Table S1. List of all marine vertebrate species known to co-occur with or be a component of EG and SB polar bear diets that were used for primer design and construction of the custom reference database.

Common name(s)	Scientific name(s)	Clade/Infraorder
Arctic species:		
Bowhead whale	*Balaena mysticetus*	Cetacea
Beluga whale, white whale, sea canary	*Delphinapterus leucas*	Cetacea
Narwhal, narwhale	*Monodon monoceros*	Cetacea
Walrus	*Odobenus rosmarus*	Pinnipediformes
Bearded seal, square flipper seal	*Erignathus barbatus*	Pinnipediformes
Ringed seal, jar seal, netsik, nattiq	*Pusa hipsida, Phoca hispida*	Pinnipediformes
Subarctic species:		
Ribbon seal	*Phoca fasciata, Histriophoca fasciata*	Pinnipediformes
Harp seal, saddleback seal, Greenland Seal	*Pagophilus groenlandicus*	Pinnipediformes
Spotted seal, larga seal, largha seal	*Phoca largha*	Pinnipediformes
Hooded seal	*Cystophora cristata*	Pinnipediformes
Additional cetacean species occasionally accessible to/overlapping with polar bears:		
Common minke whale, northern minke whale	*Balaenoptera acutorostrata*	Cetacea
Sei whale	*Balaenoptera borealis*	Cetacea
Blue whale	*Balaenoptera musculus*	Cetacea
Fin whale, finback whale, common rorqual, herring whale, razorback whale	*Balaenoptera physalus*	Cetacea
Humpback whale	*Megaptera novaeangliae*	Cetacea
Long-finned pilot whale	*Globicephala melas*	Cetacea
White-beaked dolphin	*Lagenorhynchus albirostris*	Cetacea
Killer whale, orca	*Orcinus orca*	Cetacea

Table S2. Cetacean and pinniped cytochrome oxidase 1 (CO1) and cytochrome b (cytb) prey-specific primer sequences and associated range of melting temperatures. F = forward direction , R = reverse direction.

Primer	**Direction**	**Primer Sequence (5'-3')**	Melting Temperature			Fragment size (bp)	Optimal annealing temperature (°C)
			Min. (°C)	Mean (°C)	Max. (°C)		
Pinniped_CO1_17	F	SGGRACYGGRTGAACCG	60.4	63.8	67.5	261	59
Pinniped_CO1_17	R	RRYATRGTRATRCCAGC	48.8	56.2	64.4		
*Cetacea_CO1_F4_R5	F	TAGCACATGCAGGAGC	60.7	60.5	60.8	269-273	62
*Cetacea_CO1_F4_R5	R	CCTCCDCCYGCMGGGTC	64	67.7	72.3		
Pinniped_Cytb1_2	F	YCAYCAGCACCCAAAGC	61	62.4	64.3	103-391	62
Pinniped_Cytb1_2	R	GCTTATATGCATGGGGC	-	58.7	-		
Cetacea_cytb_Forward_1	F	RYACAAATYYTAACAGG	48.8	52	56.3	266-269	51
Cetacea_cytb_Forward_1	R	ACRTARCCYACGAATGC	55.3	59	63.1		

*The Cetacea_CO1_F4_R5 primer set successfully amplified pure cetacean DNA extract in earlier lab trials, however following amplification issues after the addition of CS1 and CS2 tags (see main text) it was dropped from the current study

Table S3. List of prey DNA extracts used for prey primer testing and optimization.

Sample origin	Sample ID	DNA Extract ID	Order	Genus	Species	Common name
East Greenland	Harp seal F	H.Seal F	Pinniped	*Pagophilus*	*groenlandicus*	Harp seal
East Greenland	Harp seal G	H.Seal G	Pinniped	*Pagophilus*	*groenlandicus*	Harp seal
SIMEP	ARRB-17-0001	RS 0001	Pinniped	*Pusa*	*hispida*	Ringed seal
SIMEP	ARRB-17-0004	RS 0004	Pinniped	*Pusa*	*hispida*	Ringed seal
SIMEP	ARIQ-DFO-2134	Walrus 1	Pinniped	*Odobenus*	*rosmarus*	Walrus
SIMEP	ARIQ-DFO-2126	Walrus 2	Pinniped	*Odobenus*	*rosmarus*	Walrus
East Greenland	GM-14	PW-14	Cetacea	*Globicephala*	*melas*	Pilot whale
East Greenland	GM-15	PW-15	Cetacea	*Globicephala*	*melas*	Pilot whale
East Greenland	Mn-17	H.Whale	Cetacea	*Megaptera*	*novaeagliae*	Humpback whale
SIMEP	ARRB-XX-1403	Narwhal 1	Cetacea	*Monodon*	*monocerus*	Narwhal
SIMEP	ARRB-XX-1411	Narwhal 2	Cetacea	*Monodon*	*monocerus*	Narwhal
SIMEP	ARRB-XX-1415	Beluga 2	Cetacea	*Delphinapterus*	*leucas*	Beluga whale
SIMEP	ARRB-XX-1408	Beluga 2	Cetacea	*Delphinapterus*	*leucas*	Beluga whale
East Greenland	Ba-0007	Minke 1	Cetacea	*Balaenoptera*	*acutorostrata*	Minke whale
East Greenland	Ba-0008	Minke 2	Cetacea	*Balaenoptera*	*acutorostrata*	Minke whale
East Greenland	Orca 1 (Killer whale 1)	Orca 1	Cetacea	*Orcinus*	*orca*	Killer whale
East Greenland	2017_0003	Orca 2	Cetacea	*Orcinus*	*orca*	Killer whale
East Greenland	Mn_002	H. Whale 002	Cetacea	*Megaptera*	*novaeagliae*	Humpback whale
East Greenland	Mn_005	H. Whale 005	Cetacea	*Megaptera*	*novaeagliae*	Humpback whale

Table S4. Summary of sequence reads obtained after sequence processing and quality check (QC) from the starting 93 polar bear fecal samples and percentages of samples for which ringed seal and bearded seal prey were detected using the Pinniped Cytochrome b [Pinniped Cytb] and Pinniped Cytochrome Oxidase 1 [Pinniped CO1]) primer sets. Individual and combined results are both listed.

Primer set	Starting # of Samples	# Samples after QC	Total # reads obtained	Average # Reads/Sample	Min count	Max count	%samples prey detected	% ringed seal detected	% bearded seal detected	%No prey detected
Pinniped Cytb	93	86	1,589,939	729± 350	0	116,521	26.7	25.6	2.33	73.3
Pinniped CO1	93	90	1,147,797	11,183± 3849	0	226,272	51.1	50	6.67	48.9
Combined Primer Sets	93	92	2,737,736	11,913± 4019	0	234,686	53.3	52.2	6.52	46.7

Table S5. Comparison of the proportions (%) of each prey in the diet based on DNA metabarcoding (i.e., prey DNA relative abundances) versus quantitative fatty acid signature analysis (QFASA) for a subset of Southern Beaufort Sea (SB) polar bears for which prey DNA was detected.

