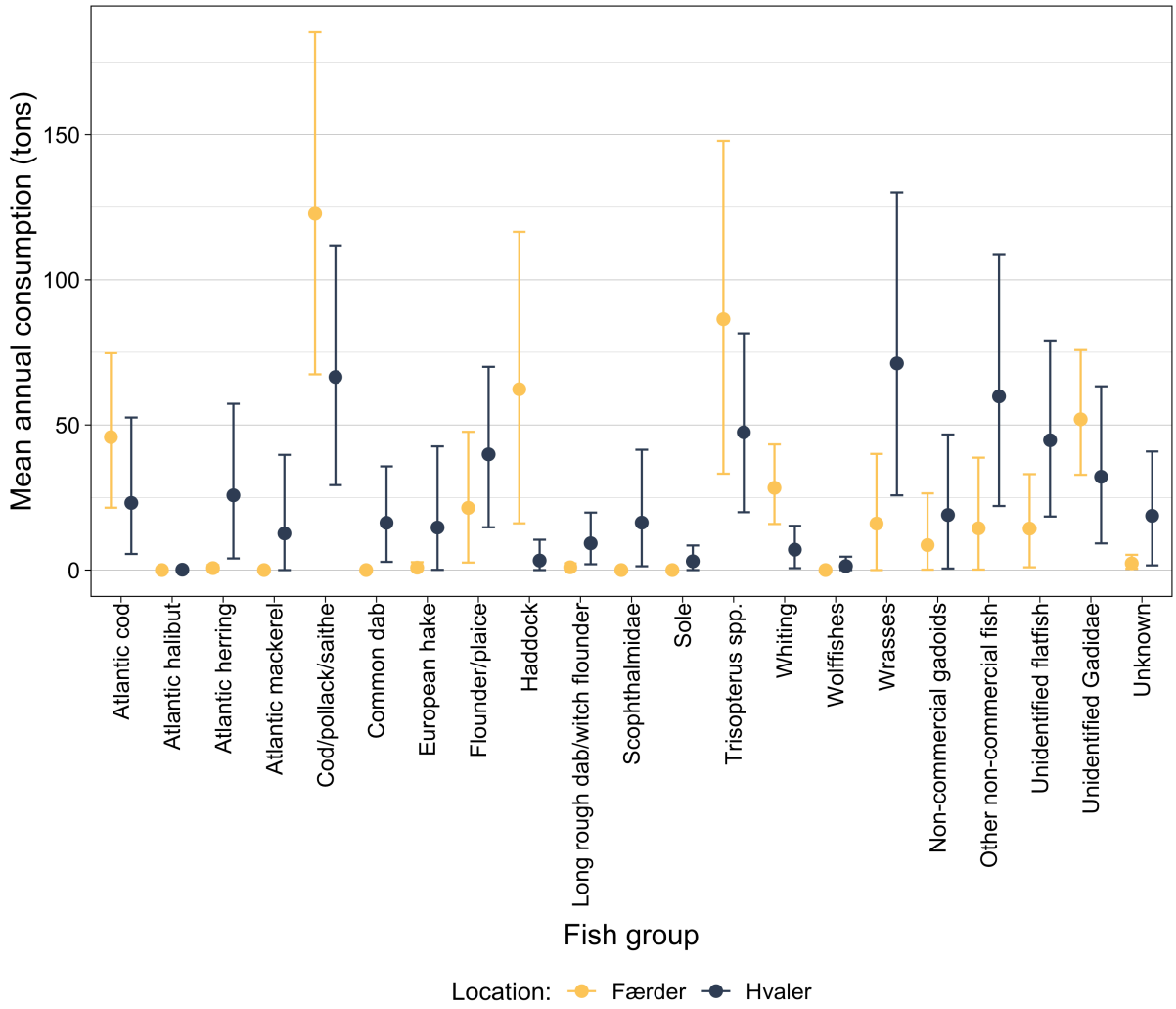

Figure 12. Estimated 2019 annual consumption of different fish prey groups by harbor seals in the Færder and Hvaler areas of outer Oslofjord, based on relative diet contributions from reconstructed biomass. Standard Monte Carlo resampling methods with 1000 bootstrap simulations were used to include some uncertainty in the prey consumption estimates with respect to relative diet composition and harbor seal population size. Mean consumption estimates are presented; error bars show standard deviation.

By location, the three most consumed prey groups at Færder, on average, were cod/pollack/saithe (122.8 tons ± 30.2 SD), *Trisopterus* spp. (686.4 tons ± 28.4 SD), and haddock (62.3 tons ± 26.2 SD) (Figure 12). The three most consumed prey groups at Hvaler, on average, were the Labridae wrasses (71.2 tons ± 25.6 SD), cod/pollack/saithe (66.5 tons ± 21.4 SD), and *Trisopterus* spp. (47.5 tons ± 15.9) (Figure 12).

Table 3. Estimated annual fish consumption by harbor seals at Færder and Hvaler in the outer Oslofjord, followed by annual commercial fisheries landings of select species in this region. All amounts in tons. 'Other non-commercial fish' includes prey belonging to the fish families Belonidae, Gobiidae, Callionymidae, Cottidae, Gaidropsaridae, Lotidae, Pholidae, Stichaeidae, and Zoarcidae. Landings data come from ICES division IIIa, subdivision 09, areas 16-22 (see map in Appendix E).

Prey fish	Seal consumption at Færder	Seal consumption at Hvaler	Total seal consumption in outer Oslofjord	2019 fisheries landings in outer Oslofjord
Clupeidae				
Atlantic herring	0.7	25.7	26.4	234.3
Flatfishes				
Atlantic halibut	0.0	0.1	0.1	2.8
Common dab	0.0	16.3	16.3	0.15
Flounder/plaice	21.4	39.9	61.3	4.3*
Long rough dab/witch flounder	1.0	9.2	10.2	5.1
Scophthalmidae	0.0	16.3	16.3	5.6
Sole	0.0	3.0	3.0	1.3
Unidentified flatfish	14.3	44.7	59.0	
Total flatfish	**36.7**	**129.6**	**166.3**	
Gadidae				
Atlantic cod	45.8	23.1	68.9	106.1**
Cod/pollack/saithe	122.8	66.5	189.3	
Haddock	62.3	3.3	65.6	17.8
Trisopterus spp.	86.4	47.5	133.9	0.7
Whiting	28.3	7.0	35.4	2.4
Non-commercial gadoids	8.6	19.0	27.6	
Unidentified gadoids	51.9	32.1	84.1	
Total Gadidae	**406.2**	**198.5**	**604.7**	
Merlucciidae				
European hake	0.8	14.7	15.5	
Scombridae				
Atlantic mackerel	0.0	12.6	12.6	35.7
Wolffishes	0.0	1.4	1.4	0.2
Wrasses	16.0	71.2	87.2	53.0***
Other non-commercial fish	14.4	59.8	74.2	
Unknown fish prey	2.3	18.7	21.0	
Total	**477.1**	**532.2**	**1009.3**	

*Flounder not included, as there were no landings data for this species.

**Given that fishing of Atlantic cod was banned in Oslofjord in 2019, 2018 statistics are provided for this species; in 2019, a total catch of 55.8 tons of Atlantic cod was reported.

***Bycatch statistics; captures of wrasses for use as cleaner fish in aquaculture are not provided.

Total annual consumption of Atlantic cod by harbor seals was estimated to be 68.9 tons (45.8 tons ± 13.8 SD at Færder and 23.1 tons ± 13.0 SD at Hvaler), but annual Atlantic cod consumption could have exceeded 250 tons when taking into account the consumed biomass of fish placed into the cod/pollack/saithe group or remaining in unidentified Gadidae (Figure 12; Table 3). Given that incidence of saithe was low (FO_i = 9.1%, mean RC_i = 1.35%) and pollack was not detected in the DNA metabarcoding, while Atlantic cod was found to be very important (FO_i = 97.7%, mean RC_i = 25.5%), it is plausible that much of the fish in group cod/pollack/saithe that were unidentifiable to species through the hard-parts analysis (e.g., due to degradation of the otoliths) were Atlantic cod. However, this assumption should be verified through DNA analysis of individual otoliths that could not be identified to species, as done in a recent study of grey seal diet (Nilssen et al. 2019). Due to the crisis of the cod stock collapse, fishing of Atlantic cod was banned in Oslofjord in 2019 (Taylor 2019), so harbor seal cod consumption – as determined from the 2019 scat samples – was compared to 2018 fisheries landings data, which report landings of 106.1 tons of Atlantic cod (in 2019, 55.8 tons were landed) in the region (ICES division IIIa, subdivision 09, areas 16-22; see Appendix E) (Norwegian catch statistics, Directorate of Fisheries, 2018 & 2019). Thus, Atlantic cod consumed by seals amounts to a minimum of ~65% of the annual cod landings by commercial fisheries in the region but could be more than 235% when considering all prey fish that are possibly cod. Without further exploration, the results operating under the assumption that Atlantic cod comprised the majority of the cod/pollack/saithe group must be regarded with considerable care.

4 Discussion

4.1 Methodological consideration

All methods available for use in the estimation of animal diet composition have biases. One critical issue with the traditional method of reconstructing pinniped diet using otoliths and other hard remains is the differential erosion and digestion of otoliths from different species, leading to differential underestimation of size and number of prey (e.g., Prime 1979, da Silva and Neilson 1985, Jobling and Breiby 1986, Murie and Lavigne 1986, Jobling 1987, Harvey 1989, Pierce and Boyle 1991). Importance of fish species with larger otoliths – such as gadoids – may be overestimated in the diet due to the higher recovery rates of larger otoliths, while importance of fish species with smaller or more fragile otoliths – such as herring – may be underestimated (e.g., Prime 1979, da Silva and Neilson 1985, Jobling and Breiby 1986, Harvey 1989, Tollit et al. 1997a, Grellier and S. Hammond 2005, Grellier and Hammond 2006, Phillips and T. Harvey 2009, Wilson et al. 2017). Conversely, fish length is more likely to be underestimated for larger prey with larger otoliths since they show greater levels of partial digestion, despite higher recovery rates (Tollit et al. 1997a, Grellier and Hammond 2006, Phillips and T. Harvey 2009). In addition to otolith size and robustness, the rate of otolith digestion may also be affected by other factors, including – but not limited to – meal size, meal composition, meal frequency, seal activity level, food intake rate, and individual seal biology (Pierce and Boyle 1991, Marcus et al. 1998, Bowen 2000, Tollit et al. 2003, Casper et al. 2006, Phillips and T. Harvey 2009).

