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Remote biopsy darting and marking of polar bears

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ABSTRACT

Remote biopsy darting of polar bears (*Ursus maritimus*) is less invasive and time intensive than physical capture and is therefore useful when capture is challenging or unsafe. We worked with two manufacturers to develop a combination biopsy and marking dart for use on polar bears. We had an 80% success rate of collecting a tissue sample with a single biopsy dart and collected tissue samples from 143 polar bears on land, in water, and on sea ice. Dye marks ensured that 96% of the bears were not resampled during the same sampling period, and we recovered 96% of the darts fired. Biopsy heads with 5 mm diameters collected an average of 0.12 g of fur, tissue, and subcutaneous adipose tissue, while biopsy heads with 7 mm diameters collected an average of 0.32 g. Tissue samples were 99.3% successful (142 of 143 samples) in providing a genetic and sex identification of individuals. We had a 64% success rate collecting adipose tissue and we successfully examined fatty acid signatures in all adipose samples. Adipose lipid content values were lower compared to values from immobilized or harvested polar bears, indicating that our method was not suitable for quantifying adipose lipid content.

Key words: biopsy sampling, darting, DNA, fatty acid, genetics, lipid content, polar bear, population estimation, *Ursus maritimus*.

Physical capture provides the basis of much of the scientific knowledge on polar bears (*Ursus maritimus*) and typically involves darting bears with an immobilizing drug from a helicopter (Stirling *et al*. 1989). Capture studies allow for the deployment of radio collars for habitat and movement studies (*e.g.*, Ferguson *et al*. 1999, Mauritzen *et al*. 2003), for documentation of changes in body condition (*e.g.*, Stirling *et al*. 1999, Rode *et al*. 2012), and for the study of population ecology (*e.g.*, Stirling *et al*. 1980, Taylor *et al*. 2009). Yet, capture of polar bears is logistically demanding, often requires long recovery times (Haigh *et al*. 1985, Stirling *et al*. 1989, Cattet *et al*. 1999), and can be either unsafe or infeasible when bears occupy areas near open water or thin ice (Larsen 1971, Ramsay and Stirling 1988, Ramsay and Farley 1996).

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Because capture efforts can be time-consuming, the method can limit sample size and the geographic extent of studies, potentially introducing bias. The capture of bears is also considered invasive (Ramsay and Stirling 1986; Cattet *et al*. 2006, 2008), and local aboriginal groups have expressed concerns over, and denied permission for, the physical capture of polar bears (Semple *et al*. 2000, Peacock *et al*. 2011). Lack of research access, whether due to permitting issues or logistical and cost demands, is of particular concern for science-based conservation of polar bears, as more monitoring is needed for increasingly stressed polar bear populations (Vongraven *et al*. 2012). Remote biopsy darting (Karesh *et al*. 1987, Karesh 2008) could be used as an alternative or in addition to physical capture for a variety of studies. However, it has only recently been used on polar bears[2] and other ursids (Olson 2009) and thus is in the early stages in development of techniques.

Remotely collected genetic information has been used in other animals to examine population structure and movements (Baker *et al*. 1993, Witteveen *et al*. 2009), examine genetic diversity (Schmidt *et al*. 2009), determine sex ratios (Curtis *et al*. 2007), and estimate abundance (Palsbøll *et al*. 1997, Woods *et al*. 1999). Other studies have used remote biopsy darts to collect tissues to test for contaminants (Ross *et al*. 2000, Wiig *et al*. 2000), conduct stable isotope and fatty acid analyses (Hooker *et al*. 2001, Witteveen *et al*. 2009), and estimate individual ages (Herman *et al*. 2008, 2009; Pauli *et al*. 2011).

A number of commercial manufacturers produce biopsy darts, particularly for use on cetaceans. However, many of these darts require the use of a crossbow, which is unwieldy in a helicopter. In autumn 2010, we field tested two of these types of biopsy darts on polar bears and found that neither were particularly well suited for darting polar bears from a helicopter. The darts were drab in color, making them difficult to recover. Darts had no marking ability, making it difficult to identify individuals that had previously been sampled; and most darts required landing of the helicopter for retrieval.

