2023

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Vol. 704: 131–147, 2023
https://doi.org/10.3354/meps14228

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published January 26

Fecal DNA metabarcoding shows credible short-term prey detections and explains variation in the gut microbiome of two polar bear subpopulations

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ABSTRACT: This study developed and evaluated DNA metabarcoding to identify the presence of pinniped and cetacean prey DNA in fecal samples of East Greenland (EG) and Southern Beaufort Sea (SB) polar bears *Ursus maritimus* sampled in the spring of 2015–2019. Prey DNA was detected in half (49/92) of all samples, and when detected, ringed seal *Pusa hispida* was the predominant prey species, identified in 100% (22/22) of EG and 81% (22/27) of SB polar bear samples with prey DNA detected. Bearded seal *Erignathus barbatus* DNA was found in 19% (5/27) of SB polar bear samples for which prey DNA was detected. Prey DNA detection frequencies and relative abundances were compared to estimates from quantitative fatty acid signature analysis (QFASA) for a subset of SB polar bears. Ringed seal and bearded seal were the main prey identified by both methods, but QFASA also identified 2 cetacean prey species not found by prey DNA. Differences in DNA metabarcoding vs. QFASA results were likely related to the different dietary timescales captured by each approach, i.e. short-term vs. long-term diet, respectively. Prey DNA detection, sex/age class, and subpopulation significantly explained variation in polar bear gut bacterial composition. Polar bear samples with prey DNA detected were associated with higher abundances of the bacterial classes Clostridia and Bacilli and lower abundances of Negativicutes. Fecal DNA metabarcoding is thus useful for identifying recent prey of polar bears, complementing quantitative and likely longer-term QFASA estimates, and may help understand variation in the polar bear gut microbiome.

KEY WORDS: Diet composition · DNA metabarcoding · QFASA · Fatty acid signatures · Marine mammal · Genomics · Arctic marine ecology

1. INTRODUCTION

Understanding temporal, including seasonal, variation in the trophic ecology of apex predators can provide nuanced insight into the structure and function of food webs, as well as into an individual's nutritional status, which can be an important predictor of reproductive success and survival (McCann 2007, Nielsen et al. 2018). Variation in feeding habits also influences the composition and diversity of the

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gut microbiome, a key aspect of health and immunity for wildlife host species (Sugden et al. 2020, van Leeuwen et al. 2020, Góngora et al. 2021). Several methods exist to assess predator diets, including direct observation, analysis of stomach contents, scats and hard parts, as well as chemical tracers like stable isotopes and fatty acid (FA) signatures (Nielsen et al. 2018). Observational methods provide a glimpse into recent consumption but are biased against unobservable predation events and prey with soft tissues that degrade during digestion (Dyck & Romberg 2007, Jeanniard-du-Dot et al. 2017). Stable isotope analysis of tissues can provide coarse resolution data on more long-term feeding habitats (e.g. $\delta^{13}C$ can differentiate benthic vs. pelagic feeding) and trophic position (e.g. $\delta^{15}N$ increases through food webs), but tends to offer limited species-level resolution of prey items (Nielsen et al. 2018). Quantitative fatty acid signature analysis (QFASA) can produce proportional estimates of prey consumed over perhaps the previous few weeks to months (Iverson et al. 2004, Thiemann et al. 2022), yet may still be subject to false negatives or positives depending on the completeness and prey-distinguishability, respectively, of the prey library used (Iverson et al. 2004). To overcome limitations of applying a single diet analysis approach that, e.g., only captures a particular timeframe, it has been recommended that multiple methods be used (Bowen & Iverson 2013, Hambäck et al. 2016, Nielsen et al. 2018).

Molecular approaches to assessing the diets of wildlife species can provide short-term (previous few days to weeks), species-level identification of prey — and possibly somewhat reflect their relative abundances in the diet — and may help to overcome challenges in detecting low-abundance prey species that may be missed by other methods (Bohmann et al. 2014). Recently, Michaux et al. (2021) performed DNA-based prey identification using a universal mitochondrial cytochrome *b* (*Cytb*) gene region target to amplify vertebrate DNA in polar bear *Ursus maritimus* scat opportunistically collected from the M'Clintock Channel (MC) subpopulation. Ringed seal *Pusa hispida* detections predominated, similar to what was reported previously for MC polar bears using QFASA (Thiemann et al. 2008), but DNA of other prey species was also identified. Nonetheless, there remain limitations to the universal primer approach, including over-representation of predator sequences, the inability to differentiate among some taxonomic groups, challenges in estimating biomass of prey consumed, and possible environmental and human DNA contamination (Michaux et al. 2021). The development of group-specific primers for DNA metabarcoding could reduce the amount of predator DNA amplified and improve species-level prey identifications, particularly when predator and prey are closely related species with higher genetic similarity (Deagle et al. 2009), such as is often the case for polar bears and their prey.

Certain FAs, which are synthesized at base levels of the food web, are incorporated from prey to predator adipose tissues in proportions that are relatively unmodified or predictably modified, making them useful as dietary indicators (Budge et al. 2006). The incorporation of FA signature data into the QFASA model allows for accurate proportional diet estimates of prey consumed in the previous perhaps several weeks to months by predators and is a well-established quantitative approach for polar bears (Iverson et al. 2004, McKinney et al. 2013, 2017). Despite some limitations (Thiemann et al. 2008, 2022, Iverson et al. 2018), QFASA estimates may provide useful, complementary long-term diet estimates when used alongside the more short-term diet information obtained from DNA-based methods. Such complementary diet information could shed light on the mechanisms by which climate-driven diet shifts in seasonal availability of prey may be impacting polar bear health, including in relation to changing contaminant exposures, infectious agents, and gut microbiota (Fagre et al. 2015, McKinney et al. 2017).

Polar bears are currently identified as vulnerable on the International Union for Conservation of Nature (IUCN) red list due to the reduced extent and seasonal duration of their sea ice habitat and consequent altered access to traditional ice seal prey and increased movement requirements (Wiig et al. 2015, Regehr et al. 2016). Changes in foraging behaviors have been observed in some subpopulations, such as the southern Beaufort Sea (SB) polar bears of Alaska (McKinney et al. 2013, 2017, Bourque et al. 2020). SB polar bears inhabit the divergent ice ecoregion (Amstrup et al. 2008), wherein the seasonal formation of a large open water space between the continental shoreline and sea ice edge has led to increased use of land and land-based food resources by some polar bears during the summer and fall; while ringed seal, followed by bearded seal *Erignathus barbatus* are the main prey, bowhead whale *Balaena mysticetus* carcasses left onshore from subsistence harvests in the fall are available (Herreman & Peacock 2013, Atwood et al. 2016, 2017, McKinney et al. 2017). Along with marked seasonal variation in habitat use, SB polar bears may be experiencing springtime food reductions and short-term nutri-

tional stress (Rode et al. 2010, 2018). Polar bears of the East Greenland (EG) subpopulation inhabit the convergent ice ecoregion, wherein the outflow of sea ice along the shoreline currently continues to allow for some year-round access to ice seals, largely ringed seal, at least in the northern parts of their range. Still, consumption of harp seal *Pagophilus groenlandicus* and hooded seal *Cystophora cristata* appears to be increasing (McKinney et al. 2013, Laidre et al. 2015). Dietary alterations of any kind have the potential to impact polar bear population dynamics and individual health (Atwood et al. 2017, Florko et al. 2020). For example, the gut bacterial composition of polar bears was recently shown to differ between SB polar bears using terrestrial habitats relative to those remaining on the sea ice during the reduced ice season (Watson et al. 2019), likely related to variation in feeding habits (Franz et al. 2022).

