Canadian Journal of Zoology

Article

A comparative methodological approach to studying the diet of a recovering marine predator, the grey seal (*Halichoerus grypus*)

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Abstract

Anthropogenic influences caused depletion and subsequent recovery of marine predators, but ecological consequences of altered predator abundance are not well understood. Although many methods are used to study predator diets, methodological biases and logistical challenges preclude robust sampling schemes. We aimed to compare two non-invasive methods: metabarcoding scat-derived deoxyribonucleic acid and hard parts analysis of scat for the Northwest Atlantic grey seal (*Halichoerus grypus* (Fabricius, 1791)), a species that rebounded after near extirpation. We hypothesized that metabarcoding would detect a greater diversity and frequency of prey, and that notable differences in diet will be detected since prior studies. Grey seal scat samples ($N = 247$) were collected between 2018 and 2019 from Monomoy Island, Massachusetts, USA. Metabarcoding detected greater prey richness on average, with more frequent detections of clupeids (Clupeidae) and flatfish (Pleuronectiformes), whereas hard parts analysis more frequently detected phycid hakes (*Urophycis* spp. Gill, 1863). Combining methods increased detections of 13 prey taxa, with 32 prey taxa identified overall. Skates (Rajidae), flatfish, clupeids, and sand lance (*Ammodytes* spp. Linnaeus, 1758) were top-occurring prey. Our study highlights the importance of using multiple methods to characterize generalist predator diets using non-invasive techniques and suggests grey seal diet has changed since the early 2000s.

Key words: diet, grey seal, *Halichoerus grypus* (Fabricius, 1791), hard parts analysis, metabarcoding, next generation sequencing

1. Introduction

Predators play a vital role in shaping the structure and function of ecosystems as they influence prey abundance and induce prey behavioral changes (Creel and Christianson 2008). These predators can traverse long distances, distributing their effects on communities across broad spatial scales (McCauley et al. 2012). In addition, top predators often exploit similar resources to that of humans, increasing human–wildlife conflict and competition for resources (Boyd et al. 2006). In marine environments, human activities have led to a worldwide decline in top predators, but the ecological consequences of changes in predator abundance are not always well understood (Boyd et al. 2006; Heithaus et al. 2008).

Predicting the ecological impacts of changes in predator abundance requires intricate knowledge of ecosystem connectivity (e.g., predator–prey relationships; Heithaus et al. 2008). However, diet information for marine predators is rarely available via direct observation. Indirect or non-invasive methods have therefore been developed and adopted to understand the diets of marine predators. For example, analyses of hard prey remains deposited in scat have been used to provide dietary data on a large number of individuals without any handling, capturing, or interactions with the predator (Bowen 2000; Waits and Paetkau 2005). Additionally, hard parts analysis can provide quantitative information on diet, allowing for an estimation of the number and size of prey consumed (Bowen 2000). However, this method tends to be biased toward prey with robust remains that survive digestion, leaving prey with small or fragile remains underrepresented (Jobling and Breiby 1986).

Non-invasive genetic samples can be subjected to metabarcoding methods to obtain dietary information from marine predators. This method involves extracting deoxyribonucleic acid (DNA) from scat samples to concurrently identify DNA from multiple prey usually at the genus or species level of taxonomic resolution (Deagle et al. 2009). Benefits of metabarcoding DNA include an increased probability of detecting any prey, increased consistency of DNA signals, and the ability to detect rare or novel dietary items that other methods like hard parts analysis often miss (Casper et al. 2007*a*; Deagle et al. 2009; Tollit et al. 2009; Brassea-Pérez et al. 2019). However, genetic analyses introduce their own set of limitations, because results can depend on DNA quality, erroneous DNA sequences can arise via contamination, samples may be prone to amplification and sequencing errors, and DNA sequence quantity is an unreliable proxy for amount of prey

Table 1. Timeframe, geographic area, and methods used in examples of studies on Northwest Atlantic grey seal (*Halichoerus grypus*) diet.

Citation	Timeframe	Geographic area	Method
Bowen et al. (1993)	1988–1990	Scotian Shelf, Canada	Stomach contents analysis
Rough (1995)	1988, 1994	Muskeget Island, Massachusetts (MA), US	Hard parts analysis of scat
Bowen and Harrison (1994)	1991–1993	Sable Island, Nova Scotia, Canada	Hard parts analysis of scat
Bowen and Harrison (2006)	1991–1998	Sable Island, Nova Scotia, Canada	Hard parts analysis of scat
Beck et al. (2007)	1993–2000	Sable Island, Nova Scotia, Canada	Fatty acid analysis of blubber
Ampela (2009)	2004–2007	(1) Muskeget and Monomoy Islands, MA, US; (2–3) Gulf of Maine to mid-Atlantic Bight, US	(1) Hard parts analysis of scat; (2) Stomach content analysis; (3) Fatty acid analysis of blubber
Hernandez et al. (2019)	2013	Chatham Harbor, MA, US	Stable isotope analysis of skin, fur, blood
Hernandez et al. (2021)	2014–2017	Muskeget and Monomoy Islands, MA, US	Stable isotope analysis of pup lanugo
Lerner et al. (2018)	2016	Monomoy Island, MA, US	Stable isotope analysis of pup vibrissae and lanugo
Dufault et al. (2021)	2016–2018	Muskeget and Monomoy Islands, MA, US	Species-specific PCR assay
Flanders et al. (2020)	2017	Monomoy Island, MA, US	Metabarcoding prey DNA in scat

consumed (Waits and Paetkau 2005; Pompanon et al. 2012; Matejusová et al. 2013). Despite their limitations, metabarcoding prey DNA and hard parts analysis are more attractive for studying a federally protected marine mammal as other methods (i.e., stable isotopes or fatty acid analysis), require invasive biological samples (e.g., blubber and vibrissae) that can limit sample sizes, may only resolve dietary information at higher order taxonomies, or may be inadequate at estimating the amount of prey consumed (e.g., Beck et al. 2007; Lerner et al. 2018; Hernandez et al. 2019).

Given that diet information is affected by the biases of the method selected, numerous studies have suggested combining diet analyses to improve accuracy in marine predator diet descriptions (Casper et al. 2007*b*; Tollit et al. 2009; Pompanon et al. 2012). Those that have taken such an approach have reported an increase in the number of prey taxa detected and an increase in resulting niche breadth (Casper et al. 2007*a*, 2007*b*; Jeanniard-Du-Dot et al. 2017; Brassea-Pérez et al. 2019).

Grey seals (*Halichoerus grypus* (Fabricius, 1791)) in the Northwest Atlantic Ocean exemplify a unique case of altered marine predator abundance with unknown ecological consequences. Grey seals are generalist near-top predators in the marine environment that feed on a diverse range of prey (Ampela 2009). Their distribution ranges from New Jersey, USA to Labrador in Canada where they were historically abundant until severe hunting pressure in the 17th century led to local extirpation (Hayes et al. 2020; Wood et al. 2020). Grey seals began to recover in the mid-1900s following a series of local protections and implementation of the Marine Mammal Protection Act by the US federal government (Lelli et al. 2009; Wood et al. 2020). In addition to federal protection, immigration from recovering Canadian pupping sites resulted in an increased presence of grey seals along the northeastern US coast, which has led to perceived conflict with fishermen who have voiced concerns about seal predation on commercial and forage fish (Lavigne 2003; Gruber 2014; Wood et al. 2020), a perception that is common to pinnipeds across the globe (Butler et al. 2011; Pont et al. 2016; Johansson and Waldo 2021).