Attempts have been made to use correction factors to correct for both reduced otolith size due to erosion and complete digestion of otoliths (e.g., Bowen 2000, Grellier and S. Hammond 2005, Wilson et al. 2017), but there are limitations to such correction factors. Correction factors have been determined through feeding experiments with captive seals, but there is uncertainty in the applicability of such correction factors to wild seal populations; for example, average digestion rates are expected to be different between captive and wild seals due to differences in food intake, meal composition, and activity state (Pierce and Boyle 1991, Bowen 2000, Casper et al. 2006, Phillips and T. Harvey 2009) and have been shown to be artificially high in captive seals (Tollit et al. 1997a, Grellier and Hammond 2006). Grade-specific digestion coefficients and grade boundaries/descriptions differ between studies (Tollit and Thompson 1996, Tollit et al. 1997a, Grellier and Hammond 2006) and due to the subjectivity of such measures, the level of degradation assigned to otoliths can vary significantly amongst lab personnel – even when they have access to the same reference materials (Wilson et al. 2017). Number correction

factors that account for the loss of otoliths due to complete digestion, based on recovery rates, differ between seal species and between experimental studies (Tollit et al. 1997a, Grellier and S. Hammond 2005, Wilson et al. 2017). In addition to the many factors that affect the results of captive feeding studies (e.g., Jobling and Breiby 1986, Harvey 1989, Marcus et al. 1998, Bowen 2000, Grellier and S. Hammond 2005, Wilson et al. 2017), there is also a lack of standardization in such studies that confounds the interpretation of results from the relevant captive feeding experiments and the decision on which correction factors to use (Bowen 2000, Tollit et al. 2003). Thus, due to unsatisfactory existing correction factors, data in the present study has not been corrected in these ways. It is important to acknowledge the potential sources of uncertainty and error in the diet estimates based on otoliths alone, though here importance of prey as determined by otoliths and the biases therein have been balanced by the addition of genetic analyses. Interestingly, the hard-parts analysis revealed higher frequency of occurrence of clupeids than the DNA metabarcoding, despite the tendency for these species to be underrepresented in diet composition estimates based on otoliths due to otolith fragility and loss to digestion. This indicates that clupeids may indeed not be very important prey species for the harbor seals in the area or during the time period studied, and that low occurrence relative to other prey families (i.e., gadoids) is not just a factor of otolith biases.

Morphological hard-parts diet assessment is also restricted by challenges with the identification or differentiation of otoliths and other remains. Otolith morphology can be very similar across several genera (Jobling and Breiby 1986), or, alternatively, otoliths of the same species can differ drastically in shape and character at different life stages (Campana and Casselman 1993). Hard-parts analysis of otoliths does not allow for the identification of prey species lacking sagittal otoliths (e.g., dogfish, skates, birds) (Pierce and Boyle 1991), nor does it allow for the identification of prey in instances where seals consume only soft tissues of fish (i.e., belly-biting) or where the head and/or otoliths may not be consumed (e.g., removing particularly spiny heads, ripping apart large prey) (Pierce and Boyle 1991, Moore 2003, Orr et al. 2004, Chouinard et al. 2005, Phillips and T. Harvey 2009). Prey recovery rates and representation in morphological hard-parts analyses could potentially be improved with the identification of other hard parts (e.g., vertebrae, jaw bones, teeth, scales, and eye lenses) in addition to otoliths (Pierce and Boyle 1991, Olesiuk 1993, Cottrell et al. 1996, Brown and Pierce 1998, Browne et al. 2002, Tollit et al. 2003, Tollit et al. 2004), but this requires more time, specialized skill, and extensive reference collections thus limiting the application of such “all-structures” methodologies. In the present study, the simultaneous application of DNA-based identification

was able to increase taxonomic resolution of prey for certain prey families (e.g., Gadidae, Labridae, Gobiidae, Zoarcidae) and groups. DNA was better able to parse groups of gadoids that resulted in the hard-parts analysis, notably Atlantic cod, pollack, and saithe. DNA identification also tended to be higher resolution for family Labridae (delineating genus *Labrus* and genus *Symphodus*) compared to hard-parts identification. Yet DNA identification was not able to resolve all prey to species and some unspecific identifications remained (e.g., species in genus *Trisopterus* were not distinguishable in either method of analysis). While pleuronectid species long rough dab and witch flounder, as well as European flounder and European plaice were identified to species in the DNA analysis (compared to the groups long rough dab/witch flounder and flounder/plaice in hard-parts analysis), the incidence of unidentified Pleuronectidae was also higher in the DNA identification suggesting that this method could not consistently increase taxonomic resolution of these flatfishes.

Molecular methods have shown promising capabilities when used to investigate pinniped diet in prior studies (Purcell et al. 2004, Deagle et al. 2005, Parsons et al. 2005, Casper et al. 2007, Deagle and Tollit 2007, Matejusová et al. 2008, Deagle et al. 2009, Méheust et al. 2015, Thomas 2015, Granquist et al. 2018). While molecular methods – such as the DNA metabarcoding analysis used in this study or stable isotope and fatty acid analyses – can correct for some of the aforementioned problems with morphological diet reconstruction (e.g., erosion or complete digestion of otoliths, inconclusive identifications), they also yield their own biases (Symondson 2002, King et al. 2008, Tollit et al. 2009). One problem with scatological DNA diet analyses is that old or desiccated scats may not yield prey DNA, or may amplify less prey DNA than fresh scats (Tollit et al. 2009). DNA metabarcoding is sensitive to contamination and methodological considerations (De Barba et al. 2014); differential detection of prey may result from subsampling from different parts of the feces (Deagle et al. 2005, Matejusová et al. 2008). Biases in diet composition may also arise in the metabarcoding PCR processes where primers may more readily or efficiently amplify the DNA of certain prey species/groups causing sequence counts to be disproportionate to the amount of prey ingested or counts of prey individuals consumed (Pompanon et al. 2012, Bowen and Iverson 2013, Deagle et al. 2013, Thomas et al. 2014). However, conserved PCR primers have generally been used in metabarcoding of fish mixtures (as in the present study assessing primarily piscivorous harbor seal diet) and recovery biases are moderate (Deagle et al. 2019). The primers utilized here were tested and proven to amplify efficiently all bony fish species, so such biases should be negligible (Granquist et al. 2018). DNA metabarcoding methods have more recently been

applied as semi-quantitative instead of strictly presence-absence (Deagle et al. 2010, Deagle et al. 2019), and biases have been found to be consistent between samples (Thomas et al. 2014) – though there is no routine method of interpretation of read counts and this discussion is ongoing (De Barba et al. 2014). Restricting the analysis to only the presence-absence occurrence index may overestimate the importance of prey consumed in small quantities, or taxa from low-level contamination or secondary ingestion (Deagle et al. 2019). Restricting occurrences to prey representing >1% of sample sequences and using a secondary index of relative read abundance can help to give a more accurate view of population-level diet, but entirely ruling out secondary predation in occurrence data may require further information about prey co-occurrence or expert knowledge (Jarman et al. 2013, McInnes et al. 2017, Deagle et al. 2019). Coupling the DNA data with hard-parts-based biomass estimates in this study helps to overcome the challenges with quantification inherent in both methods. The use of genetic methods enables future analyses coupling diet composition of samples with concurrent demographic information, since archived feces soft-matrix could be used to gather information on sex, reproductive status and even individual (Tollit et al. 2009).

The two methods employed had different capacities to detect prey other than bony fishes. The primers used in the DNA metabarcoding were designed to amplify DNA of vertebrates including bony fishes, cartilaginous fishes (i.e., skates), and birds (Granquist et al. 2018). While the hard-parts analysis could not detect the latter two groups, it was able to capture cephalopod prey through beaks remaining in the feces, unlike the DNA analysis. DNA metabarcoding does have the capacity to detect cephalopod prey (Deagle et al. 2009, Deagle et al. 2010, McInnes et al. 2017), thus future studies using DNA identification to assess the diet of harbor seals in outer Oslofjord could be designed to include primers that would amplify cephalopod DNA.

Additionally, the occurrences of prey species and groups may differ by method of assessment because the DNA metabarcoding analysis tends to assess diet over a shorter timescale than the hard-parts analysis, since prey present in the scat soft-matrix represent the most recent feeding events of the past one or two days while the hard-parts may appear in the scats for up to seven days (Deagle et al. 2005, Casper et al. 2007, Tollit et al. 2009). Subsampling different sections of the feces when gathering the fraction of starting material for the DNA extraction and analysis is another potential cause of differences between results of the DNA and hard-parts analyses (Deagle et al. 2005, Matejusová et al. 2008, De Barba et al. 2014), since the hard-parts were collected from the entirety of the sample but DNA was not.

Besides the biases associated with the specific scatological methods of diet assessment, there are important considerations to note about the use of feces to estimate diet in the first place. When using harbor seal scats collected at haul-out sites to assess diet, the unit of sampling is assumed to be a scat. However, a single scat is not likely to represent a single meal (or feeding event), but rather a composite of multiple past meals (Tollit et al. 2003, Phillips and T. Harvey 2009, Tollit et al. 2009). Since the defecation events were not observed, it is possible that multiple scats were left by the same individual and resulted from the same meal; thus, pseudo-replication cannot be ruled out. This may not be a problem; provided the remains from all prey are eventually defecated, Pierce et al. (2003) posited that the fragmentation of prey from different feeding bouts across scat samples increases the variation in composition between scats and reduces the changes of detecting significant differences between sets of scats, thus making comparisons more conservative. Nevertheless, scat sampling remains a valid method suitable for estimating the diet composition of harbor seals in a noninvasive manner, and the coupling of morphological and genetic methods can provide an informative dietary assessment. Given the importance of understanding dietary habits, food webs interactions, and ecological roles of predators in effective conservation of ecosystems and ecosystem services, efforts to improve accuracy of diet analysis should continue.

4.2 Feeding ecology

4.2.1 Consumption of fish

The high diversity of prey consumed by harbor seals in the outer Oslofjord further confirms the generalist foraging behavior of harbor seals from this and other areas (e.g., Härkönen 1987, Härkönen and M.-P. Heide-Jørgensen 1991, Olesiuk 1993, Olsen and Bjørge 1995, Tollit and Thompson 1996, Brown and Pierce 1998, Wilson et al. 2002, Pierce and Santos 2003, Andersen et al. 2004, Andersen et al. 2007, Lance et al. 2012, Scharff-Olsen et al. 2019, Wilson and Hammond 2019). The harbor seals preyed predominantly on small fish (less than 30 cm in length) from families Gadidae and Pleuronectidae, which appears typical for the region (Härkönen 1987, Härkönen and M.-P. Heide-Jørgensen 1991, Olsen and Bjørge 1995, Scharff-Olsen et al. 2019, Sørlie et al. 2020) and elsewhere (Wilson et al. 2002).

Both molecular and morphological analyses showed that gadoids were the most frequently occurring prey species in the scats of the harbor seals studied. However, the prey classification based upon the morphological analysis of hard remains tended to be less taxonomically refined

than that determined molecularly. The molecular DNA metabarcoding indicated that Atlantic cod was an important species in the harbor seal diet where the identification of otoliths was often indeterminate between Atlantic cod, pollack, and saithe. While the hard-parts analysis alone found the cod/pollack/saithe group to be most important, it did confirm that Atlantic cod was an important prey species. Given that Atlantic cod was considerably more dominant than saithe in the DNA metabarcoding, both in terms of frequency of occurrence and relative read abundance, it seems fair to assume that the proportions of cod, pollack, and saithe found in the DNA analysis apply for the hard-parts analysis as well. In that case, group cod/pollack/saithe in the hard-parts identification is dominated by Atlantic cod. Though multiple prey species were important, when operating under this assumption Atlantic cod was found to be the greatest component of the harbor seal diet across the entire study period. It is important to acknowledge that this assumption is speculative due to lack of further genetic analyses of the otoliths that were unidentifiable to species. The uncertainty in this assumption must not be forgotten when considering the following conclusions regarding seal predation on cod.