Our objective was to develop and test a variety of biopsy darting systems for remote sampling of polar bears from a helicopter. We required a dart that, when fired from a helicopter, could simultaneously dye-mark individuals to avoid resampling, was brightly colored to aid in retrieval, could float to allow for sampling bears in the water, and was magnetic to aid in remote retrieval of darts in areas where it would be unsafe to land a helicopter (*e.g.*, thin ice). We provide details and success rates of these biopsy systems, and examine their ability to provide genetic and sex identification, fatty acid signatures, and quantify adipose lipid content.

Methods

Study Area

Our study area was the spring-time sea ice of the southern Beaufort Sea adjacent to northern Alaska along with the coastline, barrier islands, and inland areas within approximately 30 km of the coast of Alaska between Barrow, Alaska and the Canadian border (Fig. 1). We darted adult and subadult polar bears in autumn 2010 and spring and autumn 2011 (Fig. 1). To minimize disturbance of family groups, we did

[2]Unpublished data from Stephen Atkinson, Department of Environment, Government of Nunavut, Nunavut, Canada, September 2011.

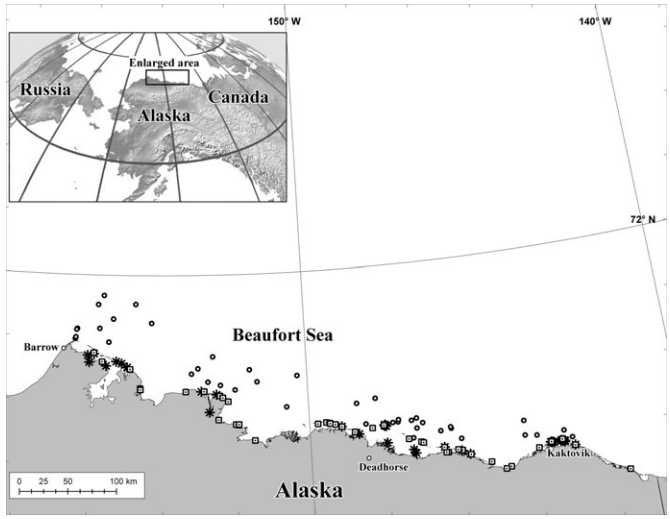

Figure 1. Study area and locations where adult and subadult polar bears were biopsy darted in the southern Beaufort Sea in autumn 2010 (asterisks, $n = 48$), spring 2011 (circles, $n = 41$), and autumn 2011 (squares, $n = 70$).

not dart dependent cubs. During the spring we used a Hughes 500 helicopter, and in autumn we used a Bell 206 LongRanger helicopter.

Previously Available Biopsy Darts

In autumn 2010, we used Pneu-dart, Inc. (Williamsport, PA) type C biopsy darts (PD, Table 1), and punched biopsy darts (PC, Table 1) developed by Palmer Cap-Chur Equipment, Inc. (Douglasville, GA) both fired from a Pneu-dart model 196 rifle. We typically fired PD and PC darts at power settings 3 and 4, respectively. The PD darts included an internal biopsy needle that was 23 mm long. We spray painted the body of the PD darts fluorescent orange to aid in recovery. The PC darts consisted of a punched biopsy head screwed onto a 10 mL aluminum dart body. We used a dental broach inside of the biopsy head to increase sample retention (Karesh 2008). We filled the 10 mL dart body with foam ear plugs to add weight to the dart to ensure the dart flew properly and to hold the dental broach in place. The PC darts were silver in color and could not be painted as they would not fit in either the Pneu-dart or Palmer Cap-Chur gun barrel when painted.

Newly Developed Biopsy Darts

In 2011, we worked with two dart manufacturers to develop darts tailored to our specifications. We worked with Palmer Cap-Chur Equipment, Inc. to develop a brightly colored biopsy dart with dye marking capabilities (Table 1, PC). This dart was a combination of a marking dart body and biopsy head, joined together by an adaptor (Fig. 2a). We attached the punched biopsy head to a 0.5 mL aluminum dart body (Fig. 2a). During the spring, we used a dental broach inside of the biopsy head and filled the 0.5 mL dart body with foam ear plugs (Karesh 2008). The marking

Table 1. Biopsy darts used to sample adult and subadult polar bears in the southern Beaufort Sea in autumn 2010, spring 2011, and autumn 2011.