Here, we identify prey presence (i.e. 'prey detected' or 'not detected', as well as type of prey species detected) for EG and SB polar bears using newly designed group-specific prey primer sets. Due to the seasonal use of terrestrial habitats and food resources, as well as implications of reduced springtime availability of prey for SB but not EG polar bears, we hypothesize that prey detection frequencies (i.e. the proportion of polar bears where a given prey species was detected) and total number of prey species detected will differ for each subpopulation. We then assess the utility and possible limitations of this new prey-specific primer approach by comparing DNA metabarcoding and QFASA prey detection frequencies for a subset of individuals from the SB subpopulation. We further investigate the differences in dietary timescale, and thus seasonal variation in foraging patterns of EG and SB polar bears, that these 2 diet analysis approaches capture. We predict that the 2 methods will yield different yet complementary estimates, due to different method-specific biases and the expected differences in dietary timeframe represented by each approach (i.e. DNA metabarcoding will provide short-term estimates of springtime foraging, while QFASA will provide longer-term estimates including fall and winter foraging, given that both the adipose and fecal samples were collected in March/April). Finally, we test for associations between short-term, springtime prey presence (i.e. 'prey DNA detected' vs. 'prey DNA not detected') and gut microbiome diversity and composition for these 2 polar bear subpopulations, using existing bacterial 16S rRNA data from Franz et al. (2022).

2. MATERIALS AND METHODS

2.1. Fecal and adipose tissue sample collection

Collection of EG and SB fecal samples and subsequent DNA extraction was previously described in Franz et al. (2022). Briefly, a sterile latex glove was used to obtain fecal residue from the rectum of captured and anesthetized EG polar bears (n = 34) in 2017 and SB polar bears (n = 59) in 2015, 2016, 2018, and 2019. Gloves containing fecal residue were stored at −20°C immediately following collection, and then stored at −80°C until laboratory analysis. Sample collection occurred in the spring (13 March to 23 April) for both subpopulations as part of routine annual population assessments and from all available sex/age classes (adult females [AF], adult males [AM], subadults [S], and cubs [C] — yearlings and dependent 2-yr olds only, not cubs of the year). SB samples were collected as part of the U.S. Geological Survey (USGS) Polar Bear Research Program (U.S. Fish and Wildlife Service Permit # MA690038) under capture protocols approved by the USGS Institutional Animal Care and Use Committee. EG samples were collected by the Greenland Institute of Natural Resources as part of a long-term monitoring program under case number 2017-5446, document 4710596 from the Department of Fisheries and Hunting. Collection of adipose tissue from a rump biopsy for a subset (n = 46) of the same SB polar bears in 2016, 2018, and 2019 was previously described in Franz et al. (2022). Adipose biopsies were collected from AF, AM, and S sex/age classes only (no cubs), stored at −20°C immediately following collection, and then stored at −80°C until laboratory analysis.

2.2. DNA extraction

DNA extraction from fecal samples was performed using a modified protocol from the QIAamp Mini Kit Buccal Swab Spin Protocol (QIAamp DNA Mini and Blood Mini Handbook), as described previously (Franz et al. 2022). Samples were eluted in a final volume of 100 µl elution buffer (buffer AE, Qiagen) and used for group-specific prey DNA amplification (see Section 2.3), as well as in 16S rRNA gene amplification analysis for gut bacteria (Franz et al. 2022).

2.3. Design and optimization of group-specific primer sets

Group-specific primer sets to amplify pinniped and cetacean DNA were developed using custom

Python (Van Rossum & Drake 1995) and Biopython (Cock et al. 2009) scripts (Menning & Talbot 2017, Menning et al. 2020). Complementary DNA sequences of mitochondrial loci, *Cytb* and cytochrome oxidase 1 (*CO1*) were compiled from the National Centre for Biotechnology Information (NCBI) GenBank nucleotide repository (www.ncbi.nlm.nih.gov) for all marine vertebrate species known to co-occur with, or be a component of, EG and SB polar bear diets and used for primer design (species listed in Table S1 in the Supplement at www.int-res.com/articles/suppl/m704p131_supp.pdf). Sequences were aligned using MEGA7 (Kumar et al. 2016), and 4 primer sets were designed: *Cytb* and *CO1* pinniped primer sets and *Cytb* and *CO1* cetacean primer sets (Table S2). These primers captured all pinniped and cetacean species of interest for this study and amplified similar-sized DNA fragments.

The selected primer pairs were tested for successful amplification using cetacean and pinniped DNA extracts that were available in the lab from earlier projects (Table S3), as well as polar bear DNA extract to test for the extent to which host DNA might be amplified by these primer sets. Prey and polar bear DNA was extracted using the DNeasy blood and tissue kit (Qiagen). A series of temperature gradient PCRs were conducted to determine the optimal annealing temperature for each primer set (Table S2, Fig. S1). PCR reactions were performed in 25 µl volumes containing: 0.4 mg ml^{-1} BSA (bovine serum albumin), 0.6 µg $µl^{-1}$ of each primer, 1X Kapa Hifi Hot Start Ready Mix (Roche Diagnostics), and 2.5 µl template prey DNA. Thermocycler conditions were 95°C for 5 min, followed by 30 cycles of 98°C for 30 s, primer set annealing temperature (i.e. 51 to 62°C; see Table S2) for 30 s, 72°C for 40 s, concluding with 72°C for 5 min. After successful amplification of the prey DNA extracts and testing for amplification of polar bear DNA (Figs. S1–S3), additional PCRs were conducted on a subsample of randomly selected polar bear fecal DNA extracts to confirm successful amplification of prey DNA from desired samples using the PCR conditions described above (Fig. S4).

2.4. Prey DNA amplification, library preparation and sequencing

A 96-well plate containing the polar bear fecal DNA extracts and 4 positive control prey DNA extracts (cetacean positive controls: humpback whale *Megaptera novaeangliae* [Mn_005] and long-finned pilot whale *Globicephala melas* [GM-15]; pinniped positive controls: ringed seal *Pusa hispida* [ARRB-17-0001] and harp seal [Harp seal F]) were sent to Genome Quebec for amplicon library preparation and sequencing (see Table S3, 'Sample ID' column for additional information on positive control samples referenced here). CS1 (5′-ACA CTG ACG ACA TGG TTC TAC A-3′) and CS2 (5′-TAC GGT AGC AGA GAC TTG GTC T-3′) tags were added to primer sequences to each amplicon in addition to an adapter and unique, single index barcodes. Amplification of prey DNA was performed separately for each of the 4 primer sets, with the appropriate corresponding humpback whale and pilot whale (cetacean) or ringed seal and harp seal (pinniped) positive controls. Due to issues amplifying the humpback whale (HW 005) and pilot whale (PW 15) positive controls using the Cetacea_CO1_F4_R5 primer set with CS1 and CS2 tags added, this primer set was not used further in the study (Fig. S5).

PCR amplifications followed the above protocols for the remaining 3 primer sets (Table S2) with 2 exceptions: BSA was not included and the template DNA was decreased from 2.5 to 1 µl. Verification of successful PCR amplification was visually confirmed on 2% agarose gel. Excess dNTPs and primers were removed with sparQ PureMag Beads (Quantabio) prior to the index PCR step. Index PCR reactions were in 20 µl volume and contained: 0.125 U $µl^{-1}$ FastStart High Fi (Roche Diagnostics), 1X Buffer (Roche Diagnostics), 1.8 mM $MgCl_2$ (Roche Diagnostics), 5% dimethylsulfoxide (DMSO; Roche Diagnostics), 0.2 mM dNTP mix, 1.0 µl of PCR product per reaction well and 0.2 µM LNA™ modified custom primers (Exiqon). Thermocycler conditions were as follows: 95°C for 10 min, 15 cycles of 95°C for 15 s, 60°C for 30 s, 72°C for 60 s, concluding with 72°C for 3 min. Verification of barcode incorporation for each sample was done on a 2% agarose gel. Amplicons were quantified using a Quant-iT™ PicoGreen dsDNA Assay Kit (Life Technologies). The library was generated by pooling the same quantity of each amplicon and with excess primers and dNTPs removed using sparQ PureMag Beads (Quantabio). The pooled libraries were quantified using Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Kapa Biosystems). The average fragment size was determined using a LabChip GX (PerkinElmer) instrument. Libraries (9 pM final concentration with 12% PhiX control) were sequenced on an Illumina MiSeq using a v3 600 cycle kit.