		Ringed seal (%)		**Bearded seal (%)**		**Beluga whale (%)**		**Bowhead whale (%)**	
Sample ID	**Sex/Age Class**	DNA-based	QFASA	DNA-based	QFASA	DNA-based	QFASA	DNA-based	QFASA
SB_5	AM	ND	33	100	60	-	0	-	7
SB_6	AM	0.2	44	99.8	35	-	0	-	21
SB_7	AM	8	27	92	17	-	0	-	56
SB_9	AM	100	38	0	58	-	0	-	4
SB_40	AM	100	7.4	0	73	-	0	-	19
SB_54	AM	100	18	0	80	-	0	-	18
SB_58	AM	100	26	0	23	-	0	-	51
SB_38	AF	1	42	99	50	-	0	-	8
SB_44	AF	100	0.2	0	98	-	0	-	1
SB_45	AF	100	20	0	73	-	0	-	8
SB_46	AF	100	89	0	10	-	0	-	2
SB_14	AF	100	48	0	1	-	8	-	42
SB_17	AF	100	77	0	10	-	0	-	13
SB_20	AF	100	73	0	0	-	27	-	0
SB_21	AF	100	69	0	28	-	3	-	0
SB_48	AF	100	56	0	42	-	0	-	1
SB_47	S	14	59	86	10	-	0	-	31
SB_39	S	100	83	0	17	-	0	-	0
SB_22	S	100	81	0	2	-	0	-	17
SB_43	S	100	55	0	30	-	0	-	15
Mean proportion consumed		76.1 ± 9.5	47.3 ± 5.9	23.8 ± 9.5	35.8 ± 6.6	-	1.9 ± 1.4	-	15.7 ± 3.8

Table S6. Summary of linear regression models showing that none of the explanatory variables explained a significant amount of the variation in Shannon and Inverse Simpson indices of alpha diversity for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears. There was a near-significant effect of sex/age class in linear regression model for Faith's phylogenetic diversity (FPD) indicating lower FPD in subadults compared to other sex/age classes, however the overall model is not significant. Models incorporate DNA diet profiles (categorical diet variable: 'prey DNA detected' or 'prey DNA not detected').

Shannon Diversity (Adj. $R^2 = 0.00$, $p = 0.76$)					
Full Model:	~ DNA diet profile + Sex/Age Class + Subpopulation + Body Condition + Sex/Age Class*Subpopulation + Body Condition*Subpopulation				
	Coefficients	Estimate	Std.Error	tvalue	Pr(>|t|)
	(Intercept)	2.53	0.33	7.62	0.00
	DNA diet profile (Prey DNA detected)	-0.01	0.11	-0.11	0.92
	Sex/age class (Adult male)	0.01	0.57	0.02	0.99
	Sex/age class (Cub)	0.11	0.27	0.39	0.70
	Sex/age class (Subadult)	-0.03	0.25	-0.11	0.91
	Subpopulation (SB)	0.27	0.43	0.63	0.53
	Body Condition	0.00	0.00	0.30	0.77
	Subpopulation (SB): Sex/age class (Adult male)	-0.08	0.64	-0.13	0.90
	Subpopulation (SB): Sex/age class (Cub)	-0.07	0.41	-0.16	0.87
	Subpopulation (SB): Sex/age class (Subadult)	-0.36	0.32	-1.11	0.27
	Subpopulation (SB): Body Condition	0.00	0.00	-0.16	0.87
Inverse Simpson Diversity (Adj. $R^2 = 0.00$, $p = 0.82$)					
Full Model:	~ DNA diet profile + Sex/Age Class + Subpopulation + Body Condition + Sex/Age Class*Subpopulation + Body Condition*Subpopulation				
	Coefficients	Estimate	Std.Error	tvalue	Pr(>|t|)
	(Intercept)	5.59	2.79	2.01	0.05
	DNA diet profile (Prey DNA detected)	0.10	0.93	0.11	0.91
	Sex/age class (Adult male)	-1.88	4.82	-0.39	0.70
	Sex/age class (Cub)	0.82	2.26	0.36	0.72
	Sex/age class (Subadult)	-0.41	2.14	-0.19	0.85
	Subpopulation (SB)	3.25	3.64	0.89	0.38
	Body Condition	0.01	0.01	0.91	0.37
	Subpopulation (SB): Sex/age class (Adult male)	1.35	5.37	0.25	0.80
	Subpopulation (SB): Sex/age class (Cub)	-0.06	3.42	-0.02	0.99
	Subpopulation (SB): Sex/age class (Subadult)	-1.66	2.69	-0.62	0.54
	Subpopulation (SB): Body Condition	-0.01	0.02	-0.59	0.56

Faiths Phylogenetic Diversity (Adj. $R^2 = 0.05$, $p = 0.18$)					
Full Model:	~ DNA diet profile + Sex/Age Class + Subpopulation + Body Condition + Sex/Age Class*Subpopulation + Body Condition*Subpopulation				
	Coefficients	Estimate	Std.Error	tvalue	Pr(>|t|)
	(Intercept)	13.65	1.83	7.47	0.00
	DNA diet profile (Prey DNA detected)	-0.01	0.61	-0.02	0.99
	Sex/age class (Adult male)	1.17	3.16	0.37	0.71
	Sex/age class (Cub)	-0.28	1.48	-0.19	0.85
	Sex/age class (Subadult)	-2.48	1.40	-1.77	0.08
	Subpopulation (SB)	1.13	2.39	0.48	0.64
	Body Condition	0.00	0.01	-0.29	0.77
	Subpopulation (SB): Sex/age class (Adult male)	-3.30	3.52	-0.94	0.35
	Subpopulation (SB): Sex/age class (Cub)	-1.33	2.24	-0.59	0.56
	Subpopulation (SB): Sex/age class (Subadult)	-0.42	1.76	-0.24	0.81
	Subpopulation (SB): Body Condition	0.00	0.01	0.24	0.81

Table S7. Results of analysis of composition with bias correction (ANCOMBC) showing the bacterial classes that differed or showed patterns of differential abundance based on DNA diet profile (i.e. increased/decreased in 'prey DNA detected' individuals compared to 'prey DNA not detected' individuals) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears. (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05). Coef. = log-transformed change in abundance, SE = standard error of the coefficient, W = Coef./SE.

Class	*Coef. (Prey DNA detected vs. Prey DNA not detected)*	*SE*	*Test statistic (W)*	*p-value*	*Adj. P-value*	*Diff. Abun*
Clostridia	**1.15**	**0.34**	**3.41**	**<0.001**	**0.009**	**TRUE**
Negativicutes	**-1.26**	**0.50**	**-2.52**	**0.01**	**0.12**	**FALSE**
Bacilli	0.90	0.44	2.07	0.04	0.41	FALSE

Table S8. Results of analysis of composition with bias correction (ANCOMBC) showing the bacterial genera that differed or showed patterns of differential abundance based on DNA diet profile (i.e. increased/decreased in 'prey DNA detected' individuals compared to 'prey DNA not detected' individuals) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears. (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05). Coef. = log-transformed change in abundance, SE = standard error of the coefficient, W = Coef./SE.

	Genus	*Coef. (Prey DNA detected vs. Prey DNA not detected)*	*SE*	*Test statistic (W)*	*p-value*	*Adj. P-value*	*Diff. Abun*
1	***Terrisporobacter***	**2.55**	**0.60**	**4.27**	**0**	**0.002**	**TRUE**
2	***Halomonas***	**-1.26**	**0.35**	**-3.57**	**0**	**0.034**	**TRUE**
3	***Clostridium_sensu_stricto_1***	**2.04**	**0.61**	**3.34**	**0.001**	**0.08**	**FALSE**
4	*Blautia*	2.02	0.62	3.23	0.001	0.117	FALSE
5	*Erysipelatoclostridium*	1.66	0.52	3.23	0.001	0.118	FALSE
6	*Megasphaera*	-2.16	0.67	-3.21	0.001	0.123	FALSE
7	*Romboutsia* (	1.95	0.63	3.07	0.002	0.199	FALSE

Table S9. Results of analysis of composition with bias correction (ANCOMBC) showing the bacterial amplicon sequence variants (ASVs) that differed in abundance based on DNA diet profile (i.e. increased/decreased in 'prey DNA detected' individuals compared to 'prey DNA not detected' individuals) for East Greenland (EG) and Southern Beaufort Sea (SB) polar bears. (False discovery rate (FDR) adjusted *p*-value cutoff: 0.05). Coef. = log-transformed change in abundance, SE = standard error of the coefficient, W = Coef./SE.