Although the literature on grey seal diet uses a variety of technologies (see Table 1 for examples of studies on Northwest Atlantic grey seals), gaps remain in our knowledge. First, studies tend to use only one method, with subsequent diet inferences subject to biases associated with each method. Second, studies that involve sampling within multiple seasons include samples from the late 1990s (Bowen and Harrison 2006) or early 2000s (Ampela 2009). The Gulf of Maine is warming at a rate 3× greater than that of the global ocean average (GMRI 2023), and there are documented shifts in species distributions as a result (see Pershing et al. 2021 for a review), suggesting there may be changes in the diet of a generalist predator such as the grey seal since prior studies have been conducted.

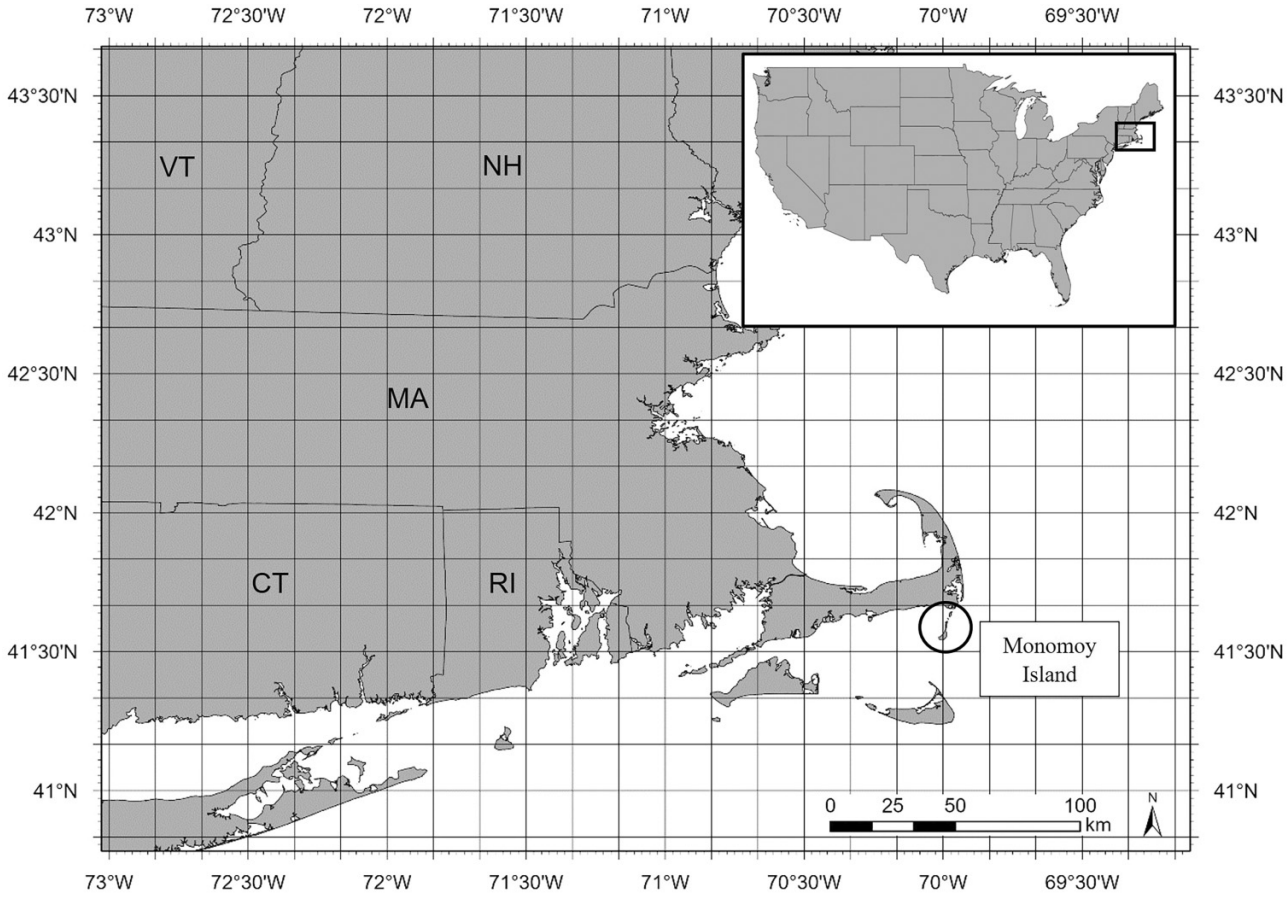
We aimed to characterize grey seal diet through the use of two non-invasive methods, metabarcoding scat-derived DNA and hard parts analysis of scat to determine whether combining methods yields a more complete description of diet for the Northwest Atlantic grey seal. We included samples from multiple seasons throughout the year to highlight differences across methods that arise with seasonal prey availability. We hypothesized that metabarcoding scat-derived DNA would detect a greater diversity and frequency of prey compared to hard parts analysis, and that differences in method specificity will vary by season. In addition, we hypothesize that grey seal diet composition in 2018–2019 will differ from prior comprehensive grey seal diet studies.

2. Materials and methods

2.1. Study site and sample collection

Wild grey seal scat samples were collected from the southeastern beach of Monomoy Island, a 13 km barrier island off the southwest tip of Cape Cod, MA, US (Fig. 1), on 29 October 2018 ($n = 64$), 5 May 2019 ($n = 140$), 2 August 2019 ($n = 35$), and 30 October 2019 ($n = 58$). Monomoy Island is one of the largest grey seal haul out sites and is estimated to be one of the fastest growing pupping sites in the US, with more than

Fig. 1. Location of the study site, Monomoy Island, Massachusetts, where wild grey seal (*Halichoerus grypus*) scat samples were collected. Inset map depicts Massachusetts and the Cape Cod region relative to the United States. Map created in ArcGIS Pro 2.7.0 using "United States State Boundaries 2018" layer data (source: Esri TomTom).



19 000 seals hauling out on the island (based on 2011 surveys) and a minimum of 1190 pups present in 2019 (Herland et al. 2016; Wood et al. 2020). The presence of grey seals year-round makes Monomoy Island an ideal location for repeated scat sampling over the course of a year. Collection of fresh scat samples, i.e., those with high moisture content and odor (Vynne et al. 2012), was prioritized over samples that were dried out. Scat samples were handled with separate disposable items and placed into separate Cryovac® or Boardwalk® re-sealable plastic bags. All scat samples were placed on ice and brought to the University of New England (Biddeford, ME, US) where samples were manually homogenized and subsampled upon arrival. Subsamples (approximately 5 mL) were collected from each scat sample using disposable items and stored in 70% ethanol, in a −20 °C freezer until DNA extraction. The remainder of each scat sample was stored in a −20 °C freezer until processed for hard prey remains.

2.2. DNA extraction

Scat subsamples were removed from ethanol and placed in disposable paper cups for evaporation at room temperature until visibly dry. Each sample was handled using separate disposable items, while reusable items, such as spatulas, were soaked in ethanol and flame sterilized between handling each scat sample. DNA was extracted using QIAamp Fast DNA Stool Mini Kit (Qiagen Inc., Germantown, MD, US) according to manufacturer protocols except for an initial incubation period of ~24 h at 60 °C to facilitate cell rehydration and lysis and the addition of 1 μL of carrier RNA after the second incubation period to improve DNA recovery. Final concentrated DNA was eluted to a volume of 100 μL and stored at −20 °C until further analysis. Extraction blanks consisted of all reagents and no scat to monitor for cross-contamination in each round of DNA extractions. Scat sample DNA extraction was performed using filtered pipette tips in a separate building within which no prey tissues were previously extracted or amplified to control for cross-contamination. All pre- and post-amplification procedures were carried out in separate labs and lab personnel did not re-enter the pre-amplification lab unless they showered and changed clothes (Waits and Paetkau 2005).

