Evaluation of environmental DNA (*e*DNA) collected from tracks in the snow as a means to monitor individual polar bears (*Ursus maritimus*) in the Chukchi and Beaufort Seas

Andrew L. Von Duyke, Elisabeth Kruger, Nicklas Wijkmark, Johan Näslund, Pähr Hellström, and Micaela Hellström

October 2019

Evaluation of environmental DNA (*e*DNA) collected from tracks in the snow as a means to monitor individual polar bears (*Ursus maritimus*) in the Chukchi and Beaufort Seas

Andrew L. Von Duyke[1]*, Elisabeth Kruger[2], Nicklas Wijkmark[3], Johan Näslund[3], Pähr Hellström[3], and Micaela Hellström[3]

[1]North Slope Borough, Department of Wildlife Management
P.O. Box 69, Utqiaġvik, AK 99723

[2]World Wildlife Fund, US Arctic Program
406 G Street #301, Anchorage, AK 99501

[3]AquaBiota Water Research AB
Löjtnantsgatan 25, SE-115 50 Stockholm, Sweden

*Corresponding author – andrew.vonduyke@north-slope.org

©2019 North Slope Borough
Department of Wildlife Management
P.O. Box 69, Utqiaġvik, AK 99723

AquaBiota

This research was supported by the North Slope Borough Department of Wildlife Management (NSB-DWM), through the Shell Baseline Studies Program and under USFWS Permit #MA 134907-1; the Alaska Department of Fish and Game, through the Coastal Impact and Assessment Program Grant #F12AF70139 and under Institutional Animal Care and Use Permit (IACUC #2016-23); and the World Wildlife Fund, through the Arctic Home Grant. This PRELIMINARY report was produced to comply with the NSB-DWM's commitments to publically disseminating research funded by the Shell Baseline Studies Program, and to keep North Slope Communities apprised of recent research findings.

DISCLAIMER

Preliminary Research Reports serve as recent "status updates" of ongoing research activities conducted along Alaska's North Slope by the North Slope Borough Department of Wildlife Management and its partners. This Preliminary Research Report details the objectives, methods, data, and preliminary findings (to date) for this research project. Also included is a description of anticipated future work related to furthering/completing this research. The intent of this preliminary research report is to provide affected communities within and outside of the North Slope Borough and interested stakeholders with timely feedback on the progress of research pertaining to the management of subsistence resources.

This preliminary research report is not considered FINAL. Additional data collection and analyses will be forthcoming. Also note that this report has not been subjected to a thorough review. Upon completion of this research project, a FINAL report will be generated that includes the full extent of the methods, results, analyses, and conclusions, and will undergo internal and peer review prior to public release. Note that Final Research Reports may also be subject to further data analysis, which could result in future adjustments to any conclusions herein. As such, care should be taken with citing Preliminary or Final Research Report findings, and it is highly recommended that the author(s) be contacted prior to citing materials.

REPORT AVAILABILITY

Research Reports (final or otherwise) are available from:
North Slope Borough Department of Wildlife Management
P.O. Box 69, Barrow, Alaska 99723
(907) 852-0350
www.north-slope.org/departments/wildlife-management

Cover Photo: Fresh polar bear tracks on the sea ice in the Beaufort Sea. Photo was taken during the early spring sampling period (mid-March 2019) when day length is nearly 12 hours.

©2019. Photo by Nicklas Wijkmark (used with permission).

INTRODUCTION

Traditionally, surveys of polar bear (*Ursus maritimus*) populations have involved invasive methods (i.e., collaring and/or permanently marking individuals) that require the physical handling of chemically immobilized animals. These invasive methods are stressful for the target animal, and risky for researchers. Furthermore, the extreme expense and logistical challenges of working in the Arctic often result in low sample sizes. Meanwhile, much concern for the welfare of polar bears has been expressed by Inuit communities in the Alaskan and western Canadian Arctic, as have requests for the development and use of alternative, less invasive research methods.

Less invasive and stressful approaches to physically handling polar bears typically rely upon collecting individual genetic samples—taken from skin, hair, and/or blood (Viengkone et al. 2016). This approach has been most often accomplished using methods such as biopsy darts (shot from a helicopter) and hair traps (e.g., barbed wire fence around bait). These methods are not without their own challenges (Von Duyke et al. 2016). A promising newer and completely non-invasive method has been developed that relies upon DNA sampled from the surrounding environment (eDNA). Thus far, eDNA has been successfully used to identify presence/absence at the species level only—particularly in aquatic environments (Kelly et al. 2014; Sformo et al. 2015)—but, to date, has been unsuccessful at identifying individuals.

Our project proposed to collect eDNA that had been shed by individual polar bears in their footprints in the snow (hereafter 'tracks'). Conceptually simple, this approach still has its challenges. For example, in previous efforts, we have had some success in collecting eDNA from snow samples, though our results were inconsistent and it became evident that we needed to better understand the factors that affect the quantity (i.e., yield) and quality of eDNA sampled from polar bear tracks (VonDuyke et al. 2017).

To achieve our ultimate goal of using eDNA to monitor polar bears at broad spatial and temporal scales, and to allow for eDNA sampling to be performed by non-technical field assistants (e.g., locally hired Native hunters in the villages), it was necessary to work out the technical details of this method. With this in mind, we assembled a team of experts, with funding provided by the World Wildlife Fund, AquaBiota, and the Animal Welfare Institute (the Christine Stevens Wildlife Award), to investigate this process in greater detail, answer some fundamental questions about factors that may affect eDNA yield and quality, and to map out a path forward based on these results.