2.5. DNA-based prey detections

Sequence counts were processed and annotated using the ANCHOR pipeline and custom reference database for each primer set, separately, and output as exact sequence variants (ESVs) (Gonzalez et al. 2019). Sequence data have been submitted to the NCBI Sequence Read Archive (SRA; accession bioproject ID: PRJNA773176). Only paired-end sequences with primer sequences present were selected, aligned, and dereplicated before selection of ESVs using a count threshold of 9 (i.e. a minimum of 3 ESV counts in at least 3 samples) across all samples. Annotation queried 2 sequence repositories with strict BLASTn criteria (>99% identity and coverage): NCBI nucleotide (nt) and either *CO1* or *Cytb* custom made database. *CO1* and *Cytb* databases were constructed from NCBI by downloading sequence IDs that were annotated as either *CO1* or *Cytb*. The *CO1* and *Cytb* databases contained 3 369 178 and 577 858 sequences, respectively, assigning to the prey species of interest (Table S1). Database versions were from August 2021. Note that all annotation was considered putative and subject to improvement as database errors are resolved and new species are characterized. Following taxonomic assignment and generation of ESV count data, 'Unknown' sequences (i.e. those that did not meet the 99% identity and coverage thresholds) were spot-checked against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure no potential species of interest went undetected in the final datasets. Unknown ESVs and ESVs that assigned to '*Ursus maritimus*' and '*Ursus arctos*' were removed from the datasets. Read counts of ESVs that assigned to the same prey species were collapsed or merged into a single summed count for a given species for subsequent analyses and prior to merging datasets (done for the pinniped primer sets only). MicrobiomeAnalyst Web Version (Chong et al. 2020) was used to explore and compare primer performance in detecting prey DNA. Given the ANCHOR pipeline's strict filtering parameter settings, no additional filtering steps were applied to raw count data. However, the PCR blank for the Pinniped CO1 primer dataset contained 2 seal prey DNA counts; therefore a minimum count of 10 prey DNA sequence reads (5× the PCR blank) was applied to samples from that dataset. Pinniped prey DNA read counts were converted to relative abundances within each polar bear fecal sample to compare the pinniped primer performances (*CO1* vs. *Cytb*). Pinniped primer performance (*CO1* vs. *Cytb*) was qualitatively assessed and reported as a comparison of the frequencies of detection of pinniped prey in the same set of polar bear fecal samples.

2.6. Fatty acid analysis and QFASA diet estimates

In the present study, previously generated FA data were used (Franz et al. 2022) to estimate the diets of the SB polar bears using QFASA, as previously described for this subpopulation (McKinney et al. 2017). In brief, adipose samples were lipid extracted, and the resulting FAs were converted to fatty acid methyl esters (FAMEs) and quantified by gas chromatography with flame ionization detection (Franz et al. 2022), producing proportions of each FA (mass% of total). A preexisting prey library was used consisting of bearded seal (n = 20), beluga whale *Delphinapterus leucas* (n = 29), bowhead whale (n = 64), and ringed seal (n = 64) (McKinney et al. 2017). To account for the effects of metabolism and biosynthesis on predator FA signatures, calibration coefficients (CCs) from mink *Mustela vison* that had been fed a marine diet were used (Iverson et al. 2004). The model was run in R Studio (R Core Team 2019) using the QFASA package (Iverson et al. 2018) with a subset of 30 dietary FAs (as per Iverson et al. 2004). The prey library used in the model was previously shown to identify prey with >90% accuracy (McKinney et al. 2017). To test the accuracy of predator diet estimates, a pseudo-predator diet was created and tested to see how well the QFASA estimate output matched a 'known' predator diet (25% bearded seal, 25% beluga whale, 25% narwhal, and 25% ringed seal) after 100 simulations (Fig. S6).

2.7. Microbial Community 16S rRNA analysis

16S rRNA gene library preparation and sequencing were previously described for these samples (Franz et al. 2022), and sequence data are available (NCBI SRA; accession number: PRJNA773176). Universal bacterial primer set 341F (5′-CCT ACG GGN GGC WGC AG-3′) and 805Rmod (5′-GAC TAC NVG GGT WTC TAA TCC-3′) with overhanging Illumina adaptors were selected to amplify a 460 base pair (bp) region of the 16S gene from metagenomic DNA extracted from EG and SB polar bear fecal residue. The final prepared 16S rRNA library was sequenced using v2 chemistry on an Illumina MiSeq 2 × 250 bp run at McGill University. In brief, sequence reads were processed (filtered, trimmed, de-replicated, and

merged) using the DADA2 pipeline (Callahan et al. 2016). Contaminant amplicon sequence variants (ASVs) were removed using Decontam (Davis et al. 2018), and ASVs with zero variance or <2 counts were removed at an initial filtering step in the MicrobiomeAnalystR (Chong et al. 2020, Franz et al. 2022). See Franz et al. (2022) for further detail.

2.8. Data analyses

Differences in prey detection frequency (i.e. whether prey DNA was detected or not) between subpopulations and among polar bear sex/age classes were assessed using Pearson's chi-square tests with an alpha-value cutoff of 0.05. R 4.0.3 (R Core Team 2020) was used for this analysis as well as subsequent statistical tests conducted throughout this study, unless stated otherwise.

Both DNA-based and QFASA prey detection frequencies (i.e. the proportion of polar bears where a given prey species was detected) and proportional prey estimates were determined and subsequently compared for the subset of SB samples for which seal prey DNA was detected (n = 20). We compared relative read abundances of prey detected from DNA-based analysis to proportional diet estimates from the QFASA approach to assess any similarities or differences in the percentages obtained, and to verify likely timescale differences captured by each method. The mean proportional estimates obtained using each approach were also visually compared among polar bear sex/age classes.

To examine the influence of diet on gut microbiota, we tested associations between prey presence and both microbiome diversity and composition by creating a new categorical variable: 'prey DNA detected' or 'no prey DNA detected'. This was done due to the insufficient sample size of polar bears that had consumed each prey item in each subpopulation (i.e. bearded seal was only detected in SB polar bears). For gut microbiome analyses, the Shannon, inverse Simpson, and Faith's phylogenetic alpha diversity indices were calculated at ASV-level for EG and SB polar bears as per Franz et al. (2022). Multiple linear regression models (LMs) were then used to examine relationships between feeding and alpha diversity indices. Other variables included subpopulation, sex/age class (AF, n = 36; AM, n = 32; S, n = 15; C, n = 10), body mass as an indicator of body condition (Rode et al. 2020), and all relevant first-order interactions. Capture year was not included as an explanatory variable as the EG samples were collected in a single year, which did not overlap the SB collection years. To test for variation in gut bacterial composition at multiple taxonomic levels (i.e. phylum, class, genus, and ASV), separate PERMANOVAs were run using the Bray-Curtis distance method and the same ecological variables and interaction terms as per the alpha diversity LMs. Homogeneity of group dispersions (PERMDISP; Anderson 2006) was assessed using the 'betadisper' function from the 'vegan' R package. For significant categorical variables, a linear regression framework based on log-transformed taxa count (ANCOMBC) was used for differential abundance (ANCOMBC; Lin & Peddada 2020). Spearman's ranked correlation tests were done to assess correlations between bacterial taxa and a given continuous variable determined from PERMANOVA models.

3. RESULTS

3.1. Prey detection for EG and SB polar bears based on DNA analysis

For both the pinniped *Cytb* and *CO1* primer sets, ringed seal and bearded seal were the only 2 prey species detected in EG and SB polar bear fecal samples. After combining the pinniped *Cytb* and *CO1* datasets, pinniped prey DNA was detected in 53.3% (49/92) of all fecal samples — 64.7% (22/34) of EG fecal samples and 46.6% (27/58) of SB fecal samples. Prey detection frequencies were not statistically different between subpopulations (χ^2 = 2.84, p = 0.09, df = 1) (Table 1, Fig. 1). Cetacean DNA was not detected in any EG or SB fecal samples. Performance of the different prey-specific primer sets (i.e. in how many samples each primer set detected prey DNA, how effectively each primer set minimized predator DNA amplification, etc.) is reported in the Supplement (Text S1, Table S4, Figs. S7– S10).

