New insights on polar bear (*Ursus maritimus*) diet from faeces based on Next Generation Sequencing technologies.

JOHAN MICHAUX*[$], MARKUS DYCK[‡], PETER BOAG[§],

STEPHEN LOUGHEED[§] & PETER VAN COEVERDEN DE GROOT[§]

* *Conservation Genetics Laboratory, Institut de Botanique, Université de Liège, 4000 Liège (Sart Tilman), Belgique, $* CIRAD, UMR CIRAD-INRA ASTRE, *F-34398 Montpellier, France, ‡ Government of Nunavut, Department of Environment, Box 209, Iglulik, NU X0A 0L0 Canada; §Department of Biology, Queen's University, 99 University Avenue, Kingston, Ontario K7L 3N6, Canada.*

Corresponding author: Johan Michaux

E-mail: johan.michaux@uliege.be

Conservation Genetics Laboratory, Institut de Botanique, Université de Liège, 4000 Liège (Sart Tilman), Belgique.

Authors affirm that any submission to *Arctic* has not been and will not be published or submitted elsewhere while under consideration by *Arctic*.

Abstract

Practical tools to quantify range-wide dietary choices on the polar bear have not been well developed 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 polar bear faeces from captive polar bears fed known diets at the Cochrane Polar Bear Habitat (Canada, n = 5 faeces from 1 bear) and Metro Toronto Zoo (Canada, n =19 from 3 polar bears); and from wild (unfed) polar bears from a holding facility in Churchill (Canada; n=7 from 7 polar bears). 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 (MC) polar bear Management Unit (Canada) (n =117 from an unknown number of bears). These data point to a diet unsurprisingly dominated by ringed seal (*Phoca hispidia*) while including evidence of bearded seal (*Erignathus barbatus),* harbor seal *(Phoca vitulina),* muskox (*Ovibos* spp.), Arctic foxes (*Alopex lagopus*), wolves (*Canis lupus*), herring gull (*Larus argentatus*) and willow ptarmigan (*Lagopus lagopus).* We found low levels pf contamination (<3% of sequences when present), suggesting *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.

Keywords : 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 (*Alopex lagopus*), le loup (*Canis lupus*), le goéland argenté (*Larus argentatus*) et le lagopède alpin (*Lagopus lagopus)* étaient également présents dans leur

71 régime alimentaire. Les risques de contaminations lors de l'utilisation de ces technologies NGS
72 sont également discutés.
73 **Mots clés** : Ours polaire, *Ursus maritimus*, régime alimentaire, séquençage de dernière
74 génération, changement climatique, gène mitochondrial cytochrome b, phoque annelé
75

INTRODUCTION

The anticipated changes in the Arctic climate and concomitant reduction in sea-ice quantity and quality is hypothesized to affect polar bear diet (Derocher *et al.* 2004). While reduced access to seals, the main prey of polar bears, is expected to negatively affect polar bears, their reproductive rates, and ultimately their persistence (Derocher *et al.* 2004; Gitay *et al.* 2002); 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& Romberg 2007), morphological identification of prey remains from their scats (Iversen 2011; Gormezano and Rockewell 2013), biochemical analyses of fatty acids (FA) and or stable isotopes profiles from harvested tissue or biopsy plugs (Thiemann *et al.* 2007; Hobson *et al.* 2009, Galicia *et al*. 2015; Mc Kinney *et al.* 2017. These data collectively indicate that polar bears have a varied vertebrate diet including among others: ringed seal (*Pusa hispida),* bearded seal (*Erignathus barbatus),* walrus (*Odobenus rosmarus),* harp seal (*Pagophilus groenlandicus*), hooded seal (*Cystophora cristata*), harbor seal (*Phoca vitulina*) (Iversen, 2011), beluga (*Delphinapterus leucas*) (Mc Kinney *et al.* 2017), narwhal (*Monodon monocerus*) (McKinney *et al.* 2017), birds (Mc Kinney *et al.* 2017) and reindeer (*Rangifer tarandus platyrhyncus)* (Mc Kinney *et al.* 2017).

While informative, the above methods have two major limitations impeding their application in large scales 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 prey and/or predator have identical FA profiles, no discrimination is possible (Thiemann *et al.* 2007). Further, while some polar bear prey, for example bearded seals

100 (*E. barbatus*), harbor seals (*Phoca vitulina*) and Atlantic walruses (*Odobenus rosmarus)*, can
101 be identified based on their non-methylene-interrupted FA profiles, those of other pinniped
102 prey, for example harp seals (*Pagophilus groenlandicus*) and hooded seals (*Cystophora*
103 *cristata*), as well as beluga whales (*Delphinapterus leucas*) and narwhals (*Monodon*
104 *monoceros*) cannot be distinguished using FAs or they are present at low levels (ringed seals)
105 (Thiemann *et al.* 2007, Galicia *et al.* 2015). While hairs of seals can be easily distinguished
106 from hairs of reindeer and the guard hairs of polar bears based on morphology, no there are no
107 species-specific features among the different seal species that polar bears consume (Iversen
108 2011). At the extreme, soft/digestable dietary items that leave no/few hard traces in the gut or
109 faeces will be less likely to be identified using non-molecular methods (Pompanon *et al.* 2012).
110 Although molecular assays of faeces hold potential for species level detection, the
111 evaluation of these possibilities for the study of polar bears is in its infancy. To date, species
112 specific oligonucleotide primers for some seals have been designed allowing the detection of
113 different seal species in polar bear faeces (Iversen 2011), but this method fails to detect non-
114 seal prey. The optimization of molecular methods that allow for the species-level detection of
115 polar bear prey and plant food choices in as few as possible assays would enhance monitoring
116 of real time polar bear dietary responses in a changing Arctic.
117 As part of efforts to develop non-invasive polar bear monitoring methods (Wong *et al.*
118 2011, Van Coverden de Groot et al. 2013, Van Coeverden de Groot, 2019), we detail the
119 optimization of a next generation sequencing (NGS) method that allows the identification of
120 most vertebrate species comprising the diet of polar bears from their faeces. We tested a 136
121 base pairs (bp) segment of the mitochondrial cytochrome b (*cytB*) sequence (Teletchea *et al.*
122 2008; Galan *et al*., 2012) amplified from polar bear faeces using 454 pyrosequencing for
123 vertebrate prey identification. We evaluated: a) the efficacy of obtaining target *cytB* amplicons

from faeces collected under a variety of 'field' conditions using this method, b) the repeatability of our method in diagnosing the same vertebrate prey ID 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 at two zoos with known diets. After demonstrating the validity of this method, we profile 117 polar bear faeces collected from the M'Clintock Channel (MC) polar bear management unit in Nunavut, Canada during May of 2007-2011 and describe the vertebrate dietary choices of this population of bears.

METHODS

Faecal Samples:

We collected two sets of captive polar bear faeces (n=24) 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 5 years from 2007-2011 (n=117, Figure 1).

Faecal samples from captive polar bears fed known diets.

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

148 & 20, for the regular diet on day 14, and for seal (D&E) diet on days 10 & 20. These samples
149 were used in the initial piloting of our methods.

150 To further assess the accuracy of our method, we assayed a second set from captive
151 polar bears comprising 19 faecal samples from 3 captive polar bears housed at the Metro
152 Toronto Zoo (MTZ in Canada). A sample was collected from each bear for each of six weeks
153 with one bear having a 7th sample collected at a later date. These bears were each fed diets fairly
154 typical of most captive polar bears which consisted mainly of horse meat with herring, smelt,
155 rabbit, hard-boiled eggs, dog food and vegetables.

156

157 *Faecal samples from wild polar bears with unknown diets.*

158 We also analyzed faeces from 7 polar bears temporarily housed in a holding facility in Churchill
159 Manitoba – Polar Bear 'Prison" (PBP) in 2007. These were 'nuisance' bears held in PBP until
160 the Hudson Bay ice sheet forms at which time they were released. In contrast to the PBH and
161 MTZ bears – they are not fed and our diet determinations reflect feeding before 'incarceration'.
162 The bears are provided with only water during their stay. The collection of these faeces relative
163 to initial date of 'incarceration' is unknown. Finally, we used our method to quantify the recent
164 dietary choices of 117 free ranging polar bears from the MC subpopulation in Nunavut collected
165 from 2007-2011 (Figure 1). These samples were collected by Inuit collaborators using snow-
166 machines as part of efforts to optimize ground based non-invasive methods of monitoring polar
167 bears (Van Coeverden de Groot *et al.* 2013; Wong *et al.* 2011). The faecal samples were stored
168 in freezer bags, kept frozen with snow and transported frozen to the Gjoa Haven Hunters and
169 Trappers Association (HTA) freezer and finally to Queen's University in Kingston, Canada.

