ARCTIC
VOL. 74, NO. 1 (MARCH 2021) P. 87–99
https://doi.org/10.14430/arctic72239

New Insights on Polar Bear (*Ursus maritimus*) Diet from Faeces based on Next-Generation Sequencing Technologies

Johan Michaux,[1] Markus Dyck,[2] Peter Boag,[3] Stephen Lougheed[3] and Peter van Coeverden de Groot[3]

(*Received 20 September 2019; accepted in revised form 27 October 2020*)

ABSTRACT. Practical tools to quantify range-wide dietary choices of the polar bear have not been well developed, thus impeding the monitoring of this species in a changing climate. Here we describe our steps toward non-invasive polar bear diet determination with the optimization of 454 pyrosequencing of a 136 base pair (bp) mitochondrial cytochrome *b* (*cytb*) fragment amplified from the extracts of captive and wild polar bear faeces. We first determine the efficacy, reliability, and accuracy of our method using five faecal samples from a captive polar bear fed a known diet at the Canadian Polar Bear Habitat in Cochrane, Ontario, Canada; 19 samples from three polar bears at the Metro Toronto Zoo, Toronto, Ontario, Canada; and seven samples from seven wild (unfed) polar bears from a holding facility in Churchill, Manitoba, Canada. We report 91% overall success in amplifying a 136 bp *cytb* amplicon from the faeces of polar bears. Our DNA analyses accurately recovered the vertebrate diet profiles of captive bears fed known diets. We then characterized multiyear vertebrate prey diet choices from free-ranging polar bears from the sea ice of the M'Clintock Channel polar bear management unit, Nunavut, Canada (n = 117 from an unknown number of bears). These data point to a diet unsurprisingly dominated by ringed seal (*Pusa hispida*) while including evidence of bearded seal (*Erignathus barbatus*), harbour seal (*Phoca vitulina*), muskox (*Ovibos moschatus* ssp.), Arctic fox (*Vulpes lagopus*), wolf (*Canis lupus*), Herring Gull (*Larus argentatus*), and Willow Ptarmigan (*Lagopus lagopus*). We found low levels of contamination (< 3% of sequences when present) and suggest specific process improvements to reduce contamination in range-wide studies. Together, these findings indicate that next-generation sequencing-based diet assessments show great promise in monitoring free-ranging polar bears in this time of climate change.

Key words: polar bear; *Ursus maritimus*; diet; next generation sequencing; climate change; mitochondrial cytochrome *b*; ringed seal

RÉSUMÉ. La réduction de la calotte glaciaire arctique suite au changement climatique risque d'avoir un effet direct sur la capacité des ours polaires à capturer les phoques, leurs principales sources de nourriture. Une surveillance précise des changements alimentaires des ours polaires s'avère ainsi essentielle pour mieux cerner l'impact des changements climatiques sur la survie de cette espèce. Nous détaillons dans cette étude, l'optimisation d'une méthode non invasive basée sur le séquençage de dernière génération (next generation sequencing - NGS) d'un fragment du gène mitochondrial cytochrome b (cytB) de 136 bp à partir de fèces d'ours polaires sauvages collectées en milieu naturel. Pour déterminer l'efficacité, la fiabilité et l'exactitude de notre méthode, nous avons analysé des fèces d›ours polaires en captivité dont le régime alimentaire était connu (Zoo Cochrane (n = 5), Toronto (Ontario, Canada) (n = 17) et des fèces d'ours polaires sauvages provenant de la ville de Churchill (Manitoba, Canada) (n= 7)) ainsi que de la région située au niveau du détroit de M'Clintock (Nunavut, Canada) (n= 117). Ces dernières fèces ont été analysées pour mieux cerner les choix alimentaires pluriannuels des ours polaires sauvages. Les profils alimentaires des ours captifs nourris avec des aliments connus ont été estimés avec précision et ont validé notre méthode. Notre étude sur les ours polaires sauvages du détroit de M'Clintock a révélé que même si le phoque annelé (*Phoca hispidia*) constituait la majorité de leur régime alimentaire, le phoque barbu (*Erignathus barbatus*), le phoque commun (*Phoca vitulina*), le boeuf musqué (*Ovibos* spp.), le renard arctique (*Vulpes lagopus*), le loup (*Canis lupus*), le goéland argenté (*Larus argentatus*) et le lagopède alpin (*Lagopus lagopus)* constituaient également des proies. Les risques de contaminations lors de l'utilisation de ces technologies NGS sont également discutés. De faibles degrés de contamination ont été observés (< 3 % des séquences lorsque la contamination était présente). Différentes stratégies sont proposées pour diminuer encore ces risques de contaminations. En conclusion notre étude démontre que les techniques de séquençage de dernière génération s'avèrent trés prometteuses pour l'étude de l'impact du changement climatique sur le régime alimentaire des ours polaires sauvages.

Mots clés : ours polaire; *Ursus maritimus*; régime alimentaire; séquençage de dernière génération; changement climatique; gène mitochondrial cytochrome *b*; phoque annelé

[1] Conservation Genetics Laboratory, Institut de Botanique, Université de Liège, 4000 Liège (Sart Tilman), Belgique, and CIRAD, UMR CIRAD-INRA ASTRE, F-34398 Montpellier, France; johan.michaux@uliege.be
[2] Government of Nunavut, Department of Environment, Box 209, Iglulik, Nunavut X0A 0L0, Canada
[3] Department of Biology, Queen's University, 99 University Avenue, Kingston, Ontario K7L 3N6, Canada

INTRODUCTION

The anticipated changes in the Arctic climate and concomitant reduction in sea ice quantity and quality are hypothesized to affect polar bear diet (Derocher et al., 2004). Reduced access to seals, the main prey of polar bears, is expected to negatively affect polar bears, their reproductive rates, and ultimately their persistence (Gitay et al., 2002; Derocher et al., 2004). However, dietary responses to a changing environment are unknown and practical tools to monitor these choices have not been well developed. Polar bear diet investigations have been largely based on direct observation (Dyck and Romberg, 2007), morphological identification of prey remains from their scats (Iversen, 2011; Gormezano and Rockewell, 2013), biochemical analyses of fatty acids (FA), and stable isotopes profiles from harvested tissue or biopsy plugs (Thiemann et al., 2007; Hobson et al., 2009; Galicia et al., 2015; McKinney et al., 2017). These data collectively indicate that polar bears have a varied vertebrate diet including ringed seal (*Pusa hispida*), bearded seal (*Erignathus barbatus*), walrus (*Odobenus rosmarus*), harp seal (*Pagophilus groenlandicus*), hooded seal (*Cystophora cristata*), harbour seal (*Phoca vitulina*) (Iversen, 2011), beluga (*Delphinapterus leucas*), narwhal (*Monodon monoceros*), birds, and reindeer (*Rangifer tarandus platyrhyncus*) (McKinney et al., 2017).

While informative, the above methods have two major limitations impeding their application in large-scale studies. First, those requiring tissue from biopsy studies such as FA analyses or direct observations are labour intensive, costly, and can be stressful for the animal. Second, prey identification to the species level is not always possible. For example, FA techniques are based on the identification of FA structures that are transferred unaltered across trophic levels; however, if the prey and predator have identical FA profiles, no discrimination is possible (Thiemann et al., 2007). Further, while some polar bear prey (e.g., bearded seal, harbour seal, and Atlantic walruses) can be identified based on their non-methylene-interrupted FA profiles, those of other prey (e.g., harp seals, hooded seals, beluga whales, and narwhals) cannot be distinguished using FAs or FAs are present at low levels as in ringed seals (Thiemann et al., 2007; Galicia et al., 2015). While hairs of seals can be easily distinguished based on morphology from hairs of reindeer and the guard hairs of polar bears, no species-specific features exist among the different seal species that polar bears consume (Iversen, 2011). At the extreme, soft, digestable dietary items that leave few or no hard traces in the gut or faeces will be less likely to be identified using non-molecular methods (Pompanon et al., 2012).

Although molecular assays of faeces hold potential for species-level detection, the evaluation of these possibilities for the study of polar bears is in its infancy. To date, the design of species-specific oligonucleotide primers for some seals has allowed the detection of different seal species in polar bear faeces (Iversen, 2011), but this method fails to detect non-seal prey. The optimization of molecular methods that allow for the species-level detection of polar bear prey and plant food choices in as few as possible assays would enhance monitoring of real-time polar bear dietary responses in a changing Arctic.

As part of efforts to develop non-invasive polar bear monitoring methods (Wong et al., 2011; Van Coeverden de Groot et al., 2013; Van Coeverden de Groot, 2019), we detail the optimization of a next-generation sequencing (NGS) method that allows the identification of most vertebrate species comprising the diet of polar bears from their faeces. We tested a 136 base pair (bp) segment of the mitochondrial cytochrome *b* (*cytb*) sequence (Teletchea et al., 2008; Galan et al., 2012) amplified from polar bear faeces using 454 pyrosequencing for vertebrate prey identification. We evaluated a) the efficacy of obtaining target cytochrome *b* (*cytb*) amplicons from faeces collected under a variety of field conditions using this method, b) the repeatability of our method in identifying the same vertebrate prey from repeated dilutions and extractions of the same faeces, and c) the accuracy of correctly detecting the vertebrate prey species consumed by polar bears using 24 faeces collected from polar bears with known diets at two zoos. After demonstrating the validity of this method, we profile 117 polar bear faecal samples collected from the M'Clintock Channel (MC) polar bear management unit in Nunavut, Canada, during May of 2007 – 11 and describe the vertebrate dietary choices of this population of bears.