	ASV	Class	Genus	*Coef. (Prey DNA detected vs. Prey DNA not detected)*	*SE*	*Test statistic (W)*	*p-value*	*Adj. P-value*	*Diff. Abun*
1	**ASV_36**	**Clostridia**	***Terrisporobacter***	**2.04**	**0.58**	**3.54**	**< 0.001**	**0.01**	**TRUE**
2	**ASV_79**	**Clostridia**	***Paeniclostridium***	**1.69**	**0.45**	**3.73**	**< 0.001**	**0.05**	**TRUE**
3	**ASV_356**	**Bacilli**	***NA***	**1.19**	**0.33**	**3.62**	**< 0.001**	**0.07**	**FALSE**
4	ASV_3	Negativicutes	*Megasphaera*	-2.17	0.66	-3.31	< 0.001	0.23	FALSE
5	ASV_60	Clostridia	*Clostridium sensu stricto 1*	1.68	0.50	3.35	< 0.001	0.20	FALSE
6	ASV_62	Bacilli	*Erysipelatoclostridium*	1.58	0.49	3.20	< 0.001	0.34	FALSE
7	ASV_285	Clostridia	*Clostridium sensu stricto 1*	1.12	0.36	3.15	< 0.001	0.39	FALSE
8	ASV_321	Clostridia	*NA*	1.10	0.35	3.15	< 0.001	0.39	FALSE
9	ASV_220	Gammaproteobacteria	*Conchiformibius*	-1.03	0.35	-2.98	< 0.001	0.69	FALSE
10	ASV_150	Clostridia	*Clostridium sensu stricto 1*	1.14	0.40	2.89	< 0.001	0.91	FALSE

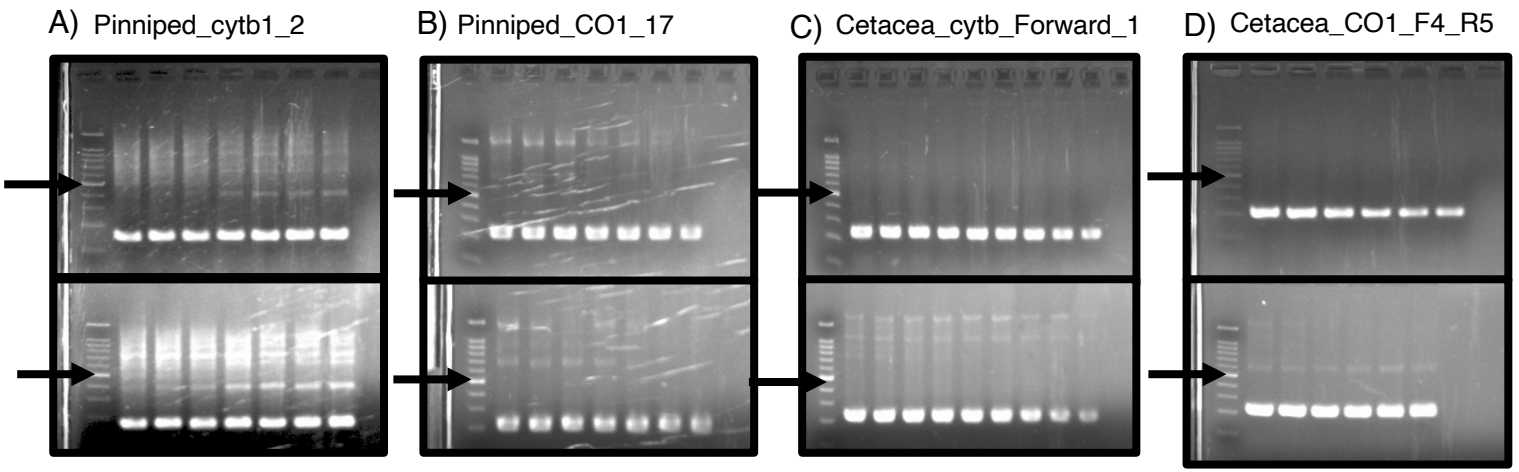

Figure S1. Gel electrophoresis images showing results of separate temperature gradient polymerase chain reaction (PCR) reactions to find optimal primer annealing temperature for each polar bear prey DNA group primer set. **A)** Pinniped_cytb1_2: 51-57 °C gradient (Annealing temperatures, left to right: 51.1, 52.4, 53.7, 54.8, 55.8, 56.5, 56.9 °C). Top gel: harp seal (*Pagophilus groenlandicus*), bottom gel ringed seal (*Pusa hispida*); **B)** Pinniped_CO1_17: 55-60 °C gradient (Annealing temperatures, left to right: 55.0, 55.2, 55.8, 56.7, 57.8, 59.1, 60.4 °C). Top gel: harp seal (*Pagophilus groenlandicus*), bottom gel: ringed seal (*Pusa hispida*); **C)** Cetacea_cytb1_Forward_1: 47-55 °C gradient (Annealing temperatures, left to right: 47.1, 47.3, 47.9, 48.8, 49.9, 51.1, 52.4, 53.7, 54.8 °C). Top gel: Humpback whale (*Megaptera novaeagliae*), bottom gel: Long-finned pilot whale (*Globicephala melas)* **D)** Cetacea COI_F4_R5: 60-65 °C gradient (Annealing temperatures, left to right: 60.4, 61.7, 62.9, 63.9, 64.6, 64.9 °C). Top gel: Humpback whale (*Megaptera novaeagliae*), bottom gel: Long-finned pilot whale (*Globicephala melas).* 100 base pair (bp) ladder used in each image. Black arrow indicates 500bp fragment band.