While all eukaryotic cells have 1-2 thousand copies of both mitochondrial DNA (mtDNA) and ribosomal DNA (rDNA), there is only a single copy of nuclear DNA (nDNA), which is used for individual identification (genotyping). As such, eDNA studies have, so far, mostly focused on the mitochondrial region (mtDNA). Thus, given the relatively lower number of nDNA copies available per sample, collecting high quality DNA in sufficient quantities is the primary challenge associated with using nuclear eDNA to genetically fingerprint individual polar bears.

Sampling captive polar bear tracks at two different Scandinavian zoos (Orsa Bearpark, Sweden and Ranua Zoo, Finland), AquaBiota, a collaborator in this research, developed and fine-tuned a method to retrieve high-quality DNA in sufficient quantities to allow for genetic fingerprinting (Hellström et al. 2019). In particular, the genetic materials sampled consisted of a large quantity of high-quality mitochondrial DNA (mtDNA) and ribosomal DNA (rDNA), where ribosomal and nuclear markers detected the bears. Our intention for this project was to apply these methods to the sampling of eDNA from wild polar bear tracks in their natural habitat as a proof of concept, and to establish a baseline for further studies that would help to further refine these methods. Based on previous work (Bellemain 2017), we had specific questions concerning the influence of environmental factors (e.g., UV light damage to DNA, and cellular damage from wind abrasion, desiccation, and freeze-thaw cycles). This investigation marks an early phase in the development of what we hope will lead to a non-invasive method that is (1) sensitive enough to identify individual bears and their sex, (2) efficient, and (3) useful for the large scale study of polar bear population biology.

METHODS

Figure 1 shows a conceptual workflow chart that highlights the steps required for the entire process; beginning with sampling polar bear tracks in the snow and concluding with the genetic fingerprinting of individual polar bears. This investigation proceeded through all but step six.

Field sampling occurred in the vicinity of Utqiaġvik, AK (Figs. 2-4); the northernmost point of Alaska and the US (71.3°, -156.8°) and an area known for an abundance of polar bears. All field work occurred in close collaboration with experienced Iñupiat guides and experienced Arctic researchers. Polar bear tracks were sampled in January and March of 2019. January is characterized by 24 hrs/day of darkness, whereas March has day lengths of approximately 12 hrs/day. The aim of the two sampling periods was to test for the effect of ultraviolet (UV) light on eDNA yield and quality.