170

171

172 *Molecular & Analytical Methods – Pilot study with faeces from a single captive bear:*

173 DNA was extracted from all faeces using the QIAamp Fast DNA Stool Mini Kit
174 (Qiagen, Inc; Netherlands) following manufacturer protocols at Queen's University and the
175 American Museum of Natural History. In the pilot study, only 2 dilutions - 1 µl & 2 µl - from
176 each of the extractions of the 5 faeces (from the single PBH bear) were used as template in the
177 PCR. These two dilutions were assessed to find the best compromise between potential
178 inhibitors and target DNA amplicon (Teletchea *et al.,* 2008; Galan *et al.,* 2012) concentration.
179 The 136 bp amplicon of *cytB* was chosen because: i) it discriminates among most vertebrate
180 species including those that show close evolutionary affinity (Teletchea *et al.,* 2008; Galan *et*
181 *al.,* 2012), ii) its short length is suited for the PCR amplification of degraded DNA (Murphy *et*
182 *al.,* 2000; Taberlet *et al.,* 1997), and iii) it has been successfully used in studies with degraded
183 DNA extracted from non-invasive, museum and archaeological samples (Galan *et al*., 2012,
184 Pagès *et al.,* 2010; Pagès *et al.,* 2008; Teletchea *et al.,* 2008). Primers used to amplify this
185 cytochrome b fragment were: (5′-
186 CCATCTCATCCCTGCGTGTCTCCGACTCAGNNNNNNNGAYAAARTYCCVTTYCAY
187 CC-3′) and H15546R (5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGNNNNNNN
188 AARTAYCAYTCDGGYTTRAT-3′) (Galan et al. 2012).

189 Following Galan *et al.* (2010), PCR amplicons were individually tagged with fusion
190 primers and then pooled for 454 pyrosequencing. The fusion primers consist of an additional 7
191 bp. sequence (the tag) and a 30 bp. A titanium adaptor at the 5' ends are necessary for emulsion-
192 based clonal amplification (emPCR) and 454 GS-FLX pyrosequencing using Lib-L Titanium
193 Series reagents. The combination of the forward and the reverse tagged-primers produces a

194 unique barcode for each amplicon. PCR blanks containing only water were used systematically
195 to check for possible cross contaminations among samples.
196 The SESAME package for genotyping multiplexed individuals based on NGS amplicon
197 sequencing (Meglécz *et al.* 2010) was used to characterize sequences., Sequences differing by
198 at least one base-pair substitution were identified "variants" (Galan et al. 2010). We followed
199 Galan *et al.* 2012 to classify all variants as "artefactual variants" (*i.e.* variants that resulted from
200 polymerase errors during PCR and emPCR, and pyrosequencing errors) or "true variants" (*i.e.*
201 variants that were retained after our validation procedure). "True variants" are henceforth
202 referred to as "haplotypes". Species identification of *cytB* haplotypes obtained from the
203 different faecal DNA extracts was performed using the NCBI's BLASTN program (Zhang *et*
204 *al.,* 2000) against the database from GenBank (EMBL, DDBJ and PDB sequences).
205
206 *Molecular & Analytical Methods*
207 We quantified *efficacy* in two ways: a) by calculating the probability of successfully
208 amplifying *cytB* amplicons from captive and wild polar bear faeces, and b) via an estimate of
209 prey identification repeatability where the same dietary item is identified across two dilutions
210 from the same extract. Based on the results of our pilot assay of 5 faeces from the same bear
211 held in Cochrane (PBH), we analyzed 1µl & 2µl dilutions from the initial extractions of
212 individual faeces from each of 7 different bears PBP bears, 19 faeces from 3 different MTZ
213 bears, and 117 faeces from an unknown number of MC bears. These dilutions were amplified
214 with the 136 bp *cytB* primers as above. All PCR products were run out on 1.5% agarose gel
215 stained with Ethidium bromide, and scored as FAIL – no product detected in agarose, WEAK
216 – small amounts of PCR product detected and OK – appreciable amount of PCR product. To
217 improve the diet characterization of those samples that performed poorly in the initial 2 PCRs

(one each of 1µl & 2µl dilutions of their initial extractions), those PBP, MTZ and MC samples that yielded only WEAK or FAIL in their first two PCRs were re-extracted. We performed PCR on these new extracts with 1µl & 2µl dilutions (as above) and scored them as FAIL, WEAK and 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 4 PCRS (of 2 dilutions of 2 extracts) and those that had at least one WEAK PCR result across a maximum of 4 PCR's. The effect of re-extraction of PBP, MTZ and MC faecals 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 faecals (described above), molecular food item identification was performed on all WEAK or OK PCRs with the same protocol used for the pilot with PBH faecals (described above). By comparing the genetic identifications for MTZ polar bears using our *2 dilution – 2 extraction protocol* with their known diets, we conducted an evaluation of the accuracy of our method. We used the *2 dilution – 2 extraction* method with 454 pyrosequencing to characterize vertebrate dietary choice of free ranging polar bears from M'Clintock Channel, Nunavut.

RESULTS

Polar bear diet determination from NGS assays of their faeces – initial evidence from a single captive bear:

241 Our pilot study of 454 diet determination from a 136 bp *cytB* sequence amplified from
242 extracts of 5 polar bear faeces from a single bear (PBH) fed three different diets for three weeks
243 over 9 weeks – suggests that our molecular diagnoses are accurate to vertebrate genus level
244 (Table 1). Three out of 5 PBH faecals (A, B, & F) samples worked across both dilutions in the
245 initial *cytB* PCR. [Neither extract for PBH D &E amplified across both dilutions and unlike the
246 process followed for all other initial FAIL or WEAK PCRS (see above), they were not re-
247 extracted nor were PCRs repeated in this pilot]. Across the 6 successful dilutions (from 3 PBH
248 extracts) a total of 657 'reads' were obtained with between 1 - 4 different *haplotypes* in each of
249 the 6 dilutions (Table 1 & Figure 2). *CytB* sequences of the host (polar bear) represented a high
250 proportion of the haplotypes for each of the 6 dilutions: 54% - 100% of the total and only polar
251 bear DNA was recovered from both dilutions of PBH A.
252 The species ascribed to the non-polar bear haplotypes matched those of the ingested
253 food items for PBH B & F. In the case of PBH B (bear fed a char diet), 39 & 46% (dilution 1
254 & 2) of the total number of sequences were assigned to the correct genus for Arctic Char -
255 *Salvelinus* (Salmonidae). For PBH F (collected when the bear was fed a harp seal diet), 9% &
256 14% of the total number of sequences were identified as the harp seal *Phoca groenlandica*.
257 PBH F also showed small traces of chicken and deer DNA. Both "exotic" chicken and deer
258 sequences were found in a very low frequency (0.9 & 1.3%) but correspond to actual diet items
259 the bear was fed as part of the regular diet preceding the collection of faecal F. In PBH F
260 *Salvelinus* sequences were also obtained at a very low frequency (0.7%) likely reflecting char
261 fed before the onset of the harp seal diet.
262 Our PBH pilot survey showed: a) accurate diet determination from polar bear faeces, b)
263 host DNA always represented the largest fraction of recovered haplotypes, c) trace quantities
264 of earlier diets may still be detectable after >3 weeks of consumption, d) the PCR of 1µl & 2µl

265 dilutions from a single extract of a target faeces yielded *cytB* amplicons were 60% successful,
266 and e) the single extraction from a faeces does not always lead to the amplification of *cytB*
267 haplotypes that can be sequenced and this failure would seem to be independent of diet. The
268 above suggests that while 454 diet analysis of our larger dataset is effective, a second extraction
269 should be attempted when the *cytB* PCR of the first extraction yields a FAIL or WEAK result.
270 The PCR of 1µl & 2µl dilutions from between one and two extracts of the same faeces was
271 followed for all subsequent 454 assays.
272
273 *Efficacy and accuracy of amplification and 454 sequencing of a 136 bp cytB amplicon for*
274 *genetic determination of vertebrate prey from polar bear faeces*
275 Using our *2 Dilution – 2 extraction* method we estimated our *efficacy* of generating 454
276 amplicons that could be sequenced at 90.8% for the 143 polar bear faeces that we assayed herein
277 (Table 2). The 'source' of the faeces affected the amplification success rates with most success
278 achieved (100%) with the 7 faeces from the 7 bears held in the Churchill facility, followed by
279 93% success with the 117 faeces collected in MC from an unknown number of bears, to 74%
280 of the 19 MTZ faeces from 3 bears (Table 2).
281 From the 143 polar bear faeces we generated a total of 250 successful *cytB* PCR
282 amplifications from the corresponding 1µl & 2µl dilutions. Our 454 sequencing of these PCR
283 products yielded a total of 53 732 *cytB* reads corresponding to 3,010 distinct variants. These
284 were subsequently assigned to the 250 dilutions (220 from 110 DNA extracts which worked
285 with two dilutions; 30 which only worked for one dilution). The artefactual variants were sorted
286 and discarded manually using SESAME. After this validation step (*i.e.* Substitution, Indel and
287 Chimera excluded), the mean number of reads per successful PCR was 188.00. More than 50

288 validated reads were obtained for 91.88% of the samples, more than 100 for 80.77% of the
289 samples.

290 From the above, we computed of a second estimate of *efficacy*, the proportion 1µl &
291 2µl dilutions from the same extraction where the same prey items were identified when the host
292 animal's sequences (polar bear, Arctic fox, wolf/dog) was excluded. Across all PBP, MTZ and
293 MC samples, prey identification repeatability was = 84.6% across paired dilutions of the same
294 extracts. Included in this estimate are those cases where only polar bear DNA was amplified
295 in one of the paired extracts, while a prey item was identified in the other.