Ringed seal was found in 98.0% (48/49) of all samples where prey DNA was detected (Fig. 1). Bearded seal DNA was detected in only 12.2% (6/49) of samples, comprising 1 individual from the EG subpopulation and 5 from the SB subpopulation (Fig. 1). For the 1 EG individual, bearded seal was only detected in trace amounts, i.e. <0.001% relative read abundance (RRA), while ringed seal was at >99.99% RRA. Thus, the detection of bearded seal DNA in this individual may reflect contamination of the sample with environmental DNA or trace amounts of DNA from a previous meal. Given this, essentially all bearded seal detection was in the SB subpopulation. Bearded

Table 1. Percentages of seal prey detected (RS: ringed seal; BS: bearded seal; ND: no prey DNA detected) among sex/age classes (AF: adult females; AM: adult males; S: subadults; C: cubs) of polar bears from the East Greenland (EG) and Southern Beaufort Sea (SB) subpopulations after summing prey DNA count data from the pinniped cytochrome *b* and the pinniped cytochrome oxidase 1 primer sets. *n*: initial sample; QC: 'quality check', refers to samples that met sample filtering parameters

Sub-population	*n*	Sample *n* post-QC	% *n* prey detected	Overall (EG: *n* = 34, SB: *n* = 58)			AF (EG: *n* = 16, SB: *n* = 20)			AM (EG: *n* = 7, SB: *n* = 24)			S (EG: *n* = 5, SB: *n* = 10)			C[a] (EG: *n* = 6, SB: *n* = 4)		
				%RS	%BS	%ND	%RS	%BS	%ND	%RS	%BS	%ND	%RS	%BS	%ND	%RS	%BS	%ND
EG	34	34	64.7	64.7	0	35.3	62.5	6.3	37.5	28.6	0	71.4	80.0	0	20.0	100	0	0
SB	59	58	46.6	44.8	8.6	53.4	55.0	5.0	45.0	29.2	12.5	66.7	60.0	10.0	40.0	50.0	0	50.0
Combined	93	92	53.3	52.2	6.5	46.7	58.3	5.6	41.7	29.0	9.7	67.7	66.7	6.67	33.3	80.0	0	20.0

[a]Yearlings and dependent 2 yr old cubs only, not cubs of the year

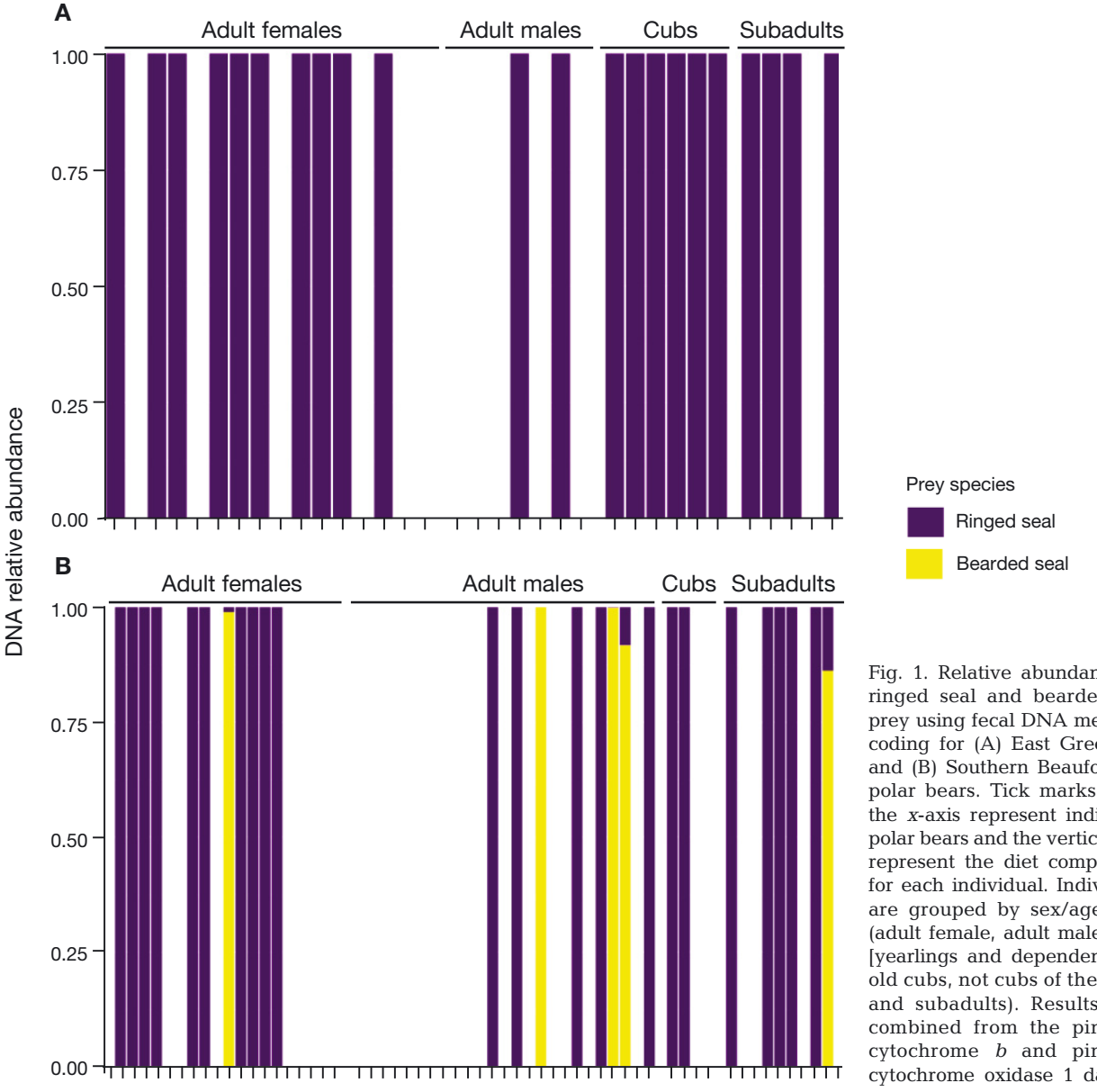

Fig. 1. Relative abundances of ringed seal and bearded seal prey using fecal DNA metabarcoding for (A) East Greenland and (B) Southern Beaufort Sea polar bears. Tick marks along the *x*-axis represent individual polar bears and the vertical bars represent the diet composition for each individual. Individuals are grouped by sex/age class (adult female, adult male, cubs [yearlings and dependent 2 yr old cubs, not cubs of the year], and subadults). Results were combined from the pinniped cytochrome *b* and pinniped cytochrome oxidase 1 datasets

seal detection was nonetheless infrequent in SB polar bears as well, with DNA being detected in only 8.6% (5/58) of SB individuals (Table 1). For individuals for which both ringed and bearded seal were detected, the RRA of one of the prey species was consistently far higher (>85%) than the other prey species (Fig. 1).

For both subpopulations combined, patterns of prey DNA detection (i.e. prey presence/absence, not the specific prey species) were similar among all sex/age classes ($\chi^2 = 7.26$, $p_{adj} = 0.07$, df = 3) (Table 1). Patterns of prey DNA detection frequencies were also similar among sex/age classes within each of the subpopulations (EG: $\chi^2 = 7.50$, $p_{adj} = 0.06$, df = 3; SB: $\chi^2 = 2.80$, $p_{adj} = 0.42$, df = 3). Within the SB subpopulation, while bearded seal DNA detections were most frequent in adult males, no statistical analysis could be done to assess this pattern due to small sample sizes with bearded seal prey detected (AM: $n = 3$; AF: $n = 1$, S: $n = 1$) (Table 1, Fig. 1).