Cod has previously been shown to be an important prey species for harbor seals in the region (Härkönen 1987, Härkönen and M.-P. Heide-Jørgensen 1991), but there is some variation in the importance of cod between studies that may be driven by regional and interannual availability (Sørlie et al. 2020). It was an exceptionally strong year-class for Atlantic cod in the outer Oslofjord in 2019 – the strongest year-class since 1996 – and there was therefore an unusually high availability of cod recruits within the size range targeted by harbor seals during the study period as shown by the beach seine time series conducted annually on fixed stations in Skagerrak (Institute of Marine Research, unpublished data). According to the small size of the Atlantic cod consumed, it is evident that the harbor seals were preying upon 0-group juvenile cod from this robust cohort during the summer and autumn of 2019. Thus, enhanced importance of Atlantic cod in the present study is most likely correlated to increased abundance of cod, further supporting the generally accepted opportunistic feeding strategy of harbor seals as predators feeding on the species that are most available (Härkönen 1987, Härkönen and M.-P. Heide-Jørgensen 1991, Olsen and Bjørge 1995, Tollit et al. 1997b, Andersen et al. 2004).

Other important prey for the harbor seals in the outer Oslofjord included gadoids haddock, genus *Trisopterus*, and whiting as well as pleuronectid flatfishes. These prey have also been found to be important for harbor seals other studies and areas (Härkönen 1987, Härkönen and M.-P. Heide-Jørgensen 1991, Olsen and Bjørge 1995, Tollit and Thompson 1996, Brown et al. 2001, Scharff-Olsen et al. 2019, Sørlie et al. 2020). Most of these important prey species are

demersal or benthopelagic, suggesting that the harbor seals studied have a preference for foraging along the seabed, sometimes at depth – a phenomenon that has previously been observed (Olsen and Bjørge 1995, Scharff-Olsen et al. 2019). The presence of deep-water species that are rarely seen in shallow waters (e.g., *Anarhichas* spp., blue whiting, ling, Norway pout, witch flounder; see Appendix J for scientific names) indicates that harbor seals sometimes forage at depth – as was also discussed by Scharff-Olsen et al. (2019).

Herring was not found to be an important prey species contributing only occasionally in this study, despite featuring prominently or showing seasonal importance in other harbor seal diet assessments (Härkönen 1987, Härkönen and M.-P. Heide-Jørgensen 1991, Brown and Pierce 1998, Brown et al. 2001, Pierce and Santos 2003, Lance et al. 2012, Granquist et al. 2018). The reduced importance of herring seen here may be attributable to seasonal variation as reduced herring consumption during the time period studied has been reported. Previous studies in the region have shown reduced relative importance of herring during August (Olsen and Bjørge 1995) and November (Härkönen 1987, Olsen and Bjørge 1995), or during summer/autumn (Sørlie et al. 2020). It is also possible that reduced herring importance reflects the greater importance of Atlantic cod in the present study. Other investigations have also found sandeels (Ammodytidae) to be important prey for harbor seals (Härkönen 1987, Tollit and Thompson 1996, Brown and Pierce 1998, Brown et al. 2001, Lance et al. 2012, Granquist et al. 2018), which contrasts with the absence of Ammodytidae in the presently observed diets. However, not all studies find Ammodytidae to be important and low importance of sandeel has also been reported (Berg et al. 2002, Pierce and Santos 2003). Seasonal variation in the contribution of Ammodytidae to harbor seal diet has been seen in the outer Oslofjord, and trends of decreasing importance of Ammodytidae in the diet and after August/September likely explain the lack of these species in the present study (Olsen and Bjørge 1995).

Whether or not there are regional differences in the harbor seal diet between Færder and Hvaler remains inconclusive. The DNA analysis did not reveal any significant regional variability between the two areas, while the hard-parts analysis showed regional differences in occurrence of prey and prey importance by biomass. In the hard-parts analysis, greater diversity of fish prey was found in the scat samples from Hvaler. Flatfish in family Pleuronectidae and wrasses in family Labridae were relatively more important in terms of biomass consumed at Hvaler than they were at Færder, where the most important prey were all in family Gadidae. Regional differences in prey importance may be driven by local availability/abundance of fish species (Härkönen 1987) or by local differences in harbor seal foraging behavior (e.g., foraging

techniques, habitat use, prey preferences) between the two seal populations (Tollit et al. 1998). Some of the differences seen between the two locations may actually represent seasonal variation in prey availability, since the samples from Hvaler were collected in August (late summer) while the samples from Færder were collected in November (autumn). Because the two study areas are geographically quite close and given that the habitat is quite similar in the two locales, it may indeed be more likely that the data reveal temporal rather than spatial differences. Regardless, no samples were collected from both locations during the same time period, so it is not possible to distinguish seasonal variability from regional variability. Furthermore, though there may exist some differences in diet composition between Færder and Hvaler, the differences observed here are likely to be an artifact of sample size. Hard parts were identified from a greater number of samples from Hvaler (45) than from Færder (32). Conversely, DNA analysis was conducted for fewer samples from Hvaler (20) than from Færder (24), though the difference in number of samples between the two locations was lesser (which may explain the lack of significant regional differences in this dataset). In order to better detect and more accurately elucidate geographical as well as seasonal differences, sufficient scat samples must be collected and analyzed. It is also important to note that there was considerable variation in prey composition between the scat samples, especially in the DNA analysis, which further emphasizes the need to analyze a sufficient number of samples in order to reveal significant trends and accurately characterize diet.

4.2.2 Other prey

In addition to bony fishes, the harbor seal diet in the outer Oslofjord was found to occasionally contain cephalopods and skates in the genus *Amblyraja (*family Rajidae), though these prey were not particularly important or common. Cephalopod beaks were occasionally recovered in the scat samples during hard-parts analysis. Cephalopods, which have been documented as prey in other harbor seal diet assessments (Härkönen and M.-P. Heide-Jørgensen 1991, Olesiuk 1993, Tollit and Thompson 1996, Brown et al. 2001, Pierce and Santos 2003, Lance et al. 2012, Wilson and Hammond 2019), were only a minor prey item for seals at Hvaler. No cephalopod prey was found at Færder. A recent harbor seal diet study in nearby areas of the Norwegian Skagerrak did not find any cephalopod prey (Sørlie et al. 2020), so it is possible there are fine-scale local differences in the harbor seal foraging behaviors that result in only select seals predating cephalopods. Although cephalopods may instead represent secondary prey due to the low frequencies at which they occurred (Härkönen and M.-P. Heide-Jørgensen 1991), the beaks recovered in the present study were not small enough to definitely draw that conclusion.

While cephalopods were only found in diet samples from Hvaler, skates occasionally occurred as prey in diet samples from Færder during the DNA metabarcoding analysis. As previously mentioned, skates were not detectable in the hard-parts analysis since they lack otoliths. Though not included in the hard-parts methodology used here, it may be possible to identify skates in family Rajidae morphologically using denticles recovered in feces (Pierce et al. 1991, Tollit and Thompson 1996). Skates have been observed in other investigations of harbor seal diets (Tollit and Thompson 1996, Browne et al. 2002, Andersen et al. 2004, Lance et al. 2012).

In addition to bony fishes and skates, the DNA metabarcoding analysis found presence of birds from families Anatidae (ducks and waterbirds, including common eiders) and Turdidae (genus *Turdu*s, the thrushes) in the scat samples. Anatidae occurred in scats from both locations, while *Turdus* spp. only occurred in samples from Færder. There exists evidence of harbor seals feeding on seabirds such as common eiders (Kirkham 2008) as well as harlequin ducks (Tallman and Sullivan 2004), but this phenomenon does not appear to be very common. Bird DNA was previously detected in harbor seal scat samples from Iceland in the study by Granquist et al. (2018) in which the same PCR primers were used. Predation on birds by other pinniped species (such as penguin predation by pinnipeds in the southern hemisphere) has been documented as well (Moore and Moffat 1992, Crawford and Cooper 1996, Long and Gilber 1997, du Toit et al. 2004, Raya Rey et al. 2012, Morrison et al. 2017). It is possible that the occurrence of bird DNA is an artifact of field contamination from the presence of bird droppings on the rocks from which the seal feces were collected. If the bird DNA were due to simultaneous collection of bird droppings during the harbor seal scat collection, it would be expected that many samples would contain low levels of bird DNA. Alternately, birds as prey would be expected to be reflected in the DNA differently than bird DNA coming from exogenous sources; if seals were occasionally to consume birds, greater relative contribution of these bird sequences would be expected in fewer samples instead of low levels of bird sequences across many samples.

Contamination from exogenous sources may explain the presence of the *Turdus* birds found in samples from Færder, since this genus had extremely low relative importance. Yet, contamination seems unlikely to explain the presence of Anatidae in the samples, since this bird family had considerable relative importance overall. Anatidae bird species are prevalent in the outer Oslofjord but were found to be less important in the diet at Hvaler than at Færder, where the family was one of the top five most important prey in terms of relative diet contribution. As explained above, if seals were occasionally to consume birds select scats would be expected to

contain a high proportion of the bird DNA. Indeed, when samples were categorized according to the dominant prey group (the group represented by >70% of the sequences) one sample was dominated by Anatidae, suggesting that Anatidae was prey for at least one seal.

4.3 Competition with fisheries

Harbor seals as predators exert top-down pressure on marine ecosystems. Mortality due to harbor seal predation has the potential to impact fisheries (Beverton 1981, Beddington et al. 1986, Bjørge 2002, Drake et al. 2010) and fish populations and may limit stock recovery. The results of the dietary assessment indicate overlap between harbor seal prey and species targeted by fisheries, though some prey species are not commercially important. In terms of biomass removed annually, the estimated harbor seal consumption exceeds local fisheries landings for all commercially important prey except Atlantic halibut, Atlantic herring, Atlantic mackerel, and – possibly – Atlantic cod. The annual consumption estimates for individual prey species and the subsequent inferences made upon these values must be regarded with caution because they rely upon the precarious assumption that results from scats sampled in August and November are representative for the entire year. The contribution of species in the harbor seal diet is expected to change seasonally, as prey availability fluctuates (Härkönen 1987, Olsen and Bjørge 1995, Sørlie et al. 2020). Thus, extrapolating consumption data from samples collected during two months to the entirety of 2019 may be unwise.

In order to investigate the potential for competition between harbor seals and commercial fisheries in the region, prey size estimates based on otolith measurements were compared to minimum allowed landing sizes. When making such comparisons, it is important to remember that because correction factors have not been applied here, the size distribution of prey is inevitably biased towards smaller sizes. Nevertheless, some individuals of the more common prey species Atlantic herring, common dab, European flounder, European plaice, haddock, and wrasses were consumed within the allowable landing size, so direct competition with these fisheries is possible. However, it was only for Atlantic herring and wrasses that a majority of individuals consumed exceeded minimum allowed landing size. Though more than 50% of the herring consumed by the seals were above the minimum allowed landing size and therefore of the same sizes targeted by the fishery, the estimated biomass of herring consumed by the seals in Færder and Hvaler in the outer Oslofjord during 2019 was only 11.3% of the fishery take. Thus, harbor seals do compete with the local herring fishery by targeting herring of the same

sizes, but they take considerably less biomass. A similar relationship between harbor seals and the herring fishery was reported in Limfjord, Denmark (Andersen et al. 2007).