METHODS

Faecal Samples

We collected two sets faeces (n = 24) from captive polar bears to evaluate the accuracy of our 454 pyrsosequencing *cytb* assay. We then applied this technique to faeces of wild polar bears held in a polar bear holding facility in Churchill, Manitoba (n = 7) and to faeces of the wild polar bears of MC collected during the month of May over five years from 2007 – 11 (n = 117, Fig. 1).

Five faecal samples (A, B, D, E, and F) were collected from an adult male polar bear held in captivity at the Canadian Polar Bear Habitat (PBH) in Cochrane, Ontario. Sample C was excluded from our analysis because it was contaminated. As part of a diet study (Dyck and Morin, 2011), this bear was fed three different diets for three weeks at a time: 1) a regular "zoo" diet comprising grass, water melon, grapes, lettuce, chicken, deer, herring, and chow pellets; 2) a diet composed of harp seal flesh and blubber (approximately 1:1 ratio); and 3) a diet composed of mainly Arctic char (*Salvelinus alpinus*) (Table 1; see Dyck and Morin, 2011 for details). The faeces were collected at different times during the three weeks the bear was fed a specific diet. During the period that the bear was fed the char diet (A and B), faeces were collected on days 10 and 20; for the regular diet (D), on day 14; and for the seal diet

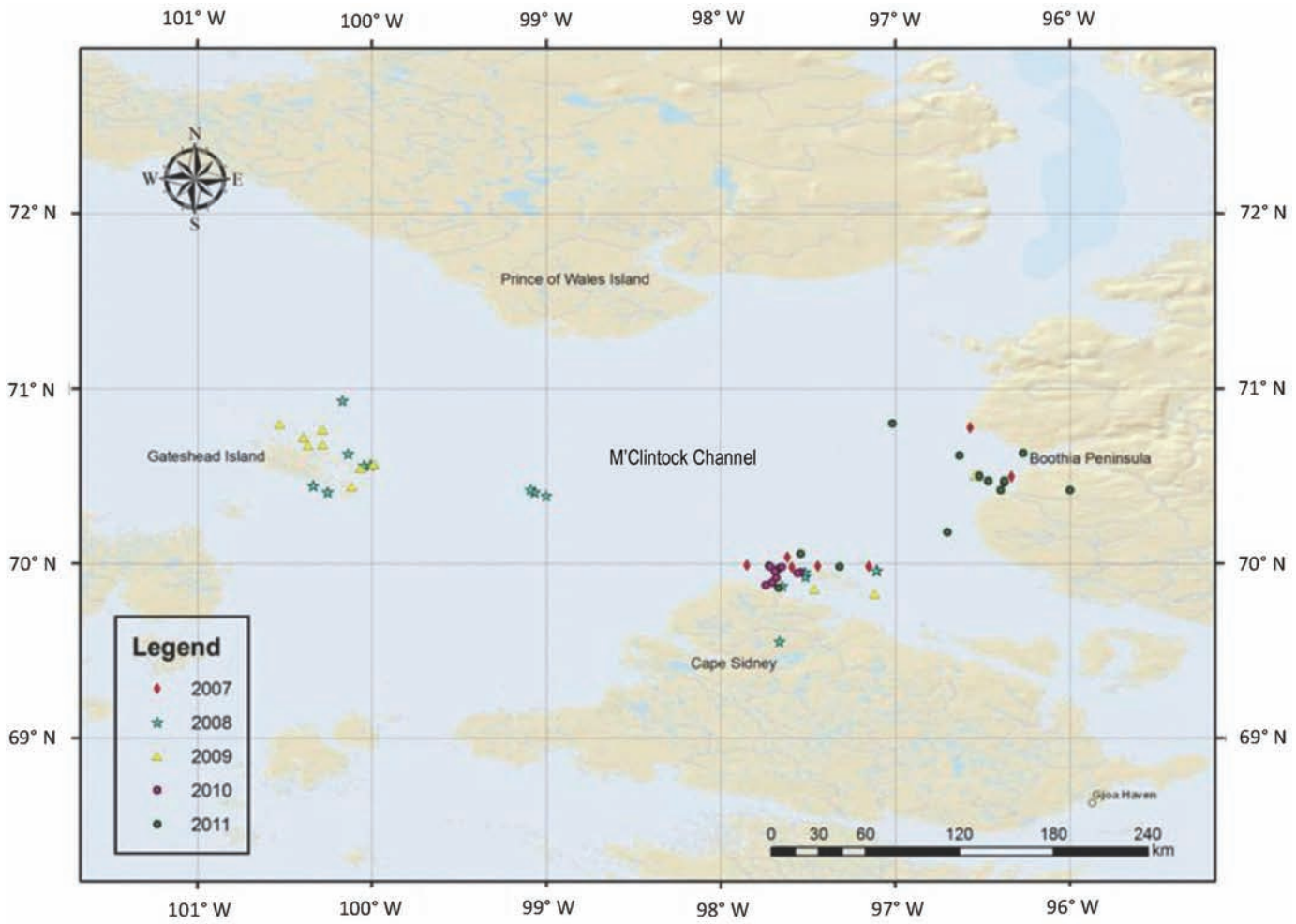

FIG. 1. Distribution of 103 polar bear faecal samples in M'Clintock Channel, Nunavut, Canada, collected during May 2007–11 and used in this study to genetically determine the bears' most recent vertebrate meal. The majority of faecal samples indicated that the most recent meal was the ringed seal (*Pusa hispida*; see Fig. 2).

(E and F) on days 10 and 20. These samples were used in the initial piloting of our methods.

To further assess the accuracy of our method, we assayed a second set of 19 faecal samples from three captive polar bears housed at the Metro Toronto Zoo (MTZ) in Toronto, Ontario. A sample was collected from each bear for each of six weeks with one bear having a seventh sample collected at a later date. These bears were each fed diets fairly typical of most captive polar bears—mainly horse meat, with herring, smelt, rabbit, hard-boiled eggs, dog food, and vegetables.

We also analyzed faeces from seven polar bears temporarily housed in 2007 in a holding facility, the "Polar Bear Prison" (PBP) in Churchill, Manitoba. These were nuisance bears held in PBP until the Hudson Bay ice sheet formed at which time they were released. In contrast to the PBH and MTZ bears, these bears were not fed and our diet determinations reflect feeding before incarceration. The bears were provided with only water during their stay. The collection of these faeces relative to initial date of incarceration is unknown. Finally, we used our method to quantify the recent dietary choices of 117 free-ranging polar bears of the MC subpopulation in Nunavut from samples collected from 2007–11 (Fig. 1). These samples were collected by Inuit collaborators using snowmobiles as part of efforts to optimize ground-based, non-invasive methods of monitoring polar bears (Wong et al., 2011; Van Coeverden de Groot et al., 2013). The faecal samples were stored in freezer bags, kept frozen with snow, and transported frozen to the Gjoa Haven Hunters and Trappers Association (HTA) freezer and finally to Queen's University in Kingston, Ontario, Canada.

Molecular and Analytical Methods

DNA was extracted from all faeces using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Inc., Netherlands) following manufacturer protocols at Queen's University and the American Museum of Natural History. In the pilot study, only two dilutions, 1 µl and 2 µl, from each of the extractions of the five faecal samples from the single PBH bear were used as template in the polymerase chain reaction (PCR). These two dilutions were assessed to find the best compromise between potential inhibitors and the target DNA amplicon concentration (Teletchea et al.,

2008; Galan et al., 2012). The 136 bp amplicon of *cytb* was chosen because 1) it discriminates among most vertebrate species including those that show close evolutionary affinity (Teletchea et al., 2008; Galan et al., 2012), 2) its short length is suited for the PCR amplification of degraded DNA (Taberlet et al., 1997; Murphy et al., 2000), and 3) it has been successfully used in studies with degraded DNA extracted from non-invasive museum and archaeological samples (Teletchea et al., 2008; Pagès et al., 2009, 2010; Galan et al., 2012). Primers used to amplify this *cytb* fragment were L15411F (5′-CCATCTCATCCCTGCGTG TCTCCGACTCAGNNNNNNNGAYAAARTYCCVTT YCAYCC-3′) and H15546R (5′-CCTATCCCCTGTGTGC CTTGGCAGTCTCAGNNNNNNNAARTAYCAYTCD GGYTTRAT-3′) (Galan et al., 2012). Following Galan et al. (2010), PCR amplicons were individually tagged with fusion primers and then pooled for 454 pyrosequencing. The fusion primers consist of an additional 7 bp (the tag) and a 30 bp sequence. A titanium adaptor at the 5' ends is necessary for emulsion-based clonal amplification (emPCR) and 454 GS-FLX pyrosequencing using Lib-L Titanium Series reagents. The combination of the forward- and the reverse-tagged-primers produces a unique barcode for each amplicon. PCR blanks containing only water were used systematically to check for possible cross contamination among samples.