3.2. Comparison of prey DNA metabarcoding to QFASA

Prey detection frequencies (i.e. the proportion of polar bears where a given prey species was detected) determined by the DNA-based and the QFASA-based approaches were compared for the subset of SB polar bears for which both sets of diet data were available (Table 2). For both techniques, ringed seal was the predominant prey species, detected in 95% (19/20) of samples by DNA metabarcoding and in 100% (20/20) of samples using QFASA. Bearded seal was the second most detected prey by both approaches, but the detection frequency of bearded seal among samples was much higher at 95% (19/20) for QFASA versus 25% (5/20) of samples by DNA metabarcoding. No cetacean DNA was detected by DNA metabarcoding; however, with QFASA, bowhead whale was detected as a prey item in 85% (17/20) of samples and beluga whale in 15% (3/20) of samples.

Table 2. Percentage of polar bear scats with positive prey detections using either prey DNA metabarcoding or quantitative fatty acid signature analysis (QFASA) for the subset of Southern Beaufort Sea (SB) polar bears for which prey DNA was detected (AF: adult females; AM: adult males; S: subadults)

Prey type	% Detections ($n = 20$)	% AF ($n = 9$)	% AM ($n = 7$)	% S ($n = 4$)
Prey DNA metabarcoding				
Ringed seal	95.0	100	85.7	100
Bearded seal	25.0	11.1	42.9	25.0
Beluga whale	0	0	0	0
Bowhead whale	0	0	0	0
QFASA				
Ringed seal	100	100	100	100
Bearded seal	95.0	88.9	100	100
Beluga whale	15.0	33.3	0	0
Bowhead whale	85.0	77.8	100	75.0

To preliminarily explore the utility of the DNA approach for quantifying prey biomass and to qualitatively assess the different timescales captured by DNA vs. QFASA methods, pinniped prey DNA relative abundances were compared to QFASA proportional diet estimates for individuals from the SB subpopulation for which both diet datasets existed (Table S5). Although comparisons of the proportional estimates using the 2 different approaches should be done with caution given the compositional nature of both datasets, it did appear that for both the DNA and QFASA approaches, the mean proportional prey estimates exhibited some consistencies in terms of rank order of the main pinniped prey consumed. That is, ringed seal was estimated to be the predominant prey by both methods, with bearded seal second (Table 3). Estimates using both methods were similar in that they detected minimal or no contribution of cetacean species. Nonetheless, ringed seal proportional estimates were higher for DNA metabarcoding than for QFASA (76 vs. 47%), while the reverse seemed to be the case for bearded seal and the cetacean species proportional estimates (Table 3). Broadly similar patterns of prey consumption among polar bear sex/age classes were found using both diet approaches; for both, adult females and subadults were estimated to consume qualitatively more ringed seal than adult males, and adult males were estimated to consume more bearded seal than adult females and subadults (Fig. 2). Given the small sample sizes among polar bear sex/age classes after reducing to a sample size of 20 individuals, statistical analyses could not be done to further assess these diet patterns.

3.3. Influence of diet estimates from DNA metabarcoding on EG and SB gut microbiota

Results detailing the sequencing efficiency of the 16S rRNA library and all subsequent data processing and filtering step results for the gut bacterial data have previously been reported (Franz et al. 2022) and are briefly summarized in the present study (Text S2 in the Supplement).

Table 3. Permutational analysis of variance (PERMANOVA) results showing differences in gut bacterial composition (at bacterial phylum, class, genus, and ASV levels) related to DNA diet profile (polar bear diet profile from DNA metabarcoding, with 2 categorical diet variables: 'prey DNA detected' and 'prey DNA not detected'), sex/age class, subpopulation and body condition for polar bears from East Greenland (EG) and the Southern Beaufort Sea (SB) combined. Values in **bold** indicate significant terms

Variable	df (factor, total)	SS	MS	F.model	R^2	p
Phylum-level						
DNA diet profile	1, 92	0.11	0.11	1.67	0.02	0.18
Sex/age class	3, 92	0.24	0.08	1.24	0.04	0.28
Subpopulation	1, 92	0.15	0.15	2.29	0.02	0.09
Body condition	1, 92	0.06	0.06	0.95	0.01	0.40
DNA diet profile × Sex/age class	3, 92	0.22	0.07	1.13	0.04	0.33
DNA diet profile × Subpopulation	1, 92	0.04	0.04	0.63	0.01	0.58
Subpopulation × Body condition	1, 92	0.08	0.08	1.28	0.01	0.29
Residuals	81	5.29	0.07		0.85	
Class-level						
DNA diet profile	**1, 92**	**0.45**	**0.45**	**3.28**	**0.03**	**0.01**
Sex/age class	**3, 92**	**0.81**	**0.27**	**1.95**	**0.06**	**0.03**
Subpopulation	**1, 92**	**0.47**	**0.47**	**3.40**	**0.03**	**0.01**
Body condition × Sex/age class	1, 92	0.10	0.10	0.71	0.01	0.60
DNA diet profile	3, 92	0.65	0.22	1.57	0.05	0.09
DNA diet profile × Subpopulation	1, 92	0.10	0.10	0.70	0.01	0.61
Subpopulation × Body condition	1, 92	0.20	0.20	1.46	0.01	0.21
Residuals	81	11.17	0.14		0.80	
Genus-level						
DNA diet profile	**1, 92**	**0.77**	**0.77**	**2.97**	**0.03**	**0.003**
Sex/age class	**3, 92**	**1.67**	**0.56**	**2.15**	**0.06**	**<0.001**
Subpopulation	**1, 92**	**1.14**	**1.14**	**4.40**	**0.04**	**<0.001**
Body condition	1, 92	0.15	0.15	0.59	0.01	0.87
DNA diet profile × Sex/age class	3, 92	1.01	0.34	1.30	0.04	0.12
DNA diet profile × Subpopulation	1, 92	0.17	0.17	0.65	0.01	0.81
Subpopulation × Body condition	1, 92	0.28	0.28	1.07	0.01	0.36
Residuals	81	20.99	0.26		0.80	
ASV-level						
DNA diet profile	**1, 92**	**0.79**	**0.79**	**2.99**	**0.03**	**<0.001**
Sex/age class	**3, 92**	**1.75**	**0.58**	**2.21**	**0.07**	**<0.001**
Subpopulation	**1, 92**	**1.30**	**1.30**	**4.93**	**0.05**	**<0.001**
Body condition	1, 92	0.16	0.16	0.61	0.01	0.84
DNA diet profile × Sex/age class	3, 92	0.95	0.32	1.20	0.04	0.20
DNA diet profile × Subpopulation	1, 92	0.20	0.20	0.75	0.01	0.69
Subpopulation × Body condition	1, 92	0.27	0.27	1.02	0.01	0.41
Residuals	81	21.39	0.26		0.80	

The DNA-based diet profiles did not significantly explain variation in Shannon, inverse Simpson, and Faith's phylogenetic alpha diversity indices (Table S6). In contrast, gut bacterial composition was found to vary significantly with DNA-based diet profile category ('prey DNA detected' vs. 'prey DNA not detected') at the bacterial class (PERMANOVA: $R^2 = 0.03$, $p = 0.01$; Fig. S11), genus ($R^2 = 0.03$, $p = 0.003$; Fig. S11), and ASV ($R^2 = 0.03$, $p < 0.001$; Fig. S11) levels (Table 3). It should be noted that the homogeneity of group dispersion assumption was not met at the ASV-level for the Bray-Curtis distance, with 'prey DNA detected' individuals having higher group dispersion (distance to centroid: 0.59 ± 0.01, ±SE) compared to 'prey DNA not detected' individuals (distance to centroid: 0.55 ± 0.01) (PERMDISP: $F = 3.96$, $p = 0.06$); thus, these model results should be interpreted with caution. Sex/age class and subpopulation were also significant in the models at bacterial class (sex/age class: $R^2 = 0.06$, $p = 0.03$; subpopulation: $R^2 = 0.03$, $p = 0.01$), genus (sex/age class: $R^2 = 0.06$, $p \leq 0.001$; subpopulation: $R^2 = 0.04$, $p \leq 0.001$), and ASV (sex/age class: $R^2 = 0.06$, $p = 0.003$; subpopulation: $R^2 = 0.05$, $p = 0.001$) levels (Table 3).