296 The initial accuracy estimates from the PBH sample were corroborated with our assay
297 of the MTZ polar bears faeces (Figures 3 and 4). Of the 14 MTZ faecal samples that worked,
298 two were discarded as results indicated only human haplotypes in the 4 extracts. The correct
299 vertebrate dietary items were identified in the remaining 12 MTZ samples. We detected all
300 known food items (*i.e.* horse, herring, rabbit, hard boiled eggs, cow, and smelt) fed to the 3
301 MTZ bears in the 12 faeces – this despite the MTZ bears having a more varied diet than the
302 single PBH bear fed the same diet for 3 consecutive weeks at time. Also, the assay showed high
303 specificity discriminating between different species of fish consumed (*e.g.* capelin, *Mallotus*
304 *villosus;* rainbow smelt, *Osmerus mordax;* walleye, *Sander vitreus*) (Figures 2 and 3). The
305 sensitivity of the technique was further exemplified by our detection of DNA from two
306 "contaminating" species in our 12 study faeces. We found a single read of *Canis*
307 *lupus*/*familiaris* (in a single extract out of 2 "positives' from 2 independent extracts from the
308 same faeces). This were never a diet item but wolves were in the next enclosure at the MTZ
309 likely leading to this "contaminated" result. We also found 2 reads (0.0045%) of
310 *Brachyramphus marmoratus* (Alcidae, marbled murrelet) in a single extract from a different
311 faeces. This species was previously studied in one of our laboratories and although below

312 0.010% of all sequences in the sample, previous taxa studied in our laboratories would appear
313 to represent a source of contamination in this sensitive assay (see below).
314
315 *454 PyrosSequencing and determining wild polar bears diets*
316 Of the 117 collected faecals from wild MC polar bears, 8 did not work across 4 dilutions
317 of 2 extractions and 6 were excluded from further diet analysis because they were not polar
318 bear. One sample (PBF07-05) yielded mainly *Canis* reads (131 and 110 reads for each replicate,
319 see Figure 2) and may have come from the dog that accompanied the expedition that year but
320 may have come from a wolf. Repeat assays of three samples (PBF08-10, -16 and -26) were
321 shown to be from an arctic fox (numerous reads of *Alopex lagopus* associated with seal reads).
322 Two samples (PBF10-03 and PBF10-08) were considered as unknown 'predator' origin as only
323 prey seal sequences could be retrieved associated with human DNA (there were no polar bear
324 sequences) .
325 The ringed seal comprises the main prey item in wild bears from M'Clintcok Channel
326 during May of 2007-2011 (*P. hispida)* DNA was in 86.14% of the faeces of wild bears; Figures
327 2 & 4). Two other seals, the harbour seal, *Phoca vitulina* and the bearded seal, *Erignathus*
328 *barbatus* form smaller but substantial portions of the polar bear diet with their DNA found in
329 3.96% and 4.95% of MC faeces respectively). While these species are the most common
330 vertebrate prey DNA reads found in MC faeces, our method points to other vertebrates
331 contributing to the polar bear diet at this time of year in M'Clintock Channel. These include:
332 carnivores, arctic foxes and wolves (5.94% and 0.99% of extracts had *Alopex* and *Canis lupus*
333 DNA respectively – these faeces are distinguished from those believed to have come from foxes
334 or wolves [see above] by the predominance of polar bear DNA reads in them); ungulates for
335 example muskox, *Ovibos moschatus* (1.98% of extracts have *Ovibos* DNA reads); and birds for

example the Ptarmigan *Lagopus lagopus* and gulls *Larus* sp. (1.98% and 0.99% of extracts had *Lagopus* and *Larus* DNA reads, respectively). Unfortunately, the mini-barcode did not allow the discrimination among three putative gull species (*L. thayeri, L. hyperboreus, L. argentatus*).

DISCUSSION

With our *2 dilution - 2 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 136bp *cytB* amplicon. Specifically we: a) determined the success of obtaining *cytB* amplicons for NGS sequencing across variety of polar bears faeces varies from 73.6% to 100% with a mean of 90.8% across all faeces, b) show our technique to be reliable and accurate by evaluating 4 captive polar bears fed known diets, and c) through the assay of 117 polar bear faeces from an unknown number of bears provide strong evidence that while the primary prey of these MC bears during the months of May 2007-2011 is the ringed seal - diverse vertebrate taxa comprise the diet of polar bears at this time. Below we discuss these findings as well as the use of our results to counter contamination in this sensitive assay, limitations of our findings and next steps given these finding and more recent technological advances.

The efficacy, reliability & accuracy of our method.

We showed decreased *cytB* amplification success - *efficacy* - in captive vs. wild polar bear faeces despite the improved collection and handling of the faeces of the captive polar bears by trained technicians. An explanation of these different success rates may be 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

359 "subzero" storage. This interpretation is consistent with other studies showing PCR success
360 from faecal extracts are correlated with the freshness of fecal samples (McInnes *et al*. (2017).
361 While the *efficacy* of generating prey *cytB* amplicons using extractions from wild polar
362 bear faeces is 93%, our early estimate of *reliability* for pooled MTZ, PBP and MC bears is
363 lower at 84.6%. This is not unexpected as one of the dilutions contains 50% more DNA than
364 the other and the two 'sampling events' of the same DNA will likely be different particularly
365 with respect to prey template DNA which are already in low numbers. In addition, the faecal
366 DNA extract contains DNA from the host, prey, bacteria, viruses, and other commensal and
367 pathogenic taxa further reducing the likelihood of replicate sampling in dilutions differing by
368 50%. The more useful estimate of reliability will come from the faeces of the *same* wild polar
369 bears. This analysis awaits increased genotyping success (see below).
370 Our recovery of the (genetic identification) of the vertebrate diet items fed to the captive
371 PBH and MTZ bears indicates our method is *accurate*. Although the 'accuracy' of our method
372 is difficult to estimate - we cannot resolve to species level for all *cytB* amplicons from taxa in
373 the same genus (see *Limitations* below) – the results of the captive bear assay means that we
374 can accept with confidence the identification of vertebrate prey items including seal and other
375 taxa that are known to be part of the polar bear diet including birds (see *Larus* difficulties below)
376 and fish (Russel, 1975, Stempniewicz 2006; Dyck & Romberg 2007, Gormezano *et al*., 2013,
377 Iversen et al. 2013). The above validation means our detection of prey items believed to be
378 uncommon in polar bear diet like muskox (*Ovibos* spp.), Artic foxes (*Alopex lagopus*) and
379 wolves (*Canis vulpus*) is likely correct and the future detection of other vertebrate taxa not eaten
380 by the polar bears in our study sample but known to have been consumed by polar bears in the
381 wild for example: reindeers (*Rangifer tarandus*) (Iversen 2011) belugas (*Delphinapterus*

382 *leucas*), narwhals (*Monodon monoceros*) walruses (*Odobenus rosmarus*) (Derocher *et al.* 2004)
383 or marine birds (*Larus* spp. (Stempniewicz 2006) is possible.
384
385 *Faceal NGS Contamination – a Road Map to Improved Quality Control.*
386 Our results suggest an acceptable level of contamination so as to not invalidate the
387 results and importantly the identification of reads resulting from contamination allow for
388 targeted quality control and specific method improvement steps that can reduce contamination,
389 This will allow for more robust inferences of polar bear dietary patterns from polar bear faeces.
390 Some of our faecals were clearly contaminated and not of polar bear origin: for example two
391 MTZ faeces yielded only human DNA and the MC samples PBF08-10, PBF08-16 and PBF08-
392 26 were from Arctic Fox and PBF07-05 was likely a wolf. These samples were excluded from
393 further analysis. In all other instances, the contaminating sequences in captive and wild bear
394 samples (Figure 2) were present at less than 3% frequency. This ratio is low and appears
395 characteristic in similar amplicon sequencing studies using Next Generation Sequencing (NGS)
396 (Pompanon *et al.* 2012). The characterization of these contaminating sequences in this study
397 assists in reducing the effects of contamination in future studies by pointing to contaminating
398 source at all our steps - from faecal collection in zoos and the wild to the final 454
399 pyrosequencing step.
400 As in other high throughput sequencing (Shehzad *et al.* 2012), human haplotypes were
401 identified in 2.50% (1,039 human reads out of 40,062 total reads including human non-
402 functional nuclear paralogs) of the validated reads and found in 56.64% of the samples. The
403 source of these human contaminating sequences could have occurred at all steps during the
404 collection of these data and suggest a general improvement in sterile technique when handling
405 faeces. The detection of cow, pig and herring sequences in wild polar bear faecals, however,

406 reflects faecal collection and storage procedures in our earlier field sampling. In our earlier
407 fieldwork, the faecal storage coolers used in the field trips were first used to store meat for
408 consumption during field work. This was changed in the later fieldwork. Similarly, the presence
409 of wolf DNA in MTZ faecals likely represents contamination from animals in nearby exhibits.
410 Finally, the presence of marbled murrelet (*Brachyramphus marmoratus)* in polar bear feces
411 also represent contamination at the stage of DNA extraction as this taxa had been previously
412 analyzed in our laboratories. The presence of these contaminating species clearly pointed to all
413 steps in our method that needed improvement – in this case from field collection, to storage,
414 through to lab sterilization.