Conceptual flow-chart for eDNA sampling and analyses

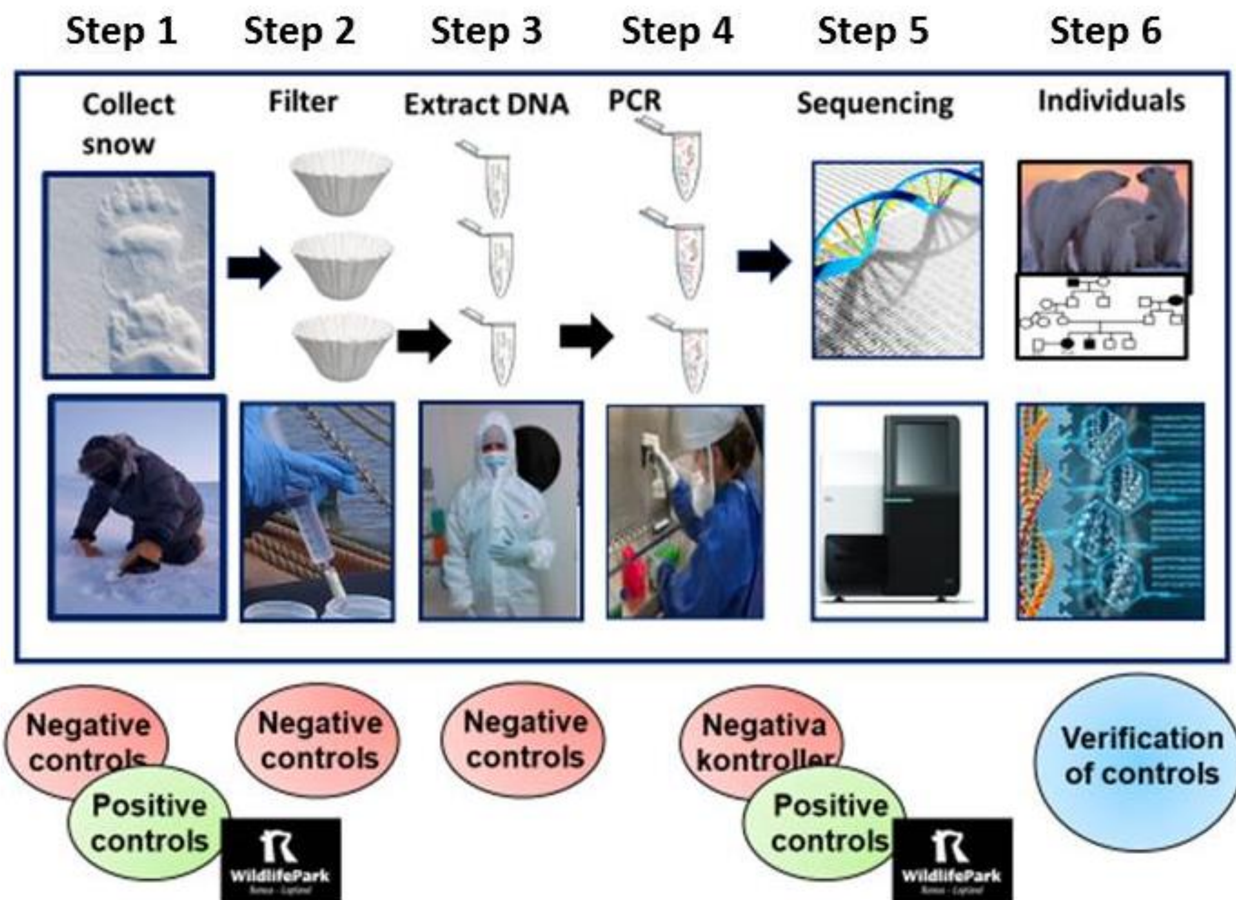

Figure 1. Conceptual flowchart of the complete process of eDNA sampling from snow. This investigation is based in part by questions posed in captive research (Hellström et al. 2019) and in a wild setting (Bellemain 2017; Von Duyke et al. 2017). Note that negative controls consist of clean snow that has been sampled and analyzed using the same methods as for the polar bear tracks.

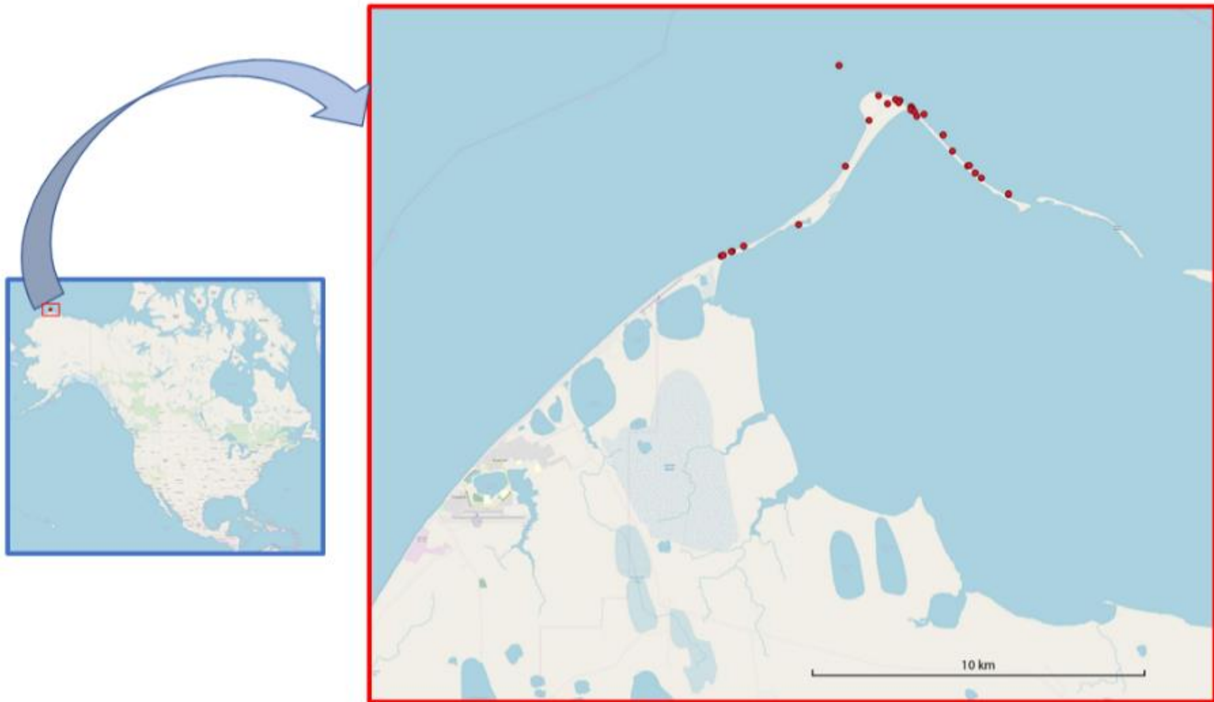

Figure 2 – Sampling locations. (left) Utqiaġvik (formerly Barrow), Alaska is the northernmost point in the United States. (right) Polar bears and their tracks are common on the long spit of land that ends at Point Barrow (Nuvuk). Red dots indicate a sampling location. Near the terminus of Point Barrow is a bone pile with the remains from fall subsistence harvested bowheads, which served as an attractant for polar bears.

Figure 3 – Sampling conditions in January. (left) Bone pile, as seen during the darkest hours of sampling. (right) Wind not only made travel difficult, but also concealed polar bear tracks. Under these conditions, it was challenging to assess the "freshness" of a set of tracks. Often, we relied upon the skills of experienced Iñupiaq colleagues to help with these assessments.

Figure 4 – Track sampling. (A) Bone pile from subsistence whaling attracted polar bears. (B) Ice fog illustrates the extreme cold conditions (-33° C) during sampling in January. (C) Heading out to the ice in March. (D) Twilight at about noon in January. (E) Andy Von Duyke sampling bear tracks. (F) Making plans for return to base. (G) Polar bear tracks at twilight. (H) Andy Von Duyke keeps an eye out for polar bears in broken up sea ice.