Dart type[a]	Total length (mm)	Weight (g)	Biopsy head length (mm)	Biopsy head width (mm)	Brightly colored dart body	Dye marking ability	Floats
Previously available biopsy darts:							
PD	144	12	15	4	Yes	No	No
PC	192	24	15	5	No	No	No
Newly developed biopsy darts:							
PC	194	33[b]	15	5	Yes	Yes	No
PX	186	38[b]	15	5	Yes	Yes	Yes
PX	186	38[b]	15	7	Yes	Yes	Yes

[a]PD = Pneu-dart, Inc. type C biopsy darts; PC = Palmer Cap-Chur Equipment, Inc. punched biopsy darts; PX = Paxarms N.Z. Ltd. biopsy darts.
[b]Weight of dart loaded with dye.

dart had a 7 mL aluminum body that was anodized bright green. It was designed with eight equally spaced holes approximately 15 mm from one end to release the marking solution similar to the marking dart described by Turner (1982). The dart was assembled, similar to typical drug loaded darts, with a dart tail, lubed plunger, and Cap-Chur charge (Talbot 1960, Wright 1962, Green 1963, Bush 1992; Fig. 2a). We filled the dart with approximately 4.0 mL of either ethanol mixed with Nyanzol dye (Fitzwater 1943, Belmar Co., North Andover, MA) or tree marking paint (Nelson Paint Company, Kingsford, MI). A lubed metal dye tip was inserted into the dart body to hold the dye or paint in the dart until impact (Fig. 2a). We then attached the adaptor to the marking dart body and the biopsy dart syringe was attached to the other end of the adaptor (Fig. 2a). We used the Palmer Cap-Chur extra-long range projector to fire these PC darts.

We worked with Paxarms N.Z. Ltd. (Timaru, New Zealand) to develop a bright red colored biopsy dart with dye marking capabilities that could float, and was recoverable using a magnet (Table 1). This dart (PX) was similar to the Paxarm's flotation biopsy dart described by Krützen *et al*. (2002), but incorporated dye marking capabilities and a marine-grade stainless steel biopsy head that was positively attracted to magnets. We used two dimensions of biopsy heads to evaluate whether we could obtain greater samples of adipose tissue with wider biopsy heads (Table 1). The biopsy heads have three internal barbs designed for tissue retention (Fig. 2b). These heads screwed onto polycarbonate floatation dart bodies that were sealed on one end and designed with three equally spaced holes for marking bears with dye (Fig. 2b). A metal plunger was used to hold up to 3 mL of marking solution in the dart body until impact with the bear (Fig. 2b). The plunger would move forward upon impact and release the solution through the three holes. A polycarbonate screw capped the dart body to ensure a watertight seal (Fig. 2b). A polycarbonate tail piece screwed into the back of the dart body (Fig. 2b). The dart was buoyant in water and floated with the dart tail upright. We used a collapsible 1.2–3.7 m long pool net to retrieve darts in the water from the helicopter. We used tree marking paint or livestock marking solution (LA-CO Industries, Inc., Elk Grove, IL) for marking bears with this dart. We used an MK24C 0.745 projector (Paxarms N.Z. Ltd.) to fire the PX darts. For all dart types, we cleaned darts with soap and boiling water and used a 10% bleach solution as a disinfectant.