The SESAME package for genotyping multiplexed individuals based on NGS amplicon sequencing (Meglécz et al., 2010) was used to characterize sequences. Sequences differing by at least one base-pair substitution were identified as "variants" (Galan et al., 2010). We followed Galan et al., (2012) in classifying all variants as "artefactual variants" (i.e., variants that resulted from polymerase errors during PCR and emPCR and pyrosequencing errors) or "true variants" (i.e., variants retained after our validation procedure). True variants are henceforth referred to as "haplotypes." Species identification of *cytb* haplotypes obtained from the different faecal DNA extracts was performed using the National Center for Biotechnology Information's BLASTN program (Zhang et al., 2000) using the database from GenBank (EMBL, DDBJ, and PDB sequences).

We quantified efficacy by calculating the probability of successfully amplifying *cytb* amplicons from captive and wild polar bear faeces and by estimating prey identification repeatability when the same dietary item is identified across two dilutions from the same extract. Based on the results of our pilot assay of five faeces from the same bear at the PBH facility, we analyzed 1µl and 2µl dilutions from the initial extractions of individual faeces from each of seven different PBP bears, 19 faeces from three different MTZ bears, and 117 faeces from an unknown number of MC bears. These dilutions were amplified with the 136 bp *cytb* primers as above. All PCR products were checked on 1.5% agarose gel stained with Ethidium bromide and scored as FAIL (no product detected in agarose), WEAK (small amounts of PCR product detected), and OK (appreciable amounts of PCR product detected). To improve the diet characterization of those samples that performed poorly in the initial two PCRs (one each of 1 µl and 2 µl dilutions of their initial extractions), those PBP, MTZ, and MC samples that yielded only WEAK or FAIL in the first two PCRs were re-extracted. We performed PCR on these new extracts with 1 µl and 2 µl dilutions as above and scored them as FAIL, WEAK, or OK. The WEAK and OK PCRs across original and subsequent extractions were sequenced.

As part of the evaluation of the efficacy of genetic prey identification from polar bear faeces collected under captive conditions and on the sea ice in May, we calculated the percentage of faeces that failed to amplify our target *cytb* amplicon across a maximum of four PCRS (of two dilutions of two extracts) and those that had at least one WEAK PCR result across a maximum of four PCRs. The effect of re-extraction of PBP, MTZ, and MC faecal samples on the determination of genetic prey identification is reported as the percentage of samples that improved from FAIL+FAIL in the first extract to at least one WEAK/OK in the two PCRs of the second extract.

Upon the completion of the PCRs from the first and second extraction of the PBP, MTZ, and MC faecal samples, molecular food item identification was performed on all WEAK or OK PCRs with the same protocol used for the pilot study PBH samples. By comparing the genetic identifications for MTZ polar bears using our two dilution – two extraction protocol with their known diets, we evaluated the accuracy of our method and used the two dilution – two extraction method with 454 pyrosequencing to characterize vertebrate dietary choice of free-ranging polar bears from M'Clintock Channel, Nunavut.

RESULTS

Pilot Study Results

Our pilot study of 454 pyrosequencing diet determination from a 136 bp *cytb* sequence amplified from extracts of five faecal samples from a single bear at PBH that had been fed three different diets for three weeks over nine weeks suggests that our molecular diagnoses are accurate to vertebrate genus level (Table 1). Three out of five PBH faecal samples (A, B, F) worked across both dilutions in the initial *cytb* PCR. Neither extract for PBH D and E amplified across both dilutions and, unlike the process followed for all other initial FAIL or WEAK PCRS (see above), they were not re-extracted nor were PCRs repeated in this pilot. Across the six successful dilutions (from three PBH extracts) a total of 657 reads were obtained with between one and four different haplotypes in each of the six dilutions (Table 1, Fig. 2). *Cytb* sequences of the host (polar bear) represented a high proportion of the haplotypes for each of the six dilutions: 54% – 100% of the total, and only polar bear DNA was recovered from both dilutions of PBH A (Table 1).

TABLE 1. Pilot study results of next-generation sequencing of extracts from faecal samples of a single captive polar bear held at the Canadian Polar Bear Habitat (PBH) in Cochrane, Ontario. Dilution of initial extraction refers to 1 µl or 2 µl of extract used in the *cytb* PCR; OTU (operational taxonomic unit) = the number of true sequence variants identified after validation (see text for details) of the products of each PCR; Total # of sequences = the number of unique sequences assigned to all OTUs. *Cytb BLAST match* = taxon to which *cytb* is assigned, and *BLAST Identity* = % match to BLAST sequence.

PBH sample			A		B		D		E		F	
Diet			Char		Char		Regular		Seal		Seal	
Dilution of initial extraction			1 µl l	2 µl l	1 µl l	2 µl l	1 µl l	2 µl l	1 µl l	2 µl l	1 µl l	2 µl
Total # of OTUs			1	1	2	2	0	0	0	0	4	4
TOTAL # of sequences			93	206	41	50	0	0	0	0	116	151
Haplotype	*cytb* BLAST match	% Blast identity										
1	*Ursus maritimus*	99	93 (100%)	206 (100%)	25 (61%)	27 (54%)					96 (82.8%)	134 (88.7%)
2	*Phoca groenlandica*	99									17 (14.7%)	14 (9.3%)
3	*Salvelinus* sp.[1]	100			16 (39%)	23 (46%)						1 (0.7%)
4	*Gallus gallus*	100									1 (0.9%)	
5	*Odocoileus* sp.[1]	100									2 (1.6%)	2 (1.3%)

[1] Identification to species was impossible because different species among the genus share the same mini-barcode.

The species ascribed to the non-polar bear haplotypes matched those of the ingested food items for PBH B and F. In the case of PBH B (bear fed a char diet), 39% and 46% (dilutions 1 and 2) of the total number of sequences were assigned to the correct genus for Arctic char—*Salvelinus* (Salmonidae). For PBH F (collected when the bear was fed a harp seal diet), 9% and 14% of the total number of sequences were identified as harp seal. PBH F also showed small traces of chicken and deer DNA. Both "exotic" chicken and deer sequences were found in a very low frequency (0.9 and 1.3%) but correspond to actual diet items the bear was fed as part of the regular diet preceding the collection of faecal sample F. In PBH F, *Salvelinus* sequences were also obtained at a very low frequency (0.7%), likely reflecting char fed before the onset of the harp seal diet.

Our PBH pilot survey showed 1) accurate diet determination from polar bear faeces, 2) host DNA always represented the largest fraction of recovered haplotypes, 3) trace quantities of earlier diets may still be detectable after more than three weeks of consumption, 4) the PCR of 1 µl and 2 µl dilutions from a single extract of a target faeces yielded *cytb* amplicons were 60% successful, and 5) the single extraction from a faeces did not always lead to the amplification of *cytb* haplotypes that can be sequenced and this failure would seem to be independent of diet. The above suggests that while 454 pyrosequencing diet analysis of our larger dataset is effective, a second extraction should be attempted when the *cytb* PCR of the first extraction yields a FAIL or WEAK result. The PCR of 1 µl and 2 µl dilutions from between one and two extracts of the same faeces was followed for all subsequent 454 pyrosequencing assays.

Efficacy and Accuracy of Prey Determination

Using our two dilution–two extraction method, we estimated our efficacy of generating 454 pyrosequencing amplicons that could be sequenced at 90.8% for the 143 polar bear faecal samples that we assayed herein (Table 2). The source of the faecal samples affected the amplification success rates with most success (100%) achieved with the seven faeces from the seven bears held in the Churchill facility, followed by 93% success with the 117 faeces collected in MC from an unknown number of bears, to 74% of the 19 MTZ faeces from three bears (Table 2).

From the 143 polar bear faeces, we generated a total of 250 successful *cytb* PCR amplifications from the corresponding 1 µl and 2 µl dilutions. Our 454 sequencing of these PCR products yielded a total of 53 732 *cytb* reads corresponding to 3010 distinct variants. These were subsequently assigned to the 250 dilutions (220 from 110 DNA extracts that worked with two dilutions; 30 that only worked for one dilution). The artefactual variants were sorted and discarded manually using SESAME. After this validation step (i.e., Substitution, Indel, and Chimera excluded), the mean number of reads per successful PCR was 188.00. More than 50 validated reads were obtained for 91.88% of the samples and more than 100 for 80.77% of the samples.