Prior to field work, all sampling supplies (e.g., plastic containers and snow spades) were cleaned using 10% hypochlorite (i.e., bleach) to remove all possible traces of DNA and rinsed with 70% molecular grade ethanol (EtOH).

We opportunistically sampled from polar bear tracks that were of varying "freshness" because we were also interested in whether and to what extent the age of a set of tracks affected the yield and quality of eDNA sampled (Fig. 5). These assessments were sometimes not straightforward due to wind and drifting snow. On several occasions we relied upon the Indigenous knowledge of our Iñupiaq colleagues to help make these assessments. Nevertheless, there is always some subjectivity in assessing wild polar bear tracks, unless the bear that made them has been observed (Fig. 6). Whenever we observed a live bear, we attempted to sample its tracks (if we could do so safely) so that we could be completely confident about the age of the sampled tracks. Additionally, snow samples without footsteps and water were used as negative controls to detect possible contamination.

Figure 5 – Polar bear track variability. (top) Fresh polar bear tracks on hard crusty snow. Note the marks in the snow from the hair that hangs down from the bear's legs. (bottom) Older and weathered polar bear tracks. Though it was preferable to sample fresher tracks, we did sample suboptimal tracks such as these in order to better understand the factors associated with eDNA yield and quality.

Figure 6. (A) Close-up of captive polar bear at zoo. (B) Wild polar bear on the sea ice near Utqiaġvik. (C) Andy and Micaela sampling a set of fresh polar bear tracks. (D) Elisabeth and Pähr sampling tracks on hard crusty snow in January. (E and F) Andy Von Duyke and Pähr Hellström wearing full Arctic gear during January field operations. (G) Back at the base, making plans. (H) The team in January: Eben Hopson Jr., Bobby Sarren, Pähr Hellström, Andy Von Duyke, and Elisabeth Kruger (Micaela Hellström not in photo).

Upon our return from the field, we allowed the snow samples to thaw at room temperature. Previous experience suggests that thawing quickly is best. Once thawed, the water/eDNA solution was filtered to capture whatever epidermal cells the polar bear may have shed in its tracks. We used encapsulated double filters (5/0.7 µm, NatureMetrics Ltd. UK; TM-GP 0.22 µm, MerckMillipore, France). We did not assess different filter pore sizes because previous work on captive polar bears indicated that there was no difference in the relative amounts of DNA filtrate based on different pore size (Hellström et al. 2019). For this investigation, we opted to use the filters with the larger pore size.

A preservative solution was injected into the filters to protect the sampled eDNA during its shipment to the lab. Clean protocols were used to ensure that no genetic cross-contamination occurred (Fig. 7).

At the lab in Sweden, the genetic materials (i.e., eDNA) were extracted from the filtrate following Spens et al (2017). The concentration of eDNA (ng/µL) was measured using Qubit Fluorometric Quantitation System (Fisher Scientific).

Figure 7. Pähr Hellström (left) and Micaela Hellström (right) prepare the filtered snow samples from polar bear tracks (presumably containing their eDNA) for shipment to the lab. Under remote field settings, it was a challenge to ensure that genetic cross-contamination did not occur.

Five microsatellites (out of 18 sufficient loci) were selected and tested on five polar bear samples (including one fecal sample) in order to determine whether nuclear markers can be retrieved from DNA traces left in snow in the wild. The samples were analyzed for microsatellites following the molecular protocols in Peacock et al. (2015). The PCR products were run out on an agarose gel in order to determine right sizes of bands on the gels.

RESULTS

DNA extractions

A total of 51 snow samples were collected from wild polar bear tracks (Table 1) in January (n = 21) and March (n = 30) of 2019. When all samples were pooled together, the mean concentration of eDNA = 0.98 ng/µL (σ = 1.45). The range of eDNA concentrations from all samples was 0.02 – 7 ng/µL, which was much lower in comparison to the eDNA concentrations collected from the tracks of captive polar bears in the two zoos (range = 2-50 ng/µL) (Hellström et al. 2019). All negative controls (i.e., clean snow that was melted, filtered, and analyzed using the same methods as for the track samples) confirmed that our field collections and laboratory methods were clean and that no genetic cross-contamination had occurred. This is in contrast to the captive studies, which showed large amounts of DNA that was dominated by food items. Though there was no significant difference in eDNA concentration between the winter (January) and early spring (March) sampling periods, the concentration of eDNA, as expected, was significantly higher when more tracks were sampled (Fig. 8).

