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Authors: Morden, C-Jae C., Weladji, Robert B., Ropstad, Erik, Dahl, Ellen, and Holand, Øystein

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Original article

Use of faecal pellet size to differentiate age classes in female Svalbard reindeer *Rangifer tarandus platyrhynchus*

C-Jae C. Morden, Robert B. Weladji, Erik Ropstad, Ellen Dahl & Øystein Holand

Proper management of threatened populations requires prior knowledge of population sizes and structures, however, current techniques to gather this information are generally impractical, costly, and can be physically stressful for the animals. Non-invasive methods (e.g. faecal sampling) that can produce high quality and accurate results are better alternatives. Using faecal samples collected from a Svalbard reindeer *Rangifer tarandus platyrhynchus* population in the winters of 2008 (N = 158) and 2009 (N = 161), we investigated and validated the feasibility of using faecal pellet sizes to differentiate between female calves, yearlings and adults. We found that pellets from adult females were longer than those from calves, and pellets from adults and yearlings were clearly wider than those from calves. With an accuracy of 91% correct classification, we did show that a combination of faecal pellet dimensions (length, width and depth), rather than a single dimension alone, can allow managers to clearly differentiate between age classes if pellets already identified as being from females are used. We also found a positive relationship between live weight and pellet size of the reindeer. Combined with DNA analysis to identify the gender of the animal that produced the faecal pellet, this information may provide important population parameters and be a valuable tool for the monitoring of various ungulate species including wild reindeer.

Key words: age, faecal pellet morphometry, Rangifer tarandus platyrhynchus, Svalbard reindeer, validation

C-Jae C. Morden & Robert B. Weladji, Department of Biology, Concordia University, 7141 Sherbrooke St. West, Montreal, Quebec, H4B 1R6, Canada - e-mail addresses: cjaemorden@gmail.com (C-Jae C. Morden); rweladji@alcor.concordia.ca (Robert B. Weladji)
Erik Ropstad & Ellen Dahl, Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science, P.O. Box 8146 Dep. N-0033 Oslo, Norway - e-mail addresses: erik.ropstad@veths.no (Erik Ropstad); Ellen.Dahl@veths.no (Ellen Dahl)
Øystein Holand, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway - e-mail: oystein.holand@umb.no

Corresponding author: Robert B. Weladji

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Knowledge of demographic parameters including density indices, age and sex composition is essential for the monitoring and management of wild animal populations (Mysterud & Ostbye 2006), and may be used to predict future population dynamics. Indeed, in many ungulates, the population structure will affect their population dynamics (Coulson et al. 2001, Gaillard et al. 2003). For example, under low hunting risk, a population composed of > 15% of caribou *Rangifer tarandus* calves would be indicative of growth (Bergerud 1992). Thus, knowledge of the age structure of a population is essential for exploring trends in recruitment, population growth, mortality and reproductive status of the population (Reilly 2002).

Unfortunately, traditional techniques to gain population information of certain wildlife, especially those that are elusive and rare, are generally impractical and costly (Haigh 1979, Valkenburg et al. 1983) and have been criticized as being harmful to the animals (Côté et al. 1998, Cattet et al. 2008, Omsjoe et al. 2009). It is, therefore, in our best

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interest to seek out methods that are economically practical and safe, yet can still produce similar high quality, accurate results. Non-invasive monitoring techniques, for example DNA microsatellites, sex hormones, and faecal pellet morphology are currently being studied to replace the more risky alternatives (Southgate 2005, Morden et al. in press).

Faecal pellet group sampling has been used successfully by many researchers to gather information on the population size and biomass, the sex structure, habitat use of a species, diet composition and selectivity to predict the weight of the individual defecator, and to identify individual animals using DNA techniques for capture-mark-recapture studies (Putman 1984, Loft & Kie 1988, Edge & Marcum 1989, Hettinga 2010, Wam & Hjeljord 2010). Unfortunately, age estimation to predict population structure is still in the initial phase of research. It has been argued that faecal pellet size can also be used as a non-invasive technique to identify age classes (Southgate 2005).