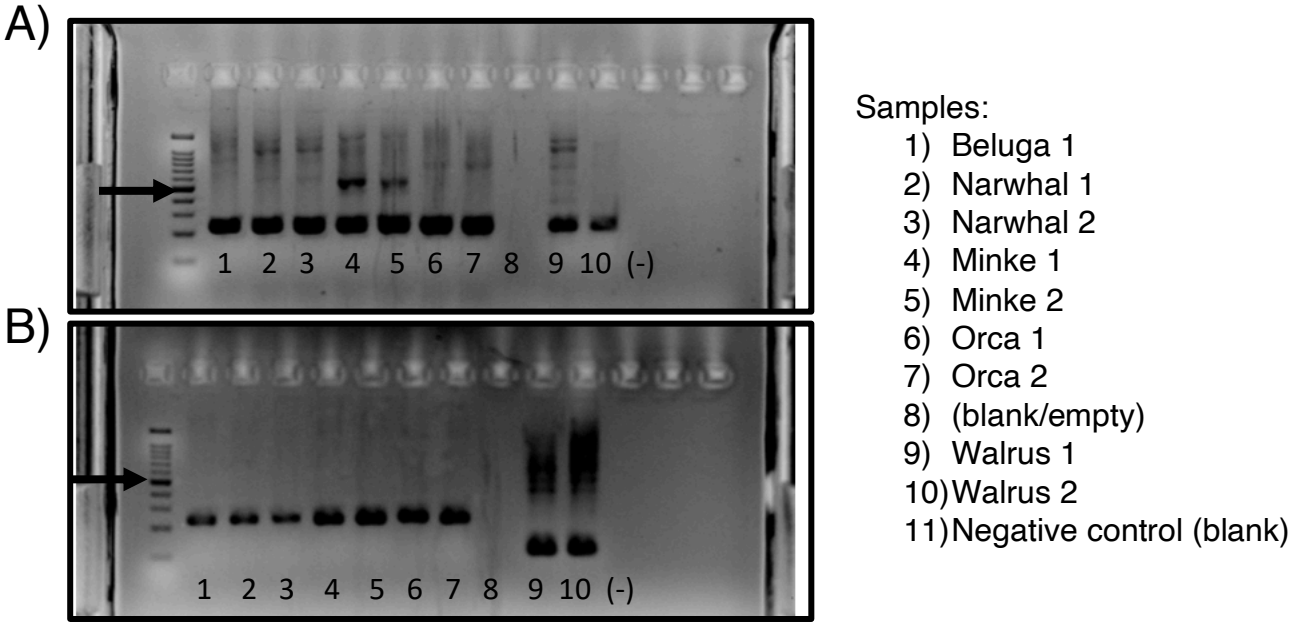

Figure S2. Gel electrophoresis images showing successful amplification of remaining available prey DNA extracts using the optimal annealing temperatures determined previously for the newly developed prey-specific primer sets . A) Samples 1-7 were amplified using the cetacea_cytb_Forward_1 primer set and samples 9-10 were amplified using the pinniped_cytb1_2 primer set B) Samples 1-7 were amplified using the cetacea_CO1_F4_R5 primer set and samples 9-10 were amplified using the pinniped_CO1_17 primer set. A 100 base pair (bp) ladder was used in both images and black arrows indicate the 500bp fragment size. All PCR reactions were run using the shared optimal annealing temperature of 54 °C. Well 11 contained an Rnase-free water negative control sample.

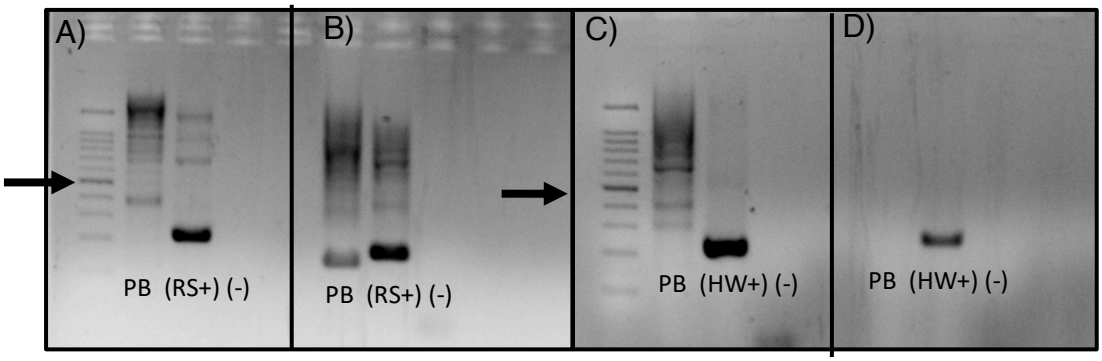

Figure S3. Gel electrophoresis images showing polymerase chain reaction (PCR) test results for polar bear DNA extract (PB) using the **A)** pinniped_CO1_17 primer set **B)** pinniped_cytb1_2 primer set **C)** cetacea_CO1_F4_R5 primer set and **D)** cetacea_cytb_Forward_1 primer set. For both pinniped primer sets the ringed seal 1 positive control (RS+) was used and for both cetacea primer sets the humpback whale 005 positive control (HW+) was used. All negative controls (-) were Rnase-free water PCR blanks. A 100 base pair (bp) ladder was used in all images and black arrows indicate the 500bp fragment size band. All PCR reactions were run using a shared optimal annealing temperature of 54 °C.

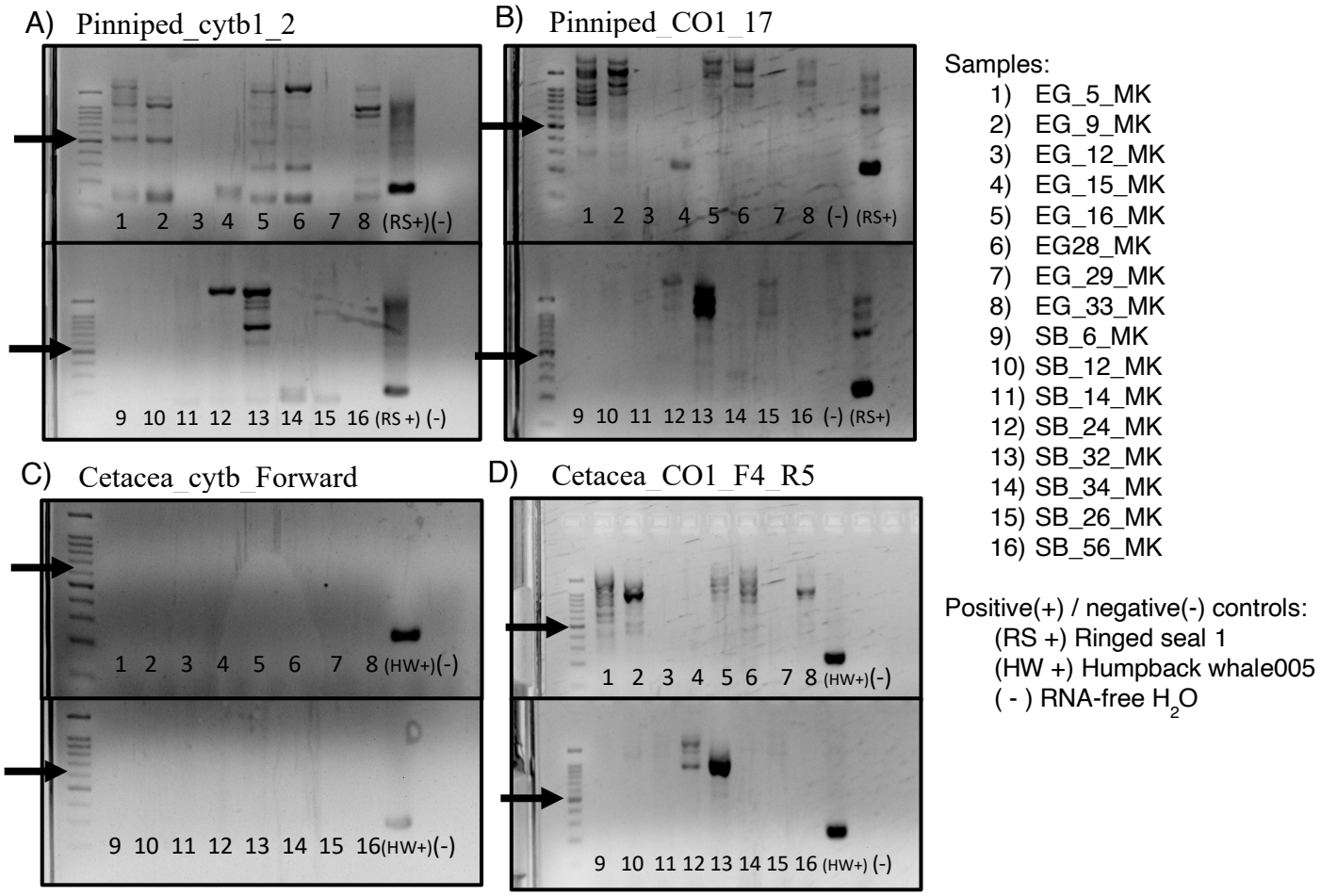

Figure S4. Gel electrophoresis images showing polymerase chain reaction (PCR) test results for sixteen randomly selected East Greenland (EG) and Southern Beaufort Sea (SB) polar bear fecal extract samples using the **A)** pinniped_cytb1_2 primer set **B)** pinniped_CO1_17 primer set C) cetacea_cytb_Forward_1 primer set and **D)** cetacea_CO1_F4_R5 primer set. A 100 base pair (bp) ladder was used in all images and black arrows indicate the 500bp fragment size band. All PCR reactions were run using a shared optimal annealing temperature of 54 °C.