2.3. Determining seal sex

For full details of methods used for determining grey seal sex from scat samples, see Flanders et al. (2020). Briefly, that method involved a grey seal microsatellite primer that targets a 150–160 base pair (bp) fragment of nuclear DNA to ensure that scat samples were of grey seal origin and to control

for DNA quality (i.e., all grey seal samples should amplify this fragment; Allen et al. 1995; Reed et al. 1997). The microsatellite primers were multiplexed into a single reaction with primers that amplified a ~69 bp fragment of the Y homolog of the zinc finger protein, which, if detected, indicated the sample originated from a male (Matejusová et al. 2013). All grey seal scat samples collected in October 2018 and August and October 2019 were assayed for sex. DNA was extracted and tested for grey seal sex from the May sampling occasion until ~100 samples (out of 140 samples) with DNA of sufficient quality were obtained, as determined by microsatellite amplification.

2.4. Prey DNA amplification and sequencing

A universal chordate primer pair that targeted ~100 bp mitochondrial (mtDNA) 16S fragment, primer set B from Deagle et al. (2009) was multiplexed into a single reaction with a grey seal blocking primer to amplify prey DNA from the phylum chordata following methods of Flanders et al. (2020). PCR reactions (25 μL) consisted of 12.8 μL distilled H_2O, 2.0 μL of 10 μmol/L forward primer, 2.0 μL of 10 μmol/L reverse primer, 2.0 μL of 100 μmol/L blocking primer, 5.0 μL MyTaq™ reaction buffer, 0.2 μL MyTaq™ DNA polymerase (Meridian Bioscience, Cincinnati, OH, US), and 1 μL of scat-derived DNA extract. Thermocycler conditions were: 95 °C for 3 min, 35 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s, along with a final extension at 72 °C for 10 min and a hold at 4 °C. Negative controls consisted of all reagents and nuclease-free water, while tissue-extracted fish DNA at a concentration of 1 ng/μL (as assessed using NanoDrop 2000c Spectrophotometer, ThermoFisher Scientific, Waltham, MA, US) served as positive controls. PCR reactions were visualized on an ethidium stained 2% agarose gel. Samples were tested for prey DNA up to three times, with dilutions and (or) re-extractions as necessary following protocols used for determining grey seal sex (Flanders et al. 2020).

PCR products were cleaned using a GeneJET™ PCR purification kit (ThermoFisher Scientific) following manufacturer protocols, unless the final PCR product contained DNA fragments larger than the target fragment. In such cases, the target fragment was extracted and cleaned using a GeneJET™ gel extraction kit (ThermoFisher Scientific) following manufacturer protocols. Cleaned PCR products were eluted to a final volume of 30 μL, of which 15 μL were sent to the University of New Hampshire Hubbard Center for Genome Studies (Durham, NH, US) for library preparation and sequencing using the Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA, US) with 2 × 250 bp chemistry.

DNA from a "fish mix" that consisted of equal amounts (10 μL each) of tissue-extracted DNA from three fish species known to be grey seal prey items (sand lance (*Ammodytes* spp. Linnaeus, 1758), red hake (*Urophycis chuss* (Walbaum, 1792)), and witch flounder (*Glyptocephalus cynoglossus* (Linnaeus, 1758))) was used as template DNA for positive controls during prey DNA amplification. A total of 10 positive control samples using the "fish mix" were sent for sequencing alongside our wild grey seal scat samples. Prey DNA from 13 wild grey seal scat samples were randomly chosen from the October 2018 sampling occasion to be amplified and sequenced in duplicate to serve as technical replicates to monitor consistency.

2.5. Bioinformatics

Following sequencing, the facility provided two FASTQ files for each sample containing demultiplexed forward and reverse reads. Initial sequence processing was carried out through QIIME 2™ where sequence reads were imported in the Casava 1.8 paired-end demultiplexed fastq.gz format (Bolyen et al. 2019). Primer and adapter sequences were trimmed using Cutadapt (Martin 2011) with default parameters. DADA2 (Callahan et al. 2016) was used to trim forward and reverse reads, denoise reads, filter chimeric sequences, and join reads together. The beginning (5' end) of reads were not trimmed as initial base pairs exhibited high quality scores, but the 3' ends of both forward and reverse reads were truncated where reads displayed a general decrease in quality score.

Subsequent sequence analyses were performed in R Studio v. 3.5.1 (R Core Team 2018) using Bioconductor packages phyloseq (version 1.30.0; McMurdie and Holmes 2013) and Biostrings (v. 2.54.0; Pagès et al. 2019). Reads with one occurrence were removed from the data set under the assumption that any read that occurred once is more likely to be a sequencing error rather than a unique grey seal prey item. Remaining sequences were extracted into a FASTQ file.

A BLAST search (Altschul et al. 1990) against GenBank's nucleotide database (of the National Center for Biotechnology Information; Benson et al. 2008) was conducted to taxonomically identify each sequence based on the expect value (e-value), with a maximum threshold of $1e^{-20}$ following Thomas et al. (2014). If multiple species shared the same lowest e-value, any species whose geographic distribution was outside of the study area (i.e., northwest Atlantic Ocean) was discarded. If two geographically plausible species shared the same lowest e-value, the sequence was identified to the lowest shared taxonomic level, such as to genus or family. If all species with the same lowest e-value had a geographic distribution outside of the study area, the lowest shared taxonomic level that contained a species in the study region was selected. For example, one sequence had leopard searobin (*Prionotus scitulus* Jordan & Gilbert, 1882) as the top hit, but this species is typically found south of Virginia, US. That sequence was identified as "sea robin *Prionotus* spp. Lacepède, 1801" as the genus "*Prionotus*" contains multiple species that are present near Cape Cod. A little skate (*Leucoraja erinaceus* (Mitchill, 1825)) sequence returned the lowest e-value for skate DNA, but other rough skate species within the genus *Leucoraja* Malm, 1877 that are found in the Cape Cod region (winter skate (*Leucoraja ocellata* (Mitchill, 1815)) and rosette skate (*Leucoraja garmani* (Whitley, 1939)) are underrepresented in the database. Therefore, these sequences were classified to as rough skate *Leucoraja* spp. In one instance, the top hit for a sequence was identified to "*Dipturus* spp. Rafinesque, 1810", a genus of skates. However, only one species of skate within this genus is present in the northwest Atlantic Ocean, barndoor skate (*Dipturus laevis* (Mitchill,

1818)), so this was presumed to be the identity of the prey DNA. Similarly, a genus of smoothhound sharks "*Mustelus* spp. Linck, 1790" was detected, but there is only a single species within this genus whose geographic range encompasses the Cape Cod region, smooth dogfish (*Mustelus canis* (Mitchill, 1815)), and that was assumed to be the identity of the prey DNA.

Flatfish (Pleuronectiformes) were approached conservatively due to general underrepresentation in GenBank and classified to family as either a large-tooth flounder (Paralichthyidae) or a righteye flounder (Pleuronectidae). Windowpane flounder (*Scophthalmus aquosus* (Mitchill, 1815)), however, is the only species in the family Scophthalmidae found in the Cape Cod region, so it remained classified to species.