From the above, we computed a second estimate of efficacy—the proportion 1 µl and 2 µl dilutions from the same extraction where the same prey items were identified when the host animal's sequences (polar bear, Arctic fox, wolf/dog) were excluded. Across all PBP, MTZ, and MC samples, prey identification repeatability was 84.6% across paired dilutions of the same extracts. Included in this estimate are those cases where only polar bear DNA was

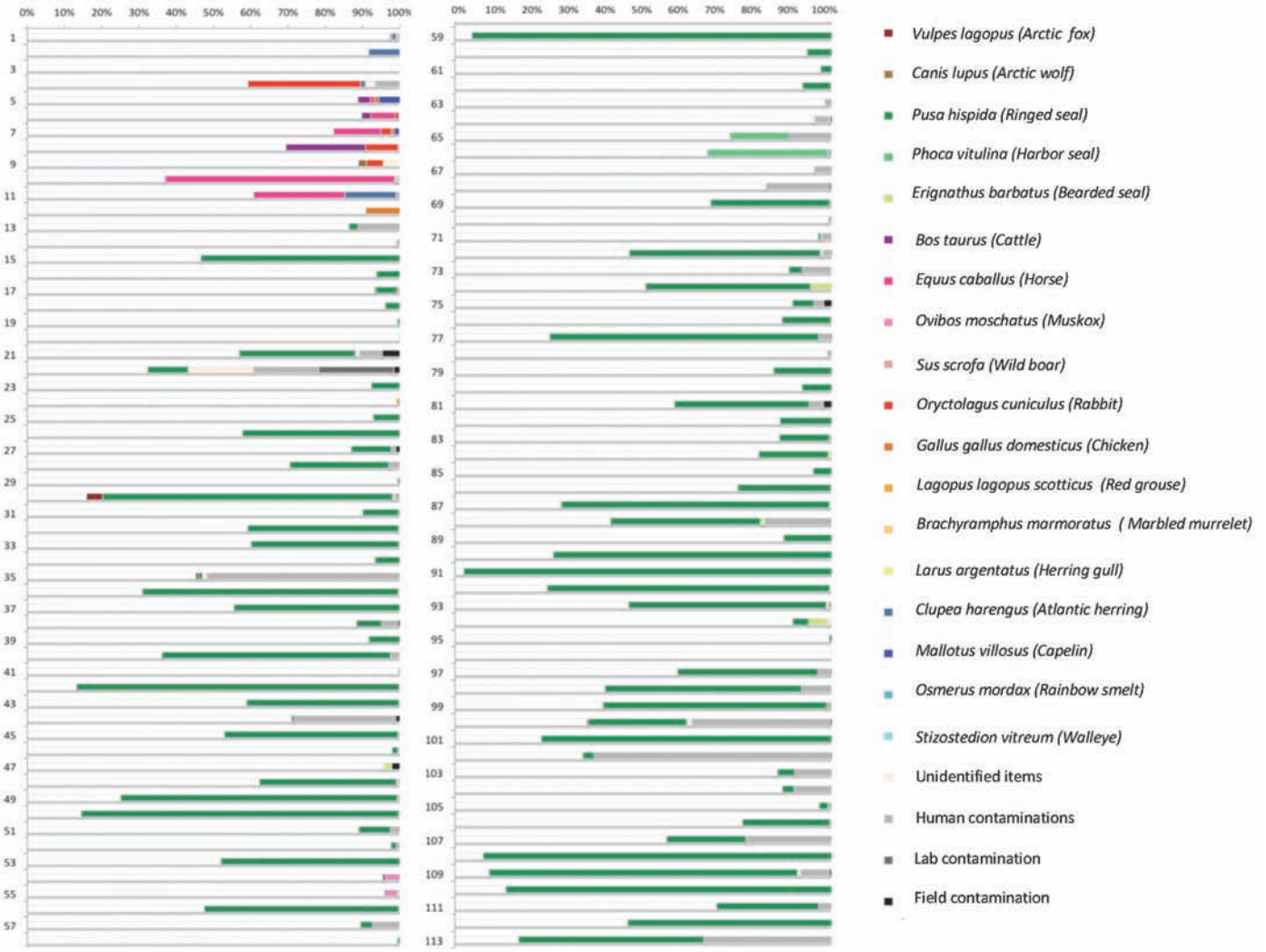

FIG. 2. Difference in identified polar bear vertebrate prey items based on proportion of *cytb* sequences generated from 454 pyrosequencing of WEAK and OK PCRs from 12 captive MTZ and 101 wild MC polar bear faeces. All sequences from the PCRs of up to four dilutions (1 µl or 2 µl dilutions for each of two possible extracts) for each faeces are combined in these calculations. The different colours in a bar represent the sequence count (%) of the vertebrate taxa identified in the faeces. The figure shows the predominance of host polar bear and ringed seal sequences.

amplified in one of the paired extracts, while a prey item was identified in the other.

The initial accuracy estimates from the PBH sample were corroborated with our assay of the MTZ polar bears faeces (Figs 3, 4, and 5). Of the 14 MTZ faecal samples that worked, two were discarded as results indicated only human haplotypes in the four extracts. The correct vertebrate dietary items were identified in the remaining 12 MTZ samples. Despite the MTZ bears having a more varied diet than the single PBH bear fed the same diet for three consecutive weeks at a time, we detected all known food items (i.e., horse, herring, rabbit, hard boiled eggs, cow, and smelt) fed to the three MTZ bears. Also, the assay showed high specificity discriminating between different species of fish consumed (e.g., capelin, *Mallotus villosus*; rainbow smelt, *Osmerus mordax*; walleye, *Sander vitreus*) (Figs. 2 and 3). The sensitivity of the technique was further exemplified by our detection of DNA from two "contaminating" species in our 12 study faecal samples. We found a single read of *Canis lupus/familiaris* (in a single extract out of two "positives" from two independent extracts from the same faeces). *Canis* sp. was never a diet item but the presence of wolves in the next enclosure at the MTZ likely led to this contaminated result. We also found two reads (0.0045%) of Marbled Murrelet (*Brachyramphus marmoratus*) in a single extract from a different faeces. This species was previously studied in one of our laboratories, and although below 0.010% of all sequences in the sample, previous taxa studied in our laboratories would appear to represent a source of contamination in this sensitive assay (see below).

Determination of Wild Polar Bear Diet

Of the 117 collected faecals from wild MC polar bears, eight did not work across four dilutions of two extractions, and six were excluded from further diet analysis because they were not polar bear. One sample (PBF07-05) yielded

TABLE 2. Summary statistics of our efficacy in obtaining a mitochondrial 136 *cytb* bp amplicon from the faeces of wild and captive polar bears using our two dilution – two extraction method. The overall (weighted) success rate was 91%. # of Bears = number of known bears from which faeces were collected; Faeces per bear = # of faeces from each bear; Total # of faeces = total number of faeces from the Metro Toronto Zoo (MTZ), the Churchill holding facility (PBP), and M'Clintock Channel (MC); Original extract = number of extracts with *cytb* PCR results characterized as FAIL, WEAK, or OK across 1 µl and 2 µl dilutions; # faeces re-extracted = number of faeces with FAIL and WEAK results that were re-extracted; 2nd extract = number of second extracts with *cytb* PCR results characterized as FAIL, WEAK, or OK across 1 µl or 2 µl dilutions; Combined success of two dilution – two extraction method = number of extracts with *cytb* PCR results characterized as FAIL, WEAK, or OK across two dilutions and two extractions; Combined probability of success = combined probability of WEAK or OK *cytb* amplification success for MTZ, PBP, and MC polar bear faeces; Increased success - second extraction = % of re-extracted faeces that resulted in WEAK or OK *cytb* PCRs.

	Number of bears	Faeces per bear	Total number of faeces	Original extract			Number faeces re-extracted	Second extract			Combined success of two dilution – two extraction method			Combined probability of success	Increased success of second extraction
				FAIL[1]	WEAK[2]	OK[3]		FAIL	WEAK	OK	FAIL	WEAK	OK		
MTZ	3	6 or 7	19	11 (0.58)[4]	6 (0.32)	2 (0.11)	17	6 (0.35)	7 (0.41)	4 (0.24)	5 (0.26)	8 (0.42)	6) (0.32)	74%	65%
PBP	7	1	7			7	0						7 (1.00)	100%	NA
MC	?	?	117	16 (0.14)	10 (0.09)	91 (0.78)	24[5]	11 (0.46)	4 (0.17)	9 (0.38)	8 (0.07)	7 0.06)	102 (0.87)	93%	54%

[1] No *cytb* amplicon detectable in PCRs with 1 µl and 2 µl dilutions of polar bear faecal extracts.
[2] *Cytb* amplicon weakly amplified in PCRs with 1 µl and 2 µl dilutions of polar bear faecal extracts.
[3] *Cytb* amplicon strongly amplified in PCRs with 1 µl and 2 µl dilutions of polar bear faecal extracts.
[4] Bracketed values are the associated cell number as a percentage of the number of faecal extracts.
[5] Although 26 faecal samples had FAIL or WEAK *cytb* amplifications, we only re-extracted and reprocessed 24 of them.

mainly *Canis* reads (131 and 110 reads for each replicate, see Fig. 2) and may have come either from the dog that accompanied the expedition that year or from a wolf. Repeat assays of three samples (PBF08-10, -16, and -26) were shown to be from an Arctic fox (numerous reads of fox associated with seal reads). Two samples (PBF10-03 and PBF10-08) were considered of unknown predator origin as only prey seal sequences could be retrieved associated with human DNA (there were no polar bear sequences).

The ringed seal comprises the main prey item in wild bears from M'Clintock Channel during May of 2007 – 11 (DNA from this species was found in 86.14% of the faeces from wild bears; Figs. 2 and 4). Two other seals, the harbour seal and the bearded seal, form smaller but substantial portions of the polar bear diet with their DNA found in 3.96% and 4.95% of MC faeces, respectively. While these species are the most common vertebrate prey DNA reads found in MC faeces, our method points to other vertebrates contributing to the polar bear diet at this time of year in M'Clintock Channel. Arctic fox and wolf DNA accounted for 5.94% and 0.99% of extracts, respectively. These faeces are distinguished from those believed to have come from foxes or wolves (see above) by the predominance of polar bear DNA reads in them. Muskox DNA reads were found in 1.98% of extracts, and Willow Ptarmigan and *Larus* sp. gull DNA reads came from 1.98% and 0.99% of extracts, respectively. Unfortunately, the mini-barcode did not allow the discrimination among three putative gull species (*L. thayeri*, *L. hyperboreus*, and *L. argentatus*).