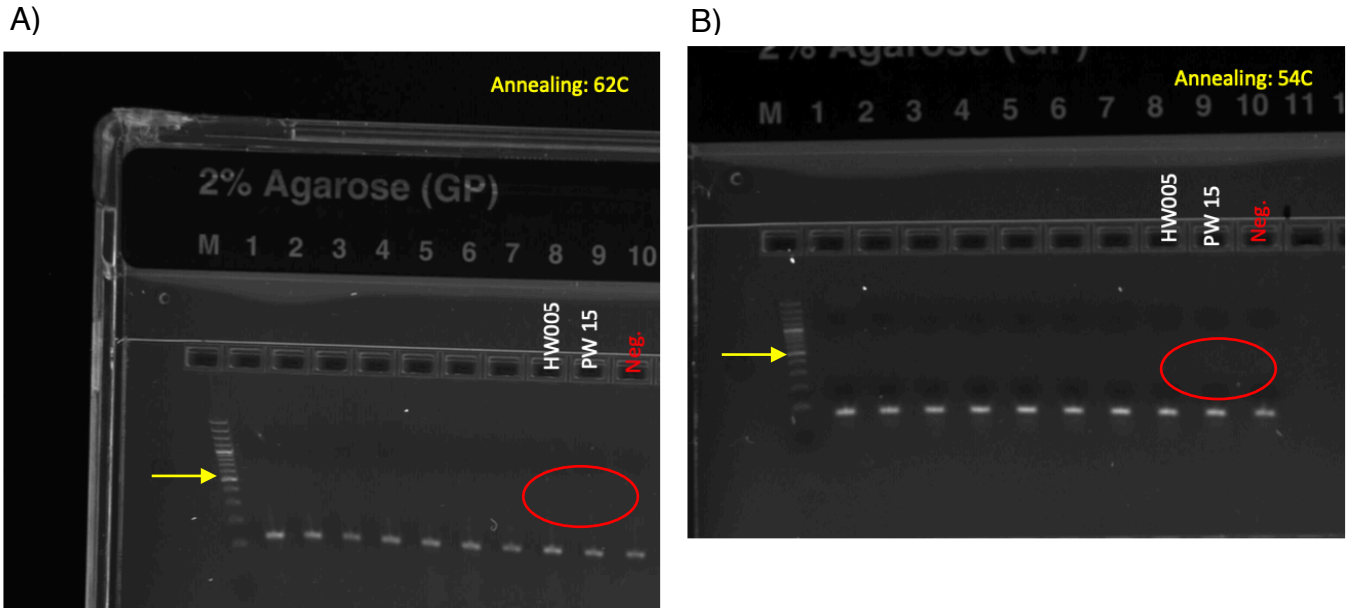

Figure S5. Gel electrophoresis images showing results of two test polymerase chain reaction (PCR) experiments run at a A) 62 °C annealing temperature and a B) 54 °C annealing temperature on a few randomly selected polar bear fecal extracts and using humpback whale (HW005) and pilot whale (PW 15) PCR positive controls. Absence of positive control bands at the expected ~260 base pair (bp) fragment size (red circles) indicates failed PCR amplification. A 100bp ladder was used in both images. Bands that appear at the bottom of each gel image indicate primer-dimer formation. Yellow arrows indicate 500bp fragment size.

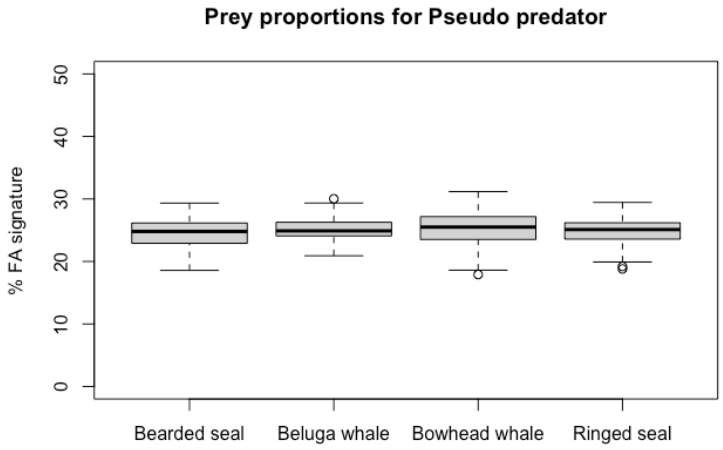

Figure S6. Boxplots showing results of quantitative fatty acid signature analysis (QFASA) pseudo predator analysis. Psuedo-predator diet prey proportions were set at 25% Bearded seal, 25% Beluga whale, 25% bowhead whale, and 25% ringed seal prior to running the simulation and were estimated with high accuracy.