2.6. Hard parts analysis

The remainder of each scat sample was thawed in lukewarm water and washed through a series of graduated sieves with mesh sizes 4, 2, 1, and 0.5 mm to remove all fecal material and reveal hard prey remains. Prey remains were categorized, enumerated, and stored in separate vials for each remain type in each sample. Soft prey remains that were used for prey identification, such as echinoderm (Echinodermata) tube feet and crustacean (Decapoda) exoskeleton remains, were preserved in 70% ethanol. Sagittal otoliths were identified to the lowest possible taxonomic level based on otolith morphology using standard references (Campana 2004; McBride et al. 2010). For otoliths not identifiable by our lab, we consulted an expert in the field.

2.7. Grey seal diet composition: metabarcoding and hard parts analysis

While metabarcoding and hard parts analysis can yield information about prey abundance, the process to estimate abundance varies and is often subject to biases associated with each method (Deagle et al. 2019). Therefore, we used presence/absence of prey in each sample in each method for our comparative analysis on grey seal diet. To do so, we calculated the frequency of occurrence (FO; Ahlbeck et al. 2012) for each prey as $\%FO_i = (n_i/n)*100$, where n_i = number of grey seal scat samples containing taxon i and n = number of grey seal scat samples analyzed. Methods to obtain read abundance and prey abundance through either method can be found in Supplementary file 1. Additionally, for details on prey species FO and abundance data obtained through each method within each sampling occasion, see Supplementary Tables S3–S6.

2.8. Comparing diet analysis methods: metabarcoding versus hard parts analysis

Due to differing taxonomic resolutions provided by each method, three groups of prey were combined to avoid overestimating prey richness in scat samples. Some otoliths could only be identified to the family Clupeidae, so all clupeid detections were combined and classified as Clupeidae, including American shad (*Alosa sapidissima* (Wilson, 1811)), Atlantic herring (*Clupea harengus* Linnaeus, 1758), Atlantic menhaden (*Brevoortia tyrannus* (Latrobe, 1802)), blueback herring (*Alosa aestivalis* (Mitchill, 1814)), and river herring (*Alosa* spp. Linck, 1790). Some flatfish otoliths could not be identified past order, so all flatfish were combined and classified as Pleuronectiformes: American plaice (*Hippoglossoides platessoides* (Fabricius, 1780)), fourspot flounder (*Hippoglossina oblonga* (Mitchill, 1815)), large-tooth flounder, righteye flounder, smallmouth flounder (*Etropus microstomus* (Gill, 1864)), windowpane flounder, winter flounder (*Pseudopleuronectes americanus* (Walbaum, 1792)), witch flounder, and yellowtail flounder (*Myzopsetta ferrugineus* (Storer, 1839)).

Skate denticles could not be identified to species, so metabarcoding detections of rough skate and barndoor skate were combined and classified as skate (Rajidae) to match the resolution for these taxa from hard parts analysis. The metabarcoding primer implemented in our study did not amplify invertebrate DNA, so invertebrate prey was recovered solely from hard parts analysis.

Comparison of grey seal diet across methods occurred by identifying prey that was detected in each sample through both data sets (i.e., a joint presence), and noting instances where prey were identified in one or the other data set, but not in both (i.e., mismatches). Mismatches took two forms: (1) an identifiable prey hard part was detected via hard parts analysis without evidence of matching DNA and (2) prey DNA was present without evidence of matching prey hard parts.

Our data did not violate the assumption of homogeneity of variances (Fligner–Killeen test, $p = 0.05$), but did violate the assumption of normality (Shapiro–Wilk test, $p < 0.01$); therefore, we used a Wilcoxon–Mann–Whitney test to determine whether the method—metabarcoding or hard parts analysis—affected average prey richness (α-diversity) per sample, where prey richness was the total number of prey taxa present within each sample. To determine whether method affected β-diversity, defined as variation in prey composition among scat samples (Anderson et al. 2011), in grey seal scat samples, a site $\times$ species matrix was constructed using FO data where the presence/absence of prey obtained from metabarcoding was considered a separate set of samples from presence/absence of prey obtained through hard parts analysis. A dissimilarity matrix was then created using *betadiver* from package vegan (v. 2.5-4; Oksanen et al. 2018) with dissimilarity defined as 1 − Sørensen's index (Sørensen 1948).

The *adonis* function was used to conduct a permutational multivariate analysis of variance (PERMANOVA) on group (method) dissimilarity. Group dispersions were modeled with *betadisper* to determine whether the assumption of equal dispersions had been met under *adonis* and principal coordinate analysis was used relative to PERMANOVA results to visualize group dispersions around the centroid. Significant dissimilarity was further investigated using similarity percentage (SIMPER) analysis (Clarke 1993). Species identified by SIMPER to be influential in group dissimilarity were further investigated using log-linear models to determine whether the presence of each prey was dependent on the method used. Post-hoc analyses were performed using Fisher's exact tests with p values corrected using the sequential Bonferroni method (Holm

Table 2. Number of wild grey seal scat (*Halichoerus grypus*) samples tested for seal sex and classified as male, female, or inconclusive (failed microsatellite amplification) in each sampling period and overall.

Sampling period	Male	Female	Inconclusive	Total
October 2018*	54 (*54*)	9 (*9*)	1 (*1*)	64 (*64*)
May 2019*	72 (*72*)	26 (*26*)	23 (*1*)	121(*99*)
August 2019	20 (*20*)	10 (*10*)	5 (*0*)	35 (*30*)
October 2019*	45 (*45*)	9 (*9*)	4 (*0*)	58 (*54*)
Overall*	191 (*191*)	54 (*54*)	33 (*2*)	278 (*247*)

Note: Numbers in parentheses indicate samples included from each category in our study and asterisks (*) indicate sampling groups that exhibited significant differences in the number of male and female scat samples.

1979). All statistical analyses were performed in R Studio v. 3.5.1 (R Core Team 2018) with $\alpha = 0.05$ for all statistical tests.

2.9. Combining diet analysis methods: metabarcoding + hard parts analysis

To obtain an overall description of grey seal diet across methods, presence/absence data from hard parts analysis were combined with metabarcoding DNA detections (hereafter termed "combined data set"). Any prey taxa that were detected by at least one method in each sample were considered "present" in that sample in combined data set. Dietary information was summarized using FO for each prey as previously described and qualitatively compared to prior studies on grey seal diet.

3. Results

3.1. DNA extraction and seal sex determination

All extraction blanks yielded no evidence of DNA through DNA extract quantification by the NanoDrop and visualization of PCR products following prey DNA amplification. From the 297 wild grey seal samples collected, 278 were tested for sex (Table 2). 88.1% of samples tested ($n = 245$) exhibited grey seal microsatellite amplification and were classified as male or female. Two inconclusive samples were used for prey amplification and sequencing to determine whether they originated from harbor seals (*Phoca vitulina* Linnaeus, 1758). Both inconclusive samples produced prey reads without evidence of harbor seal DNA, and one sample contained grey seal DNA. Therefore, both samples were assumed to be of grey seal origin and included in prey analysis, while remaining sex-inconclusive samples were excluded from prey amplification and further analysis.