DISCUSSION

With our two dilution – two extraction method we have optimized a next-generation sequencing method to determine non-invasively the vertebrate prey of wild polar bears from their faeces using a 136 bp *cytb* amplicon. Specifically, we 1) determined that the success of obtaining *cytb* amplicons for NGS sequencing across a variety of polar bear faeces varies from 73.6% to 100% with a mean of 90.8% across all samples, 2) have shown our technique to be reliable and accurate by evaluating four captive polar bears fed known diets, and 3) through the assay of 117 polar bear faeces from an unknown number of bears, provided strong evidence that while the primary prey of these MC bears during the months of May 2007 – 11 was the ringed seal, diverse vertebrate taxa comprise the diet of polar bears at this time.

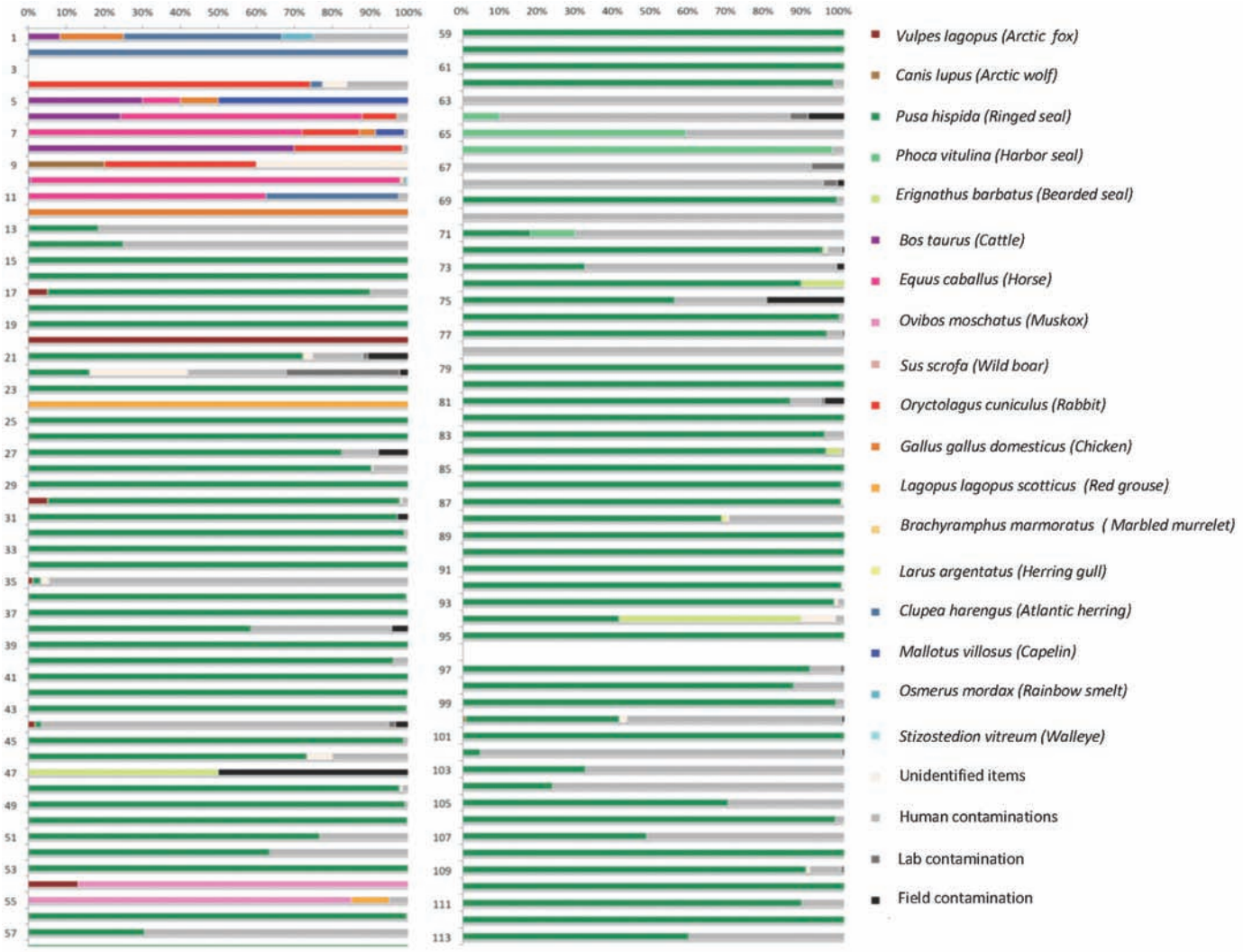

FIG. 3. The difference in identified polar bear vertebrate prey items, but with the polar bear sequences removed to better display the relative proportion of prey items. As in Figure 2, all sequences from the PCRs of up to four dilutions (1 µl or 2 µl dilutions for each of two possible extracts) for each faeces are combined in these calculations. The different colours in a bar represent the sequence count (%) of the vertebrate taxa identified in the faeces. Of the 113 samples, the first 12 entries are from Metro Toronto Zoo bears and the remaining 101 samples are from wild bears (8 from 2007, 28 from 2008, 29 from 2009, 18 from 2010 and 18 from 2011).

Method Efficacy, Reliability and Accuracy

We showed decreased *cytb* amplification success (i.e., efficacy) in captive versus wild polar bear faeces despite the improved collection and handling of the faeces of the captive polar bears by trained technicians. These different success rates may be explained by the better preservation of wild faeces in cold Arctic ambient temperatures. The samples from the captive bears were deposited in above zero temperatures although they were likely collected and frozen within 24 hours of defecation. In contrast, the wild faecals were immediately deposited into subzero storage. This interpretation is consistent with other studies showing PCR success from faecal extracts is correlated with the freshness of faecal samples (McInnes et al., 2017).

While the efficacy of generating prey *cytb* amplicons using extractions from wild polar bear faeces is 93%, our early estimate of reliability for pooled MTZ, PBP, and MC bears is lower at 84.6%. This result is not unexpected as one of the dilutions contains 50% more DNA than the other, and the two sampling events of the same DNA will likely be different, particularly with respect to prey template DNA, which is already in low numbers. In addition, the faecal DNA extract contains DNA from the host, prey, bacteria, viruses, and other commensal and pathogenic taxa, which further reduces the likelihood of replicate sampling in dilutions differing by 50%. The more useful estimate of reliability will come from the faeces of the same wild polar bears. This analysis awaits increased genotyping success (see below).

Our recovery of the genetic identification of the vertebrate diet items fed to the captive PBH and MTZ bears indicates our method is accurate. Although the accuracy of our method is difficult to estimate—we cannot resolve to species level for all *cytb* amplicons from taxa in the same genus (see Limitations below)—the results of the captive