415 *Limitations of the method*

416 Although the detection of diverse array of prey species from faeces of anonymous polar
417 bears suggest our method surpasses other molecular methods based on species-specific primers
418 (Iversen 2011) and FA methods, there are limitations to current faecal extract molecular
419 methods including our own. More specifically these limitations are: a) the inability to quantify
420 the amount of prey ingested (Pinol *et al.,* 2018), b) the preferential amplification of polar bear
421 mitochondrial DNA, c) inability of our 136 *cytB* amplicon to discriminate among some species
422 of potential prey species, d) the lack identity of the defecating polar bear (meaning that we do
423 not know how many individuals are encompassed by our field sampling), and e) the need to
424 optimize the genetic identification of the plant diet of wild polar bears.

425 Unlike FA, we cannot provide quantitative estimates of the amount of prey items
426 ingested from the amplicons amplified. More specifically, the number of reads obtained per
427 sample cannot be directly linked to the quantity of ingested preys because: 1) preferential
428 amplification of some species to the detriment of others is possible when dealing with mixtures
429 of DNA templates as found in faecal DNA extracts (Polz and Cavanaugh 1998; Pompanon *et*

430 *al.* 2012); 2) tissues do not have the same density of mitochondrial DNA across species
431 prohibiting quantitative PCR with these DNAs (Deagle *et al.* 2005); and 3) scat samples
432 correspond to the end of the assimilation process and do not always reflect accurately food
433 intake (*i.e.* difference survival of DNA during digestion). The quantification of ingested prey
434 with 454 pyrosequencing and other NGS platforms of polar bear faecal extracts will remain a
435 challenge.

436 The amplification of relatively larger amounts of host (polar bear) DNA will impede the
437 amplification of prey DNA. In this regard, the reduction in the amplification of polar bear DNA
438 amplicons may be achieved with addition of blocking oligonucleotides (Shehzad *et al.* 2012;
439 Vestheim & Jarman 2008)). These oligonucleotides bind to the host DNA and prevent PCR
440 elongation (Vestheim& Jarman 2008). While promising, the application of this technique to
441 454 pyrosequencing and other NGS assays of polar bears may not be straightforward for two
442 initial reasons.

443 The finding of an appropriate binding site for a species-specific primer next to a binding
444 site of universal primer is difficult when the amplicon is small. Polar bear vertebrate diet
445 includes related and distantly related species, which increases the difficulty of designing
446 primers to inhibit the amplification of bear amplicons while allowing amplification of prey
447 items. In addition, the amplification of the polar bear DNA ensures the faeces is from a polar
448 bear (some of our samples were revealed to come from arctic foxes and from arctic wolves or
449 dogs accompanying Inuit hunters). In this regard, it is important to know that the faeces is from
450 a polar bear as evidenced by some sequenced polar bear amplicons.

451 We are unable to discriminate among some species and could only discriminate to the
452 genus level for char, gulls, and deer. The discrimination between S*alvelinus elgyticus*, *S.*
453 *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 (*e.g. Salvelinus fontinalis* x *S. alpinus*, (Bernatchez *et al.* 1995); *S. alpinus* x *S. namaycush*, (Wilson & Bernatchez 1998); *S. malma* x *S. confluentus,* (Redenbach *et al*. 2002) meaning that mitochondrial marker could not be the most suitable tool to discriminate among char species. Similarly, with respect to the gull species *L. thayeri*, *hyperboreus*, *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 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-ing those faecal extracts which the vertebrate prey could only be resolved to genus level with (prey) species-specific primers under more stringent conditions. Also, some members of these species groups are not found within the range of polar bears and can therefore be excluded as prey. Finally, other mitochondrial gene sequences for example 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 which distinguish among different polar bears. This identification is critical to a) determine patterns of consumption of the same bears over time and location and b) 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 type 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 *2 dilution – 2 extraction* 454 pyrosequencing method, it is possible to simultaneously determine: a) baseline dietary characteristics and b) dietary response to ongoing climate change for polar bears at a scale not previously possible for polar bears and that should be part of a long-term monitoring program (Vongraven and Peacock (2012). Further, it is important to highlight the potential new NGS platforms bring to a non-invasive Inuit inclusive methods for studying polar bear diets. Illumina technology - for example MiSeq, NextSeq, NovaSeq and HiSeq sequencers - offer much larger number of sequences per faecal extract (Gillet *et al.,* 2015, Biffi *et al.,* 2017a, Biffi *et al.*, 2017b, Andriollo *et al*., 2019). Higher sequence number allows 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.

ACKNOWLEDGMENTS

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 (TEK) that enabled the collection of polar bear faeces in the wild. Field work was supported by the Polar Continental Shelf Project (PCSP), Nunavut Wildlife Management Board (NWMB) and Nunavut Tunngavik Inc. (NTI) awards to

501 the Gjoa Haven Hunters and Trappers Organization and P.v.C.d.G. and a National Sciences and
502 Engineering Research Council (NSERC) Northern Research Supplement (P.T.B.). Laboratory
503 costs were funded by the Nunavut General Monitoring Plan (P.v.C.d.G., M.P., J.M. & P.T.B.)
504 with contributing funds from an NSERC Discovery Grant (P.T.B.). We thank the Cochrane
505 Polar Bear Habitat (PBH) for their co-operation and in-kind support during the captive polar
506 bear feeding trials. Manitoba Conservation and Daryll Hedman for providing the faeces from 7
507 polar bears held in their holding cells. Finally we thank the Metro Toronto Zoo (MTZ) for their
508 cooperation and polar bear faecal sample collection at their facility.
509

510 REFERENCES

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

514 Bernatchez, L., Glémet, H., Wilson, C.C. and Danzmann, R.G. 1995. Introgression and fixation
515 of Arctic char (Salvelinus alpinus) mitochondrial genome in an allopatric population of
516 brook trout (Salvelinus fontinalis). Canadian Journal of Fisheries and Aquatic Science
517 52, 179-185. https://doi.org/10.1139/f95-018

518 Biffi M., Gillet F., Laffaille P., Aulagnier S., Blanc F., Colas F., Galan M., Némoz M.,
519 Tiouchichine M.-L, Buisson L.and Michaux J.R. 2017a. Novel insights into the diet of
520 the Pyrenean desman Galemys pyrenaicus using next-generation sequencing molecular
521 analyses. Journal of Mammalogy, 87 176-184 .
522 https://doi.org/10.1093/jmammal/gyx070

523 Biffi M., Lafaille P., Jabiol J., André A., Gillet F., Lamothe S., Michaux J.R. and Buisson L.
524 2017b. Comparison of diet and prey selectivity of the Pyrenean desman and the Eurasian
525 water shrew using next-generation sequencing methods. Mammalian Biology, 87 176-
526 184. https://doi.org/10.1016/j.mambio.2017.09.001

527 Bradley, R.D., Bryant, F.C., Bradley, L.C., Haynies, M.L. and Baker, R. 2003. Implications of
528 hybridization between white-tailed deer and mule deer. Southwestern Naturalist 48,
529 654-660. https://doi.org/10.1894/0038-4909(2003)048<0654:IOHBWD>2.0.CO;2

530 Chase, M.W., Cowan, R.S., Hollingsworth, P.M., van den Berg, C., Madrinan, S., Petersen, G.,
531 Seberg, O., Jorgsensen, T., Cameron, K.M., Carine, M., Pedersen, N., Hedderson,
532 T.A.J., Conrad, F., Salazar, G.A., Richardson, J.E., Hollingsworth, M.L., Barraclough,

T.G., Kelly, L. and Wilkinson, M. 2007. A proposal for a standardised protocol to barcode all land plants. Taxon 56, 295-299. https://doi.org/10.1002/tax.562004

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, 417-423. https://doi.org/10.1093/jhered/esi036

Derocher, A., Lunn, N. and Stirling, I. 2004. Polar bears in a warming climate. Integrative and Comparative Biology 44, 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 (Salevlinus alpinus). Pakistan Journal of Zoology 43, 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 (Myoxocephalus quadrinis). Polar Biology 30, 1625-1628. https://doi.org/10.1007/s00300-007-0338-3

Fišer Pečnikar, Ž., Buzan, E.V. 2014. 20 years since the introduction of DNA barcoding: from theory to application. J Appl Genetics 55, 43-52. https://doi.org/10.1007/s13353-013-0180-y

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., 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. 1-16. https://doi.org/10.1101/155721

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

Gilbert ,C., Ropiquet, A. and Hassanin, A. 2006. Mitochondrial and nuclear phylogenies of Cervidae (Mammalia, Ruminantia): Systematics, morphology, and biogeography. Molecular Phylogenetics and Evolution 40, 101-117. https://doi.org/10.1016/j.ympev.2006.02.017

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, 505-509. https://doi.org/10.1016/j.mambio.2015.08.002

Gitay, H., Suárez, A., Watson, R. and Dokken, D. 2002. Climate Change and Biodiversity IPCC, Geneva, Switzerland, Geneva, Switzerland.

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

Hailer, F., Kutschera, V.E. and Hallstrom, BM, Klassert, D.,. Fain, S.R. Leonard, J., Arnason, U. and Janke, A. 2012. Nuclear genomic sequences reveal that polar bears are an old and distinct bear lineage. Science 336, 344-347. https://doi.org/10.1126/science.1216424

Hobson, K.A., Stirling, I. and Andriashek, D.S. 2009. Isotopic homogeneity of breath CO2 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, University of Tromsø.