3.2. Bioinformatics

A total of 247 wild grey seal scat samples and 10 positive "fish mix" controls were sequenced and produced 35 337 723 reads, of which 91.54% passed initial filtering steps, 91.2% were denoised, 88.7% were joined, and 41.7% were non-chimeric. In total, 14 724 871 reads remained after DADA2 processing. These reads were grouped into 6904 operational taxonomic units (OTUs), which was defined as clusters of identical sequences in our study. All OTUs contained >2 reads, so no OTUs were discarded at this step. After a BLAST search, the OTUs were taxonomically identified to 87 taxa, which were categorized as potential prey DNA ($n = 54$ taxa), predator DNA (grey seal), mammalian DNA ($n = 17$ taxa), likely lab or environmental contaminants ($n = 11$ taxa), and bacteria. Remaining taxa were considered to be outside the scope of the primer used, did not reach the e-value threshold ($1e^{-20}$) for taxonomic assignment, or had no significant similarity found to any representative DNA sequences in GenBank's database, and were excluded from the study.

3.3. Positive controls

Metabarcoding successfully retrieved sand lance, red hake, and flatfish DNA in each of our 10 positive "fish mix" controls as expected. Positive controls also contained relatively small amounts (<0.03% of reads per taxa per positive control) of DNA from non-target taxa (e.g., river herring, grey seal, Atlantic cod (*Gadus morhua* Linneaeus, 1758), and skate) that were considered to be background noise and used to create a threshold for discarding presumed background noise from wild grey seal scat samples. For each positive control, the highest non-target relative read abundance (RRA; calculated as $\%RRA_i = (n_{ik}/n_k)*100$, where n_{ik} = number of reads produced for prey item i in sample k and n_k = total number of reads produced in sample k (Deagle et al. 2019) and lowest target RRA was identified and averaged. The average of those values across positive control samples was used as an RRA threshold for discarding sequences from samples in the main data set. This threshold was 0.9% of sample reads, similar to the 1% threshold suggested by Deagle et al. (2019). Analysis of our technical replicates revealed that our metabarcoding efforts successfully detected the same species in duplicate samples, along with similar RRAs for species that were detected.

3.4. Grey seal diet composition: metabarcoding

There were a total of 10 431 844 prey reads classified to 32 taxa across all sampling periods after discarding prey reads below the 0.9% threshold. (Table 3; Tables S3 and S4). Out of all 247 samples used for the study, 3.6% ($n = 9$; all of which originated from the same sampling period, August 2019) did not contain any prey reads. Through metabarcoding, rough skate was the most frequently consumed prey (FO = 47.8%), followed by sand lance (FO = 28.7%), Atlantic menhaden (FO = 26.3%), and windowpane flounder (FO = 25.1%).

3.5. Grey seal diet composition: hard parts analysis

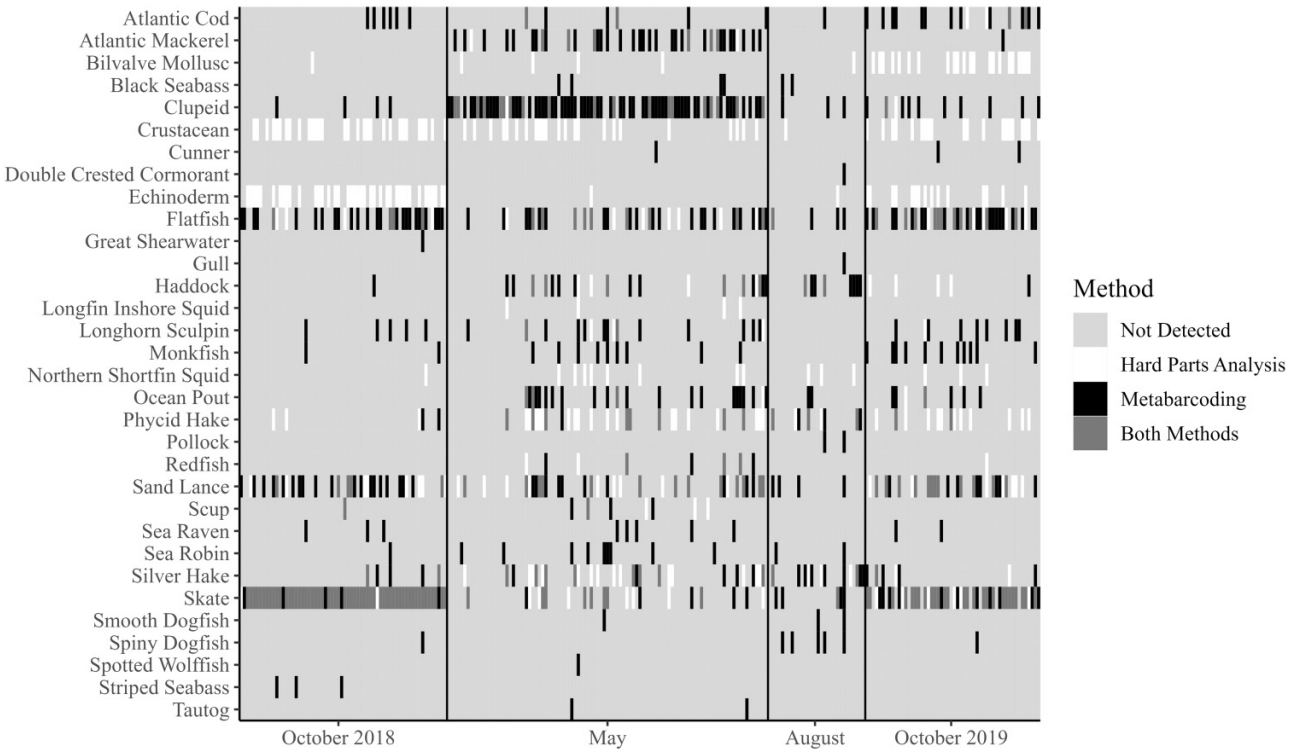
A total of 190 (76.9%) samples contained prey remains that could be taxonomically identified to 31 taxa (Table 3; Tables S4 and S5). Of the samples with identifiable remains, 63.2% ($n = 120$) contained a total of 3368 otoliths and 35 squid beaks that could be used to characterize diet in terms of minimum number of individuals and biomass consumed and 36.8% ($n = 70$; Table S5) contained only remains for which no biomass could be estimated (i.e., skate denticles, crustacean remains, echinoderm remains, and bivalve (Bivalvia) shells). Additionally, 15% ($n = 37$) of all samples contained only unidentifiable

Table 3. Frequency ofoccurrence (FO), expressed as a percentage, of prey detected in grey seal (*Halichoerus grypus*) scat samples across the sudy ($N = 247$) for metabarcoding (M), hard parts analysis (HP), and the combined data (CD) set.