Currently, field biologists spend a substantial amount of time and money to capture and tag young animals for the purpose of identifying their age later in life (Delibes-Mateos et al. 2009). Common age estimation tools include tooth sectioning, molar tooth wear, eye lens weight, the measurement of different body sections, the progression of lumbar epiphyseal fusion and body weight. All of this often requires the capture or killing of some animals, which is not always possible or good for population viability (Delibes-Mateos et al. 2009). Another common tool for age determination is by visual estimation during helicopter surveys. However, the accuracy of this method requires that the animals be in open areas, and therefore, it may not be applicable to all species (Miller 2003, Reimers 2006). Many studies have attempted to use pellet size or dimensions such as diameter, weight or volume of the pellets to predict age directly from the measurements of the pellets or by deriving an equation from a statistical relationship for the estimation of the live weight (Putman 1984, MacCracken & Van Ballenberghe 1987, Reilly 2002, Chapman 2004, Southgate 2005). However, most of these studies have used only one measure of pellet size or dimension which may work for some species, but more often makes age classification unclear due to pellet size overlap. Accordingly, we argue that a combination of dimensions may be a more promising approach. Indeed, Khan & Goyal (1993) concluded that one pellet measurement alone was not sufficient to predict the age of an individual, however, a combination of length, maximum width and weight measurements were able to predict the age in female Manipur brow-antlered deer *Cervus eldi eldi*.

Faecal pellet size has also been reported to be correlated with both body mass and age in several species. Coe & Carr (1983) reported that within a given species, specifically many bovid species, weight of dry dung pellets may be related to the weight of the individual defecator; thus allowing an age-structure to be created (Putman 1984). Since in most ungulates, body size increases as the individual ages to adulthood, there is potential that pellet size may indicate the sex and age structure of a population (see e.g. Bubenik (1982) for elk *Cervus elaphus* and MacCracken & Van Ballenberghe (1987) for moose *Alces alces*). In our paper, we investigated the possibility of using winter faecal pellet groups to identify age class of female Svalbard reindeer *Rangifer tarandus platyrhynchus* based on pellet morphometry (either single dimension or combination of measurements) and validated our model using data from another year from the same population. Svalbard reindeer have been monitored since 1994, and faecal samples have been collected from individuals of known age and body mass. Furthermore, we examined the relationship between faecal pellet morphometry and live body mass solely as a possible explanation for faecal size differences that may exist between age classes.

Materials and methods

Study area

Our study took place in Nordenskiøldland, Spitsbergen (77°50'-78°20'N, 15°00'-17°30'E), Svalbard in Norway, including Colesdalen, Semmeldalen and parts of Reindalen, with adjacent side valleys. The area is home to a population of about 1,500 wild Svalbard reindeer (Milner et al. 2003). Human presence is rare and predators are limited, so this wild population's only threat is the extreme environment that often causes large fluctuations in population sizes (Meteorologisk institutt 2009). Most reindeer feed on lichens during the winter, however, Svalbard reindeer are limited to feeding on browse of mosses and low-quality vascular plants which are often difficult to find and attain (Bjorkvoll et al. 2009, Sundset et al. 2009). Since 1994, female

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reindeer have been caught and sampled once or twice a year for individual survival and reproductive rates. Because it is a long-term, closely studied population, a large percentage of the females are marked with ear tags and neck collars for identification and are of known age (Milner et al. 2003).

Data collection

Methods of capture are as described by Milner et al. (2003). Faecal samples were collected from individual reindeer during four visits to Svalbard, during February and April in 2008 (N = 158) and 2009 (N = 161). The faeces were either gathered directly from the anus or from the snow immediately following excretion. The faecal samples remained frozen at -20°C until analysis. Our study was carried out in agreement with the provisions enforced by the Norwegian National Animal Research Authority. All activities related to capturing and sampling animals were done with permission from the authorities of Svalbard. We collected 319 different pellet groups from 272 female Svalbard reindeer (1,834 pellets total), 263 pellet groups from adults, 25 pellet groups from yearlings, and 31 pellet groups from calves.

Using the running mean approach, we found that five random pellets per individual was an adequate sample size for the analysis. Each pellet was measured for its maximum length (L), maximum width (W) and depth (D) at 90° rotation from W. Only complete, regular oval pellets were used (Zahratka & Buskirk 2007). All samples were measured by the same observer using Vernier calipers with a precision of 0.05 cm (Sanchez-Rojas et al. 2004). An approximated volume index (V) was calculated using the product of the three measurements ($L \times W \times D$). We obtained the mean value for each measurement per animal per sampling period.