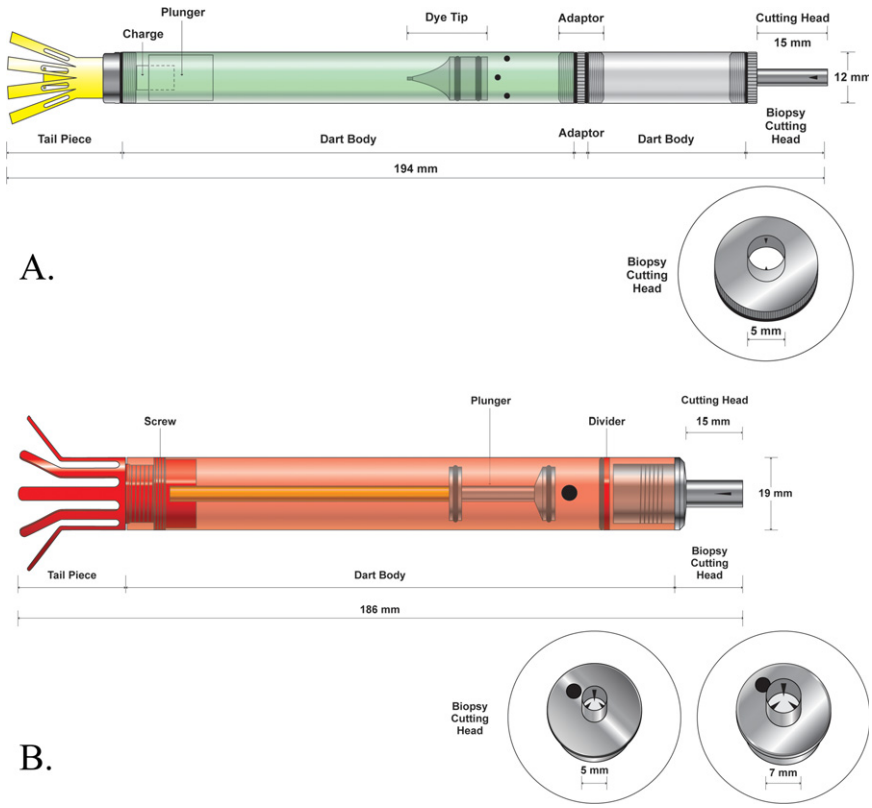

Figure 2. A. Diagram of a combination marking and biopsy sampling dart manufactured by Palmer Cap-Chur Equipment, Inc. B. Diagram of a combination marking and biopsy sampling dart that floats manufactured by Paxarms N.Z. Ltd.

When biopsy darting polar bears, we attempted to fire darts perpendicular to the body around the upper shoulder, similar to immobilization darting (Stirling *et al.* 1989). This approach was used to help ensure darts would immediately bounce out from the large muscle upon impact. We typically darted bears when they were approximately 3–6 m below the helicopter. Upon recovery of darts, we examined whether tissue samples had been collected and if not, we re-darted individuals when feasible.

We examined the amount of time required to dart bears using the time at which bears were first observed to the time the helicopter landed to recover fired darts. We weighed entire samples obtained in spring 2011 and September 2011. In August and September 2011, we separated adipose tissue from hair and skin and only submitted the hair and skin portion of the sample for genotyping. We air dried all genetic samples prior to DNA extraction. DNA was extracted from tissue samples using QIAGEN DNeasy Tissue Kits according to manufacturer's instructions by Wildlife Genetics International (WGI) Inc. (Nelson, British Columbia, Canada). WGI amplified DNA extracts at 20 microsatellite loci and the ZFX/ZFY sex identification marker (Aasen and Medrano 1990) using methods and primers as described in detail

by Paetkau (2003) and Kendall *et al*. (2009). We considered genotyping successful if the DNA extract amplified at the full suite of microsatellite loci and ZFX/ZFY.

We extracted lipids from adipose, derivatized fatty acids to their fatty acid methyl ester (FAME) analogues using the Hilditch reagent, and quantified individual FAMEs by gas chromatography with flame ionization detection (Budge *et al*. 2006). We considered fatty acid analysis of the remote biopsies successful if we were able to quantify all fatty acids routinely determined in larger biopsies from captured or harvested polar bear samples (Thiemann *et al*. 2008; McKinney *et al*. 2009, 2011).

We tested for normality in data sets using Shapiro-Wilk tests in the R programming language (http://cran.r-project.org/). We tested for differences in the mean wet weight of samples (the entire sample: hair, skin, and adipose tissue), mean adipose weight of samples, and adipose percent lipid content of samples obtained using the PC 5 mm heads, PX 5 mm heads, and PX 7 mm heads. We log transformed sample weight and arcsine transformed percent lipid content and used ANOVA and Tukey's HSD tests in R. We also similarly tested for differences in the ability of darts to obtain a tissue sample among the three dart types.

Results

Previously Available Biopsy Darts

In autumn 2010, we darted polar bears on the Alaska coast during two sampling efforts (Fig. 1): September (9 d); and October (9 d). We used PD darts to sample 30 polar bears (Fig. 3a) and PC darts to sample 18 polar bears (Fig. 3b). Two PD darts only collected hair. Three (10%) of the bears that we sampled using PD darts were darted twice because the first dart broke on impact with the bear, and one (3%) of the bears we sampled using PD darts was darted twice because the first dart failed to collect a sample. Except for missed shots, we successfully recovered all fired darts. Excluding the two darts that only collected hair that could not be genotyped, tissue samples (n = 46) were 100% effective in genotyping and sex determination of individuals. Genetic analysis revealed that none of the bears were sampled more than once in the same sampling effort whether darted with a PD or PC dart (neither type had a marking mechanism). Darting times averaged 6.8 min per bear (95% CI: 5.9–7.6 min, n = 48).