Similarly, more than 50% of the wrasses consumed – which were not consistently identifiable to species – were larger than minimum landing size for all wrasses except ballan wrasse and corkwing wrasse. Since the late 1980s, wrasses have been targeted commercially for use as cleaner fish to combat the sea lice problem in the Norwegian salmon aquaculture (Blanco Gonzalez and de Boer 2017), but these catches are reported as number of individuals rather than biomass. Unlike in the case of the herring fishery, the landings statistics reported for wrasses in the region (Table 3) are bycatch numbers, so no there will not be any further discussion of competition here.

There was no evidence for direct competition for fish of the same size between fisheries and other harbor seal prey species. Even the largest individuals consumed of Atlantic cod, European hake, turbots (Scophthalmidae), witch flounder, and whiting were smaller than the minimum allowed landing size. While harbor seals do not have a direct impact on fisheries of these species, indirect competition may exist.

Atlantic cod is a species of concern in the outer Oslofjord and adjacent areas (Svedang 2003, Cardinale and Svedäng 2004, Moland et al. 2020). Here we documented that small Atlantic cod were a significant component of harbor seal diet in the outer Oslofjord. Due to concerns over the cod stocks, fishing of Atlantic cod within Oslofjord was banned in 2019 so annual harbor seal consumption was compared to 2018 cod landings data (Norwegian catch statistics, Directorate of Fisheries, 2018). Based on the assumption that results from scats sampled in August and November are representative for the annual seal diet, the estimated total annual consumption of Atlantic cod was estimated to be 68.9 tons in 2019, or 64.9% of the 2018 fishery take of 106.1 tons. Due to the likelihood that much of the fish in the 189.3 tons of prey classified to the cod/pollack/saithe group are indeed cod, the estimated cod consumption in 2019 could expand to more than 250 tons or twice as much the 2018 fishery take. However, these estimates are unreliable because they are predicated on the fact that no seasonal variation in diet occurs and that seals have been eating 0-group cod for all 365 days of the year. As previously discussed, the considerable cod consumption documented in this study is believed to be driven by the presence of the strongest year-class in modern times and, therefore, unusually inflated cod availability (Institute of Marine Research, unpublished data). Thus, the diet assessment presented describes cod predation during August and November in a year when juveniles were

exceptionally plentiful. Since these juveniles settled to the nurseries in the spring of 2019 and became available as prey items only as the year progressed, the harbor seals could not have been eating the 0-group cod in the beginning of the year, so the annual harbor seal cod consumption estimates are unrealistic and overestimate the consumption of cod throughout 2019. Instead, it would have been better to calculate consumption for only the second half of the year (July-December 2019), multiplying daily consumption by 183 days rather than 365. In doing so, harbor seal cod consumption for the second half of 2019 would be estimated as 34.5 tons and consumption of cod/pollack/saithe would be estimated as 94.6 tons – still considerable amounts in comparison to the 2018 fishery take (Appendix I). Moreover, the abundant cod from the strong 2019 cohort will remain as small juveniles within the outer Oslofjord and adjacent areas and should be available to harbor seals as prey all year long during the coming years, so it is possible that the annual consumption estimated here may accurately reflect harbor seal cod consumption for subsequent years if cod availability remains high.

This study demonstrates a higher potential for competition between harbor seals and the Atlantic cod fishery than has previously been reported in the region (Härkönen and M.-P. Heide-Jørgensen 1991, Sørlie et al. 2020). The proportion of cod in the harbor seal diet is expected to decline as cod abundance declines (Hansen and Harding 2006). Given that cod stocks have been severely depleted in this region for many years (Cardinale and Svedäng 2004), the negligible impact of harbor seals on cod stocks and low potential for competition between seals and fisheries reported in these studies may have been an artifact of low cod population. As previously discussed, cod availability increased in 2019 due to a strong recruitment year (Institute of Marine Research, unpublished data). Because juvenile cod inhabit that shallow waters in which harbor seals forage, it is not surprising that the harbor seals studied were heavily exploiting small 0-group cod in the summer and autumn of 2019. Cod in these shallow coastal waters remain below minimum landing size (400 mm) and within the preferred prey size range for harbor seals for approximately four years (Berg and Albert 2003), implying that a high predation pressure from harbor seals on the 2019 age class of cod in the Skagerrak can be expected until ca. 2022, when this class will exceed the small size targeted by seals. Bakun (2006) described a “predator pit” type of dynamics where a species experiences refuge from predation when abundance is very low but is the target of destructive predation when abundance is high enough to attract interest from predators yet remains below the level sufficient to satiate predators. In other words, below a certain density threshold, harbor seal interest in cod as prey is very low (e.g., Sørlie et al. 2020) but harbor seal predation on cod becomes particularly heavy

(as seen in this study) when cod abundance increases above that threshold but remains below the "satiation point" (Bakun 2006). Synthesizing the results of this study with the findings of prior studies, we hypothesize that the role of harbor seals as predators in the ecosystem may be contributing to such a "predator pit" that is constraining the recovery of cod and other hampered fish stocks in the outer Oslofjord and adjacent areas. Although the cod fishery targets larger individuals than the harbor seals, this study shows that seals in the outer Oslofjord have the capacity to impose a significant negative impact on cod survival. Because the life history of cod is adapted to high mortality (from environmental variability and predation) during the egg, larvae, and juvenile phases with each female producing 1.2 million eggs on average during spring, seal predation on juvenile cod must overwhelm these coping strategies to have lasting impacts. The possibility that harbor seals may be hampering the recruitment of cod through "predator pit" dynamics cannot be ruled out, but the validity of this hypothesis must be tested with further investigation; the data presented here do not have the capacity to confirm such a phenomenon.

Wherever competition between harbor seals and fisheries may exist – directly or indirectly – the actual impacts of seal predation in terms of depletion of local fish abundance are harder to quantify and depend on numerous other factors. The targeted removal of small, juvenile cod with lower reproductive value than larger individuals may limit the harbor seal impact on future cod population growth (Hansen and Harding 2006). Additionally, predation of harbor seals on juvenile fishes may compensate for other mortalities (Andersen et al. 2007). Seal predation may reduce intraspecific competition within targeted fish populations, benefiting fisheries by increasing the likelihood that survivors live to sizes that can be commercially exploited (Andersen et al. 2007). Furthermore, removal of some predatory fishes by seals may initiate competitive release of their prey, benefitting other fisheries that target lower-trophic level organisms (Bjørge 2002, Pedersen et al. 2008). Movements of fishes through the area will affect the scale of the impacts of seal predation on local stocks (Harwood and Croxall 1988, Bjørge 2002). Studies in the region frequently report differing conclusions about the relationship between the estimated prey consumption of harbor seals and local fisheries catches (Scharff-Olsen et al. 2019), but diet assessment studies are often limited to specific areas and seasons. In the present study, our data is restricted to only two months of the year in a region where seasonal variations in harbor seal diet composition have been described (Härkönen 1987, Olsen and Bjørge 1995, Sørlie et al. 2020). Biases and limitations to such assessments include the methodological considerations discussed previously as well as potential errors in both the

bioenergetics assumption of the annual consumption estimates and the fisheries landings data. Still, this study gives reason to believe that seal predation may have significant impact on local, coastal fisheries, even if such impacts appear negligible at broader scales or on off-shore populations (Hansen and Harding 2006, Hansson et al. 2018). Further investigation is critical.

5 Concluding remarks

In this study, we show the benefits of combining taxonomically sensitive DNA metabarcoding with the traditional hard-parts analysis that offers information on prey size distribution in order to better characterize harbor seals as predators and improve understanding of the role of Atlantic cod in the harbor seal diet. Refined understanding of predator diet is important in terms of ecosystem-based management and conservation of repressed fish populations. In order to adequately investigate the impact of harbor seals on Atlantic cod, a long timeseries of harbor seal diet data or a functional response model predicting the consumption of cod as a function of local cod abundance would be required. We show that harbor seals have the capacity to consume large quantities of commercially important species and these pinniped predators should be carefully considered in ecosystem analyses; the information on seal feeding ecology and diet composition garnered in this study should be integrated into ecological and stock assessment models. Further study of harbor seal diet in the region across multiple seasons in subsequent years would give a more complete picture of the impacts of seals as predators over longer time periods and would enable exploration into how harbor seals regulate their prey (i.e., whether they can generate a "predator pit"). Our findings suggest that predation by harbor seals is potentially an important factor in preventing the recovery of the depleted coastal cod stocks. These findings underscore the importance of consistent monitoring of predator diets in order to explore predator-prey dynamics and the underlying causal mechanisms and highlight the need for continued development of methods for estimating pinniped diet.

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Appendix A

PCR product pooling scheme and normalization calculations.

Sample	Forward primer barcode	Pool	Qubit concentration of DNA in PCR product (ng/µl)	Concentration ratios	Conversion factor*	Amount used in pool (µl)	DNA contributed by each sample (ng)
Hvaler_01	GAATTC	1	1.6	2.31	3.86	8.93	14.29
Færder_23	GCCTAA	1	3.1	1.19		4.61	14.29
Hvaler_29	ACATCG	1	3.7	1		3.86	14.29
Hvaler_11	TGGTCA	1	9.6	0.39		1.49	14.29
Færder_22	CACTGT	1	12.9	0.29		1.11	14.29
Færder_08	GATCTG	2	0.25	20.8	0.75	15.59	3.90
Hvaler_02	TACAAG	2	2.5	2.08		1.56	3.90
Hvaler_18	ATTGGC	2	4.3	1.21		0.91	3.90
Færder_03	ACATCG	2	5.2	1		0.75	3.90
Færder_05	GCCTAA	2	7.1	0.73		0.55	3.90
Færder_02	CGTGAT	2	11.1	0.47		0.35	3.90
Færder_32	TGGTCA	2	20.1	0.26		0.19	3.90
Hvaler_10	CACTGT	2	36.9	0.14		0.11	3.90
Færder_11	GATCTG	3	1.4	12.93	1.04	13.43	18.80
Hvaler_60	ACATCG	3	11.5	1.57		1.63	18.80
Hvaler_63	CGTGAT	3	16.8	1.08		1.12	18.80
Færder_14	GCCTAA	3	18.1	1		1.04	18.80
Hvaler_61	TACAAG	3	21.1	0.86		0.89	18.80
Færder_43	ATTGGC	3	23.5	0.77		0.80	18.80
Hvaler_76	TGGTCA	3	32.1	0.56		0.59	18.80
Færder_39	CACTGT	3	37.1	0.49		0.51	18.80
Færder_15	ACATCG	4	13	2.1	2.17	4.55	59.19
Færder_04	GATCTG	4	16.3	1.67		3.63	59.19
Hvaler_69	CGTGAT	4	21.1	1.29		2.81	59.19
Hvaler_75	TACAAG	4	27.3	1		2.17	59.19
Hvaler_55	ATTGGC	4	31.6	0.86		1.87	59.19
Færder_27	GCCTAA	4	32.4	0.84		1.83	59.19
Færder_17	TGGTCA	4	35.6	0.77		1.66	59.19
Færder_38	CACTGT	4	40	0.68		1.48	59.19
Hvaler_49	CGTGAT	5	26.8	1.38	2.43	3.37	90.28
Hvaler_39	TACAAG	5	33.4	1.11		2.70	90.28
Hvaler_68	ACATCG	5	34.2	1.08		2.64	90.28
Færder_41	ATTGGC	5	37.1	1		2.43	90.28
Færder_52	TGGTCA	5	37.1	1		2.43	90.28
Hvaler_58	GATCTG	5	38.6	0.96		2.34	90.28
Hvaler_77	CACTGT	5	41.9	0.89		2.15	90.28
Færder_28	GCCTAA	5	46.8	0.79		1.93	90.28

Appendix A, PCR product pooling scheme *(concluded).*

Sample	Forward primer barcode	Pool	Qubit concentration of DNA in PCR product (ng/µl)	Concentration ratios	Conversion factor*	Amount used in pool (µl)	DNA contributed by each sample (ng)
Hvaler_66	TACAAG	6	34.6	1.20	2.65	3.18	110.15
Færder_48	ACATCG	6	40.7	1.02		2.71	110.15
Færder_40	ATTGGC	6	41.1	1.01		2.68	110.15
Færder_13	GATCTG	6	41.6	1		2.65	110.15
Færder_49	CGTGAT	6	45.3	0.92		2.43	110.15
Færder_35	TGGTCA	6	49.4	0.84		2.23	110.15
Hvaler_51	CACTGT	6	52	0.8		2.12	110.15
Færder_42	GCCTAA	6	55	0.76		2.00	110.15

*Conversion factor was calculated as: allowable volume (20 µl)/sum of total parts (from ratio).