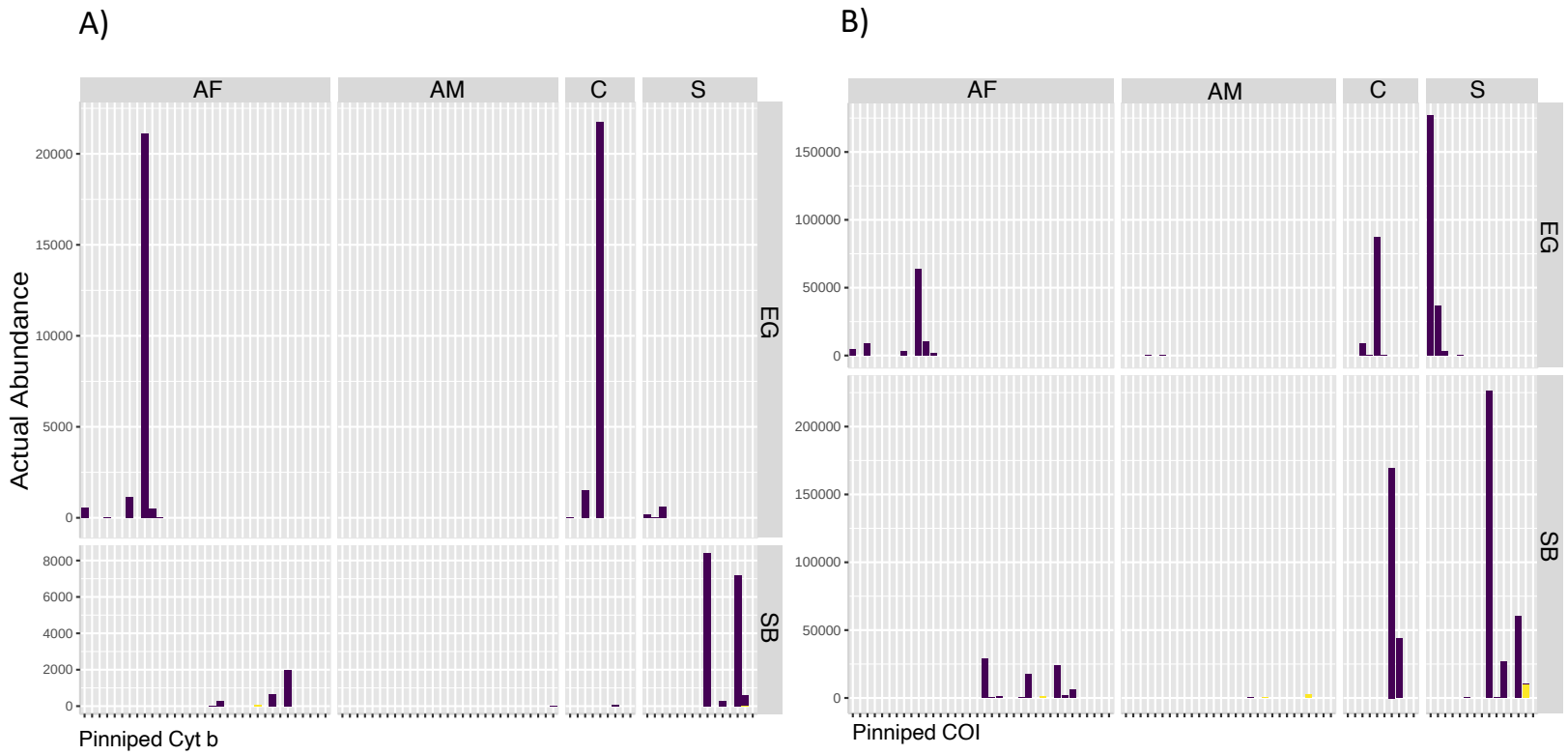

Figure S7. Grouped barplot showing the actual abundances (i.e. sample sequence coverage) of ringed seal (Pusa) and bearded seal (Erignathus) prey detected among polar bear sex/age classes (Adult female [AF], adult male [AM], cubs [C] and subadults [S] in East Greenland (EG) and Southern Beaufort Sea (SB) polar bear fecal samples using A) the Pinniped_Cytb1_2b (Pinniped Cyt b) primer set, B) the Pinniped_CO1_17 (Pinniped CO1) primer set, and C) the Cetacea_cytb_Forward_1 (Cetacea Cyt b) primer set.

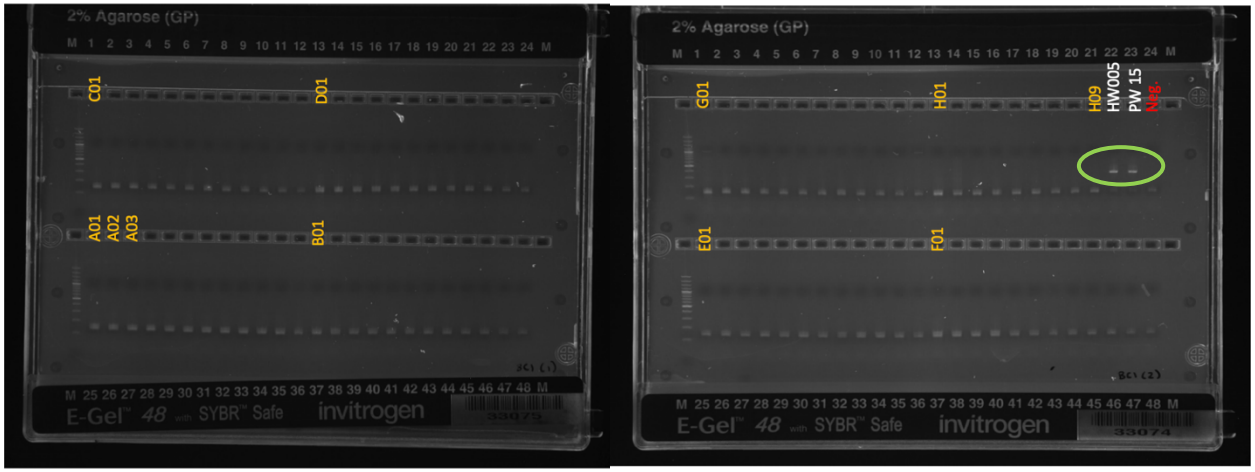

Figure S8. Gel electrophoresis image showing successful amplification of humpback whale 005 (HW005) and pilot whale 15 (PW 15) positive controls (green circle) using the Cetacea_Cytb_F1 primer set. Image also shows minimal or nonexistent amplification of cetacean DNA in the 93 polar bear fecal extracts (i.e. lack of bands at ~260 base pair (bp) fragment size for the remaining gel wells pictured). A 100bp ladder was used in both images.