ANCOMBC results revealed that bacteria within the class Clostridia were significantly differentially abundant according to DNA-based prey detection categories. At the class level, Clostridia were significantly higher (ANCOMBC: $p_{adj} = 0.009$), and Negativicutes tended to be lower, although not significantly so (ANCOMBC: $p_{adj} = 0.12$), for 'prey DNA detected' individuals compared to 'prey DNA not detected' individuals (Fig. 3A, Table S7). Two bacterial genera, *Terrisporobacter* (class: Clostridia; ANCOMBC: $p_{adj} = 0.002$) and *Halomonas* (class: Gammaproteobacteria; ANCOMBC: $p_{adj} = 0.03$), showed significantly different abundances according to DNA-based diet profile (Fig. 3B, Table S8). Two

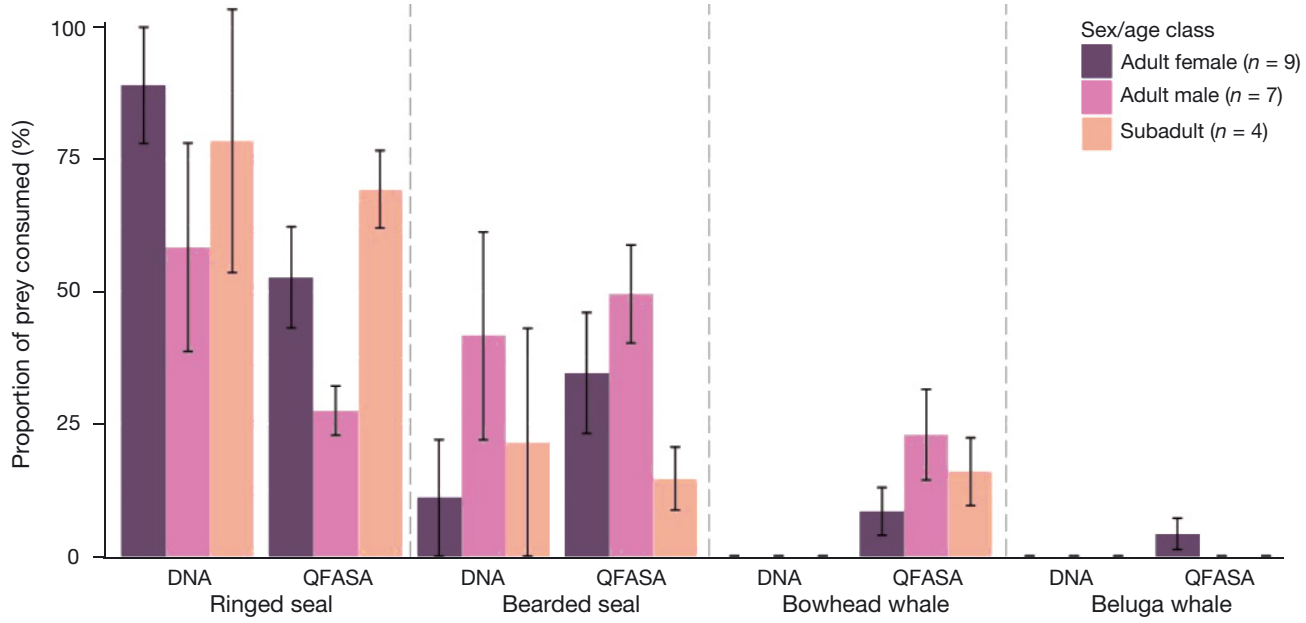

Fig. 2. Comparison of diet estimates using DNA metabarcoding and quantitative fatty acid signature analysis (QFASA) for a subset of Southern Beaufort Sea polar bears for which prey DNA was detected (n = 20). Proportions of prey consumed are grouped by diet estimate approach (DNA- or QFASA-based) and further by sex/age class (adult female, adult male, and subadult). Error bars are ±SE. Bowhead whale and beluga whale were not detected by DNA metabarcoding

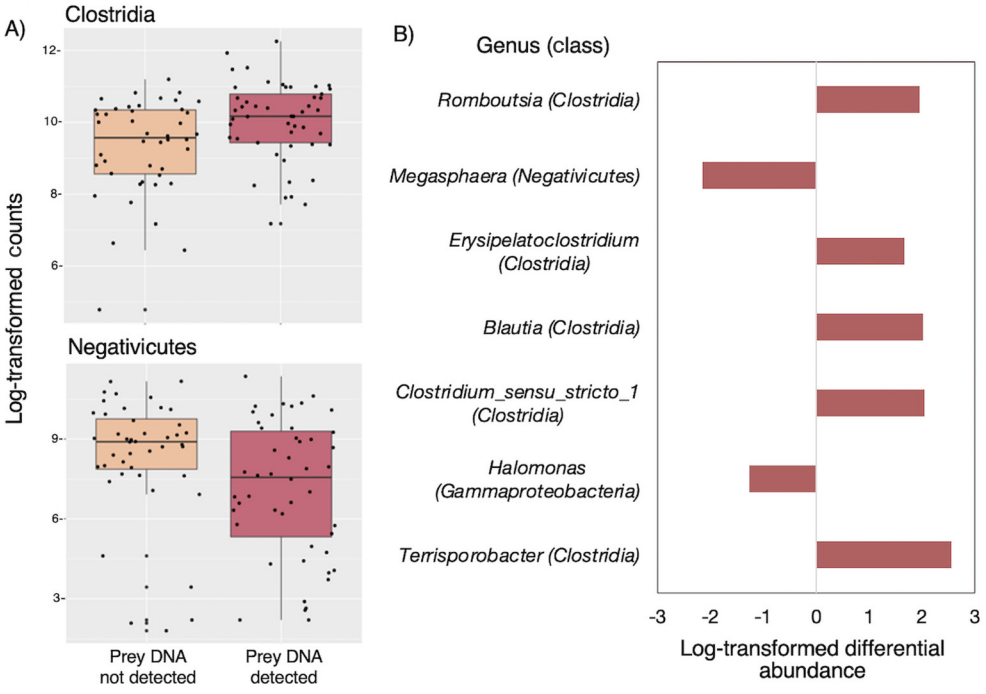

Fig. 3. Log-transformed abundances (as per Lin & Peddada 2020) of (A) bacterial classes and (B) bacterial genera that were, or showed a tendency to be, differentially abundant (i.e. increased or decreased) in polar bears with 'prey DNA detected' vs. 'prey DNA not detected' using DNA metabarcoding. Each point in panel A represents the log-transformed count of Clostridia or Negativicutes for an individual polar bear. Statistical results are summarized in Tables S5 & S6

ASVs (ASV_36, ANCOMBC: p_{adj} = 0.01; ASV_79, ANCOMBC: p_{adj} = 0.05) were significantly differentially abundant and assigned to the class Clostridia (Table S9). ASV_356, assigned to class Bacilli, tended to be higher in 'prey DNA detected' individuals compared to 'prey DNA not detected' individuals, but not significantly so (ANCOMBC: p_{adj} = 0.07).

4. DISCUSSION

Pinniped and cetacean group-specific prey primer sets were developed to amplify prey DNA in polar bear fecal extracts. To our knowledge, this is one of the few studies to use DNA-based methods to assess polar bear diets (Iversen et al. 2013, Michaux et al.

2021), and it advances the approach by using group-specific primers instead of the universal *Cytb* primer set. Use of targeted primer sets facilitated species-level resolution and reduced co-amplification of polar bear DNA. The approach was also compared to the established QFASA method, with frequency of detection results from both methods underscoring the importance of ringed and bearded seals in polar bear diets, but variability in proportional estimates between the 2 methods possibly related to differences in the time frame represented. DNA metabarcoding results also showed promise for explaining variation in the gut microbiome of polar bears.