Appendix B

B.1 QIIME 2 bioinformatics script

The following is the code used for the bioinformatics analysis in QIIME 2.

Activate the QIIME 2 conda environment.

conda activate qiime2-2021.4

Choose directory.

Both demultiplexing and DADA2 denoising assume they are looking at a single Illumina run, so everything will be done 6 times to cover each of the pools 1-6, and then data will be combined at the stage of feature table & representative set.

IMPORT the data to turn it into QIIME artifacts ready to use in the rest of the processing. Note that this requires a folder containing only reads named "forward.fastq.gz" and "reverse.fastq.gz". Therefore, within the "Raw_sequences_QIIME" folder a sub-folder for each pool containing these two files was created.

for run in pool_1 pool_2 pool_3 pool_4 pool_5 pool_6
do
**qiime tools import **
**--type MultiplexedPairedEndBarcodeInSequence **
**--input-path Raw_sequences_QIIME/$run **
--output-path QIIME_analyses/$run/multiplexed-seqs.qza
done

DEMULTIPLEX the sequences to separate them out by sample, based on the barcodes in the sequences.

for run in pool_1 pool_2 pool_3 pool_4 pool_5 pool_6
do
**qiime cutadapt demux-paired **
**--i-seqs Qiime_analyses/$run/multiplexed-seqs.qza **
**--m-forward-barcodes-file Map_files/map_$run.txt **
**--m-forward-barcodes-column BarcodeSequence **
**--o-per-sample-sequences QIIME_analyses/$run/demultiplexed_sequences.qza **
--o-untrimmed-sequences
**QIIME_analyses/$run/untrimmed_from_demultiplexed_sequences.qza **
**--p-mixed-orientation **
--verbose
done

REMOVE THE PCR PRIMERS at the 5' ends of the sequences and discard the untrimmed sequences that lacked primers at 5' ends.

for run in pool_1 pool_2 pool_3 pool_4 pool_5 pool_6

```
do
qiime cutadapt trim-paired \
--i-demultiplexed-sequences QIIME_analyses/$run/demultiplexed_sequences.qza \
--p-front-f CGTGCRAAGGTAGCG \
--p-front-r CCTYGGGCGCCCCAAC \
--p-discard-untrimmed \
--o-trimmed-sequences
QIIME_analyses/$run/demultiplexed_forward_trimmed_sequences.qza
done
```

DENOISE sequences to correct errors and merge forward and reverse reads, generating a counts per sample table (feature table) and a representative set.

```
for run in pool_1 pool_2 pool_3 pool_4 pool_5 pool_6
do
qiime dada2 denoise-paired \
--i-demultiplexed-seqs
QIIME_analyses/$run/demultiplexed_forward_trimmed_sequences.qza \
--p-trunc-len-f 185 \
--p-trunc-len-r 135 \
--p-trunc-q 2 \
--p-max-ee-f 3 --p-max-ee-r 3 \
--p-min-fold-parent-over-abundance 2 \
--p-pooling-method pseudo \
--o-table QIIME_analyses/$run/ASV_count_table.qza \
--o-representative-sequences QIIME_analyses/$run/rep_set.qza \
--o-denoising-stats QIIME_analyses/$run/denoising_log.qza \
--verbose
done
```

Combine all feature tables into one.

```
qiime feature-table merge \
--i-tables QIIME_analyses/pool_1/ASV_count_table.qza \
--i-tables QIIME_analyses/pool_2/ASV_count_table.qza \
--i-tables QIIME_analyses/pool_3/ASV_count_table.qza \
--i-tables QIIME_analyses/pool_4/ASV_count_table.qza \
--i-tables QIIME_analyses/pool_5/ASV_count_table.qza \
--i-tables QIIME_analyses/pool_6/ASV_count_table.qza \
--o-merged-table QIIME_analyses/ASV_count_table_all.qza
```

Make one master representative set of sequences.

```
qiime feature-table merge-seqs \
--i-data QIIME_analyses/pool_1/rep_set.qza \
--i-data QIIME_analyses/pool_2/rep_set.qza \
--i-data QIIME_analyses/pool_3/rep_set.qza \
--i-data QIIME_analyses/pool_4/rep_set.qza \
--i-data QIIME_analyses/pool_5/rep_set.qza \
--i-data QIIME_analyses/pool_6/rep_set.qza \
```

--o-merged-data QIIME_analyses/representative_set_all.qza

ASSIGN TAXONOMY

First, get data from NCBI to create a reference database that includes the teleostei fishes, cartilaginous fishes (Chondrichthyes), birds (Aves), and seals (Phocidae).

**qiime rescript get-ncbi-data **
--p-query '(("Teleostei"[Organism] OR teleostei[All Fields]) AND
(is_nuccore[filter] AND mitochondrion[filter])) OR
(("Chondrichthyes"[Organism] OR Chondrichthyes[All Fields]) AND
(animals[filter] AND is_nuccore[filter] AND mitochondrion[filter])) OR
(("Phocidae"[Organism] OR phocidae[All Fields]) AND (animals[filter]
AND is_nuccore[filter] AND mitochondrion[filter]) OR
("Aves"[Organism] OR aves[All Fields]) AND (animals[filter] AND
**refseq[filter] AND mitochondrion[filter]))' **
**--o-sequences QIIME_analyses/ncbi-refseqs-unfiltered.qza **
--o-taxonomy QIIME_analyses/ncbi-refseqs-taxonomy-unfiltered.qza

##Run BLAST+ with this reference database.

**qiime feature-classifier classify-consensus-blast **
**--i-query QIIME_analyses/representative_set_all.qza **
**--i-reference-reads QIIME_analyses/ncbi-refseqs-unfiltered.qza **
**--i-reference-taxonomy QIIME_analyses/ncbi-refseqs-taxonomy-unfiltered.qza **
**--o-classification QIIME_analyses/BLAST_taxonomy_all.qza **
--verbose

CLEAN RESULTS

Since there are high numbers of unassigned sequences that may be bad data or off-target reads I will filter the DADA2 results to get a set of "hits" and a set of "misses", then I can remove the "misses" from the BLAST taxonomy output.

I have put some of the most prevalent sequences through blastn (megablast) on the NCBI website and they come back either without matches, to bacteria, or inconclusive/low match to fishes

Forum posts referenced: https://forum.qiime2.org/t/too-many-unassigned-or-only-at-kingdom-level-features/2934 and https://forum.qiime2.org/t/non-target-dna-in-16s-reads-unassigned-kingdom-level-only/14858/2

Identify sequences that are very different from the target sequences ("misses").

**qiime quality-control exclude-seqs **
**--i-query-sequences QIIME_analyses/representative_set_all.qza **
**--i-reference-sequences QIIME_analyses/ncbi-refseqs-unfiltered.qza **
**--p-method blast **
**--p-perc-identity 0.65 **
**--p-perc-query-aligned 0.6 **

**--o-sequence-hits QIIME_analyses/hits.qza **
**--o-sequence-misses QIIME_analyses/misses.qza **
--verbose

Remove the the misses.qza features from the original ASV feature table.

**qiime feature-table filter-features **
**--i-table QIIME_analyses/ASV_count_table_all.qza **
**--m-metadata-file QIIME_analyses/misses.qza **
**--o-filtered-table QIIME_analyses/no_misses_ASV_count_table.qza **
--p-exclude-ids

Discard any remaining unassigned sequences.

**qiime taxa filter-table **
**--i-table QIIME_analyses/no_misses_ASV_count_table.qza **
**--i-taxonomy QIIME_analyses/BLAST_taxonomy_all.qza **
**--p-exclude Unassigned **
--o-filtered-table QIIME_analyses/fully_filtered_ASV_table.qza

Visualize a taxa bar plot with the filtered (no misses and no unassigned sequences) ASV table.

**qiime taxa barplot **
**--i-table QIIME_analyses/fully_filtered_ASV_table.qza **
**--i-taxonomy QIIME_analyses/BLAST_taxonomy_all.qza **
**--m-metadata-file DNA_sample_metadata.tsv **
--o-visualization QIIME_exports/taxa_barplot_filtered.qzv

TABULATE METADATA to associate feature IDs to ASVs and see which taxonomic assignments are found in each sample.

First transpose the ASV count table to get samples as columns and ASVs as rows.

**qiime feature-table transpose **
**--i-table QIIME_analyses/ASV_count_table_all.qza **
--o-transposed-feature-table QIIME_analyses_transposed_ASV_count_table.qza

Tabulate metadata.

**qiime metadata tabulate **
**--m-input-file QIIME_analyses/hits.qza **
**--m-input-file QIIME_analyses/BLAST_taxonomy_all.qza **
**--m-input-file QIIME_analyses/transposed_ASV_count_table.qza **
--o-visualization QIIME_exports/taxonomic_metadata.qzv

Export merged metadata to tsv.

**qiime tools export **
**--input-path QIIME_exports/taxonomic_metadata.qzv **

--output-path QIIME_exports/merged-data.tsv

COLLAPSE FILTERED ASV FEATURE TABLE to merge all features that share the same taxonomic assignment into a single feature, collapse to species (level = 7).

**qiime taxa collapse **
**--i-table QIIME_analyses/fully_filtered_ASV_table.qza **
**--i-taxonomy QIIME_analyses/BLAST_taxonomy_all.qza **
**--p-level 7 **
--o-collapsed-table QIIME_analyses/collapsed_taxonomy_filtered.qza

Export collapsed taxonomy.

**qiime tools export **
**--input-path QIIME_analyses/collapsed_taxonomy_filtered.qza **
--output-path QIIME_exports/collapsed_taxonomy_filtered.qzv

##Convert biom file to txt to view in excel.

**biom convert **
**-i QIIME_exports/collapsed_taxonomy_filtered.qzv/feature-table.biom **
**-o QIIME_exports/collapsed_taxonomy_filtered.qzv/table.from_biom.txt **
--to-tsv

#Correct erroneous assignments.

#Discard samples with fewer than 100 assigned prey sequences.

B.2 Unassigned or erroneously assigned sequences

A large number of amplicon sequence variants (75% of amplicon sequence variants representing 36% of total sequences) that did not align well to the selected reference sequences were filtered out of the dataset using the q2-exclude-seqs plugin (Camacho et al. 2009). The few remaining sequences for which taxonomy was not able to be assigned (28 amplicon sequence variants representing 116 sequences) were discarded from further analyses. A total of 669 amplicon sequence variants (1996221 sequences, 64% of the 3099842 initial sequences remaining after the DADA2 processing) remained in the dataset and were used in further analyses.