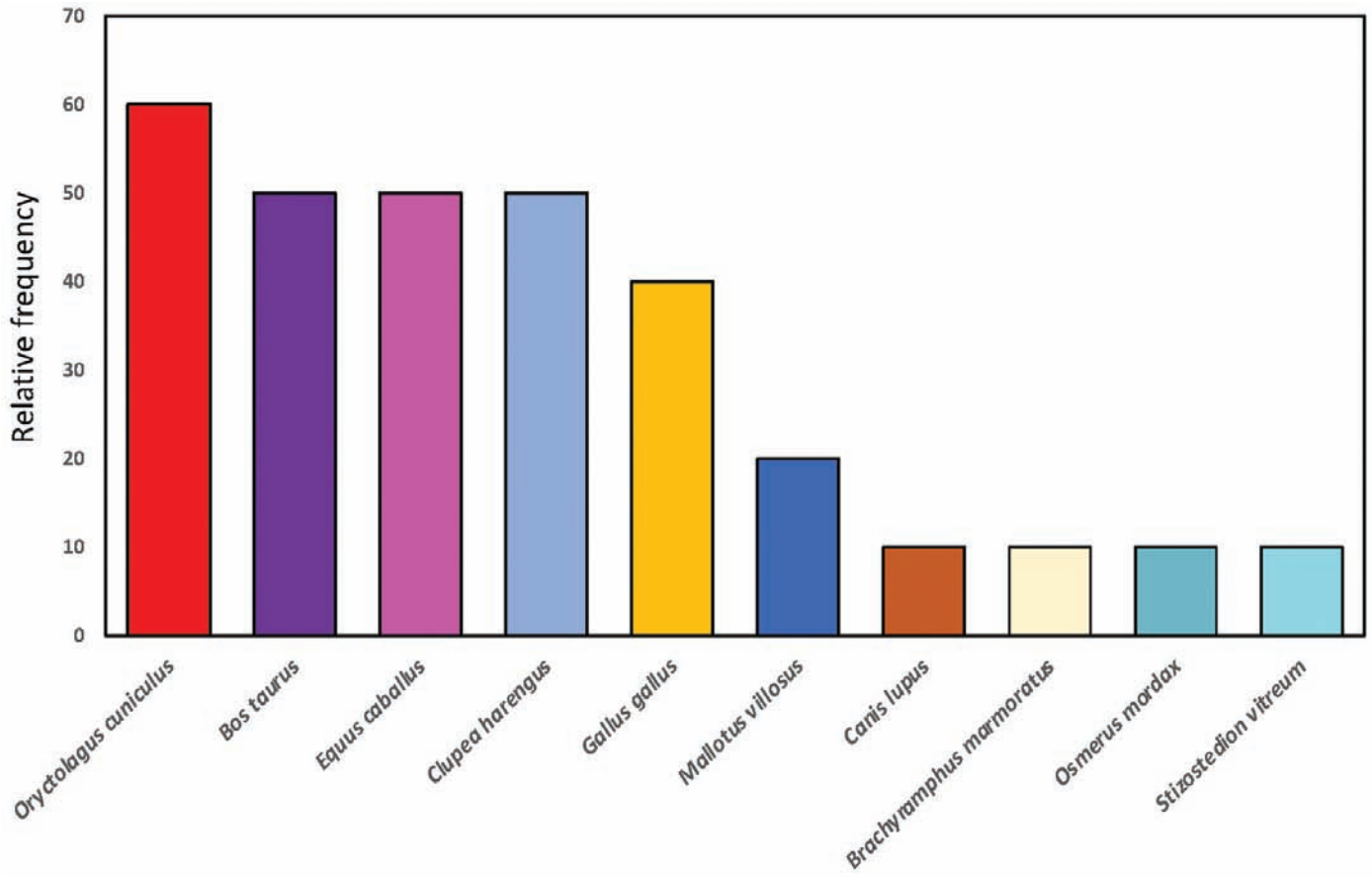

FIG. 4. The identity and relative frequency of non-polar bear vertebrates identified from the faeces of three captive polar bears in the Metro Toronto Zoo (Toronto, Ontario, Canada). The single *Canis lupus* sequence likely reflects contamination from wolves housed in the adjacent enclosure. The results from the PCRs for all faeces are pooled.

bear assay means that we can accept with confidence the identification of vertebrate prey items including seal and other taxa that are known to be part of the polar bear diet including birds (see *Larus* difficulties below) and fish (Russel, 1975; Stempniewicz, 2006; Dyck and Romberg, 2007; Gormezano and Rockwell, 2013; Iversen et al., 2013). The above validation means our detection of prey items believed to be uncommon in polar bear diet such as muskox, Arctic fox, and wolf is likely correct, and the future detection of other vertebrate taxa not eaten by the polar bears in our study sample but known to have been consumed by polar bears in the wild (e.g., reindeer (Iversen, 2011), beluga, narwhal, walrus (Derocher et al., 2004) or marine birds (*Larus* spp.; Stempniewicz, 2006) is possible.

Faecal NGS Contamination

Our results suggest that an acceptable level of contamination did not invalidate the results and, importantly, that the identification of reads resulting from contamination allowed for targeted quality control and specific method improvement steps to reduce contamination. Reduced contamination will allow for more robust inferences of polar bear dietary patterns from polar bear faeces. Some of our faecal samples were clearly contaminated and not of polar bear origin; for example, two MTZ faeces yielded only human DNA, and the MC samples PBF08-10, PBF08-16, and PBF08-26 were from Arctic fox, and PBF07-05 was likely a wolf. These samples were excluded from further analysis. In all other instances, the contaminating sequences in captive and wild bear samples (Fig. 2) were present at less than 3% frequency. This ratio is low and appears characteristic in similar amplicon sequencing studies using next-generation sequencing (Pompanon et al., 2012). The characterization of these sequences in this study assists in reducing the effects of contamination in future studies by pointing to the contaminating source at all our steps, from faecal collection in zoos and the wild to the final 454 pyrosequencing step.

As in other high-throughput sequencing (Shehzad et al., 2012), human haplotypes were identified in 2.50% (1039 human reads out of 40 062 total reads, including human non-functional nuclear paralogs) of the validated reads and found in 56.64% of the samples. The source of these human contaminating sequences could have occurred at all steps during the collection of these data, thus highlighting the need for a general improvement in sterile technique when handling faeces. The detection of cow, pig, and herring sequences in wild polar bear faecal samples, however, reflects faecal collection and storage procedures in our

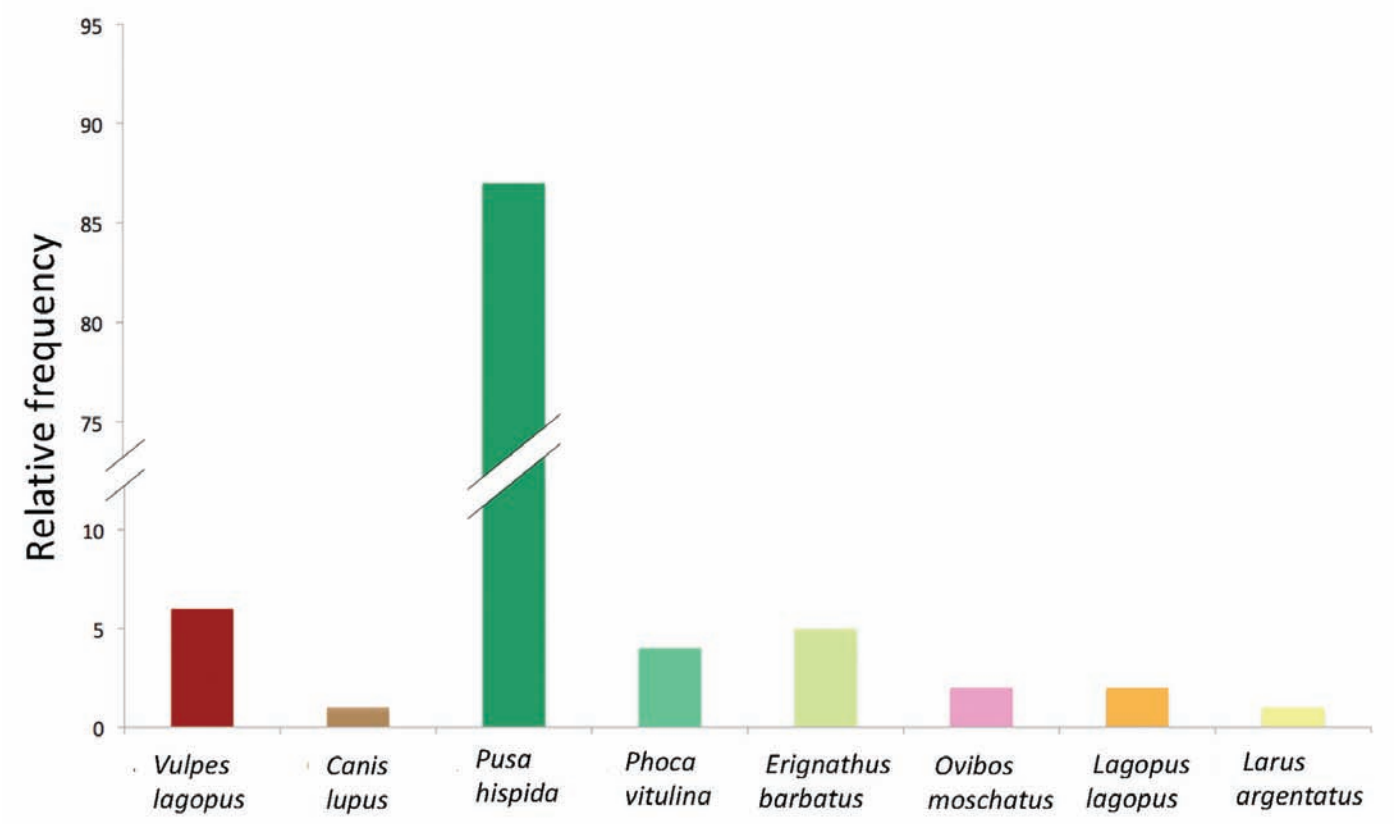

FIG. 5. The identity and relative frequency of vertebrate prey identified from 101 faeces from wild polar bears of M'Clintock Channel, Nunavut, shows the array of prey items consumed by these wild bears during May 2007–11. While the most common food items are the ringed seals (*Pusa hispida*; 86.14% of the faeces), harbor seal (*Phoca vitulina*; 3.96%), and bearded seal (*Erignathus barbatus*; 4.95%), other diet items at this time of the year include the Arctic fox (*Vulpes lagopus*; 5.94%), wolf (*Canis lupus*; 0.99%), muskox (*Ovibos moschatus*; 1.98%), Willow Ptarmigan (*Lagopus lagopus*; 1.98%) and gulls (*Larus* sp.; 0.99%). The 136 bp *cytb* sequence did not allow the discrimination of three putative gull species (*L. thayeri*, *L. hyperboreus*, and *L. argentatus*). The results from the pooling of all PCRs for 101 faeces are shown

earlier field sampling. In our earlier fieldwork, the coolers used to store our samples during the field trips were first used to store meat for consumption during fieldwork. This practice was changed in the later fieldwork. Similarly, the presence of wolf DNA in MTZ faecal samples likely represents contamination from animals in nearby exhibits. Finally, the presence of Marbled Murrelet DNA in polar bear faeces also represents contamination at the stage of DNA extraction, as this taxon had been previously analyzed in our laboratories. The presence of these contaminating species clearly pointed to the steps in our method needing improvement—from field collection, to storage, and through to lab sterilization.

Limitations of the Method

Although the detection of a diverse array of prey species from faeces of anonymous polar bears suggests that our method surpasses other molecular methods based on species-specific primers (Iversen, 2011) and FA methods, there are limitations to current faecal extract molecular methods, including our own. More specifically, these limitations are 1) the inability to quantify the amount of prey ingested (Piñol et al., 2018), 2) the preferential amplification of polar bear mitochondrial DNA, 3) the inability of our 136 *cytb* amplicon to discriminate among some species of potential prey species, 4) the lack of identity of the defecating polar bear (meaning that we do not know how many individuals are encompassed by our field sampling), and 5) the need to optimize the genetic identification of the plant diet of wild polar bears.