Taxonomy				Common name (scientific name)	M	HP	CD
Chordata	Actinopterygii	Pleuronectiformes	Pleuronectidae	Unidentified righteye flounder (Pleuronectidae)	21.9	–	–
				Witch flounder (*Glyptocephalus cynoglossus*)	–	0.8	–
				American plaice (*Hippoglossoides platessoides*)	–	4.5	–
				Winter flounder (*Pseudopleuronectes americanus*)	–	1.2	–
				Yellowtail flounder (*Myzopsetta ferrugineus*)	–	6.1	–
			Paralichthyidae	Unidentified large-tooth flounder (Paralichthyidae)	2	0.8	–
				Fourspot flounder (*Hippoglossina oblonga*)	–	0.4	–
				Smallmouth flounder (*Etropus microstomus*)	–	0.4	–
				Windowpane flounder (*Scophthalmus aquosus*)	25.1	2	–
				Unknown flatfish (Pleuronectiformes)	–	10.9	–
				All flatfish (Pleuronectiformes)	38.1	16.6	44.5
		Clupeiformes	Clupeidae	Atlantic menhaden (*Brevoortia tyrannus*)	26.3	0.4	–
				Blueback herring (*Alosa aestivalis*)	–	0.81	–
				American shad (*Alosa sapidissima*)	–	1.2	–
				Unidentified river herring (*Alosa* spp.)	20.7	3.2	–
				Atlantic herring (*Clupea harengus*)	14.2	2.4	–
				Unknown clupeid (Clupeidae)	–	4.1	–
				All clupeid (Clupeidae)	38.5	8.9	39.3
		Perciformes		Sea robin (*Prionotus* spp.)	4.9	–	4.9
				Redfish (*Sebastes* spp.)	2.4	2.4	3.6
				Black seabass (*Centropristis striata* (Linnaeus, 1758))	2.4	–	2.4
				Longhorn sculpin (*Myoxocephalus octodecemspinosus*)	10.5	2.4	12.2
				Spotted wolffish (*Anarhichas minor* Olafsen, 1772)	0.4	–	0.4
				Ocean pout (*Zoarces americanus*)	11.7	2.4	12.6
				Sea raven (*Hemitripterus americanus* (Gmelin, 1789))	4.1	–	4.1
				Striped bass (*Morone saxatilis* (Walbaum, 1792))	1.2	–	1.2
				Scup (*Stenotomus chrysops*)	2	2	3.2
		Gadiformes	Gadidae	Pollock (*Pollachius virens* (Linnaeus, 1758))	0.8	–	0.8
				Haddock (*Melanogrammus aeglefinus*)	10.5	6.5	13.8
				Atlantic cod (*Gadus morhua*)ee	10.9	2.4	11.7
				Phycid hake (*Urophycis* spp.)	7.7	19	21.5
				Silver hake (*Merluccius bilinearis*)	13	13	20.7
		Labriformes	Labridae	Tautog (*Tautoga onitis* (Linnaeus, 1758))	0.8	–	0.8
				Cunner (*Tautogolabrus adspersus* (Walbaum, 1792))	1.2	–	1.2
				Atlantic mackerel (*Scomber scombrus*)	14.6	3.2	15.4
				Monkfish (*Lophius americanus*)	8.9	–	8.9
				Sand lance (*Ammodytes* spp.)	28.7	21.9	38.5
				Unidentified Otolith	–	9.3	9.3
	Chondrichthyes	Rajiformes	Rajidae	Rough skate (*Leucoraja* spp.)	47.8	–	–
				Barndoor skate (*Dipturus laevis*)	0.8	–	–
				Unknown skate (Rajidae)	–	44.1	–
				All skate (Rajidae)	47.8	44.1	53.9
				Smooth dogfish (*Mustelus canis*)	1.2	–	1.2
				Spiny dogfish (*Squalus acanthias* Linnaeus, 1758)	2.8	–	2.8
	Aves			Double-crested cormorant (*Phalacrocorax auritus*)	0.4	–	0.4
				Great shearwater (*Ardenna gravis*)	0.4	–	0.4
				Gull (*Larus* spp.)	0.4	–	0.4
Mollusca	Cephalopoda			Longfin inshore squid (*Doryteuthis pealeii* (Lesueur, 1821))	–	1.6	1.6
				Northern shortfin squid (*Illex illecebrosus* (Lesueur, 1821))	–	6.1	6.1
				Bivalve mollusc (Bivalvia)	–	11.3	11.3
				Crustacean (Decapoda)	–	30	30
				Echinoderm (Echinodermata)	–	23.1	23.1

Note: Clupeids (Clupeidae), flatfish (Pleuronectiformes), and skates (Rajidae) are presented overall and by taxa, when appropriate. Shared taxonomy (phylum, class, order, family) is presented on the left.

Fig. 2. Presence/absence of prey items detected in grey seal (*Halichoerus grypus*) diet through metabarcoding, hard parts analysis, or both methods in all ($N = 247$) grey seal scat samples. Prey taxa on *y*-axis are grouped as in the combined data set (see Methods: Comparing diet analysis methods).



prey remains (i.e., vertebrae, spines, unidentifiable otoliths, and other structures that cannot be assigned to a species) and 8.1% ($n = 20$) did not contain any prey remains. The "empty" samples (i.e., $n = 20$) occurred in all four sampling periods, but a majority ($n = 15$) occurred in August. Through hard parts analysis, skates were again the most frequently consumed prey item (FO = 44.1%), followed by crustaceans (FO = 30%), echinoderms (FO = 23.1%), and sand lance (FO = 21.9%).

3.6. Comparing diet analysis methods: metabarcoding versus hard parts analysis

Across both methods and all 247 samples, there were 792 vertebrate prey detections, with detection defined as each time a prey taxon was detected in a sample through prey remains or prey reads. These prey detections included 223 joint presences across 149 samples (Fig. 2). Skates were detected jointly in 93 samples, followed by sand lance ($n = 30$), flatfish ($n = 25$), and 10 other prey taxa.

There were 435 metabarcoding detections across 199 samples that did not correspond with hard parts in those samples (Fig. 2). Clupeid DNA was detected in 75 samples without matching hard parts, followed by flatfish ($n = 69$), sand lance ($n = 41$), and 24 other prey taxa. On the other hand, an identifiable hard part was recovered 134 times across 82 samples without matching DNA. Phycid hake (*Urophycis* spp. Gill, 1863) remains were detected in 34 samples without matching DNA, followed by sand lance ($n = 24$), silver hake (*Merluccius bilinearis* (Mitchill, 1814)) ($n = 19$), and 10 other prey taxa.

Separately, we also considered a third category of mismatch in which identifiable prey hard parts were present, but corresponding DNA was discarded after implementation of our 0.9% threshold. From the 134 times identifiable hard parts were recovered without DNA, 53.7% ($n = 72$) of detections, across 56 samples, were cases in which DNA was originally present for a prey taxa but discarded using the threshold. Sand lance DNA that corresponded with hard prey remains was discarded in 16 samples, followed by phycid hake ($n = 13$), skates ($n = 12$), and 9 other prey taxa.

Excluding invertebrate prey, metabarcoding increased prey richness in 66% ($n = 163$) of samples, while hard parts analysis increased prey richness in 12.6% ($n = 31$) of samples. Metabarcoding detected prey in 28.3% ($n = 70$) of samples that did not contain identifiable otoliths or squid beaks, while hard parts analysis detected prey in 0.8% ($n = 2$) of samples that did not contain any vertebrate prey DNA.

The Wilcoxon–Mann–Whitney test revealed that mean prey richness determined by metabarcoding (2.66 ± 0.12) was 30% greater than mean prey richness determined by hard parts analysis (2.05 ± 0.12; $V = 13232$, $p < 0.01$). Mean sample dissimilarity between metabarcoding and hard parts analysis was 0.77 (range 0–1). The PERMANOVA revealed that metabarcoding species diverged from hard parts analysis in multivariate space ($F_{[1,426]} = 32.07$, $p < 0.01$), without a significant difference in group dispersions ($F_{[1,426]} = 3.85$, $p = 0.05$; Supplementary Fig. S1).

SIMPER identified skate as the largest contributor toward sample dissimilarity between metabarcoding and hard parts

data sets (13.6% of variation explained), followed by clupeids (11.8%). Six additional species, along with skates and clupeids, collectively contributed 71% of differences in sample dissimilarity: flatfish, sand lance, crustaceans, echinoderms, phycid hake, and silver hake.