16 amplicon sequence variants (30763 sequences) were erroneously assigned by the initial automated BLAST+ consensus search to species that were not possibly prey, since they occur either in the wrong ocean (Pacific) or in freshwater. These sequence variants were manually compared to the entire NCBI non-redundant nucleotide collection (nr/nt) database using the BLAST Megablast algorithm (Zhang et al. 2000, Morgulis et al. 2008). The top ten hits (with

both query cover and percent identity greater than 80%) were assessed and an updated secondary consensus to the lowest possible taxon was determined. (An exception to this protocol was made for one sequence variant, where the top 100 hits were considered because 82% of these hits corresponded to a possible prey species though the top 10 only belonged to an arctic endemic.) Additionally, there were a few instances where the BLAST+ consensus search assigned genus-level taxonomy, but species could subsequently be determined based upon known species distributions because only one species from the genus exists in the study region.

Appendix C

Regression equations used to estimate the fish length (FL) and weight (FW) from otolith measurements (OL = otolith length, OW = otolith width). For prey that could not be identified to species, regressions of the most likely species were used. If this was not feasible an average of relevant equations was used; or when applicable, an average weighted by proportions of species in the region from trawl data was used.

Species	OL (mm) to FL (mm)	OL (mm) to FW (mm)	Reference
Atlantic wolffish	-242.27+216.51•OL	$1.000•(OL^{5.595})$	Härkönen 1986
Spotted wolffish	-196+177.41•OL	$5.290•(OL^{4.08})$	Härkönen 1986
Garfish	(10.38+10.62•OL)•10	$(1.48•OL)^{2.91}$	Leopold 2001
Dragonet	(-5.48+8.41•OL)•10	$(0.88•OL)^{4.14}$	Leopold 2001
Atlantic horse mackerel	(-0.90+3.29•OL)•10	$(0.67•OL)^{2.98}$	Leopold 2001
Atlantic herring	(-1.93+6.29•OL)•10	$(0.93•OL)^{3.35}$	Leopold 2001
Fourhorn sculpin*	43.78+20.81•OL	$6.289•e^{0.353•OL}$	Härkönen 1986
Shorthorn sculpin	-9.95+34.84•OL	$0.2261•(OL^{3.496})$	Härkönen 1986
Atlantic cod	(-6.64+3.49•OL)•10	$(0.37•OL)^{4.04}$	Leopold 2001
Bib	(-5.4+2.99•OL)•10	$(0.33•OL)^{4.55}$	Leopold 2001
Blue whiting*	(5.65+2.66•OL)•10	$(0.30•OL)^{3.51}$	Leopold 2001
Haddock	(-3.27+2.53•OL)•10	$(0.34•OL)^{3.72}$	Leopold 2001
Norway pout	(2.28•OL)•10	$(0.41•OL)^{3.24}$	Leopold 2001
Pollack	$13.20•OL^{1.329}$	$0.01192•OL^{4.205}$	Härkönen 1986
Poor cod	(-3.84+2.61•OL)•10	$(0.35•OL)^{3.84}$	Leopold 2001
Saithe*	$8.97297•OL^{1.53}$	$0.007288•OL^{4.501}$	Härkönen 1986
Silvery pout*	$19.449•OL^{1.053}$	$0.021289•OL^{3.785}$	Härkönen 1986
Tadpole fish	-20.37+22.96•OL	$0.151155•OL^{2.912}$	Härkönen 1986
Whiting	(0.81+1.73•OL)•10	$(0.37•OL)^{2.95}$	Leopold 2001
Fivebeard rockling*	-74.6+92.29•OL	$1.0736•OL^{3.444}$	Härkönen 1986
Fourbeard rockling	-28.8+70.344•OL	$0.1752•OL^{3.482}$	Härkönen 1986
Black goby	-8.927+42.037•OW	$0.225•OW^{4.197}$	Härkönen 1986
Common goby	-10.05+44.71•OW	$0.2465•OW^{3.983}$	Härkönen 1986
Painted goby	-13.0+50.0•OW	$0.294•OW^{7.076}$	Härkönen 1986
Sand goby	-23.36+56.94•OW	$0.1677•OW^{5.369}$	Härkönen 1986
Ballan wrasse	-31.24+67.97•OL	$0.695•OL^{4.205}$	Härkönen 1986
Corkwing wrasse	3.05+63.54•OL	$3.29•OL^{3.30}$	Härkönen 1986
Cuckoo wrasse*	-4.76+52.12•OL	$0.688•OL^{3.51}$	Härkönen 1986
Goldsinny wrasse	9.50+39.36•OL	$1.23•OL^{2.88}$	Härkönen 1986
Rock cook*	28.90+41.18•OL	$3.66•OL^{2.417}$	Härkönen 1986
Ling*	-406+95.731•OL	$0.00765•OL^{4.996}$	Härkönen 1986
European hake	(-4.35+2.66•OL)•10	$(0.35•OL)^{3.41}$	Leopold 2001
Rock gunnel	(0.89+8.71•OL)•10	$(1.33•OL)^{3.39}$	Leopold 2001
Atlantic halibut**	mean of Pleuronectidae equations used		
Common dab	(-3.49+5.43•OL)•10	$(0.69•OL)^{4.01}$	Leopold 2001

Appendix C, regression equations (*concluded*).

Species	OL (mm) to FL (mm)	OL (mm) to FW (mm)	Reference
European flounder	(-3.65+5.61•OL)•10	$(0.79•OL)^{3.63}$	Leopold 2001
European plaice	(-2.07+4.85•OL)•10	$(0.79•OL)^{3.42}$	Leopold 2001
Long rough dab	-24.52+48.35•OL	$0.166•OL^{3.788}$	Härkönen 1986
Witch flounder	-100.65+78.29•OL	$0.0770•OL^{4.633}$	Härkönen 1986
Atlantic mackerel	(8.09•OL)•10	$(1.89•OL)^{2.73}$	Leopold 2001
Brill	(-2.93+6.49•OL)•10	$(1.11•OL)^{3.44}$	Leopold 2001
Norwegian topknot	(5.50•OL)•10	$(1.29•OL)^{2.91}$	Leopold 2001
Turbot	3.42+57.14•OL	$3.61•OL^{3.03}$	Härkönen 1986
Common sole	-12.622+80.901•OL	$2.535•OL^{3.444}$	Härkönen 1986
Snakeblenny	(207.11•OL)-303.76	$0.0342•FL^{1.9847}$***	OL-FL: Härkönen 1986 FL-FW: Silva et al. 2013
Eelpout	(-1.98+9.24•OL)•10	$(1.16•OL)^{3.84}$	Leopold 2001
Vahl's eelpout	21.19+37.74•OL	$1.002•OL^{1.933}$	Härkönen 1986

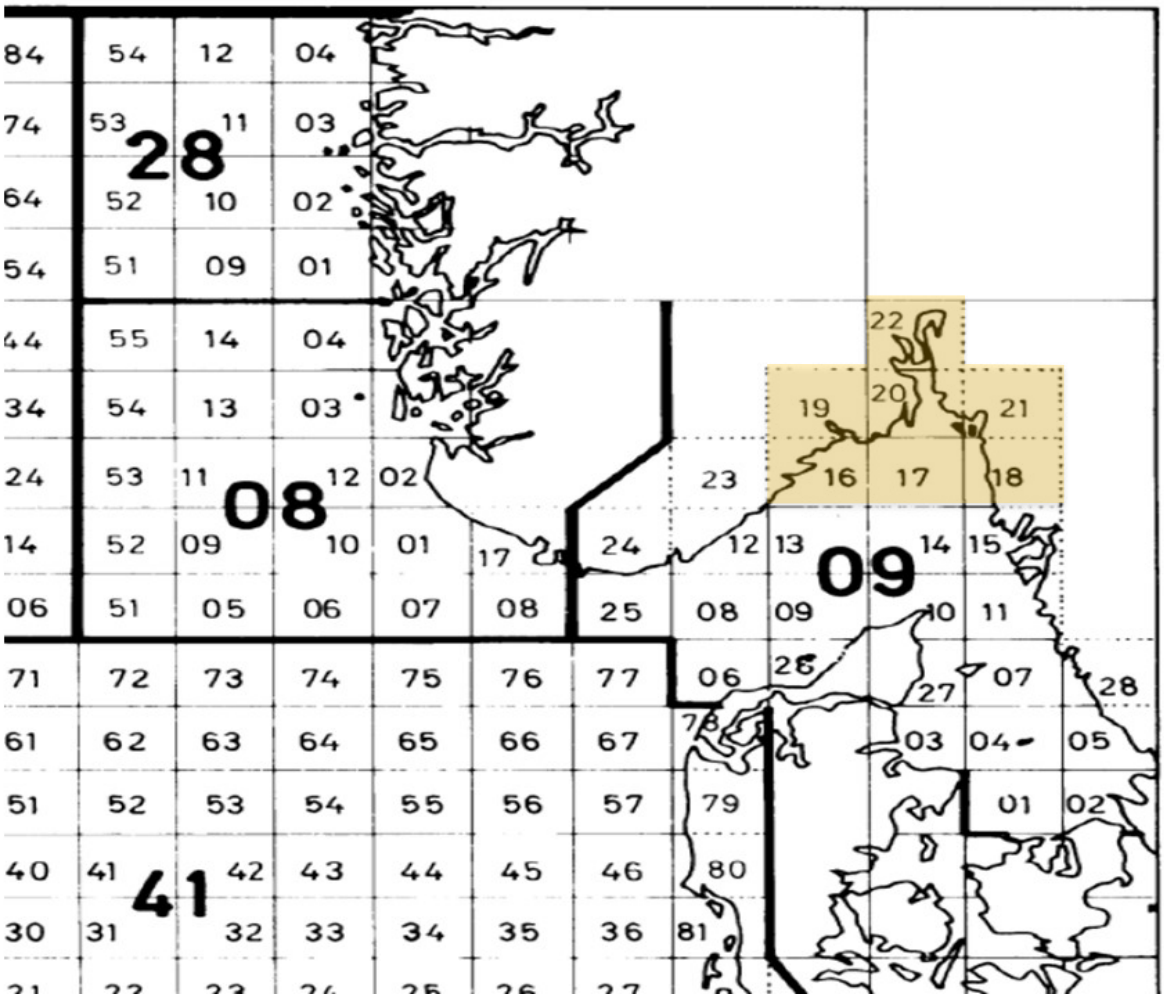
* Some otoliths of these species were outside the size range used to generate the regression equations.
** No regression equation appropriate for the size range of Atlantic halibut otoliths recovered.
*** Fish length in cm.

Appendix D

Pooled prey groups (in bold) for species that could not reliably be distinguished. Additionally, the groups 'Gobiidae', 'unidentified Gadidae', 'unidentified Gaidropsaridae', and 'unidentified Pleuronectidae' are respectively comprised of Gobiidae, Gadidae, Gaidropsaridae, and Pleuronectidae otoliths that were too degraded or otherwise not possible to identify further.