Newly Developed Biopsy Darts

In spring 2011, we darted polar bears on the sea-ice (20 d, Fig. 1). We used PC marking darts to sample and mark 41 bears (Fig. 1). These darts generally collected a small piece of skin and adipose tissue, as well as hair (Fig. 3b). Except for missed shots, we successfully recovered all fired darts. We re-darted three bears (7%) because the first dart failed to collect a sample. These samples were 100% effective in identifying sex and individual genetic identity. Genetic results indicated that we sampled one bear on two occasions. Darting times averaged 6.5 min per bear (95% CI: 5.4–7.6 min, n = 41).

In autumn 2011, we darted polar bears on the Alaska coast during two sampling efforts (Fig. 1): August (6 d) and September (6 d). We used PX marking darts to sample and mark 35 bears (11 in the water and 24 on land, Fig. 3c) and PC marking darts to sample and mark 35 bears (all on land). Nine of the PX and five of the PC

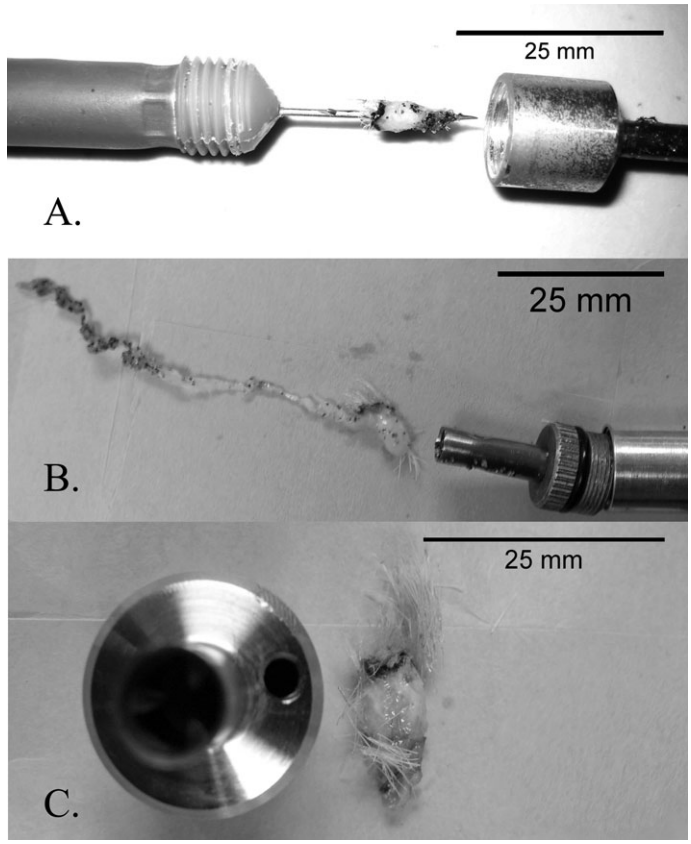

Figure 3. Representative samples obtained using biopsy darts in autumn 2010 and 2011. A. Pneu-dart, Inc. biopsy dart with a 4 mm biopsy head. B. Palmer Cap-Chur Equipment, Inc. biopsy dart with a 5 mm biopsy head. C. Paxarms N.Z. Ltd. biopsy dart with a 7 mm biopsy head.

darts only collected a hair sample. Nine (26%) of the bears we sampled using PX darts were darted twice because the first dart failed to collect a sample. We were unable to recover three PX darts from the water because rough seas made it difficult to recover and/or spot the dart. Excluding darts that only collected hair, samples ($n = 56$) were 98% effective in genotyping individuals and identifying sex. Three of the 14 samples that only collected hair were sufficient enough to genotype 12 microsatellite loci. Genetic results indicated that two bears were sampled on two occasions and one bear was sampled on three occasions during the August sampling period, while one bear was sampled on two occasions during the September sampling period. Darting times averaged 4.2 min per bear (95% CI: 3.6–4.8 min, $n = 70$).

We successfully quantified fatty acid profiles from all darts that collected adipose tissue ($n = 45$, Table 2). Mean total weights, lipid weights, and percent lipid content of biopsy samples using the PC 5 mm heads, PX 5 mm heads, and PX 7 mm heads differed (Table 2, ANOVA, $F = 22.5$, $P < 0.001$, $F = 5.6$, $P = 0.007$, $F = 4.7$, $P = 0.01$, respectively). *Post hoc* analyses indicated that PC and PX darts with 5 mm

Table 2. Mean (95% CI) total sample weights, success rates for obtaining adipose tissue, mean lipid weight (95% CI), and mean percent lipid content (95% CI) values based on biopsy dart type and sampling period of adult and subadult polar bears sampled in the southern Beaufort Sea.