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

Lindahl, T. 1993. Instability and decay of the primary structure of DNA. Nature 362, 709-715. https://doi.org/10.1038/362709a0

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, 192-202. https://doi.org/10.1111/2041-210X.12677

MC Kinney, M.,_Atwood, T., 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, 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, 951-957

603 Musser, G. and Carleton, M. 2005. Superfamily Muroidea. In Mammal species of the world A
604 taxonomic and geographic reference Volume 2. 3rd edition. Edited by: Wilson DE,
605 Reeder DM. Baltimore , Johns Hopkins University. 894-1531.
606 Pagès, M., Chaval, Y., Herbreteau, V, Waengsothorn, S., Cosson, JF, Hugot, J.P., Morand, S
607 and Michaux J.R. 2010. Revisiting the taxonomy of the Rattini tribe: a phylogeny-based
608 delimitation of species boundaries. BMC Evolutionary Biology 10, 184.
609 https://doi.org/10.1186/1471-2148-10-184
610 Pagès, M., Desse-Berset, N., Brosse, L., Hänni, C. and Berrebi, P. 2008. Historical presence of
611 the sturgeon Acipenser sturio in the Rhône basin determined by the analysis of ancient
612 DNA cytochrome b sequences. Conservation Genetics 10, 217-224.
613 https://doi.org/10.1007/s10592-008-9549-6
614 Pinol, J., Senar, M. and Symondson, W.O.C. 2018. The choice of universal primers and the
615 characteristics of the species mixture determine when DNA metabarcoding can be
616 quantitative. Molecular Ecology 28:407-419. DOI: 10.1111/mec.14776.
617 https://doi.org/10.1111/mec.14776
618 Polz, M.F. and Cavanaugh, C.M. 1998. Bias in template-to-product ratios in multitemplate
619 PCR. Appiedl Environmental Microbiology 64, 3724-3730.
620 https://doi.org/10.1128/AEM.64.10.3724-3730.1998
621 Pompanon, F., Deagle, B.E., Symondson, W.O., Brown, D., Jarman, S.N. and Taberlet, P. 2012.
622 Who is eating what: diet assessment using next generation sequencing. Molecular
623 Ecology 21, 1931-1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x
624 Redenbach, Z. and Taylor, E.B. 2002. Evidence for historical introgression along a contact zone
625 between two species of char (Pisces: Salmonidae) in northwestern North America.
626 Evolution 56, 1021-1035. https://doi.org/10.1111/j.0014-3820.2002.tb01413.x

627 Russell, F. and Richard, H. 1975. "The Food Habits of Polar Bears of James Bay and Southwest
628 Hudson Bay in Summer and Autumn". Arctic 28 (2), 117-129.
629 https://doi.org/10.14430/arctic2823
630 Shehzad, W., Riaz, T., Nawaz, M.A, Miquel, C., Poillot, C., Shah, S.A., Pompanon, F., Coissac,
631 E. and Taberlet, P. 2012. Carnivore diet analysis based on next-generation sequencing:
632 application to the leopard cat (Prionailurus bengalensis) in Pakistan. Molecular Ecology
633 21, 1951-1965. https://doi.org/10.1111/j.1365-294X.2011.05424.x
634 Stempniewicz, L. (2006) Polar bear predatory behaviour toward molting barnacle geese and
635 nesting glaucous gulls on Spitsbergen. Arctic 59, 247-251.
636 https://doi.org/10.14430/arctic310
637 Taberlet, P., Camarra, J.J., Griffin S, Uhres, E., Hanotte, O., Waits, L.P., Dubois-Paganon, C.,
638 Burke, T., Bouvet, J. 1997. Noninvasive genetic tracking of the endangered Pyrenean
639 brown bear population. Molecular Ecology, 6, 869-876. https://doi.org/10.1046/j.1365-
640 294X.1997.00251.x
641 Taberlet, P., Griffin, .S, Goossens B, Questiau, S., Manceau, V., Escaravage, N., Waits, .L.P.
642 and Bouvet, J. 1996. Reliable genotyping of samples with very low DNA quantities
643 using PCR. Nucleic Acids Research 24, 3189-3194.
644 https://doi.org/10.1093/nar/24.16.3189
645 Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A.Vermat, T.,
646 Corthier G., Brochmann, C. and Willerslev, E. 2006. Power and limitations of the
647 chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic acids research, 35(3),
648 e14. doi:10.1093/nar/gkl938. https://doi.org/10.1093/nar/gkl938
649 Teletchea, F., Bernillon, J., Duffraisse, M., Laudet, V. and Hänni, C. 2008. Molecular
650 identification of vertebrate species by oligonucleotide microarray in food and forensic

651 samples. The Journal of applied ecology doi:10.1111/j.1365-2664.2007.01415.x.
652 https://doi.org/10.1111/j.1365-2664.2007.01415.x
653 Thiemann, G.W., Budge, S.M,. Iverson, S.J. and Stirling, I. 2007. Unusual fatty acid biomarkers
654 reveal age- and sex-specific foraging in polar bears (Ursus maritimus). Canadian
655 Journal of Zoology 85, 505-517. https://doi.org/10.1139/Z07-028
656 Thiemann, G.W., Iverson, S.J. and Stirling, I. 2008. Polar bear diets and arctic marine food
657 webs: insights from fatty acid analysis. Ecology Monographs 78, 591-613.
658 https://doi.org/10.1890/07-1050.1
659 Valentini, A., Miquel, C., Nawaz, M.A., Bellemain, E., Coissac, E., Pompanon, F., Gielly L.,
660 Cruaud C., Nascetti G., Wincker, P., Swenson J.O. and Taberlet, P. 2009. New
661 perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the
662 trnL approach. Molecular Ecology Resources 9, 51-60. https://doi.org/10.1111/j.1755-
663 0998.2008.02352.x
664 Van Coeverden de Groot, P. 2019. M'Clintock Channel Polar Bear health - Baseline Estimates
665 From Non-Invasively Collected Faeces. NGMP 2011-2015. Final Report (Excel
666 Workbook).
667 Van Coeverden de Groot P., Wong, P.B.Y., Harris, C., Dyck, M., Kamookak, L., Pagès, M.,
668 Michaux, J.R. & Boag, P. 2013. Toward a Noninvasive Inuit Polar Bear Survey: Genetic
669 Data from Polar Bear Hair Snags. Wildlife Society Bulletin 37, 394-401.
670 https://doi.org/10.1002/wsb.283
671 Vestheim H. and. Jarman, S.N. 2008. Blocking primers to enhance PCR amplification of rare
672 sequences in mixed samples - a case study on prey DNA in Antarctic krill stomachs
673 Frontiers Zoology 5, 12. https://doi.org/10.1186/1742-9994-5-12

674 Vigfúsdóttir, F., Pálsson, S., & Ingólfsson, A. 2008. Hybridization of glaucous gull (Larus
675 hyperboreus) and herring gull (Larus argentatus) in Iceland: mitochondrial and
676 microsatellite data. Philosophical transactions of the Royal Society of London. Series
677 B, Biological sciences, 363(1505), 2851-2860. https://doi.org/10.1098/rstb.2008.0042.
678 Vongraven, D., Aars, J., Amstrup, S., Atkinson, S.N., Belikov, S., Born, E.W., DeBruyn, T.D.,
679 Derocher, A.E., Durner, G., Gill, M., Lunn, N.J., Obbard, M.E., Omelak, J.,
680 Ovsyanikov, N., Peacock, E., Richardson, E., Sahanatien, V., Stirling, I., and Wiig, Ø.
681 2012. A circumpolar monitoring framework for polar bears. Ursus Monograph Series
682 5: 1-66. https://doi.org/10.2192/URSUS-D-11-00026.1
683 Wilson, C.C. and Bernatchez, L. 1998. The ghost of hybrids past: fixation of arctic charr
684 (Salvelinus alpinus) mitochondrial DNA in ann introgressed population of lake trout (S.
685 namaycush). Molecular Ecology 7, 127-132. https://doi.org/10.1046/j.1365-
686 294x.1998.00302.x
687 Wong, P.B.Y., Van Coeverden De Groot P., Fekken C., Smith, H., Pagès, M. and Boag, P.T.
688 2011. Interpretations of polar bear (Ursus maritimus) tracks by Inuit hunters: Inter-rater
689 reliability and inferences concerning accuracy. The Canadian Field-Naturalist 125, 140-
690 153. https://doi.org/10.22621/cfn.v125i2.1197
691 Zhang, Z., Schwartz, S., Wagner, L., and Miller W. 2000, "A greedy algorithm for aligning
692 DNA sequences". J Comput Biol 7(1-2), 203-14.
693 https://doi.org/10.1089/10665270050081478
694
695
696
697

698 **FIGURE LEGENDS**

699 Figure 1: Distribution of 103 polar bear faecals in M'Clintock Channel (MC, Nunavut , Canada)
700 collected during May 2007 - 2011 and used in this study to genetically determine their most
701 recent vertebrate meal. Most of these faeces indicated the most recent meal was the ringed seal
702 (*Phoca hispida*; see Figure 2).