Unlike FA analysis, we cannot provide quantitative estimates of the amount of prey items ingested from the amplicons amplified. More specifically, the number of reads obtained per sample cannot be directly linked to the quantity of ingested preys because 1) preferential amplification of some species to the detriment of others is possible when dealing with mixtures of DNA templates such as those found in faecal DNA extracts (Polz and Cavanaugh, 1998; Pompanon et al., 2012), 2) tissues do not have the same density of mitochondrial DNA across species, which prohibits quantitative PCR analysis with these DNAs (Deagle et al., 2005), and 3) scat samples correspond to the end of the assimilation process and do not always accurately reflect food intake (i.e., differential survival of DNA during digestion). The quantification of ingested prey with 454 pyrosequencing and other NGS platforms of polar bear faecal extracts will remain a challenge.

The amplification of relatively larger amounts of host (polar bear) DNA will impede the amplification of prey DNA. In this regard, the reduction in the amplification of polar bear DNA amplicons may be achieved by adding blocking oligonucleotides (Vestheim and Jarman, 2008; Shehzad et al., 2012). These oligonucleotides bind to the host DNA and prevent PCR elongation (Vestheim and Jarman, 2008). While promising, the application of this technique to 454 pyrosequencing and other NGS assays of polar bears may not be straightforward for two initial reasons. The finding of an appropriate binding site for a species-specific primer next to a binding site of universal primer is difficult when the amplicon is small. Polar bear vertebrate diet includes related and distantly related species, which increases the difficulty of designing primers to inhibit the amplification of bear amplicons while allowing amplification of prey items. In addition, the amplification of the polar bear DNA ensures the faeces are from a polar bear (some of our samples were revealed to come from Arctic foxes and wolves or dogs accompanying Inuit hunters). In this regard, it is important to know that the faeces are from a polar bear as evidenced by some sequenced polar bear amplicons.

We are unable to discriminate among some species and could only discriminate to the genus level for char, gulls, and deer. The discrimination between *Salvelinus elgyticus*, *S. taranetzi*, and *S. neiva* is not possible because they share the same mini-barcode and are 100% identical to the haplotype here detected. Numerous cases of hybridization and introgression have been indeed reported among the char species complex, for example: *Salvelinus fontinalis* × *S. alpinus*, (Bernatchez et al., 1995); *S. alpinus* × *S. namaycush*, (Wilson and Bernatchez, 1998); and *S. malma* × *S. confluentus* (Redenbach and Taylor, 2002), meaning that mitochondrial markers could not be the most suitable tool to discriminate among char species. Similarly, with respect to the gull species *L. thayeri*, *L. hyperboreus*, and *L. argentatus*, the mini-barcode does not allow discrimination. Gulls are described as a group of recent origin with weak reproductive barriers (Vigfusdottir et al., 2008) and with taxonomic uncertainties. Finally, while it was impossible to discriminate between two closely related species—the mule deer, *Odocoileus hemionus*, and the white-tailed deer, *Odocoileus virginianus*—this issue is less problematic as it is unlikely that polar bears would eat these animals in the wild. This current lack of resolving power among closely related species complexes in our current method can be partly addressed by PCR analysis of those faecal extracts for which the vertebrate prey could only be resolved to genus level with prey species-specific primers under more stringent conditions. Finally, other mitochondrial gene sequences such as Cytochrome Oxydase I (Gillet et al., 2015; Galan et al., 2018) may be used to distinguish among closely related taxa (Biffi et al., 2017a and b; Andriollo et al., 2019). The complementary use of such primers would help to improve the resolving power of future NGS approaches.

The missing data for all these extracts are individual genotypes that distinguish among different polar bears. This identification is critical to determine patterns of consumption variation in diet among polar bears sampled at the same time and location. Here we describe only probabilities to obtain reliable and accurate vertebrate diet profiles using our 454 pyrosequencing method and the diversity and relative abundance of prey types of wild polar bears in the same area (MC).

Despite the above limitations, this fast, sensitive, and accurate method improved monitoring of polar bear populations in the wild. Using wild polar bear faecal samples and our two dilution–two extraction 454 pyrosequencing method, it is possible to simultaneously determine baseline dietary characteristics and dietary response of polar bears to ongoing climate change at a scale not previously possible for polar bears and that should be part of a long-term monitoring program (Vongraven et al., 2012). Further, it is important to highlight the potential new NGS platforms bring to a non-invasive Inuit-inclusive method for studying polar bear diets. Illumina technology (e.g., MiSeq, NextSeq, NovaSeq and HiSeq sequencers) offers a much larger number of sequences per faecal extract (Gillet et al., 2015; Biffi et al., 2017a, b; Andriollo et al., 2019). Higher sequence numbers allow more reads per prey items and therefore a more precise species identification. In conclusion, while our preliminary results define a baseline of polar bear feeding choices for M'Clintock Channel bears against which the impacts of future climate change and other disturbances can be measured, the application of newer NGS platforms will result in higher-resolution, real-time profiles of vertebrate diets from these and any polar bear population.

ACKNOWLEDGEMENTS

We thank M. Pagès and M. Galan for collecting much of the data and for assistance in the interpretation of the results. We thank G. Konana, W. Aglukkaq, and S. Qirniruq for their field expertise and traditional ecological knowledge that enabled the collection of polar bear faeces in the wild. Fieldwork was supported by the Polar Continental Shelf Program, the Nunavut Wildlife Management Board, and Nunavut Tunngavik Inc. awards to the Gjoa Haven Hunters and Trappers Organization and P. van Coeverden de Groot, and a National Sciences and Engineering Research Council (NSERC) Northern Research Supplement to P. Boag. Laboratory costs were funded by the Nunavut General Monitoring Plan (P. van Coeverden de Groot, M. Dyck, J. Michaux, and P. Boag) with contributing funds from an NSERC Discovery Grant to P. Boag. We thank the staff at the Canadian Polar Bear Habitat in Cochrane, Ontario, for their co-operation and in-kind support during the polar bear feeding trials, and Manitoba Conservation and Daryll Hedman for providing the faeces from seven polar bears held in the holding cells in Churchill, Manitoba. Finally, we thank the Metro Toronto Zoo for its cooperation and polar bear faecal sample collection.

REFERENCES

Andriollo, T., Gillet, F., Michaux, J.R., and Ruedi, M. 2019. The menu varies with metabarcoding practices: A case study with the bat *Plecotus auritus*. PLoS ONE 14(7): e0219135. https://doi.org/10.1371/journal.pone.0219135

Bernatchez, L., Glémet, H., Wilson, C.C., and Danzmann, R.G. 1995. Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Science 52(1):179–185. https://doi.org/10.1139/f95-018

Biffi, M., Gillet, F., Laffaille, P., Colas, F., Aulagnier, S., Blanc, F., Galan, M., et al. 2017a. Novel insights into the diet of the Pyrenean desman (*Galemys pyrenaicus*) using next-generation sequencing molecular analyses. Journal of Mammalogy 98(5):1497–1507. https://doi.org/10.1093/jmammal/gyx070

Biffi, M., Lafaille, P., Jabiol, J., André, A., Gillet, F., Lamothe, S., Michaux, J.R., and Buisson, L. 2017b. Comparison of diet and prey selectivity of the Pyrenean desman and the Eurasian water shrew using next-generation sequencing methods. Mammalian Biology 87:176–184. https://doi.org/10.1016/j.mambio.2017.09.001

Deagle, B.E., Jarman, S.N., Pemberton, D., and Gales, N.J. 2005. Genetic screening for prey in the gut contents from a giant squid (*Architeuthis* sp.). Journal of Heredity 96(4):417–423. https://doi.org/10.1093/jhered/esi036

Derocher, A.E., Lunn, N.J., and Stirling, I. 2004. Polar bears in a warming climate. Integrative and Comparative Biology 44(2):163–176. https://doi.org/10.1093/icb/44.2.163

Dyck, M.G., and Morin, P. 2011. In vivo digestibility trials of a captive polar bear (*Ursus maritimus*) feeding on harp seal (*Pagophilus groenlandicus*) and Arctic charr (*Salvelinus alpinus*). Pakistan Journal of Zoology 43(4):759–767.