Dart type[a]	Sampling period	n[b]	Sample wet weight (g)	Success rate retrieving adipose tissue	n[c]	Lipid weight (g)	Percent lipid content
PC	spring 2011	41	0.12 (0.10–0.14)	na[d]	na[d]	na[d]	na[d]
PC	autumn 2011	3	0.24 (0.19–0.28)	69%	24	0.02 (0.01–0.02)	23% (19%–27%)
PX-5	autumn 2011	4	0.08 (0.03–0.13)	44%	8	0.01 (0.004–0.02)	10% (5%–15%)
PX-7	autumn 2011	15	0.32 (0.25–0.40)	76%	13	0.02 (0.01–0.03)	19% (12%–27%)

[a]PD = Pneu-dart, Inc. type C biopsy darts; PC = Palmer Cap-Chur Equipment, Inc. punched biopsy darts; PX-5 = Paxarms N.Z. Ltd. biopsy darts with 5 mm wide biopsy heads; PX-7 = Paxarms biopsy darts with 7 mm wide biopsy heads.
[b]Number of samples weighed.
[c]Number of samples containing adipose tissue.
[d]Samples were not evaluated for adipose tissue.

heads collected samples of similar total weight (Tukey's HSD, $P = 0.18$), but samples from PC darts had greater lipid weights and percent lipid content than PX darts with 5 mm heads (Tukey's HSD, $P = 0.04$, $P = 0.01$, respectively). Samples from both the PC and the PX darts with 5 mm heads weighed less than samples obtained from the PX 7 mm heads (Tukey's HSD, both $P < 0.001$). Lipid weights between the PX 5 mm heads and the PX 7 mm heads differed (Tukey's HSD, $P = 0.005$), but percent lipid content did not (Tukey's HSD, $P = 0.14$). Neither lipid weights nor percent lipid content differed between PC darts and PX darts with 7 mm heads (Tukey's HSD, $P = 0.38$, $P = 0.51$, respectively). The ability to obtain a tissue sample among the three dart types also differed (ANOVA, $F = 17.3$, $P < 0.001$), with PC and PD darts having higher tissue sample rates than PX darts (Fig. 4).

Discussion

Biopsy darts that collected tissue were 99.3% successful in genetically identifying individuals and determining their sex; darts that collected adipose tissue were 100% successful in producing fatty acid profiles. Our 81% and 64% success rates in using a single dart to obtain tissue and adipose samples, respectively, suggest that biopsy darting can be an effective field methodology for foraging ecology studies and for studies requiring identification of polar bears, including mark-recapture population ecology. Moreover, we had an overall 89% success rate of obtaining tissue samples when adjusting for bears that were darted twice because the first dart failed to collect a tissue sample. However, our darting systems had variable success rates (Fig. 4). The angle of impact when biopsy darting has been found to strongly influence sample retention and size in other species (Brown *et al*. 1991, Barrett-Lennard *et al*. 1996, Noren and Mocklin 2012), and this was likely a strong factor, particularly with our

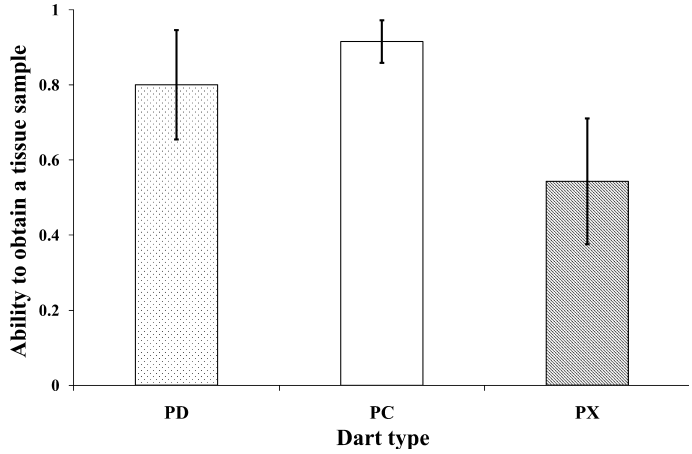

Figure 4. Mean (95% CI) tissue sample retention rates from Pneu-dart (PD), Inc. biopsy darts ($n = 30$); Palmer Cap-Chur Equipment (PC), Inc. biopsy darts ($n = 94$); and Paxarms (PX) N.Z. Ltd. biopsy darts ($n = 35$) sampling adult and subadult polar bears in the southern Beaufort Sea. Tissue sample retention rates were similar between PD and PC darts (Tukey's HSD, $P = 0.29$), but both PD and PC darts had higher retention rates than PX darts (Tukey's HSD, $P = 0.01$, $P < 0.001$, respectively).