Pooled prey group composition
***Anarhichas* spp.**
Atlantic wolffish (*Anarhichas lupus*)
Spotted wolffish (*Anarhichas minor*)
Northern wolffish (*Anarhichas denticulatus*)
Blue whiting/whiting
Blue whiting *(Micromesistius poutassou)*
Whiting (*Merlangius merlangus*)
***Callionymus* spp.**
Dragonet (*Callionymus lyra*)
Spotted dragonet (*Callionymus maculatus*)
Cod/pollack/saithe
Atlantic cod (*Gadus morhu*a)
Pollack (*Pollachius pollachius*)
Saithe (*Pollachius virens*)
Flounder/plaice
European flounder (*Platichthys flesus)*
European plaice (*Pleuronectes platessa*)
Labridae (wrasses)
Ballan wrasse (*Labrus bergylta*)
Cuckoo wrasse (*Labrus mixtus)*
Goldsinny wrasse (*Ctenolabrus rupestris)*
Rock cook (*Centrolabrus exoletus*)
Long rough dab/witch flounder
Long rough dab (*Hippoglossoides platessoides*)
Witch flounder (*Glyptocephalus cynoglossus*)
***Myoxocephalus* spp.**
Fourhorn sculpin (*Myoxocephalus quadricornis*
Shorthorn sculpin (*Myoxocephalus scorpius*)
Pollack/saithe
Pollack (*Pollachius pollachius*)
Saithe (*Pollachius virens*)
Scopthalmidae
Brill (*Scophthalmus rhombus*)
Norwegian topknot (*Phrynorhombus norvegicus*)
Topknot (*Zeugopterus punctatus*)
***Trisopterus* spp.**
Norway pout (*Trisopterus esmarkii)*
Poor cod (*Trisopterus minutus*)
Bib (*Trisopterus luscus*)
Zoarcidae
Eelpout (*Zoarces viviparus*)
Vahl's eelpout (*Lycodes gracilis*)

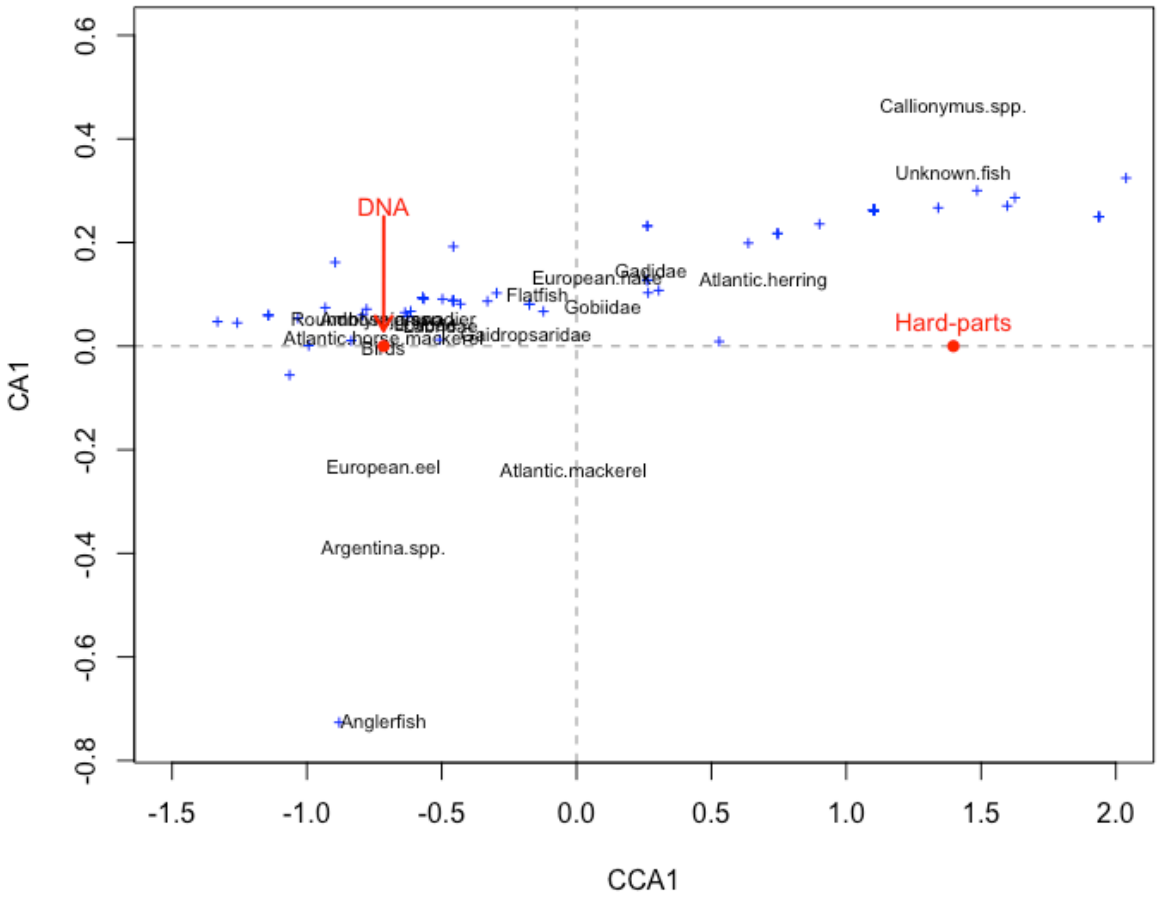
Appendix E



Map of the ICES statistical divisions of Skagerrak and the outer Oslofjord. Discussion of fisheries landings data concerns landings reported in the highlighted areas only: ICES division IIIa, subdivision 09, areas 16-22.

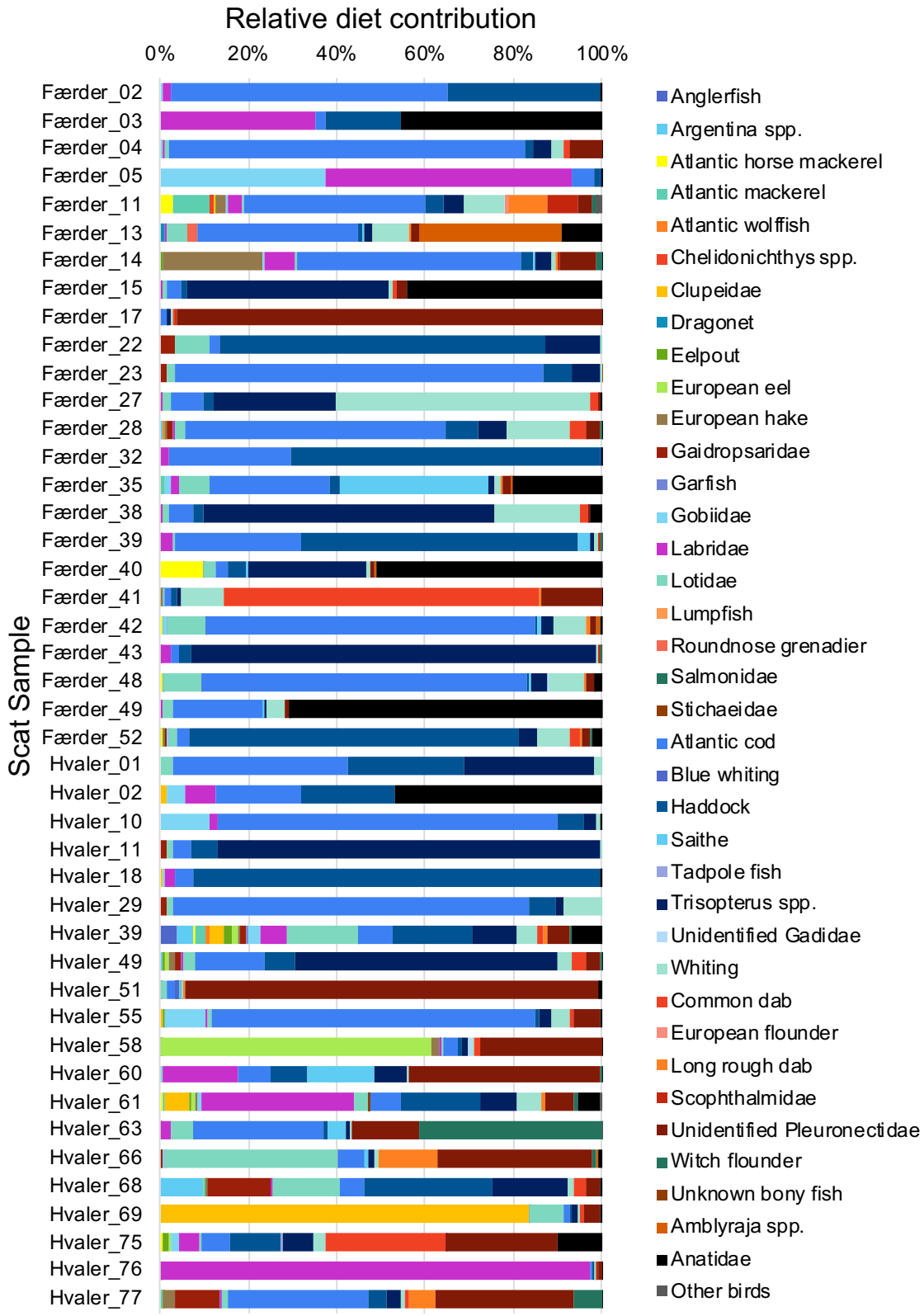
Appendix F

A constrained correspondence analysis (CCA) was run on the prey presence-absence data and, like the chi-square test on the same data, revealed that method of analysis was a significant predictor explaining variance in occurrence of prey (CCA, $F_1 = 6.5312$, $\chi^2 = 0.3278$, $P = 0.005$).



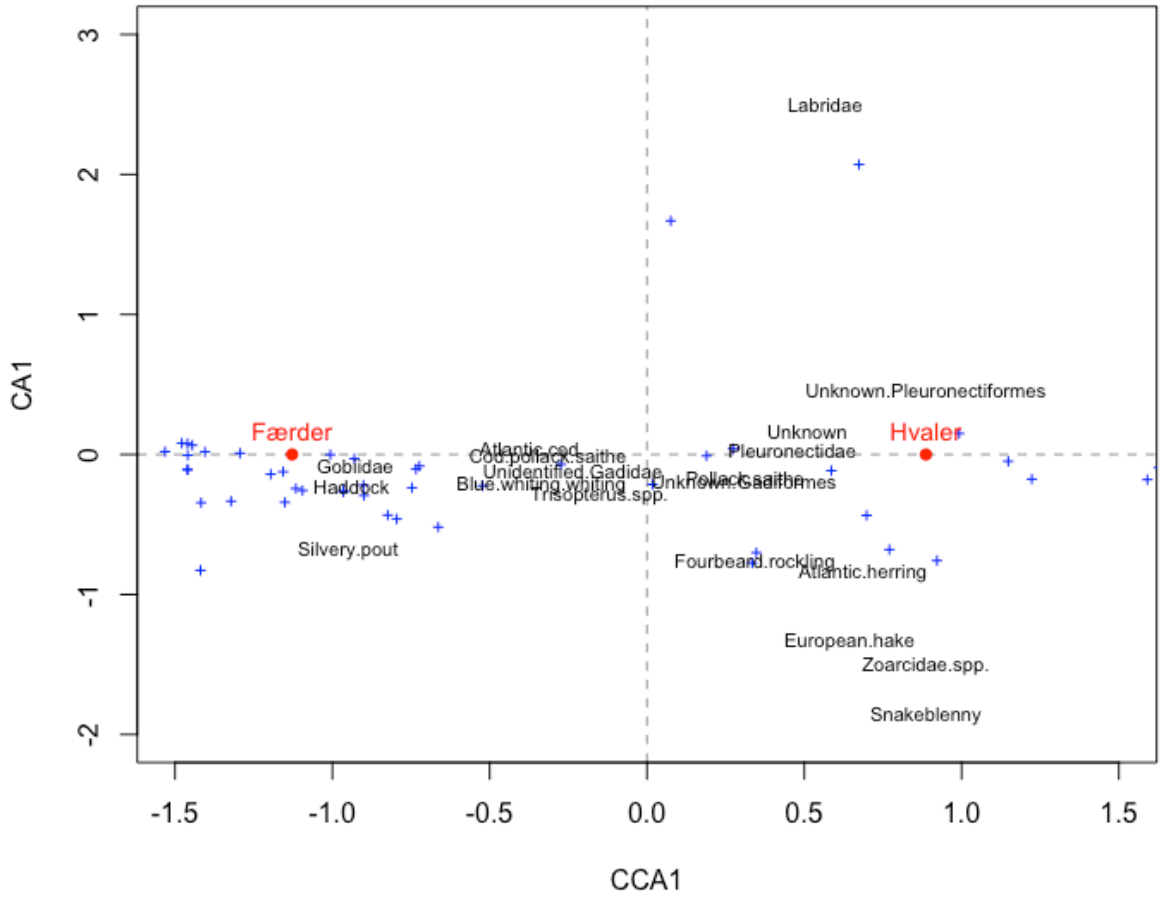
Constrained correspondence analysis (CCA) ordination biplot of presence of prey species and groups as response variables by method of analysis (red).