Dyck, M.G., and Romberg, S. 2007. Observations of a wild polar bear (*Ursus maritimus*) successfully fishing Arctic charr (*Salvelinus alpinus*) and fourhorn sculpin (*Myoxocephalus quadricornis*). Polar Biology 30:1625–1628. https://doi.org/10.1007/s00300-007-0338-3

Galan, M., Guivier, E., Caraux, G., Charbonnel, N., and Cosson, J.-F. 2010. A 454 multiplex sequencing method for rapid and reliable genotyping of highly polymorphic genes in large-scale studies. BMC Genomics 11: 296. https://doi.org/10.1186/1471-2164-11-296

Galan, M., Pagès, M., and Cosson, J.-F. 2012. Next-generation sequencing for rodent barcoding: Species identification from fresh, degraded and environmental samples. PLoS ONE 7(11): e48374. https://doi.org/10.1371/journal.pone.0048374

Galan, M., Pons, J.-B., Tournayre, M., Pierre, E., Leuchtmann, P., Pontier, D., and Charbonnel, N. 2018. Metabarcoding for the parallel identification of several hundred predators and their prey: Application to bat species diet analysis. Molecular Ecology Resources 18(3):474–489. https://doi.org/10.1111/1755-0998.12749

Galicia, M.P., Thiemann, G.W., Dyck, M.G., and Ferguson, S.H. 2015. Characterization of polar bear (*Ursus maritimus*) diets in the Canadian High Arctic. Polar Biology 38:1983–1992. https://doi.org/10.1007/s00300-015-1757-1

Gillet, F., Tiouchichine, M.-L., Galan, M., Blanc, F., Némoz, M., Aulagnier, S., and Michaux, J.R. 2015. A new method to identify the endangered Pyrenean desman (*Galemys pyrenaicus*) and to study its diet, using next generation sequencing from faeces. Mammalian Biology 80(6):505–509. https://doi.org/10.1016/j.mambio.2015.08.002

Gitay, H., Suárez, A., Watson, R.T., and Dokken, D.J., eds. 2002. Climate change and biodiversity. IPCC Technical Paper 5. Geneva, Switzerland: Intergovernmental Panel on Climate Change.

Gormezano, L.J., and Rockwell, R.F. 2013. Dietary composition and spatial patterns of polar bear foraging on land in western Hudson Bay. BMC Ecology 13: 51. https://doi.org/10.1186/1472-6785-13-51

Hobson, K.A., Stirling, I., and Andriashek, D.S. 2009. Isotopic homogeneity of breath CO_2 from fasting and berry-eating polar bears: Implications for tracing reliance on terrestrial foods in a changing Arctic. Canadian Journal of Zoology 87:50–55. https://doi.org/10.1139/Z08-137

Iversen, M. 2011. The diet of polar bears (*Ursus maritimus*) from Svalbard, Norway, inferred from scat analysis. MSc thesis, University of Tromsø, Tromsø, Norway.

Iversen, M., Aars, J., Haug, T., Alsos, I.G., Lydersen, C., Bachmann, L., and Kovacs, K.M. 2013. The diet of polar bears (*Ursus maritimus*) from Svalbard, Norway, inferred from scat analysis. Polar Biology 36:561–571. https://doi.org/10.1007/s00300-012-1284-2

McInnes, J.C., Alderman, R., Deagle, B.E., Lea, M.-A., Raymond, B., and Jarman, S.N. 2017. Optimised scat collection protocols for dietary DNA metabarcoding in vertebrates. Methods in Ecology and Evolution 8(2):192–202. https://doi.org/10.1111/2041-210X.12677

McKinney, M., Atwood, T.C., Iverson, S.J., and Peacock, E. 2017. Temporal complexity of southern Beaufort Sea polar bear diets during a period of increasing land use. Ecosphere 8(1): e01633. https://doi.org/10.1002/ecs2.1633

Meglécz, E., Piry, S., Desmarais, E., Galan, M., Gilles, A., Guivier, E., Pech, N., and Martin, J.-F. 2010. SESAME (SEquence Sorter & AMplicon Explorer): Genotyping based on high-throughput multiplex amplicon sequencing. Bioinformatics 27(10):277–278. https://doi.org/10.1093/bioinformatics/btq641

Murphy, M.A., Waits, L.P., and Kendall, K.C. 2000. Quantitative evaluation of fecal drying methods for brown bear DNA analysis. Wildlife Society Bulletin 28(4):951–957.

Pagès, M., Desse-Berset, N., Tougard, C., Brosse, L., Hänni, C., and Berrebi, P. 2009. Historical presence of the sturgeon *Acipenser sturio* in the Rhône basin determined by the analysis of ancient DNA cytochrome *b* sequences. Conservation Genetics 10:217–224. https://doi.org/10.1007/s10592-008-9549-6

Pagès, M., Chaval, Y., Herbreteau, V., Waengsothorn, S., Cosson, J.-F., Hugot, J.-P., Morand, S., and Michaux, J. 2010. Revisiting the taxonomy of the Rattini tribe: A phylogeny-based delimitation of species boundaries. BMC Evolutionary Biology 10: 184. https://doi.org/10.1186/1471-2148-10-184

Piñol, J., Senar, M.A., and Symondson, W.O.C. 2018. The choice of universal primers and the characteristics of the species mixture determine when DNA metabarcoding can be quantitative. Molecular Ecology 28(2):407–419. https://doi.org/10.1111/mec.14776

Polz, M.F., and Cavanaugh, C.M. 1998. Bias in template-to-product ratios in multitemplate PCR. Applied and Environmental Microbiology 64:3724–3730. https://doi.org/10.1128/AEM.64.10.3724-3730.1998

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., and Taberlet, P. 2012. Who is eating what: Diet assessment using next generation sequencing. Molecular Ecology 21(8):1931–1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x

Redenbach, Z., and Taylor, E.B. 2002. Evidence for historical introgression along a contact zone between two species of char (Pisces: Salmonidae) in northwestern North America. Evolution 56(5):1021–1035. https://doi.org/10.1111/j.0014-3820.2002.tb01413.x

Russell, R.H. 1975. The food habits of polar bears of James Bay and southwest Hudson Bay in summer and autumn. Arctic 28(2):85–152. https://doi.org/10.14430/arctic2823

Shehzad, W., Riaz, T., Nawaz, M.A., Miquel, C., Poillot, C., Shah, S.A., Pompanon, F., Coissac, E., and Taberlet, P. 2012. Carnivore diet analysis based on next-generation sequencing: Application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. Molecular Ecology 21(8):1951–1965. https://doi.org/10.1111/j.1365-294X.2011.05424.x

Stempniewicz, L. 2006. Polar bear predatory behaviour toward molting Barnacle Geese and nesting Glaucous Gulls on Spitsbergen. Arctic 59(3):247–251. https://doi.org/10.14430/arctic310

Taberlet, P., Camarra, J.-J., Griffin, S., Uhrès, E., Hanotte, O., Waits, L.P., Dubois-Paganon, C., Burke, T., and Bouvet, J. 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Molecular Ecology 6(9):869–876. https://doi.org/10.1046/j.1365-294X.1997.00251.x

Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann, C., and Willerslev, E. 2007. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic Acids Research 35(3): e14. https://doi.org/10.1093/nar/gkl938

Teletchea, F., Bernillon, J., Duffraisse, M., Laudet, V., and Hänni, C. 2008. Molecular identification of vertebrate species by oligonucleotide microarray in food and forensic samples. Journal of Applied Ecology 45(3):967–975. https://doi.org/10.1111/j.1365-2664.2007.01415.x

Thiemann, G.W., Budge, S.M., Iverson, S.J., and Stirling, I. 2007. Unusual fatty acid biomarkers reveal age- and sex-specific foraging in polar bears (*Ursus maritimus*). Canadian Journal of Zoology 85:505–517. https://doi.org/10.1139/Z07-028

Van Coeverden de Groot, P. 2019. M'Clintock Channel polar bear health - Baseline estimates from non-invasively collected faeces. https://www.polardata.ca/pdcsearch/PDCSearch.jsp?doi_id=12988

Van Coeverden de Groot, P., Wong, P.B.Y., Harris, C., Dyck, M.G., Kamookak, L., Pagès, M., Michaux, J., and Boag, P.T. 2013. Toward a noninvasive Inuit polar bear survey: Genetic data from polar bear hair snags. Wildlife Society Bulletin 37(2):394–401. https://doi.org/10.1002/wsb.283

Vestheim, H., and Jarman, S.N. 2008. Blocking primers to enhance PCR amplification of rare sequences in mixed samples – a case study on prey DNA in Antarctic krill stomachs. Frontiers in Zoology 5: 12. https://doi.org/10.1186/1742-9994-5-12

Vigfúsdóttir, F., Pálsson, S., and Ingólfsson, A. 2008. Hybridization of Glaucous Gull (*Larus hyperboreus*) and Herring Gull (*Larus argentatus*) in Iceland: Mitochondrial and microsatellite data. Philosophical Transactions of the Royal Society B: Biological Sciences 363(1505):2851–2860. https://doi.org/10.1098/rstb.2008.0042

Vongraven, D., Aars, J., Amstrup, S., Atkinson, S.N., Belikov, S., Born, E.W., DeBruyn, T.D., et al. 2012. A circumpolar monitoring framework for polar bears. Ursus 23(sp2):1–66. https://doi.org/10.2192/URSUS-D-11-00026.1

Wilson, C.C., and Bernatchez, L. 1998. The ghost of hybrids past: Fixation of Arctic charr (*Salvelinus alpinus*) mitochondrial DNA in an introgressed population of lake trout (*S. namaycush*). Molecular Ecology 7(1):127–132. https://doi.org/10.1046/j.1365-294x.1998.00302.x

Wong, P.B.Y., Van Coeverden de Groot, P., Fekken, C., Smith, H., Pagès, M., and Boag, P.T. 2011. Interpretations of polar bear (*Ursus maritimus*) tracks by Inuit hunters: Inter-rater reliability and inferences concerning accuracy. The Canadian Field-Naturalist 125(2):140–153. https://doi.org/10.22621/cfn.v125i2.1197

Zhang, Z., Schwartz, S., Wagner, L., and Miller, W. 2000. A greedy algorithm for aligning DNA sequences. Journal of Computational Biology 7(1-2):203–214. https://doi.org/10.1089/10665270050081478