Log-linear models indicated that method significantly affected the presence of clupeids (deviance explained = 44.53, df = 1, $p < 0.01$), flatfish (deviance explained = 15.26, df = 1, $p < 0.01$), crustaceans (deviance explained = 141.96, df = 1, $p < 0.01$), echinoderms (deviance explained = 105.05, df = 1, $p < 0.01$), and phycid hake (deviance explained = 23.66, df = 1, $p < 0.01$) in the diet. Clupeids and flatfish were detected at a significantly greater frequency in the diet through metabarcoding ($n = 95/238$ and $n = 94/238$, respectively) compared to hard parts analysis ($n = 22/187$ and $n = 41/187$, respectively; all $p < 0.01$). On the other hand, crustaceans, echinoderms, and phycid hake were detected significantly more often by hard parts analysis ($n = 74/187$, $n = 57/187$, and $n = 47/187$, respectively) compared to metabarcoding ($n = 0/238$, $n = 0/238$, and $n = 19/238$, respectively; all $p < 0.01$). Although skates and silver hake were identified by SIMPER to affect sample dissimilarity, log-linear models suggested that the presence of skates (deviance explained = 3.20, df = 1, $p = 0.07$) and silver hake (deviance explained = 1.09, df = 1, $p = 0.30$) was not significantly affected by method.

3.7. Combining diet analysis methods: metabarcoding + hard parts analysis

A total of 32 prey taxa were detected across 247 wild grey seal scat samples in the combined data set, with qualitative intra-annual trends observed in prey consumption (Table S6). In October 2018, skates were detected in all samples collected, while echinoderms were detected in 62.5% of samples. Additionally, sand lance and flatfish were each detected in half of the samples collected during October 2018. Clupeids were the dominant prey in samples collected during May (FO = 79.8%), followed by Atlantic mackerel (*Scomber scombrus* Linnaeus, 1758), flatfish, and sand lance. Silver hake was the most frequently detected prey within the August sample collection (FO = 40%), followed by haddock (*Melanogrammus aeglefinus* (Linnaeus, 1758)) and phycid hake. Lastly, in October 2019, skate was again the most frequently consumed prey taxa, although not to the same extent as it was during the October 2018 collection. Flatfish were detected in 68.5% of samples in October 2019, followed by sand lance. October 2019 prey detections were similar to October 2018, as 8 of the top 10 prey in October 2019 overlapped with the top 10 in October 2018 (i.e., 80% overlap), compared to 60% and 50% overlap with May and August, respectively. Overall, skates occurred in more than half of 247 samples, followed by flatfish (44.5%) and clupeids (39.3%; Table 3). Sand lance occurred in just over one-third of samples, with fewer occurrences by 28 other prey taxa.

Across the entire study, 27 prey taxa had the potential to be detected by both methods (i.e., excluding invertebrate prey), of which 48.2% ($n = 13$ taxa) exhibited an increase in FO when both methods were combined compared to either method separately (Table 3). For example, Atlantic cod was detected in 2.4% of samples through hard parts analysis, 10.9% through metabarcoding, but 11.7% when methods were combined. Flatfish exhibited the greatest increase in FO with methods combined, with an FO of 16.6% via hard parts analysis, 38.1% via metabarcoding, and 44.5% in the combined data set. Additional prey taxa that exhibited increases in FO included Atlantic mackerel, clupeid, haddock, longhorn sculpin (*Myoxocephalus octodecemspinosus* (Mitchill, 1814)), ocean pout (*Zoarces americanus* (Bloch & Schneider, 1801)), phycid hake, redfish (*Sebastes* spp. Cuvier, 1829), sand lance, scup (*Stenotomus chrysops* (Linnaeus, 1766)), silver hake, and skates.

4. Discussion

Understanding the impact of a rebounding population of marine predators on its environment is vital for ecosystem monitoring and management. Although there are many methods that can be used to study marine predator diets, results are often biased by limitations, including, but not limited to, taxonomic resolution and logistical challenges obtaining biological samples. Hard parts analysis of scat and metabarcoding scat-derived prey DNA are two non-invasive methods that do not require handling the animal, making them an attractive option for studying federally protected marine mammals. We sought to combine methods of diet analysis to obtain information on the diversity of prey consumed by Northwest Atlantic grey seals, and determine whether diet has changed from early 2000s to 2018.

We found that metabarcoding detected 30% more prey taxa, on average, per grey seal scat sample than hard parts analysis, supporting our hypothesis. On a per-sample basis, metabarcoding detected a greater number of prey taxa than hard parts analysis in 66% of samples, whereas hard parts analysis detected a greater number of prey taxa in only 12.6% of samples. Similar findings of increased prey detections through DNA-based methods compared to hard parts analysis have been reported in studies on other pinnipeds (Casper et al. 2007*b*; Jeanniard-du-Dot et al. 2017; Brassea-Pérez et al. 2019) as well as other marine and terrestrial generalist predators (e.g., squid, bears, and coyotes; Braley et al. 2010; Mumma et al. 2016). Variation in prey detections among methods may be the result of different passages rates of liquid and solid components through the digestive tract (Mårtensson et al. 1998), or as a result of degradation. Prey DNA is subject to degradation by digestion and external environmental conditions once the scat is deposited, leading to a decrease in prey DNA read counts (Vynne et al. 2012; Thomas et al. 2014). It is possible that our 0.9% threshold excluded true prey detections, and future research should characterize prey DNA degradation in grey seals to understand how this impacts diet interpretations. On the other hand, degradation of hard prey remains can make it more difficult or impossible to identify the species consumed, and impact estimates of prey size and biomass that rely on otolith size (Bowen 2000). Prior research has developed correction factors that account for the reduction in size and number of otoliths (e.g., Bowen 2000; Grellier and Hammond 2006), but similar efforts for ge-

netic analyses are not yet well developed (e.g., Thomas et al. 2014).

Comparisons of dietary information provided by each method suggests the detection of certain prey taxa are dependent on the method used. In our study, clupeids and flatfish contributed to sample dissimilarity between methods, with significantly greater detections through metabarcoding (38.5% and 38.1%) compared to hard parts analysis (5.7% and 16.6%, respectively). Clupeids have relatively fragile otoliths that are more likely to be broken, eroded beyond identification, or completely lost in digestion, leading to prey DNA detection in the absence of otoliths (Casper et al. 2007*a*; Brassea-Pérez et al. 2019). In the case of larger prey (e.g., flatfish), Ampela (2009) documented observations of grey seals partially consuming larger prey while discarding the heads. Without ingestion of otoliths located within the head, this type of prey would be undetectable through hard parts analysis even though their DNA may still be present. Although skates were identified by SIMPER to contribute to sample dissimilarity, our log-linear models suggested there was no significant difference between detections through metabarcoding (47.8%) and hard parts analysis (44.1%). Differences in detection of skates may have been driven by different passage rates for skate DNA and skate denticles used for identification through hard parts analysis (Casper et al. 2007*a*).

One ancillary benefit of implementing universal primers in a metabarcoding study is the ability to detect unexpected or novel dietary prey items. Metabarcoding scat-derived prey DNA provided some of the first evidence of grey seal predation on monkfish (*Lophius americanus* Valenciennes, 1837) and Atlantic menhaden in scat samples collected in 2016 and 2017 (Flanders 2018; Flanders et al. 2020). Our study supports claims that monkfish and Atlantic menhaden are grey seal prey items, with FOs of 8.9% and 26.3%, respectively, detected via metabarcoding. Additionally, smooth dogfish and three bird taxa were detected in our study, giving further insight into possible rare predation events. Smooth dogfish inhabit nearshore pupping estuaries in spring and summer, coinciding with the time they were detected in scat samples (McCandless et al. 2007; Able and Fahay 2010). Grey seals have previously been observed attacking seabirds near Nova Scotia, Canada (Lucas and McLaren 1988), which could account for the double-crested cormorant (*Phalacrocorax auritus* (Lesson, 1831)), great shearwater (*Ardenna gravis* (O'Reilly, 1818)), and gull (*Larus* spp. Linnaeus, 1758) DNA that was detected in our study. Although each bird taxa were only detected in a single sample and could represent contamination from the study site, these interactions may facilitate interspecies transmission of influenza A virus, with severe consequences for the health of Northwest Atlantic pinniped populations (Puryear et al. 2016), and may warrant further research.