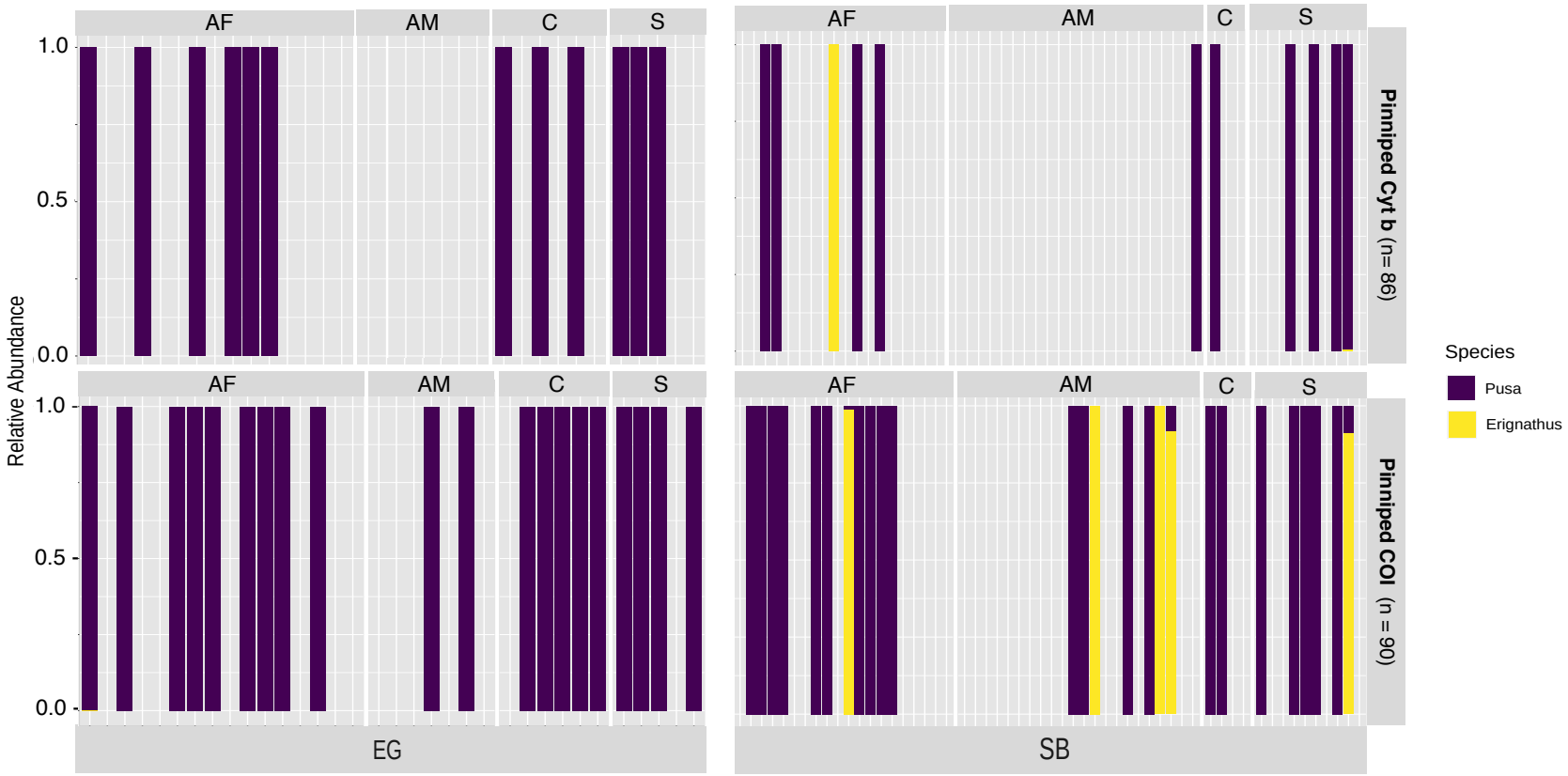

Figure S9. Grouped barplot showing the relative abundances of ringed seal (Pusa) and bearded seal (Erignathus) prey detected among different polar bear sex/age classes (Adult female [AF], adult male [AM], cubs [C] and subadults [S] in East Greenland (EG) and Southern Beaufort Sea (SB) polar bear fecal samples using A) the Pinniped_Cytb1_2b (Pinniped Cytb) primer set and B) the Pinniped_CO1_17 (Pinniped CO1) primer set.

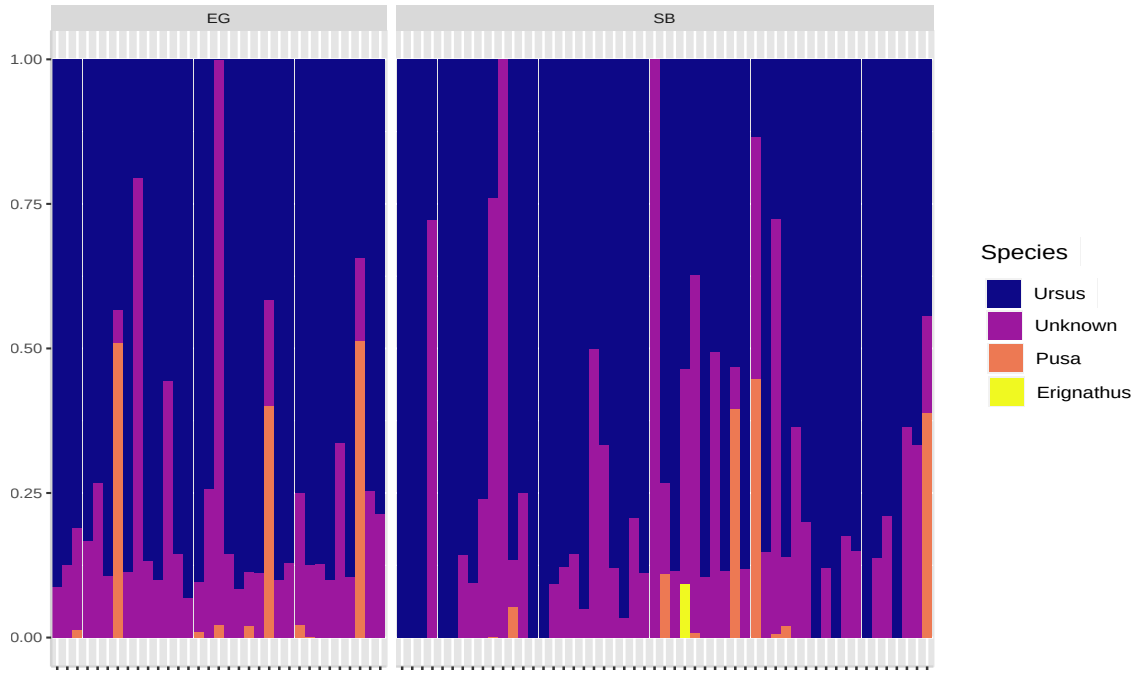

Figure S10. Grouped relative abundance barplot showing the proportions of polar bear and brown bear (grouped under 'Ursus'), unknown exact sequence variants (Unknown), ringed seal (Pusa) and bearded seal (Erignathus) sequences detected in East Greenland (EG) and Southern Beaufort Sea (SB) polar bear fecal samples using the Pinniped_Cytb1_2b (Pinniped Cyt b) primer set. Polar bear DNA was found in a majority of samples (83/86, or 96.5%). Each vertical bar represents an individual polar bear.

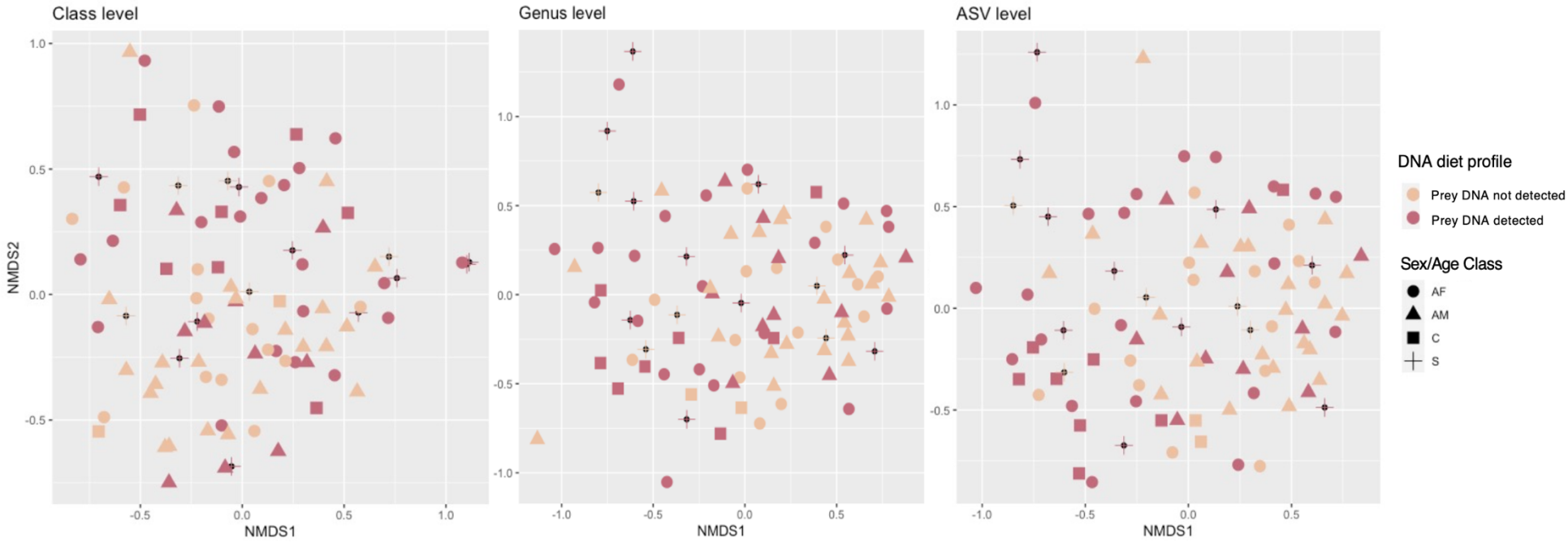

Figure S11. Non-metric multi-dimensional scaling (NMDS) plots showing gut bacterial composition at bacterial Class, Genus, and ASV levels for individuals where prey DNA was detected or prey DNA was not detected. Points represent individual polar bear fecal samples from the East Greenland and Southern Beaufort Sea subpopulations. Color denotes whether prey DNA was detected or not, and shape denotes polar bear Sex/Age Classes (adult females [AF], adult males [AM] and subadults [S] compared to cubs [C]). Bray–Curtis distances were calculated at each bacterial level (Class level: Stress = 0.22, PERMANOVA: $R^2 = 0.03$, $p = 0.01$; Genus level: Stress = 0.23, PERMANOVA: $R^2 = 0.03$, $p = 0.003$; ASV level: Stress = 0.24, PERMANOVA: $R^2 = 0.03$, $p < 0.001$)

Text S1.