Appendix G



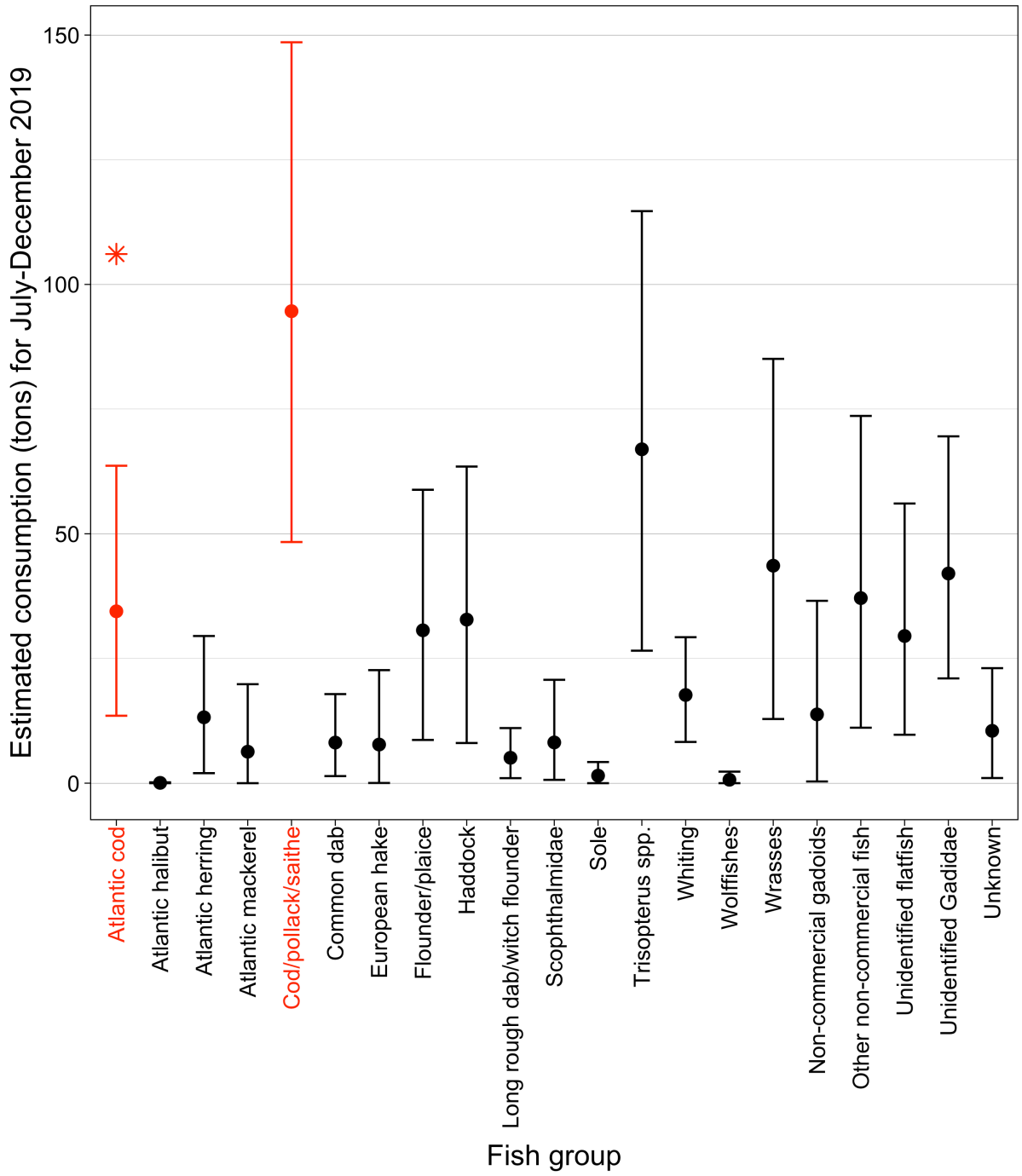
Relative diet contribution of prey groups (listed at right) for all scat samples individually; some species have been consolidated to genus or family for display purposes.

Appendix H



Constrained correspondence analysis (CCA) ordination biplot of reconstructed consumed biomass (Hellinger-transformed) of prey species and groups as response variables by location (red).

Appendix I



Estimated tons of prey consumed by harbor seals during the second half of 2019 (July to December). The red asterisk (*) shows the 2018 fishery landings for Atlantic cod, for comparison with seal cod consumption (see data highlighted in red for Atlantic cod and group cod/pollack/saithe).

Appendix J

Table of common and scientific species names accompanied by the name of the taxonomic authority followed and year of publication.

Family	Common name	Scientific name	Taxonomic authority
Anarhichadidae	Atlantic wolffish	*Anarhichas lupus*	Linnaeus, 1758
Anarhichadidae	Spotted wolffish	*Anarhichas minor*	Olafsen, 1772
Anguillidae	European eel	*Anguilla anguilla*	Linnaeus, 1758
Belonidae	Garfish	*Belone belone*	Linnaeus, 1760
Callionymidae	Dragonet	*Callionymus lyra*	Linnaeus, 1758
Carangidae	Atlantic horse mackerel	*Trachurus trachurus*	Linnaeus, 1758
Clupeidae	Atlantic herring	*Clupea harengus*	Linnaeus, 1758
Clupeidae	European spray	*Sprattus sprattus*	Linnaeus, 1758
Cottidae	Fourhorn sculpin	*Myoxocephalus quadricornis*	Linnaeus, 1758
Cottidae	Shorthorn sculpin	*Myoxocephalus scorpius*	Linnaeus, 1758
Cyclopteridae	Lumpfish	*Cyclopterus lumpus*	Linnaeus, 1758
Gadidae	Atlantic cod	*Gadus morhua*	Linnaeus, 1758
Gadidae	Bib	*Trisopterus luscus*	Linnaeus, 1758
Gadidae	Blue whiting	*Micromesistius poutassou*	Risso, 1827
Gadidae	Haddock	*Melanogrammus aeglefinus*	Linnaeus, 1758
Gadidae	Norway pout	*Trisopterus esmarkii*	Nilsson, 1855
Gadidae	Pollack	*Pollachius pollachius*	Linnaeus, 1758
Gadidae	Poor cod	*Trisopterus minutus*	Linnaeus, 1758
Gadidae	Saithe	*Pollachius virens*	Linnaeus, 1758
Gadidae	Silvery pout	*Gadiculus thori*	Schmidt, 1913
Gadidae	Tadpole fish	*Raniceps raninus*	Linnaeus, 1758
Gadidae	Whiting	*Merlangius merlangus*	Linnaeus ,1758
Gaidropsaridae	Fivebeard rockling	*Ciliata mustela*	Linnaeus, 1758
Gaidropsaridae	Fourbeard rockling	*Enchelyopus cimbrius*	Linnaeus ,1766
Gobiidae	Black goby	*Gobius niger*	Linnaeus, 1758
Gobiidae	Common goby	*Pomatoschistus microps*	Krøyer, 1838
Gobiidae	Crystal goby	*Crystallogobius linearis*	Düben, 1845
Gobiidae	Painted goby	*Pomatoschistus pictus*	Malm, 1865
Gobiidae	Sand goby	*Pomatoschistus minutus*	Pallas, 1770
Gobiidae	Transparent goby	*Aphia minuta*	Risso, 1810
Gobiidae	Two-spotted goby	*Pomatoschistus flavescens*	Fabricius, 1779
Labridae	Ballan wrasse	*Labrus bergylta*	Ascanius, 1767
Labridae	Corkwing wrasse	*Symphodus melops*	Linnaeus, 1758
Labridae	Cuckoo wrasse	*Labrus mixtus*	Linnaeus, 1758
Labridae	Goldsinny wrasse	*Ctenolabrus rupestris*	Linnaeus, 1758
Labridae	Rock cook	*Centrolabrus exoletus*	Linnaeus, 1758
Lophiidae	Anglerfish	*Lophius piscatorius*	Linnaeus, 1758

Appendix J, common and scientific species names *(concluded).*

Family	Common name	Scientific name	Taxonomic authority
Lotidae	Ling	*Molva molva*	Linnaeus, 1758
Macrouridae	Roundnose grenadier	*Coryphaenoides rupestris*	Gunnerus, 1765
Merlucciidae	European hake	*Merluccius merluccius*	Linnaeus, 1758
Pholidae	Rock gunnel	*Pholis gunnellus*	Linnaeus, 1758
Pleuronectidae	Atlantic halibut	*Hippoglossus hippoglossus*	Linnaeus, 1758
Pleuronectidae	Common dab	*Limanda limanda*	Linnaeus, 1758
Pleuronectidae	European flounder	*Platichthys flesus*	Linnaeus, 1758
Pleuronectidae	European plaice	*Pleuronectes platessa*	Linnaeus, 1758
Pleuronectidae	Long rough dab	*Hippoglossoides platessoides*	Fabricius, 1780
Pleuronectidae	Witch flounder	*Glyptocephalus cynoglossus*	Linnaeus, 1758
Scombridae	Atlantic mackerel	*Scomber scombrus*	Linnaeus, 1758
Scophthalmidae	Brill	*Scophthalmus rhombus*	Linnaeus, 1758
Scophthalmidae	Norwegian topknot	*Phrynorhombus norvegicus*	Günther, 1862
Scophthalmidae	Turbot	*Scophthalmus maximus*	Linnaeus, 1758
Soleidae	Common sole	*Solea solea*	Linnaeus, 1758
Stichaeidae	Snakeblenny	*Lumpenus lampretaeformis*	Walbaum, 1792
Zoarcidae	Eelpout	*Zoarces viviparus*	Linnaeus, 1758
Zoarcidae	Vahl's eelpout	*Lycodes vahlii*	Reinhardt, 1831