703 Figure 2: Difference in identified polar bear vertebrate prey items based on proportion of *cytB*
704 sequences generated from 454 sequencing of *WEAK* and *OK* PCR's from 12 captive MTZ and
705 101 wild MC polar bear faeces (See text for species identification). All sequences from the
706 PCR's of up to 4 dilutions – 1 & 2 ul for each of two possible extracts - for each faeces are
707 combined in these calculations. The different colours in a bar represent the sequence count (%)
708 of the vertebrate taxa identified in the faeces. The predominance of host polar bear and ringed
709 seal sequences is shown in panel A. The polar bear sequences were removed to better display
710 relative proportion of prey items in panel B. The first 12 entries are from Metro Toronto Zoo
711 bears with the next 8 =2007, 28 = 2008, 29 = 2009, 18 = 2010 and 18 = 2011 wild bears. The
712 species name for the vertebrate taxa is shown.

713 Figure 3: The identity and relative frequency of non-polar bear vertebrates identified from the
714 faeces of 3 captive polar bears in the Metro Toronto Zoo (MTZ), Canada. The single *Canis*
715 *lupus* sequence likely reflects contamination from wolves housed in the adjacent enclosure.
716 (For species identification and further detail see text). The results from the PCR's for all faeces
717 are pooled.

718 Figure 4: The identity and relative frequency of vertebrate prey identified from 101 faeces from
719 wild polar bears of M'Clintock Channel, Nunavut shows array prey items being consumed by
720 these wild bears at this time of year during 2007-2011. While the most common food items are
721 the seals *P. hispida* (86.14% of the faeces), *Phoca vitulina* (3.96%) and the bearded seal -

Erignathus barbatus (4.95%), other diet item at this time of the year include the arctic foxes - *Alopex lagopus* (5.94%), wolves - *Canis lupus* (0.99%), muskoxen, *Ovibos moschatus* (1.98%), birds, Ptarmigan - *Lagopus lagopus* (1.98%) and gulls *Larus sp.* (0.99%). [Unfortunately the 136bp *cyt*B sequence did not allow the discrimination between three putative gull species (*L. thayeri*, *L. hyperboreus, L. argentatus*)]. The results from the pooling of all PCR's for 101 faeces are shown.

728 Table 1: Pilot study results of 454 pyrosequencing from faecal extracts of a captive polar bear at the Polar Bear Habitat (PBH) in the Cochrane
729 Zoo. *PBH Sample* = bear fed Char for 3 weeks, 'Regular' diet for 3 weeks followed by Seal for the last 3 weeks. Faeces collected twice during the
730 Char (A&B) feeding, once during the Regular (C) and twice during the Seal (D&E) feedings. *Dilution of Initial extraction* = 1 or 2ul of extract
731 used in *cytB* PCR; *OTU* = the number of true sequence variants identified after validation (see text for details) of the PCR products; *Total # of*
732 *sequences* = the number of unique sequences assigned to all OTU's. *OTU's* = the identity of the sequence assigned to 5 vertebrate taxa. *CytB*
733 *BLAST match* = taxon to which *cytB* is assigned and *BLAST Identity* = % match to BLAST sequence. '*' & '**' indicates species level resolution
734 impossible as different species share the same *cytB* mini-barcode sequences.
735
736
737
738
739
740
741
742
743

PBH Sample			A		B		D		E		F	
DIET			Char		Char		Regular		Seal		Seal	
Dilution of Initial extraction			1ul	2ul	1ul	2ul	1ul	2ul	1ul	2ul	1ul	2ul
# of Otu's			1	1	2	2	0	0	0	0	4	4
Total # sequences			93	206	41	50	0	0	0	0	116	151
Otu's	Cyt b 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.**	100			16 (39%)	23 (46%)						1 (0.7%)
4	*Gallus gallus*	100									1 (0.9%)	
5	*Odocoileus sp.***	100									2 (1,6%	2 (1.3%)

750 Table 2: Summary statistics of our efficacy in obtaining a mitochondrial 136 *cytB* bp amplicon from the faeces of wild and captive polar bears.
751 using our 2 dilution- 2 extraction method The overall (weighted) success rate was 91%. *# of Bears* = number of known bears from which faeces
752 collected; *Faeces per bear* =# of faeces from each bear; *Total # of Faeces* =total number of faeces from Metro Toronto Zoo, Churchill holding
753 facility (PBP) and M'Clintock Channel; *Original Extract* = number of extracts with cytB PCR results characterized as FAIL, WEAK or OK across
754 1 & 2 ul dilutions; *# faeces re-extracted* = number of faeces with FAIL and WEAK results that were re-extracted; *2nd Extract* = number of 2nd
755 extracts with cytB PCR results characterized as FAIL, WEAK or OK across 1 & 2 ul dilutions; *Combined success of* 2 dilution - 2 extraction
756 *method* = number of extracts with *cytB* PCR results characterized as FAIL, WEAK or OK across 2 dilutions and 2 extractions; *Combined*
757 *probability of success* =combined probability of WEAK or OK cytB amplification success for MTZ, PBP and MX polar bear faecces; *Increased*
758 *success - 2nd extraction* = % of re-extracted faeces that resulted in WEAK or OK *cytB* PCR s.
759
760
761
762

	# of Bears	Faeces per bear	Total # of faeces	Original Extract			# faeces re-extracted	2nd Extract			Combined success of *2 dilution - 2 extraction* method			Combined probability of success	Increased success - 2nd extraction
				FAIL [1]	*WEAK* [2]	*OK* [3]		*FAIL* [1]	*WEAK* [2]	*OK* [3]	*FAIL* [1]	*WEAK* [2]	*OK* [3]		
MTZ [4]	3	6 or 7	**19**	11 (0.58) [7]	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 [5]	7	1	**7**			7	**0**						7 (1.00)	100%	NA
MC [6]	?	?	**117**	16 (0.14)	10 (0.09)	91 (0.78)	**24** [8]	11 (0.46)	4 (0.17)	9 (0.38)	8 (0.07)	7 (0.06)	102 (0.87)	93%	54%

Notes:

1. No *cytB* amplicon detectable in pcr's with 1 & 2ul dilutions of polar bear faecal extracts. 2. *cytB* amplicon weakly amplified in pcr's with 1 or 2ul dilutions of polar bear faecal extracts. 3. *cytB* amplicon strongly amplified in pcr's with 1 or 2ul dilutions of polar bear faecal extracts. 4. Polar bear faeces from captive bears from Metro Toronto Zoo bears. 5. Polar bear faeces from bears held at Churchill holding facility –Polar Bear Prison (PBP). 6. Polar bear faeces collected from M'Clinotck Channel Management Unit in Nunavut Canada. 7. Bracketed values are the associated cell number as a percentage of number faecals extracted. 8. Although 26 faeces had FAIL or WEAK cyt B amplifications, we only re-exracted and re-pcred 24 of them.