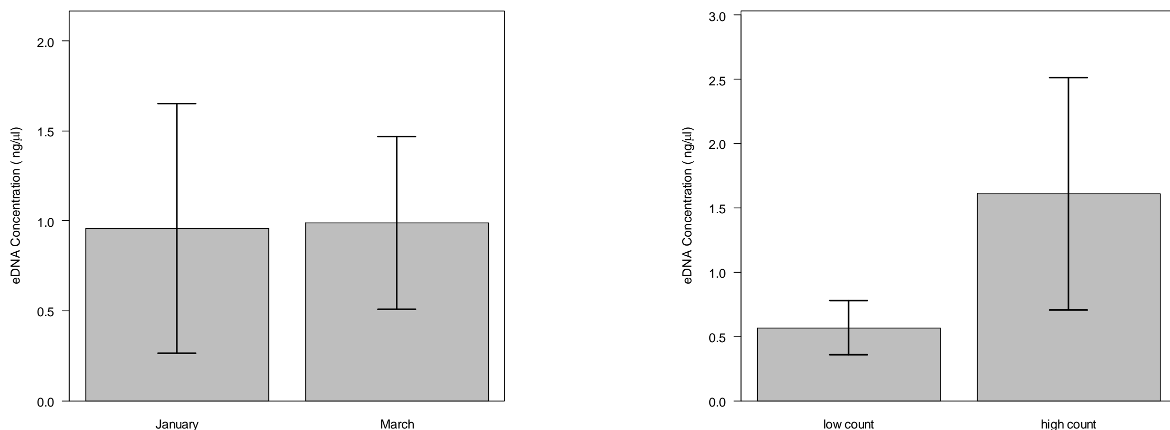

Figure 8. (left) Seasonal concentrations of eDNA (ng/µL) were not significantly different ($p = 0.94$). (right) Significantly higher concentrations of eDNA were obtained when more polar bear tracks were sampled ($p = 0.039$). Note that the category “low count” consists of samples with ≤ 20 footprints/sample, and “high count” has > 20 footprints/sample.

Table 1. List of polar bear track samples collected during the 2019 January and March field sessions. Sex or age were determined by visual observation and based on the field skills of Native Iñupiaq colleagues who assisted with the field sampling. Note that the projection for the locations is WGS84.

Sample ID	date	Temp ambient (˚C)	Temp wind chill (˚C)	Latitude	Longitude	sex	# tracks	mL filtered	DNA ng/uL
PBA_01	2019-01-04	-33°	-68°	71.356°	-156.533000°	na	20	580	0.04
PBA_02	2019-01-04	-33°	-68°	71.356°	-156.533000°	F	25	690	1.6
PBA_03	2019-01-05	-32°	-56.7°	71.381°	-156.480000°	M	14	780	0.8
PBA_04	2019-01-05	-32°	-56.7°	71.381°	-156.480000°	na	40	2160	0.8
PBA_05	2019-01-05	-32°	-56.7°	71.37°	-156.497778°	na	16	940	0.98
PBA_06	2019-01-05	-32°	-56.7°	71.356°	-156.533000°	na	40	1890	1.68
PBA_07	2019-01-05	-32°	-56.7°	71.382°	-156.444000°	na	25	590	0.5
PBA_08	2019-01-05	-32°	-56.7°	71.385°	-156.466000°	na	20	810	0.5
PBA_09	2019-01-05	-32°	-56.7°	71.385°	-156.466000°	na	30	795	7.1
PBA_10	2019-01-05	-32°	-56.7°	71.385°	-156.466000°	na	30	840	3.42
PBA_11	2019-01-06	-33°	-34°	71.386°	-156.460000°	na	20	540	0.14
PBA_12	2019-01-05	-27°	-45°	71.356°	-156.533000°	na	30	880	1.07
PBA_14	2019-01-05	-27°	-45°	71.386°	-156.460000°	na	30	685	0.03
PBS_15	2019-01-06	-33°	-49.5°	71.387°	-156.472778°	na	20	630	0.51
PBA_16	2019-01-06	-33°	-40.5°	71.363°	-156.374940°	na	30	390	0.03
PBA_18	2019-01-06	-33°	-40.5°	71.394°	-156.502500°	na	20	420	0.04
PBA_22	2019-01-08	-32°	-32°	71.394°	-156.502500°	na	20	695	0.4
PBA_23	2019-01-08	-32°	-32°	71.378°	-156.424167°	adult	15	630	0.02
PBA_24	2019-01-08	-32°	-32°	71.378°	-156.424167°	cub	13	470	0.11
PBA_25	2019-01-08	-32°	-32°	71.378°	-156.424167°	na	22	515	0.2
PBA_28	2019-01-08	-32°	-32°	71.378°	-156.424167°	na	12	300	0.14
PBB_01	2019-03-17	-15°	-20.4°	71.38533°	-156.457438°	na	>30	1010	2.1
PBB_02	2019-03-17	-15°	-20.4°	71.38578°	-156.456697°	na	11	240	0.4
PBB_03	2019-03-17	-15°	-20.4°	71.38523°	-156.457602°	na	13	50	0.7
PBB_06	2019-03-17	-15°	-20.4°	71.38395°	-156.447497°	F	7	280	0.03
PBB_08	2019-03-17	-15°	-20.4°	71.38427°	-156.448240°	na	15	170	0.4
PBB_09	2019-03-17	-15°	-20.4°	71.38384°	-156.448485°	Cub	17	160	0.13
PBB_10	2019-03-17	-15°	-20.4°	71.38341°	-156.447981°	na	6	65	0.41
PBB_11	2019-03-17	-15°	-20.4°	71.38395°	-156.447499°	Cub	13	200	1.2
PBB_12	2019-03-17	-15°	-20.4°	71.38385°	-156.448499°	na	22	435	1.1
PBB_14	2019-03-17	-15°	-20.4°	71.38351°	-156.448460°	na	23	170	1.2
PBB 19	2019-03-20	-26°	-53.4°	71.36319°	-156.374940°	na	12	215	0.71
PBB 20	2019-03-20	-26°	-53.4°	71.3632°	-156.374975°	na	hole	565	0.4
PBB 21	2019-03-20	-26°	-53.4°	71.36328°	-156.374910°	na	16	530	0.9
PBB 22	2019-03-20	-26°	-53.4°	71.36328°	-156.374952°	na	8	250	0.1
PBB 24	2019-03-20	-26°	-53.4°	71.36717°	-156.395388°	na	25	705	1.02
PBB 25	2019-03-20	-26°	-53.4°	71.36835°	-156.400083°	na	8	240	0.41
PBB 26	2019-03-20	-26°	-53.4°	71.36834°	-156.400066°	na	17	540	0.75
PBB 28	2019-03-20	-26°	-53.4°	71.37007°	-156.405685°	na	13	525	0.21
PBB 29	2019-03-20	-26°	-53.4°	71.37017°	-156.404424°	na	21	815	1.3
PBB 31	2019-03-20	-26°	-53.4°	71.37363°	-156.417134°	na	14	705	1.2
PBB_16	2019-03-18	-15°	-27°	71.38306°	-156.445706°	na	20	220	2.4
PBB_17	2019-03-18	-15°	-27°	71.38249°	-156.438564°	na	12	260	0.4
PBB_33	2019_03_21	-24°	-24.9°	71.34844°	-156.591327°	M	25	110	0.97
PBB_34	2019_03_21	-24°	-24.9°	71.34855°	-156.590673°	M	25	870	0.31
PBB_35	2019_03_21	-24°	-24.9°	71.3485°	-156.590827°	na	22	740	0.32
PBB_36	2019_03_21	-24°	-24.9°	71.34863°	-156.589681°	na	20	580	0.21
PBB_38	2019_03_21	-24°	-24.9°	71.34955°	-156.583318°	na	30	920	0.23
PBB_39	2019_03_21	-24°	-24.9°	71.34959°	-156.582953°	na	22	1080	7.2
PBB_41	2019_03_21	-24°	-24.9°	71.34948°	-156.583733°	na	15	795	2.4
PBB_42	2019_03_21	-24°	-24.9°	71.35083°	-156.574352°	na	18	590	0.07
PBB 23N	2019_03_20	-26°	-53.4°	71.36341°	-156.374911°	na	14	275	0.9