Statistical analysis

We used a generalised linear mixed model (PROC MIXED using the SAS 9.2 software; SAS Institut Inc. 2008) to explore the relationship between the collection periods (two levels: February and April) and the age classes (three levels: calves (0-1 year; C), yearlings (1-2 years; Y), and adults (2+ years; A)) of pellet sizes using 2008 data. 'Individual ID' was included in our model as a random factor to account for repeated measurement on the same individuals, thereby controlling for pseudoreplication. There is often controversy with the development of ecological indices and their effectiveness in reality. Therefore, it is essential to carry out case-specific verification and validation for each species (Delibes-Mateos et al. 2009, Rouco et al. 2009). We therefore performed a cross-validation using a subsample of our data (Conroy & Carroll 2009). Unfortunately, due to severe weather icing conditions in the winter of 2008 on Svalbard, high calf mortality occurred, therefore, samples collected in 2009 lacked any calf samples and very few yearling samples. To deal with this issue, we limited our mixed model analysis to data from February and April 2008 using a random subset of 20 calves (65% of all calves) generated by Minitab 15.0 (Minitab Inc. 2007) to analyze the pellet size data. We used the second year of data, from 2009, and the remaining 11 random calves to validate our model and to see how accurately age class can be predicted in the second year by various pellet morphometry models.

We also used a multiple discriminant analysis (MDA; PROC DISCRIM using the SAS 9.2 software; SAS Institute Inc. 2008) to investigate the difference between groups, specifically the three age classes (Ims & Yoccoz 2000). Multiple discriminant analysis is used to classify grouping dependent variables which have two or more categories, in our case age class, using multiple predictor variables such as pellet dimensions (Sanchez-Rojas et al. 2004, Garson 2008). The accuracy of the classification function was estimated as the number of animals correctly classified depending on their mean pellet size (MacCracken & Van Ballenberghe 1987). We therefore assessed all possible models to determine which most accurately identified the correct age class.

To assess the effect of age class (calves, yearlings and adults) on body mass of female reindeer, we also used a linear mixed model, with 'Individual ID' entered as a random term and the weighing date and year being entered as covariates to account for change in body mass with time. From these models, we generated least square means (lsmeans) and the corresponding standard error for each age class. Finally, we used linear mixed models to assess the relationship between live body weight and the mean dimensions (i.e. average of the five samples per individual) of the faecal pellets (Zahratka & Buskirk 2007). In these models, we controlled for the effect of 'age class', 'year' and 'month', and 'individual ID' was added as a random term. All the analyses were performed using SAS 9.2 software (SAS Institute Inc. 2008) and a significance level of 5% was adopted.

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Results

Age class vs pellet size

Adult (A) pellets were longer than those from calves (C; estimated difference: A - C = 0.146, SE = 0.044), but there was no difference in length between pellets from adults and yearlings (Y) as well as calves and yearlings (Table 1 and Fig. 1A). Adult and yearling pellets were wider than calf pellets (estimated differences: A - C = 0.119, SE = 0.018 and Y - C = 0.059, SE = 0.024). Additionally, adult pellets were wider than yearling pellets (estimated differences: A - Y = 0.060, SE = 0.019; see Table 1 and Fig. 1B). Adult pellets were deeper than both calf (estimated difference: A - C = 0.108, SE = 0.017) and yearling pellets (estimated difference: A - Y = 0.064, SE = 0.018), but there was no difference between calf and yearling pellets depth (see Table 1 and Fig. 1C). Finally, the volume of adult pellets was greater than those of both calves (estimated difference: A - C = 0.286, SE = 0.052) and yearlings (estimated difference: A - Y = 0.160, SE = 0.054), but there was no difference in volume between calf and yearling pellets (see Table 1 and Fig. 1D).

Using validation and cross-validation techniques, single dimensions were able to correctly classify pellets into age classes with a range of accuracy of 56.4% for length alone to 79.1% for depth or width alone. A combination of length and depth was able to correctly classify 89% of the pellets into age classes, but the most accurate combination of dimensions included length, width and depth with an accuracy of 90.7% of correct classification into age class.

Results of MDA revealed the best model for overall accuracy to be the length and width combination with an error of 24.4%. We also found that the combination of length, width and depth only provided intermediate accuracy with an error of 25.7%. Focusing solely on the correct assignment of the calf age class, we found that both the length and width model and the length, width and depth model were the most accurate with 80.7% accuracy (Table 2).

Body mass vs pellet size

We found body mass to differ between calf, yearling and adult female reindeer ($F_{2,116} = 210.05$, $P < 0.001$), the smallest animals being the calves (lsmeans = 29.17 kg, SE = 0.96) followed by the yearlings (lsmeans = 38.64 kg, SE = 0.96) and adults being the largest (lsmeans = 49.50 kg, SE = 0.37). We also found a positive relationship between live weight and all pellet size dimensions, the effect being non-significant only for the length (width: b = 22.063, SE = 3.2858; depth: b = 31.107, SE = 3.846; volume: b = 7.368, SE = 1.248; length: b = 1.698,