lower success rate using the PX darts. Part of this lower success rate was likely related to poor shot placement; we used PX darts in high winds (mean wind speed autumn 2011: 7.2 m/s, range = 3.6–11.9 m/s; mean wind speed autumn 2010: 5.8 m/s, range = 0–10.3 m/s) and had a new helicopter pilot. However, the PX darts are heavier than the PC darts and we frequently attempted to fine-tune the velocity on the Paxarms dart gun to ensure darts flew at a correct trajectory. In addition, we used the wider (7 mm) biopsy head on a high proportion of the PX darts; wider diameter biopsy heads have been found to have lower retention rates than smaller heads (Patenaude and White 1995). We also had no prior experience using the Paxarms dart gun, whereas we had long histories of using both Pneu-Dart and Palmer Cap-chur dart guns. Although we used a dental broach with the PC punched biopsy heads in autumn 2010 and spring 2011, we did not notice a change in our ability to obtain a tissue sample when we did not use the dental broaches in autumn 2011. Overall, we had greatest confidence in the PC punched biopsy heads to obtain samples compared to either the PX or PD biopsy heads.

Despite our lower success rate using PX darts, 16% of the bears sampled in autumn 2011 were sampled in the water using the PX darts. Not sampling these polar bears, which were mainly around small barrier islands, would decrease precision of resulting mark-recapture parameter estimates. In addition, failure to sample these animals would bias the sample toward those bears on larger parcels of land or further inland; polar bears are known to sexually segregate in coastal areas with respect to distance from shore (Clark and Stirling 1998). The use of a net from the helicopter to recover darts in the water was challenging and required an excellent pilot. Preliminary results from autumn 2012 (USGS, unpublished data) indicate PX tether darts (Best *et al*. 2005) work well for sampling polar bears in the water.

We only measured lipid content percentages for biopsy samples obtained in autumn 2011. These values were considerably lower than lipid content values

documented in other studies of polar bears using adipose tissue samples obtained from the rump of immobilized bears or harvest samples (Thiemann *et al*. 2006; Stirling *et al*. 2008; McKinney *et al*. 2010, 2011). This suggests our current method of biopsy darting should not be used to assess condition based on lipid content of adipose samples (Stirling *et al*. 2008). Other studies using remote biopsy darts on cetaceans have also reported reduced lipid concentrations in their samples (Ylitalo *et al*. 2001, Krahn *et al*. 2004). Ylitalo *et al*. (2001) speculated this may have been in part a result of samples containing higher proportions of connective tissue than samples collected from necropsied animals. This was likely also a factor in our study and preliminary results from samples obtained in spring 2012 (USGS, unpublished data) indicate that while samples from the rump had higher lipid concentrations than samples obtained from other body locations, the lipid concentrations were still lower than samples from captured bears. Krahn *et al*. (2004) suggested that reduced lipid concentrations resulted from lipids seeping away from the sample when the dart is removed from the animal. Additionally, some of our samples became encrusted with sand once darts bounced off bears. We made attempts to remove extraneous materials from samples, but any additional weight from other sources would have reduced gravimetric lipid content estimates. We also found that samples obtained using either the PX 7 mm biopsy head or PC dart had higher lipid concentrations than the PX 5 mm biopsy head. This suggests that obtaining larger samples may improve estimates of lipid concentrations. Our initial results indicate that remote biopsy darts may not be suitable for quantifying lipid content, but we recommend future studies examine the potential for using wider and longer biopsy heads (Gauthier *et al*. 1997), rinsing samples in distilled water as soon as they are recovered, and, for comparisons with other studies, targeting the same area of the body from which samples are taken from immobilized polar bears (*i.e.*, the rump).

We found the painted orange PD darts easy to recover on land despite being smaller than the other darts we tested. Both the silver PC darts and green PC darts were difficult to spot and recover on land, but the green PC darts were generally easy to spot and recover on the sea ice. The red PX darts were the easiest to spot and recover on land given their bright color and large size.