Temperatures during both sampling periods were extremely cold (Table 1; Fig. 9). The minimum temperatures were -33° C (x̄ = -32° C) in January and -26° C (x̄ = -21° C) for March. Though there was little variation in ambient temperature in January, there were high winds that led to extreme wind chills (min = -68˚C; x̄ = -48° C). March was also noteworthy for extreme wind chills (min = -55.8˚C; x̄ = -33.7˚C). No wind speed data were recorded during these sampling periods.

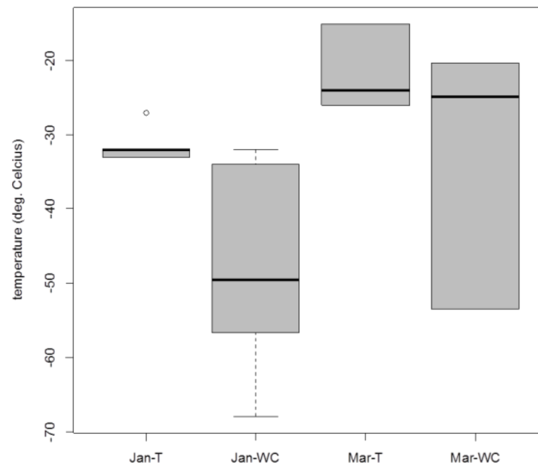

Figure 9 - Boxplot of temperatures and wind chills. The means are indicated by the bold horizontal bars, and variability is indicated by the boxes and whiskers. The three letter abbreviations "Jan" and "Mar" stand for January and March respectively. The suffix following the three letter abbreviation for month indicates whether the value is a temperature (T) or wind chill (WC).

During the January sampling period, the snow surface consistency was soft and therefore facilitated the collection of snow from polar bear tracks. In contrast, the snow surface consistency in March was characterized by a hard, wind-driven crust; making it difficult to scrape snow from the tracks (Fig. 5).

Gel electrophoresis was used to help determine whether we were able to isolate nuclear DNA from the eDNA samples. All of the five polar bear eDNA samples that were analyzed for the five different microsatellite loci yielded PCR products with correctly sized bands (Fig. 10); strongly suggesting that nuclear DNA was amplified only for the targeted polar bear DNA. Sanger sequencing, which reassembles the genetic sequence of PCR products, further indicated that nuclear DNA had been accessed and can be reassembled correctly (Fig. 11).

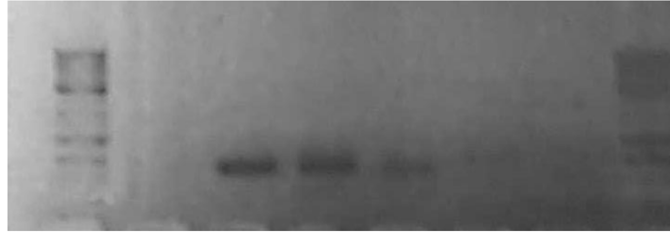

Figure 10. Gel electrophoresis of one of the microsatellite PCRs tested indicates an accurate band size, suggesting that nuclear DNA had been accessed.