We found no difference in pinniped prey DNA detection frequencies between EG and SB polar bears. Although previous work suggested increases in short-term springtime fasting and potentially reduced springtime prey availability in the SB compared to the EG subpopulation (Cherry et al. 2009, Rode et al. 2010, 2018), with the exception of an anomaly year in 2012, SB subpopulation abundance has been relatively stable since 2012 albeit depleted compared to historical numbers (Bromaghin et al. 2021). This stability in recent years coincides, to some extent, with our result of no apparent difference in prey detections between the subpopulations in the 2015–2019 timeframe.

Of the prey detected by DNA metabarcoding, ringed seal predominated for SB polar bears and was essentially the only prey item found for EG polar bears. This finding is consistent with the well-documented tight predator–prey relationship between polar bears and ringed seals (Stirling & Archibald 1977, Stirling et al. 1982, Cherry et al. 2009). The SB and EG polar bears were sampled in the spring, which is the most important feeding time for polar bears; during this period polar bears exhibit hyperphagia and, particularly for adult female polar bears and their cubs, consumption is largely in the form of adult female ringed seals and their newborn pups (Laidre et al. 2008, Iversen et al. 2013). Previous studies using QFASA have suggested EG polar bears are increasingly feeding on seasonally resident harp and hooded seals related to long-term sea ice declines (Wiig et al. 2003, McKinney et al. 2013). However, no sub-Arctic seals were observed during the spring field work for the present study, which occurred from 19 March to 14 April 2017 across a range of latitudes both north and south of 64°N (Laidre et al. 2022), consistent with the lack of detection of DNA of these sub-Arctic seals in the spring diets of EG polar bears.

The DNA metabarcoding method detected bearded seals as a minor prey species in the diets of SB but not EG polar bears, and no other seal species was detected as prey for either subpopulation. The coastal zone where the SB polar bears were sampled is characterized by shallow, productive waters, which represent the preferred habitat of bearded seals (Laidre et al. 2008, Kovacs et al. 2011). Although polar bears are thought to prey on bearded seals less often than on ringed seals across the circumpolar Arctic, some kill observations suggest a higher reliance on bearded seals in the western Arctic, including the SB subpopulation (Stirling & Archibald 1977), consistent with bearded seal DNA detection in SB but not EG polar bears. A lack of detection of bearded seal and walrus prey DNA for EG polar bears is also consistent with previous diet estimates by QFASA of <5% for bearded seals and no detection of walrus prey for the EG subpopulation (McKinney et al. 2013), despite these 2 pinnipeds being within the range of the EG polar bear subpopulation (Laidre et al. 2008). The low to no detection of bearded seals by DNA metabarcoding is consistent with reports of ringed seals predominating the spring diets, whereas bearded seals are more important in the summer (Derocher et al. 2002, Iversen et al. 2013). Aside from ringed and bearded seals, no other pinniped species are thought to be accessible to SB polar bears (Laidre et al. 2008, Rode et al. 2014), which may explain a lack of detection of other pinniped prey DNA in this subpopulation.

Cetacean DNA was not found in the spring-collected EG and SB polar bear fecal samples. Given evidence of positive control amplification, but lack of amplification in polar bear fecal samples, we conclude that cetaceans were likely not consumed by the EG or SB polar bears around the time of sample collection. The EG region is inhabited by narwhal and bowhead whales, which may be potential prey for EG polar bears (Laidre et al. 2008). However, bowhead whales would only be accessible as rare beach-cast carcasses (Laidre et al. 2018) and previous QFASA estimates of narwhal consumption were <1% for EG polar bears (McKinney et al. 2013). Beluga whales and bowhead whales are present as potential cetacean prey or scavenge for polar bears within the SB region (Laidre et al. 2008, Rode et al. 2014); however, there is limited evidence of springtime predation of SB polar bears on cetaceans (Lowry et al. 1987). Some SB polar bears have been documented hunting and consuming entrapped beluga whales in springtime (Lowry et al. 1987, McKinney et al. 2017), but these events are considered to be infrequent, which may explain the lack of beluga DNA detected. In the summer and fall, some SB polar

bears shift to using onshore food resources as the sea ice extent declines (Schliebe et al. 2008, Atwood et al. 2016). In particular, large numbers of SB polar bears access 'bone piles', which consist of remains of subsistence-harvested bowhead whales, as well as other species left in the area (Atwood et al. 2016). Since the DNA metabarcoding approach probably only recovers recently consumed prey, if bowhead whale carcass consumption is largely in the fall, then it would be unlikely that bowhead DNA would be detected in fecal samples of spring sampled SB polar bears. For this reason, it would be useful to apply the DNA metabarcoding approach to analyze polar bear fecal samples collected in fall to provide greater insight into land-based feeding and seasonal differences in diets.

For most individual polar bears, when prey DNA was detected, it was largely just from a single species. This finding could be because polar bears are known to successfully capture prey somewhat infrequently, consuming perhaps the equivalent of around 1 adult ringed seal per week (Stirling & Archibald 1977, Stirling et al. 1982). Thus, the single prey species detected via prey DNA metabarcoding likely represents a single recent meal. For a minority of polar bears, a second prey species was detected in the same individual. The second prey always represented less than 15% RRA, suggesting perhaps several days had passed since this second prey species was consumed. This finding of just 1 or 2 prey species per individual polar bear sampled diverges from the wider range of prey estimated using polar bear feces collected on the landscape and assessed by the universal *Cytb* primer set (Michaux et al. 2021). This difference could be related to differences in methodological approaches (i.e. targeted pinniped and cetacean primers vs. universal primers) and sample location differences between the present study and Michaux et al. (2021). In addition, Michaux et al. (2021) used scat samples opportunistically collected from the landscape in May, which means factors such as a larger amount of starting material (scat), greater exposure to environmental DNA sources, and seasonal or regional differences in prey availability between sampling locations could have led to a greater variety of prey species being detected relative to the present study. An alternative explanation for low prey diversity seen in the DNA-based diet profiles of the present study is differential habitat preferences among prey species. For example, ringed and bearded seals have different sea ice habitat preferences; thus, with yearly variation in sea ice conditions in the Arctic, one species might be more prevalent in a given region in one year compared to another (Olnes et al. 2020, Von Duyke et al. 2020).

DNA-based prey detection frequencies and relative abundances were compared to QFASA prey detection frequencies and proportional diet estimates, respectively, to assess the utility of the DNA metabarcoding approach and potential complementarity of both methods. Prey detection frequencies using DNA metabarcoding were consistent with QFASA as well as other established methods (stable isotopes, direct observation, prey kill sites) (Stirling & Archibald 1977, Bourque et al. 2020, Stern et al. 2021) in identifying ringed seals as the predominant prey of SB polar bears, followed by bearded seals. After converting prey DNA sequence count data to relative abundance data for comparison to QFASA proportional estimates, the pattern of higher bearded seal (or other larger prey) consumption by adult males, and higher ringed seal consumption by adult females and subadults (although not tested statistically), was consistent with the QFASA estimates by sex/age class for SB polar bears in this study and earlier work (McKinney et al. 2017) and with sex/age differences found for other subpopulations (Thiemann et al. 2008, McKinney et al. 2013, Stern et al. 2021). DNA diet profiles from this study (both presence/absence and relative abundance) also suggested that cetacean species were not consumed, in contrast to the QFASA estimates and direct observations of SB polar bear feeding habits (Lowry et al. 1987, Herreman & Peacock 2013, McKinney et al. 2017). Again, this is likely, at least in part, related to differences in the time window captured by each method and possibly limited springtime access to cetacean prey species in both the EG and SB subpopulation geographic regions at the time of fecal sample collection.

The divergence in quantitative estimates between the 2 methods is likely, in part, due to differences in the timescale represented by each method (Iverson et al. 2004, Michaux et al. 2021). In general, prey consumed more than 1 to 2 wk prior to sample collection are unlikely to be detected by DNA-based approaches, due to prey DNA degradation by digestive enzymes and stomach bile (Dunshea 2009, Deagle et al. 2019). Nonetheless, in a controlled study specifically on captive polar bears fed a known diet, trace amounts of prey items were detected using a universal *Cytb* primer set approximately 3 wk post-consumption (Michaux et al. 2021). Relative to DNA approaches, QFASA likely represents a longer feeding window, possibly on the order of months for ursids. Controlled feeding trials on captive juvenile

brown bears *Ursus arctos* found that QFASA estimates represented diet over the last 1 to 3 mo (Thiemann et al. 2022). The difference in time period represented by the 2 approaches may explain why, for instance, bowhead whales were indicated as prey by QFASA but not by DNA metabarcoding, since SB polar bears are known to feed on bowhead carcasses onshore in the fall (Schliebe et al. 2008, Atwood et al. 2016), a time window unlikely to be captured by fecal DNA samples taken in the spring.