Figure 1

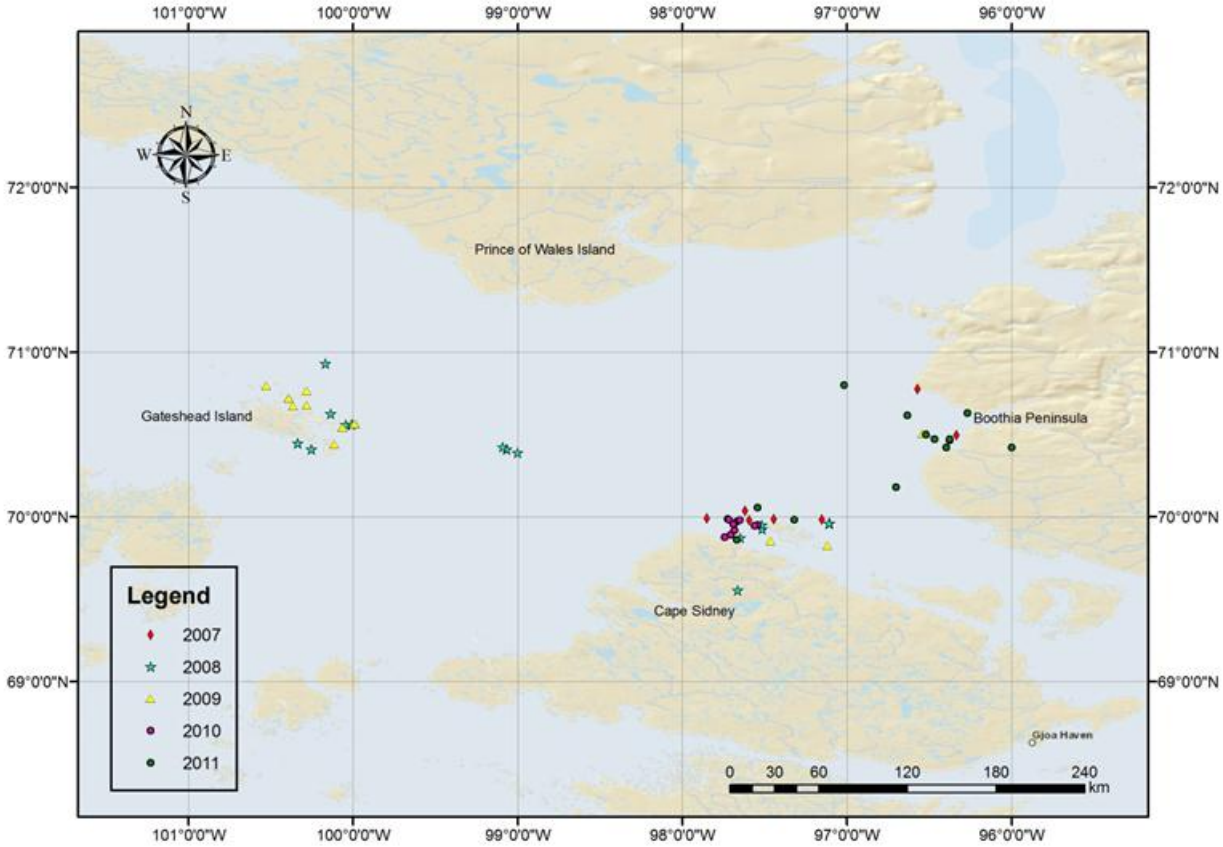
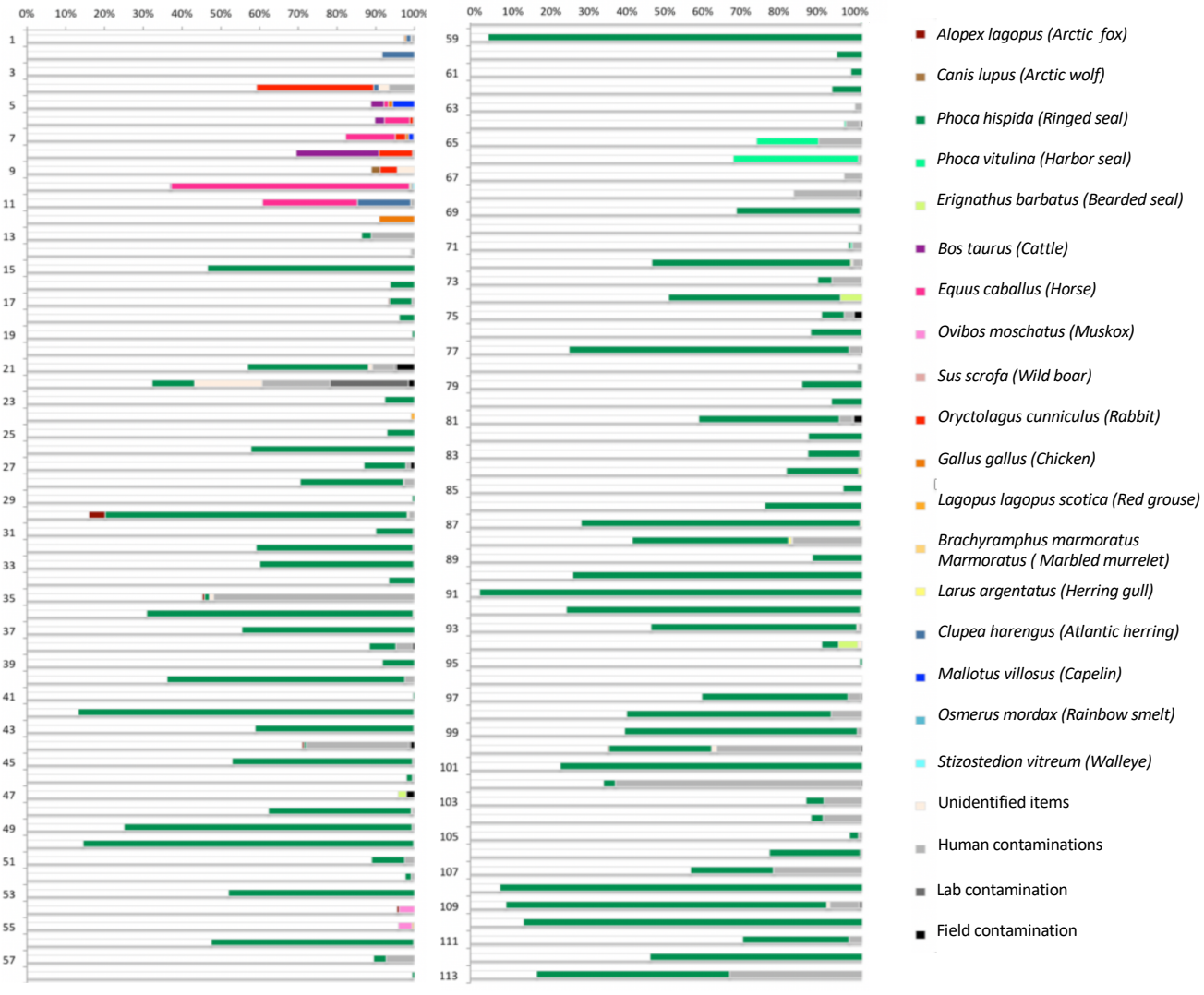
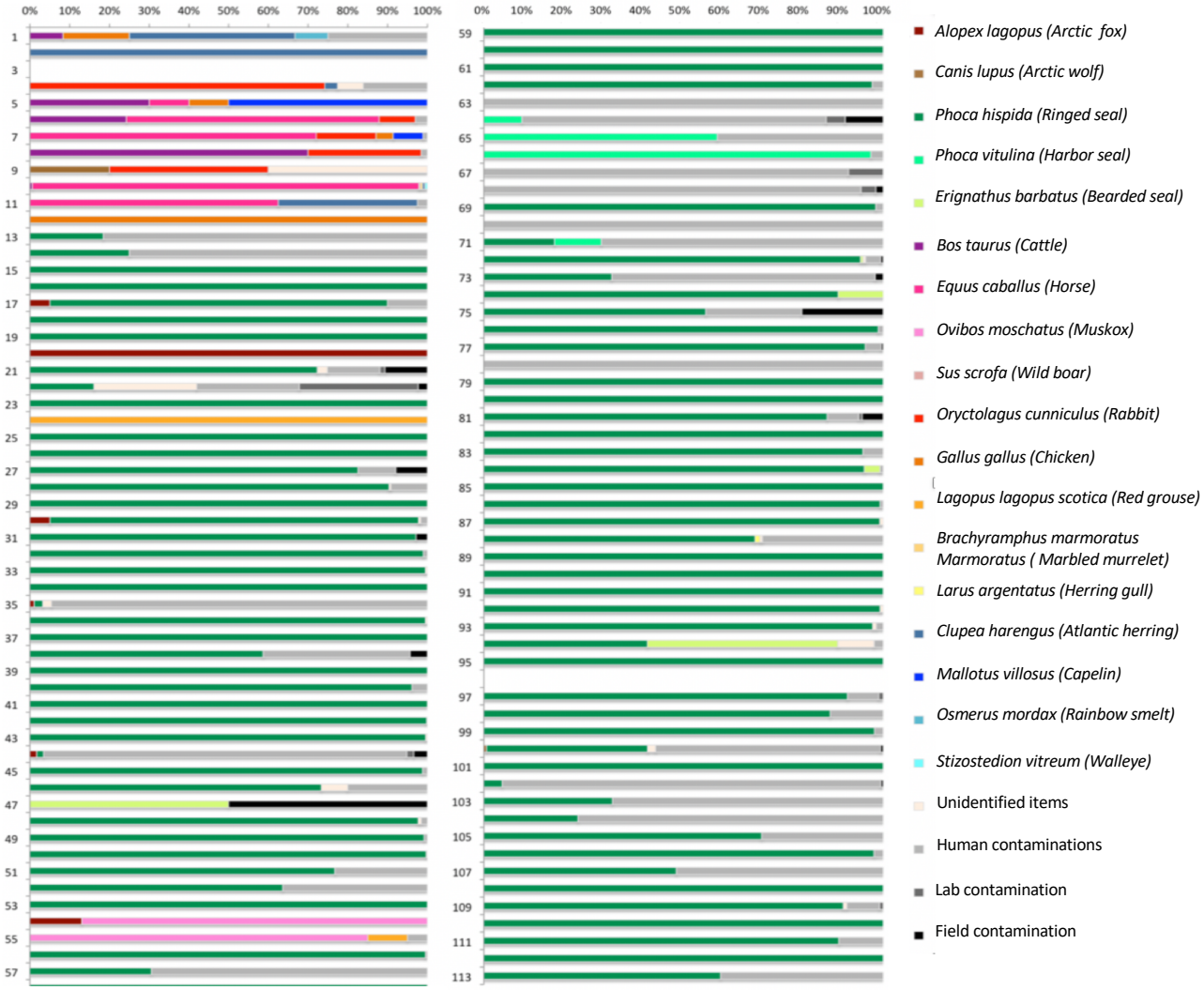

101°0'0"W
100°0'0"W
99°0'0"W
98°0'0"W
97°0'0"W
96°0'0"W
72°0'0"N
71°0'0"N
70°0'0"N
69°0'0"N
N
W
E
S
Prince of Wales Island
Gateshead Island
Boothia Peninsula
Cape Sidney
Gjoa Haven
Legend
2007
2008
2009
2010
2011
0 30 60 120 180 240
km