Figure 11. Sequence of wild polar bear microsatellite samples provide further evidence that nuclear DNA was accessed. Nuclear DNA is the portion within the cell's nucleus that is used to identify individuals through genotyping (i.e., genetic fingerprinting).

DISCUSSION

Our results indicated that, for the first time, we were able to successfully access wild polar bear nuclear DNA from eDNA samples collected from snow tracks. While our samples contained relatively lower yields of eDNA than those collected in captive studies, these small quantities of DNA in the snow samples were sufficient to target the cell's nuclear region, presumably making it possible to genetically "fingerprint" individual polar bears. The ability to uniquely identify individuals allows for the use of these data to inform investigations into polar bear population dynamics. Moreover, because large libraries of genetic data already exist, it should be possible to merge new genetic information about polar bears into these data sets.

Sampling tracks during the two different periods, each of which had a dramatically different light regime, showed no appreciable difference in DNA yield based on sampling period (Fig. 8). This could suggest that UV degradation is not closely related to DNA yield. However, it should also be noted that our samples were not random—i.e., we attempted to sample the "freshest" tracks possible—even sampling from bears immediately after observing them. Older tracks tended to be partially to completely covered with snow, which may have shielded the genetic materials below from UV light. Finally, for most of the tracks sampled, our assessments of "freshness" were qualitative and therefore subject to error. A better understanding of the

influence of UV light on yield would be possible through a more systematic approach to resampling and analyzing tracks of a known age. As it stands, a rule of thumb may be to preferentially sample tracks that appear to be as "fresh" as possible.

One concept that has been proposed to work in a more controlled manner while sampling in a wild setting is to set up baited stations that are monitored using motion-sensing trail cameras (Fig. 12). The tracks of bears that are attracted to the bait can then be sampled, and we would have a record of the exact circumstances associated with their tracks (e.g., number of bears, age of tracks, etc.). This work is planned for March-April 2020. This approach would allow for experimental manipulation of the number of tracks sampled, environmental conditions, and different filtering/extraction methods for tracks of a precisely known age.

Figure 12. Male polar bear in the Chukchi Sea is attracted to a baited station that is designed to snag hair for DNA collection (Von Duyke et al. 2016). This approach lends itself well to controlling for the age of polar bear tracks when assessing eDNA methods. Photographic evidence can also confirm how many bears were present. Finally, these stations can be shifted to areas of "pristine" snow, thereby minimizing the possibilities of genetic cross-contamination.

The effects of the other environmental factors e.g. wind, desiccation, abrasion, and freeze-thaw cycles were less straightforward to characterize based on our field sampling protocol. That we were able to extract and sequence nDNA under extremely windy conditions with blowing snow appears to suggest that desiccation and abrasion may not have had an important effect on yield (at least for fresh tracks or tracks shielded under a layer of snow). Because the temperatures were extremely cold during the 2019 sampling season, we could not ascertain the influence of freeze-thaw cycles. However, it may be profitable to test these environmental factors under more controlled lab settings, particularly if there is reason to sample in the later spring when intense sunlight and cold evenings can lead to extreme temperature swings, freeze-thaw cycles, and/or if the surrounding region continues to warm as it has over the past decade.

Assuming that there were damaged cells within our samples, it is probable that some eDNA could have passed directly through the filters. This is because the pore-size of the filters that we used was too large to capture any cellular fragments or exposed DNA, which are much too small to be captured. Further experimentation into different filtering/extraction/precipitation protocols may lead to higher yields of eDNA that ordinarily would have been lost if a large proportion of the shed epidermal cells were damaged.

Given that the number of tracks per sample is related to eDNA yield (Fig. 8), it makes sense that sampling more tracks is a good strategy. However, this can be quite time consuming and difficult depending on weather conditions. As such, identifying efficiencies through further experimentation, tools, and techniques could help to make this method more viable.

In addition to fine tuning our sample collection methods, and increased yield of eDNA may also be facilitated by improving the extraction methods. One potentially important factor is the lengthy time-lag associated with international shipping of biological samples obtained from ESA listed species[1]. This process required substantial effort and time, and we suspect that delays in lab analyses may be a factor in decreasing eDNA yield during extraction. To remedy this, our team has discussed what it would take to set up a small field laboratory in Alaska in order to extract samples and measure DNA concentration on site. This would help with fine tuning sampling and extraction through an iterative process that can be performed in days rather than months.

Upon DNA extraction, genetic fingerprinting can be accomplished via analyzing microsatellites or SNPs (small nucleotide polymorphisms; Malefant et al. 2016, Viengkone et al. 2016).

[1] Incredibly, these samples fell under CITES (Convention on International Trade in Endangered Species) regulation and therefore required a CITES permit for shipment.

Microsatellites have been a standard protocol for years, but have their drawbacks, which may be overcome through the use of SNPs. When the time comes to scale up this work to analyze a statistically robust sample, there will be a need to further fine tune the laboratory methods, which is standard procedure for optimizing molecular science workflow.

In an independent project, the North Slope Borough has been working on developing SNP panels (Ferrer et al. 2019) that will advance the ability to not only identify and count individual polar bears, but also increase the ability to answer interesting questions about movement patterns, behavior, and general biology.