Dye marking prevented accidentally resampling 96% of the bears. Although we did not resample any bears in autumn 2010 when we did not dye mark bears, we struggled to keep track of which bears had been sampled when darting bears in groups and avoided sampling some bears because we were unsure whether they had already been sampled. In addition, our on-shore survey methodology (progressively flying from east to west) made it unlikely that we would have encountered the same individuals more than once. In the spring, we randomly searched the sea ice, so we had a higher probability of re-encountering previously sampled individuals. We resighted three bears that had been previously sampled and marked earlier in the spring, and we resampled one individual whose mark was either undetectable or overlooked. We resampled a total of four bears in autumn 2011. Three of these occurred in August when we were marking with a Nyanzol dye. This was likely in part because the Nyanzol dye marks looked similar to the mud or dirt marks on polar bears during the autumn. In addition, bears may have been molting (Kolenosky 1987) and bears frequently entered the water shortly after being darted, which may have reduced the intensity of marks. Bears marked with livestock solution or tree marking paint in September appeared to quickly lose their marks upon entering the water. The only bear resampled in September 2011 had been darted in the water and marked with tree marking paint, indicating that the paint is not effective at marking

when bears are in the water. Neither the solution nor paint appeared to be suitable if marks are desired to last more than several minutes. We have subsequently tested five different dye/paint combinations of various colors, but these did not improve mark longevity (USGS, unpublished data). Further study should focus on finding appropriately colored, fast-fixing dye. The PC marking darts provided a larger and darker mark on bears than the PX darts (Fig. 5).

Two of the dart rifles we tested, the Paxarms and the Pneu-Dart dart rifles, allow for fine adjustments in the velocity of the dart, meaning rapid adjustments can be made when darting distances or wind conditions change, which should reduce the degree of tissue damage caused by the biopsy dart (Patenaude and White 1995). On a small number of occasions, we observed some bleeding around the dart wound. However, biopsy darts should cause less injury than immobilization darts, particularly rapid injection darts (Cattet *et al*. 2006). On average, biopsy darting took <7 min per bear, which is a considerable reduction in time spent disturbing animals compared to immobilization. Although it is possible to biopsy dart dependent cubs, we did not in this study because of the challenges involved in keeping family groups together during darting runs. During capture of polar bears, mothers are typically sedated first and the dependent cubs typically stay near the sedated mother. Since there is no sedation involved in biopsy darting there is an increased risk of separation while attempting to sample dependent cubs.

Remote biopsy darting provides an additional tool or an alternative to capturing polar bears and other wildlife, for the purpose of individual and sex identification and diet analysis. Although biopsy darting does not provide the detailed health and physiological information that can be attained through capture, it is less invasive than immobilization and handling and may be more acceptable to local people who live in proximity to polar bears. Finally, biopsy darting can be used without the extensive equipment required for capture-based studies, and in some areas could be conducted on the ground with snowmachines to monitor remote subpopulations of polar bears that have limited research access (Vongraven *et al*. 2012). The type of biopsy dart to use will depend on the type of habitat and season of the study.

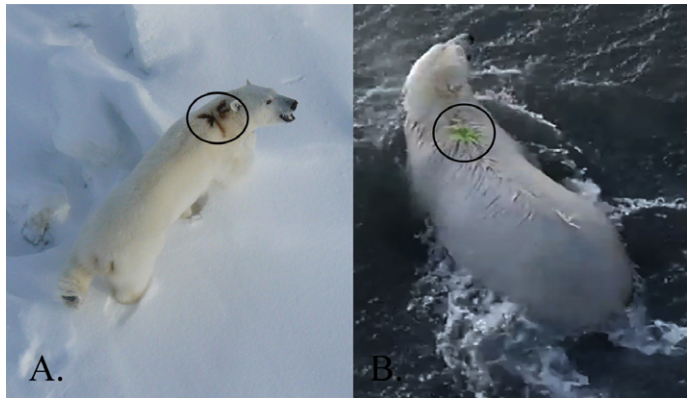

Figure 5. A. Dye mark on a polar bear on the spring-time sea ice using Nyanzol dye in a Palmer Cap-Chur Equipment, Inc. biopsy/marking dart. B. Dye mark on a polar bear in the water using tree marking paint in a Paxarms N.Z. Ltd. biopsy/marking dart.

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