On the other hand, hard parts analysis detected phycid hakes at a significantly greater frequency (19%) than metabarcoding (7.7%). Phycid hakes are gadoids with robust otoliths that may become trapped in the digestive tract and pass with DNA from subsequent meals (Tollit et al. 2003; Grellier and Hammond 2006). Grellier and Hammond (2006) reported recovery of gadoid otoliths up to 6 days after ingestion, whereas final prey DNA detections in scat have been reported to occur 31.7 ± 14.4 h after consumption (Dufault et al. 2021).

Hard parts analysis may underestimate occurrence for some prey, but it frequently provided greater taxonomic resolution in prey identification than did metabarcoding and allowed for estimations of size and number of prey consumed (see Supplementary File 1). For example, morphological analysis of otoliths was used to distinguish between American shad and blueback herring, while our metabarcoding methods could not discriminate between *Alosa* spp. Various DNA-based methods have been used to improve the resolution of metabarcoding methods, including use of multiple markers that we did not attempt in the current study. Another DNA-based method has also been used to increase assay specificity: utilization of species-specific PCR primers (e.g., Dufault et al. 2021 for flatfish species), which could be a viable method for determining the identity of flatfish and clupeid DNA detected in our study.

Invertebrates may have been underestimated by previous grey seal diet studies as well. Echinoderms have rarely been reported in Northwest Atlantic grey seal diet (Rough 1995; McCosker et al. 2020), but our study suggests echinoderms may be a notable prey item as they were detected, via their tube feet or skeleton, in 23.1% of samples overall. Other invertebrates, such as crustaceans and bivalve molluscs, are often discarded as secondary prey in grey seal diet studies because they are typically consumed by fish on which grey seals predate (e.g., gadoids) but were detected in 30% and 11.3%, respectively, of samples overall. It is possible for both genetic and morphological based techniques to detect secondary prey (Arnett and Whelan 2001; Hosseini et al. 2008). In our study, crustaceans are a common prey item for many fish detected (e.g., cod (Arnett and Whelan 2001), skates (Packer et al. 2003)), and thus the presence of crustaceans in grey seal diet should be interpreted with caution. Arnett and Whelan (2001) showed that when grey seal and cod diets overlapped, cod tended to consume prey of a smaller size, but this is difficult to ascertain without concurrent prey diet analysis. Valuable additions to future studies would be the incorporation of metabarcoding primers that target both vertebrate and invertebrate prey, as well as simultaneous morphological diet analysis of generalist predators (e.g., grey seal) and their potential prey to further understand secondary prey consumption.

Due to methodological limitations that lead to biases in prey detections, we combined results across methods to obtain a more robust description of Northwest Atlantic grey seal diet. Grey seals play a vital ecological role in the marine ecosystem through predator–prey interactions, and we found evidence that supports our hypothesis of a shift in grey seal diet from the early 2000s to 2018. For example, it is well established that skates represent a food item of grey seals, but skate detections have been limited to <25% of samples in previous studies, some of which span multiple seasons (Rough 1995; Ampela 2009; Dufault 2019; Flanders et al. 2020). Our study suggests that skates may be of greater importance than previously reported, as we detected skates in 53.9% of samples across the entire study.

Sand lance is another commonly reported prey item in grey seal diets, especially for grey seals near Cape Cod. FOs for sand lance have ranged from 1.4% to 43.9% in studies using stomach and scat content analysis (Bowen et al. 1993; Bowen and Harrison 1994; Rough 1995; Ampela 2009). An exception appears to lie within Flanders et al. (2020), with a reported sand lance FO of 85.4% through metabarcoding that may suggest genetic analysis of scats leads to greater sand lance detection rates. However, we detected an FO of 28.7% for sand lance, more similar to prior studies. This notable decrease in consumption may be due to reduced availability to a generalist predator as trawl surveys suggest a decrease in sand lance abundance between 2016 and 2019 (DMF 2017, 2020).

Haddock and herrings have repeatedly been reported as Northwest Atlantic grey seal prey but they were not thought to be of great importance as prior studies have not detected these prey in more than 3% of samples (Ampela 2009; Dufault 2019; Flanders et al. 2020). Grey seal consumption of haddock and herrings may be increasing, as we detected haddock in 13.8% of samples via both methods, and Atlantic herring and river herrings in 14.2% and 20.7%, respectively, of samples via metabarcoding. Fisheries management actions have led to an increase in local haddock since 2010, as suggested by trawl surveys (DMF2020), which could account for the increased consumption. Additionally, haddock and herrings are documented prey of harbor seals, a second pinniped species that inhabits the Northwest Atlantic but has been outnumbered in recent years by grey seals in the Cape Cod region (Kopec 2009; Pace et al. 2019). It is possible that grey seals may be outcompeting harbor seals for particular prey, and future work should be conducted to determine how the diet of two sympatric species has shifted in relation to each other.

Our study uses sample collections throughout the course of 1 year to describe overall grey seal diet, but includes one sampling period per season, excluding winter. In the Northwest Atlantic, grey seals pup and mate in the winter, so scat collection did not occur to avoid disturbing mother–pup pairs. Additionally, grey seals typically fast during the breeding season, so any scat collected in winter would provide limited dietary information. The extent of species consumed by grey seals and information regarding seasonal grey seal diet would benefit from increased sampling within seasons to more accurately capture seasonal trends. Despite this limitation, we provide valuable information regarding differences in dietary information achieved through the use of two methods of non-invasive diet analysis techniques. Additionally, the combination of these methods yielded a more robust description of grey seal diet that, when compared to prior studies, suggests that the diet of this generalist predator may have shifted over the past few decades. As the Gulf of Maine is experiencing rapid warming, studies on predator–prey dynamics will become increasingly important to assess the impacts a growing grey seal population has on local fish communities. A deeper understanding of the connection between predators, prey, humans, and the environment is critical for successful conservation of Northwest Atlantic coastal marine ecosystems.

Acknowledgements

We thank Matthew Hillman (US Fish and Wildlife Service, Eastern Massachusetts NWR Complex) for logistical support during sample collections, Frederick Wenzel (National Oceanic Atmospheric Administration Northeast Fisheries Science Center) for assistance with fish otolith identifications, and Dr. Alicia Williams for feedback throughout the project. Scat samples were collected under National Marine Fisheries permits #18849 to Dr. Ono and #17670 to the Northeast Fisheries Science Center, and under IACUC protocol #080417-002. Access to Monomoy Island was under US Fish and Wildlife Service permit #53514-12006.

Article information

History dates

Received: 3 June 2023
Accepted: 15 August 2023
Accepted manuscript online: 30 August 2023
Version of record online: 5 December 2023

Data availability

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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Writing – review & editing: CMM, ZHO, KAO

Competing interests

The authors declare there are no competing interests.

Funding information

This research was supported by the University of New England Graduate Research Fund to CMM, the Lerner-Grey Fund for Marine Research from the American Museum of Natural History to CMM, and University of New England Provost's mini-grant awarded to KAO and ZHO.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjz-2023-0104.

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