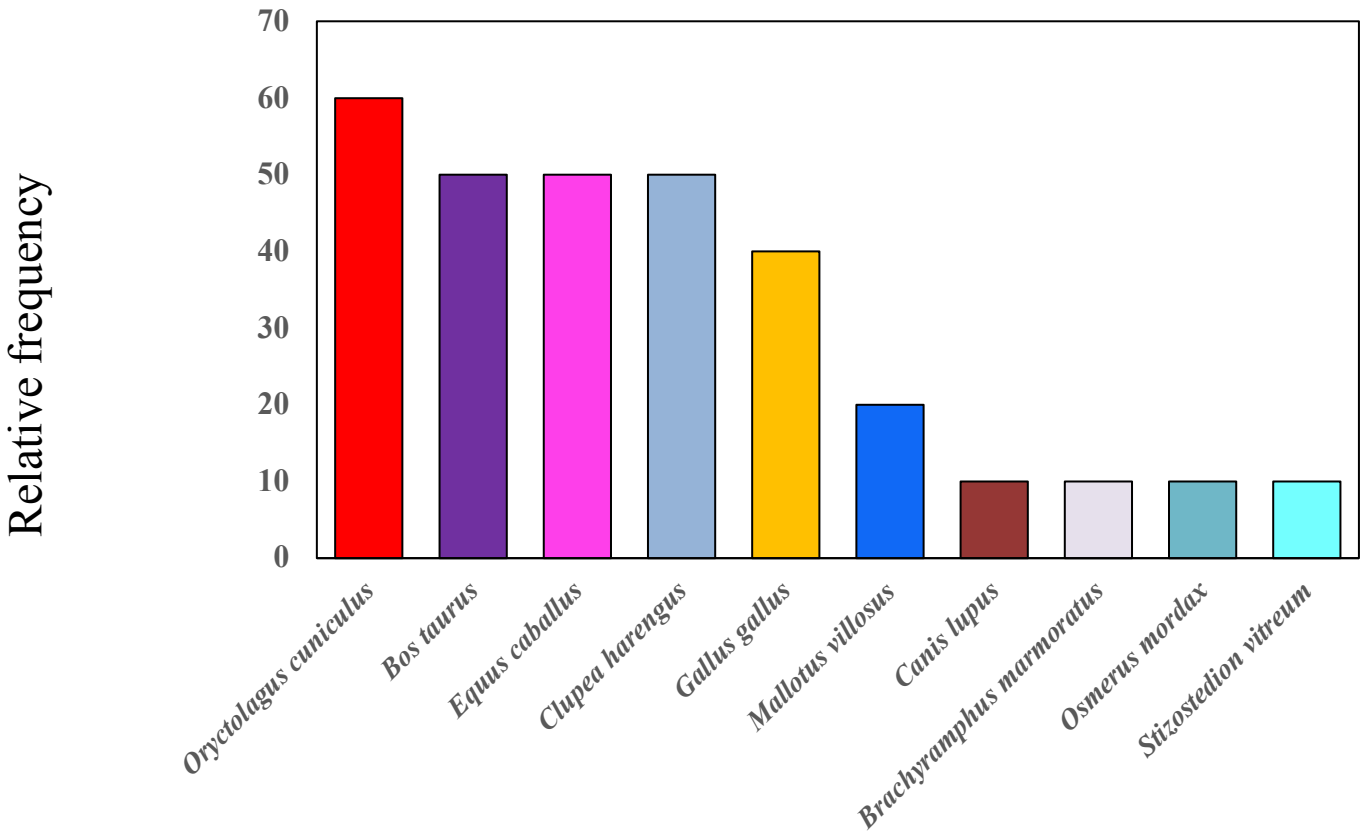
Figure 2

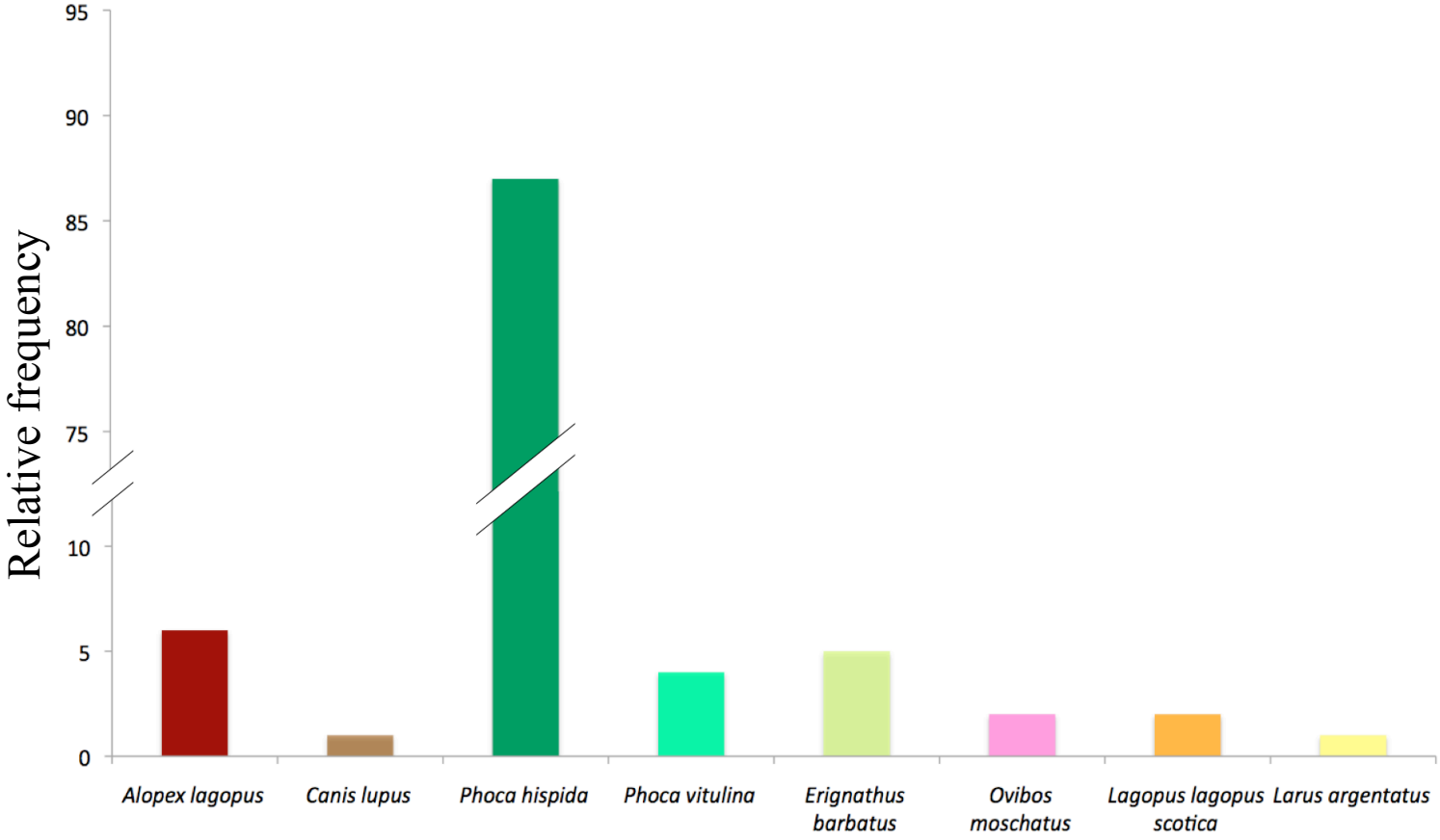

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57
59 61 63 65 67 69 71 73 75 77 79 81 83 85 87 89 91 93 95 97 99 101 103 105 107 109 111 113
Alopex lagopus (Arctic fox)
Canis lupus (Arctic wolf)
Phoca hispida (Ringed seal)
Phoca vitulina (Harbor seal)
Erignathus barbatus (Bearded seal)
Bos taurus (Cattle)
Equus caballus (Horse)
Ovibos moschatus (Muskox)
Sus scrofa (Wild boar)
Oryctolagus cunniculus (Rabbit)
Gallus gallus (Chicken)
Lagopus lagopus scotica (Red grouse)
Brachyramphus marmoratus Marmoratus (Marbled murrelet)
Larus argentatus (Herring gull)
Clupea harengus (Atlantic herring)
Mallotus villosus (Capelin)
Osmerus mordax (Rainbow smelt)
Stizostedion vitreum (Walleye)
Unidentified items
Human contaminations
Lab contamination
Field contamination

A.


0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
59 61 63 65 67 69 71 73 75 77 79 81 83 85 87 89 91 93 95 97 99 101 103 105 107 109 111 113
Alopex lagopus (Arctic fox)
Canis lupus (Arctic wolf)
Phoca hispida (Ringed seal)
Phoca vitulina (Harbor seal)
Erignathus barbatus (Bearded seal)
Bos taurus (Cattle)
Equus caballus (Horse)
Ovibos moschatus (Muskox)
Sus scrofa (Wild boar)
Oryctolagus cunniculus (Rabbit)
Gallus gallus (Chicken)
Lagopus lagopus scotica (Red grouse)
Brachyramphus marmoratus Marmoratus (Marbled murrelet)
Larus argentatus (Herring gull)
Clupea harengus (Atlantic herring)
Mallotus villosus (Capelin)
Osmerus mordax (Rainbow smelt)
Stizostedion vitreum (Walleye)
Unidentified items
Human contaminations
Lab contamination
Field contamination

B.


Relative frequency
70
60
50
40
30
20
10
0
Oryctolagus cuniculus
Bos taurus
Equus caballus
Clupea harengus
Gallus gallus
Mallotus villosus
Canis lupus
Brachyramphus marmoratus
Osmerus mordax
Stizostedion vitreum


Relative frequency
95
90
85
80
75
10
5
0
Alopex lagopus
Canis lupus
Phoca hispida
Phoca vitulina
Erignathus barbatus
Ovibos moschatus
Lagopus lagopus scotica
Larus argentatus