Our initial investigation into sampling eDNA from wild polar bears was successful in that we showed in a proof of concept that we could access the genetic materials necessary to genetically fingerprint individuals (i.e., nuclear DNA)[2]. This method is completely non-invasive, and, with optimization, can be scaled such that it will be possible to sample a large number of polar bears from across their range using methods that are cost effective and can be performed by locally hired paraprofessionals (i.e., Native community members). Conservation and management of polar bears in Alaska will be facilitated if local subsistence based community members can become involved in generating the scientific information that will be used in the decision making processes.

A final thought concerns the value of performing these assessments in a wild setting. While there are good reasons to perform captive studies, our experience has demonstrated that there are also some very tangible and important differences when implementing a new method in a wild setting under extreme conditions. Accounting for these conditions requires that we continue to move forward with work in a wild Arctic setting. As such, we are already making plans for further work to continue to develop and learn from these efforts[3].

[2] Our research was recently featured on the BBC: https://www.bbc.com/news/science-environment-49857028

[3] Note that at the conclusion of the 2018-19 funding period, the North Slope Borough Department of Wildlife Management had unspent funds. The primary reason for the unspent funds is due to the unanticipated but fortunate addition of collaborators and funding resources to this project, thereby spreading the workload and stretching our funding dollars. We are grateful to the Animal Welfare Institute's Christine Stevens Wildlife Award for supporting this project and want to ensure that entirety of these funds are put to good use. As such, we have requested and were approved for a no-cost 6-month extension so that we can add a second field season. Additional results at the conclusion of the second field season will be provided in a final report.

ACKNOWLEDGEMENTS

Funding for this investigation was provided by the Christine Stevens Wildlife Award (Animal Welfare Institute), World Wildlife Fund (Canada), World Wildlife Fund (Sweden), the North Slope Borough Department of Wildlife Management, and AquaBiota Water Research. We are grateful to our Iñupiaq colleagues Bobby Sarren and Billy Adams of Utqiaġvik, Alaska for assisting in the field, taking care of us while out on the sea ice, spotting polar bears, and sharing their expertise. We are grateful to MoRe Research Sweden AB for extracting the DNA and Cuong Tang at NatureMetrics for the initial screening and for providing laboratory facilities for further testing. Work on this project was performed under USFWS research permit # #MA80164B-0. All samples were shipped internationally under CITES permit # 19US33153D/9.

CITATIONS

Bellemain, E. 2017. The Future is Now: Science in a Spoonful of Snow. Final Report to the World Wildlife Fund US - March 2017

Ferrer, T., A.L. Von Duyke, R. Stimmelmayr, R. Suydam, T. Sformo, and G. O'Corry-Crowe. SNP genotyping as a new approach for genetic mark-recapture studies of polar bears, *Ursus maritimus*, in the Alaskan Arctic. Alaska Marine Science Symposium, Anchorage, AK, January 2019.

Hellström, M., N. Wijkmark, and J. Näslund. 2019. Footsteps in the snow – Pilot study for future monitoring of individual polar bears (Ursus maritimus) from eDNA in snow tracks. AquaBiota Report 2019:03. 14pp.

Kelly, R.P., Port, J.A., Yamahara, K.M. och L.B. Crowder. 2014. Using environmental DNA to census marine fishes in a large mesocosm. PloS One 9 (1): e86175

Malefant, M., et al. 2016. Circumpolar genetic structure and recent gene flow of polar bears: A reanalysis. PloS One 11 (3): e0148967

Peacock, E., Sonsthagen SA, Obbard ME, Boltunov A, Regehr EV, Ovsyanikov N, et al. 2015. Implications of the circumpolar genetic structure of polar bears for their conservation in a rapidly warming Arctic. PLoS ONE. 2015;10(1):e112021

Sformo, T., J.W. Bickham, J.A. Crawford, E.A. Montano, A. Minard Smith, and D.M. Stoeckel. 2015. Environmental DNA (eDNA) Assessment of Arctic Fish in the Beaufort Sea (Elson Lagoon), Barrow, Alaska. Alaska Marine Science Symposium 2015. Anchorage, AK.

Spens, J., A. R. Evans, D. Halfmaerten, S. W. Knudsen, M. E. Sengupta, S. S. T. Mak, E. E. Sigsgaard, och M. Hellström. 2017. Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. Methods in Ecology and Evolution, 8(5), 635-645

Viengkone M., et. al, 2016. Assessing polar bear (*Ursus maritimus*) population structure in the Hudson Bay region using SNPs. Ecol Evol. 6(23): 8474–8484

Von Duyke, A.L., K. Nesvacil, K. Titus, L. Quakenbush, and T. Sformo. 2016. Use of hair to non-invasively sample DNA from polar bears (*Ursus maritimus*) in the Chukchi and Beaufort Seas: Project status update 2016. North Slope Borough Department of Wildlife Management, Preliminary Research Report NSB.DWM.PRR.2016-01.01.

Von Duyke, A.L., E. Bellemain, T. Dejean, K. Nesvacil, K. Titus, A. Lyet, E. Kruger. 2017. The future is now; science in a spoonful of snow. Using eDNA to monitor a polar bear (*Ursus maritimus*) population in Chukci Sea. 22nd Biennal Conference of the Biology of Marine Mammals. Halifax, Nova Scotia, Canada.