Other possible explanations for differences in the proportional estimates generated from DNA metabarcoding vs. QFASA may be related to particularities of the methods themselves. It is currently impossible to convert DNA count data to reliable diet estimates given the multi-copy nature of the mtDNA target gene fragment in eukaryotes, and without conducting a controlled feeding study prior to implementing newly designed primers in studies on free-ranging wildlife (Deagle et al. 2009, Dunshea 2009, Pompanon et al. 2012, Michaux et al. 2021). Certain primer sets can show preferential amplification of particular prey species compared to others (Pompanon et al. 2012); they can also show index hopping, and different tissues of the same prey species often have different mtDNA densities (Veltri et al. 1990, Deagle et al. 2005, Guenay-Greunke et al. 2021). For our study, it is also possible that the probability of detection of cetacean species was diminished, given that only 1 of the 2 cetacean primer sets was effective (Pompanon et al. 2012, Deagle et al. 2019). In addition, both prey DNA and QFASA will only return prey they are set up to detect: without specially designed primers for prey DNA or particular prey in the library for QFASA, certain prey species may not be identified, such as shorebirds or their eggs, which could be a minor component of the polar bears' diets in some regions (Bourque et al. 2020). Further, for QFASA, prey species that show similar FA composition can be difficult to differentiate (Iverson et al. 2004, McKinney et al. 2013). Despite these potential caveats, these 2 diet approaches may be complementary in representing diets in different seasons, and thus may contribute new insight into temporal variation in polar bear diets. Our findings suggest that prey DNA results should be interpreted as 'short-term' presence/absence estimates, whereas QFASA better represents 'long-term' diet estimates.

The DNA diet profile term ('prey DNA detected' or 'prey DNA not detected') was significantly correlated with gut bacterial composition for EG and SB bears, but not with alpha diversity. Changes in composition but not alpha diversity of the gut microbiome have similarly been found in studies examining the inherent dietary changes due to captivity on gut microbiota for multiple mammalian wildlife species (McKenzie et al. 2017, Haworth et al. 2019). Polar bear sex/age class also significantly explained variation in gut microbiota. Morphological variation, energetic requirements, reproductive status, and spatial segregation vary among polar bear sex/age classes and thus lead to diet differences and differences in prey selection among them (Atwood et al. 2017, McKinney et al. 2017, Bourque et al. 2020, Florko et al. 2020). As such, it is likely that the interplay of diet and sex/age class differences explains some of the additional variation seen in polar bear gut bacterial composition.

For EG and SB polar bears, Clostridia abundances were higher and Bacilli showed a tendency to be higher in individuals for which prey DNA was detected (ringed or bearded seals) vs. individuals without prey DNA detected. Clostridia is a bacterial class constituting a significant proportion of the polar bear core gut microbiome (Glad et al. 2010, Watson et al. 2019), and higher Clostridia counts with recent feeding supports its suggested functional role in lipid metabolism and fat deposition (Hildebrandt et al. 2009, Watson et al. 2019). Bacilli are an important class of bacteria for maintaining gut health (Ilinskaya et al. 2017), so we speculate that consumption of traditional ice seal prey may help maintain gut homeostasis in polar bears. Bacilli were previously found to be higher in EG and adult female polar bears compared to SB and adult male polar bears, respectively (Franz et al. 2022). More EG polar bears and adult females tended to have prey DNA detected compared to SB and adult male polar bears, suggesting that previously identified patterns of Bacilli with respect to subpopulation and sex/age class may in part be related to feeding.

Although not significant, Negativicutes showed patterns of being reduced in polar bears where prey DNA was detected compared to those where no prey DNA was detected, particularly those genera and ASVs assigning to *Megasphaera* species. More Negativicutes were previously found in SB polar bears than in EG polar bears (Franz et al. 2022). Less is known about the class Negativicutes relative to Clostridia and Bacilli, yet human gut microbiome studies suggest some genera found within the class Negativicutes serve as indicator species for host disease states following perturbation of the gut flora (Könönen 2015). Other research has suggested that some Negativicutes are part of the commensal microbiota and can be involved with polysaccharide

metabolism, producing short chain FAs that help maintain gut health (Shetty et al. 2013, Zhang et al. 2013, Sergeant et al. 2014, Michaux et al. 2021). Deusch et al. (2014) found significant increases in *Megasphaera* sp. in kittens fed a mixed proteinaceous/carbohydrate diet, while Franz et al. (2022) suggested that increased Negativicutes could reflect carbohydrate or starch input from onshore food resources or protein input from increased use of bowhead whale 'bone piles'. Terrestrial foraging typically occurs in the summer and fall months for polar bears in the SB subpopulation, and the 'bone piles' are a particularly important food resource for adult male polar bears (Miller et al. 2015), which were found to have higher levels of Negativicutes compared to other sex/age classes (Franz et al. 2022). It is possible that these more long-term diet habits of some of the onshore bears within the SB subpopulation are contributing to this possible pattern of Negativicutes being reduced in individuals with prey DNA detected, as long-term diet is a known driver of gut bacterial composition (Debelius et al. 2016). Although certain gut bacteria vary in function between host species, it is plausible that there are links between higher abundance of Negativicutes and the use of land-based food resources by some SB polar bears, as well as adult male polar bear-specific foraging tendencies. We speculate that changing habitats and feeding habits may be leading to shifts in metabolic functionality of some bacteria in the gut microbiome of SB polar bears to accommodate seasonal reductions in availability of ice-associated, lipid-rich prey. Studies on long-term changes in the polar bear gut microbiome and the functional role of specific bacterial groups within their wildlife hosts are needed.

With molecular diet analysis now available, this method will likely increase in use for studying the diets of free-ranging wildlife and, as we have shown here, can be helpful in interpreting gut bacterial community data. It is also beneficial that prey DNA metabarcoding and gut microbiome data can be obtained from the same fecal DNA extract. The DNA-based diet profiles appeared to be credible, given the similar dietary patterns among sex/age classes as found by QFASA. The 2 techniques provided complementary yet uncorrelated results, likely a consequence of the approaches representing different, minimally overlapping dietary time periods — DNA metabarcoding appeared to reflect more short-term diets while QFASA appeared to reflect more long-term diets. Thus, this study underscores the importance of using complementary diet assessment techniques to reveal spatio-temporal variation in dietary patterns for wildlife species (Nielsen et al. 2018, Deagle et al. 2019, Nelms et al. 2019, Michaux et al. 2021). Given the strong influence of short-term diet on gut bacterial composition, this aspect of polar bear health should continue to be monitored as polar bears undergo large-scale climate-induced changes in foraging ecology. An important future direction could include testing for associations between long-term diet and gut bacterial composition.

Acknowledgements. Thanks to K. Simac, A. Pagano, E. Peacock, T. Donnelly, and G. Durner at the USGS Alaska Science Center for SB field sampling and sample coordination. Thanks to P. Hegelund and C. Isaksen for EG field work, and to DANCEA and the Government of Greenland for support of the EG field activities. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The laboratory work was funded by a U.S. Geological Survey grant (to M.A.M., #G19AC00335), the Canada Research Chairs Program (to M.A.M., 950–232183), a Canada Foundation for Innovation Grant (to M.A.M., 37873), and the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants Program (to M.A.M., RGPIN-2019–05330).

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Editorial responsibility: Robert M. Suryan, Juneau, Alaska, USA
Reviewed by: 3 anonymous referees

Submitted: April 25, 2022
Accepted: December 12, 2022
Proofs received from author(s): January 21, 2023