Performance of prey-specific primer sets

A total of 10,559,615 combined raw reads were obtained from the multiplexed sequencing run containing Pinniped_Cytb1_2b (pinniped Cytb), Pinniped_CO1_17 (pinniped CO1), and Cetacea_cytb_Forward_1 (cetacea Cytb) amplicons. Following sequence quality filtering and taxonomic assignment for each primer set using the reference database, 1,589,393 reads were obtained for pinniped Cytb, 1,147,797 reads for pinniped COI, and 3,000 reads for cetacea Cytb. Sequencing coverage for the pinniped primer sets was highly variable and ranged from 0 – 116,521 reads for Pinniped Cytb and 0 – 226,272 reads for pinniped COI (Supplementary Table S3; Supplementary Fig. S7).

No usable prey DNA was obtained using the cetacea Cytb primer set. After quality filtering there were 1 – 1163 reads per sample and sequence data was obtained for just 38 of the original 93 fecal samples. Five ESVs were identified, but none assigned to the cetacean species in the reference database. After searching the obtained ESV sequences in the NCBI database they were identified as being *Clostridium perfringens*, *Malassezia pachydermatis*, *Pusa hispida*, and *Streptococcus pasteurianus*, reflecting non-target cetacea Cytb primer binding and low levels of amplification of non-target DNA within the fecal sample DNA extracts, or possible PCR contamination. Although not sequenced, the humpback whale (HW005) and pilot whale (PW-15) positive controls (Supplementary Table S2) successfully amplified during library preparation using this primer set (Supplementary Fig. S8). We therefore concluded that no cetacean DNA present in the polar bear fecal samples.

For the pinniped Cytb primer set, 86 of the original 93 polar bear fecal extract samples yielded usable sequence data following filtering steps (Supplementary Fig. S9). 309 exact sequence variants (ESVs) were obtained, and 23 were identified to species-level using the curated reference database, corresponding to four species: *Ursus maritimus* (polar bear), *Ursus arctos* (brown bear), *Pusa hispida* (ringed seal) and *Erignathus barbatus* (bearded seal). When spot-checked against the NCBI blastn database, the remaining 286 unknown ESVs predominantly assigned to the same four species but were labeled as 'Unknowns' in the analysis as they did not meet the 99% coverage threshold used for taxonomic assignment. After removing 'Unknown' ESVs from the analysis and grouping sequence counts from ESVs that assigned to the same species, a total of 1,214,487 reads remained with an average of 14,122 +/- 2,684 reads per sample (Range: 0 – 106,701 reads). *Ursid* DNA predominated and was found in ~97% of samples (Supplementary Fig. S10)

For the pinniped CO1 primer set, 90 of the original 93 polar bear fecal extract samples yielded usable sequence data following filtering steps (Supplementary Fig. S9). 45 ESVs were obtained, and six ESVs assigned to species-level using the reference database: five sequence variants assigned to ringed seal and one sequence variant assigned to bearded seal. The remaining 39 'Unknown' ESVs were spot-checked using the NCBI blastn database and a majority were found to assign back to either polar bear or bacterial species. The 'Unknown' ESVs were removed prior to downstream analysis and the counts from the six remaining ESVs

were grouped according to seal prey species they assigned to. Following this, 1,028,970 reads remained with an average of 11,307 +/- 3,888 reads per sample (range: 0 – 226,272 reads)

The Cetacea_CO1_F4_R5 primer set did not amplify our humpback whale and pilot whale cetacea positive controls samples after the addition of the CS1 and CS2 tags. After checking this primer set with the added CS1/CS2 tags with the OligoAnalyzer 3.1 by Integrated DNA Technologies (http://www.idtdna.com/calc/analyzer), it appeared that the addition of these tags increased the guanine/cytosine (G/C) content of the primer sequence, thus increasing its melting temperature and preventing successful primer annealing and elongation of target cetacea CO1 mtDNA in complex fecal DNA extract mixture. This cetacea CO1 primer set should undergo re-development and preliminary testing with sequencing tags added to troubleshoot this issue.

The cetacea Cytb primer set successfully amplified cetacean prey DNA extracts (i.e. humpback whale and pilot whale positive controls), however no useable sequence data was obtained from the sequencing run as none of the ESVs identified with this primer set assigned to cetacean sequences of interest included in the custom reference database. This could be due to the primer set performing poorly in a complex metagenomic mixture (Michaux et al. 2021), but this was not verifiable in the initial testing without a positive control metagenomic extract sample.

The pinniped CO1 and pinniped Cytb primer sets produced high-quality, usable data for DNA-based diet analysis of EG and SB polar bears; however, the pinniped CO1 primer set appeared to perform better than the pinniped Cytb primer set. The CO1 detected pinniped DNA in a more polar bear fecal samples and with a higher average number of sequence reads/sample than the Cytb. CO1 also showed minimal to no host (i.e. polar bear/Ursid) DNA amplification, whereas large amounts of host DNA amplified with the Cytb primer set (Supplementary Figures S9 and S10, Supplementary Table S3). There could be greater sequence similarity at the Cytochrome b gene region between Ursid and Pinniped species which led to high amplification of host DNA that competed with and interfered with prey DNA amplification in the PCR reactions (Sonsthagen et al. 2020; Deagle et al. 2019; King et al. 2008). Thus, the CO1 gene region may be a better choice of primer in a study such as this where the prey species of interest are closely-related to the predator or host species and these gene regions are likely very similar (Michaux et al. 2021). As a result, there could be greater sequence similarity at the Cytochrome b gene region between Ursid and Pinniped species which resulted in the high amplification of host DNA that could have competed with and interfered with prey DNA amplification in the PCR reactions (Sonsthagen et al. 2020; Deagle et al. 2019; King et al. 2008). Despite these differences in primer performance, both the CO1 and Cytb pinniped primer sets detected the same two pinniped species from the same individual polar bears on a relatively consistent basis. Using both primer sets in combination ultimately enabled increased sample coverage and as such we recommend complementary use of these two group-specific primer sets to ensure maximum opportunity for prey DNA detection in polar bear fecal samples.

Text S2.

16S rRNA library sequencing efficiency and sample coverage

Briefly, 12,294,006 total reads (average of 81,960 reads per sample) were obtained from the Illumina MiSeq 2 x 250 base-pair run with v2 chemistry, assigning to both EG (n=34) and SB metagenomic fecal DNA extract samples. After preliminary processing using the DADA2 pipeline (Callahan et al. 2016), 6,172 amplicon sequence variants (ASVs) were identified. Of these 6,172 ASVs, only 1,129 remained after the identification of contaminant ASVs using the decontam package (Davis et al. 2018) and after applying the zero variance and < 2 count threshold imposed by the MicrobiomeAnalyst platform (Chong et al. 2020).

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