Table 1. Results of the mixed models assessing the effect of age class on pellet length, width, depth and volume of Svalbard reindeer in 2008. The effect of month (entered as a class variable with two levels, February and April, the latter being the reference) was controlled for and individual ID was entered as a random factor. Age class was entered as a class variable with three levels, adults (A), yearlings (Y) and calves (C) with yearling as the reference level. Significant terms are shown in italics.

	b	SE	t (df = 20)	P-value
Length (cm)				
Intercept	1.340	0.045	29.86	*≤ 0.001*
Age class : A - Y	0.036	0.046	0.80	0.435
C - Y	-0.110	0.059	-1.86	0.078
Month: February - April	0.048	0.029	1.67	0.110
Width (cm)				
Intercept	0.729	0.019	39.37	*≤ 0.001*
Age class : A - Y	0.060	0.019	3.16	*0.005*
C - Y	-0.059	0.024	-2.43	*0.025*
Month: February - April	0.039	0.011	3.42	*0.003*
Depth (cm)				
Intercept	0.685	0.018	39.08	*≤ 0.001*
Age class : A - Y	0.064	0.018	3.56	*0.002*
C - Y	-0.044	0.023	-1.92	0.069
Month: February - April	0.023	0.010	2.34	*0.030*
Volume (cm^3)				
Intercept	0.666	0.053	12.67	*≤ 0.001*
Age class : A - Y	0.160	0.054	2.99	*0.007*
C - Y	-0.126	0.069	-1.82	0.084
Month: February - April	0.108	0.034	3.17	*0.005*

Table 2. Discriminant analysis for Svalbard reindeer pellet groups showing the two potential grouping models. First number is actual number of pellet groups classified into representative age class. Number in parentheses is percent of pellet groups classified into representative age class. Italic values indicate individuals correctly grouped into the proper age class.

	Classified as		
Model	Calf	Yearling	Adult
Model 1: L × W			
Calf	*25 (80.65)*	4 (12.9)	2 (6.45)
Yearling	6 (24)	*18 (72)*	1 (4)
Adult	18 (6.84)	50 (19.01)	*195 (74.14)*
Model 2: L × W × D			
Calf	*25 (80.65)*	4 (12.9)	2 (6.45)
Yearling	7 (28)	*17 (68)*	1 (4)
Adult	17 (6.46)	51 (19.39)	*195 (74.14)*

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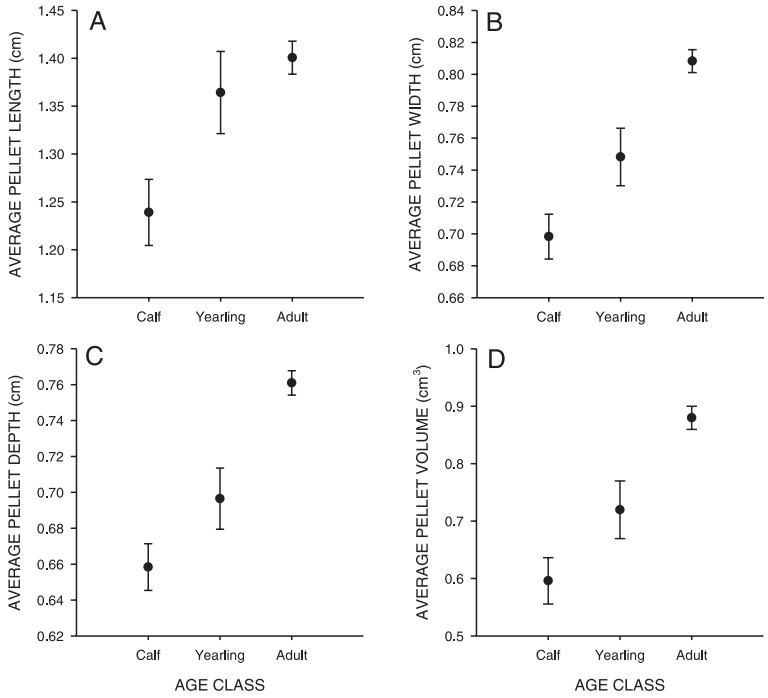


Figure 1. Least square means (± SE) (adjusted for month) of pellets length (A), width (B), depth (C) and volume (D) as a function of the three age classes for Svalbard reindeer using a random set of calves.

SE = 1.639; Table 3). There was a significant effect of age class, month and year for all pellet measurements (see Table 3).

Discussion

Age class vs pellet size

In general, the highest values across all of the pellet measurements occurred in adult samples, yearlings were intermediate, and calves had the smallest values, similar to what was found in mule deer *Odocoileus hemionus* (Sanchez-Rojas et al. 2004). Overall, we found that pellet size differed significantly between age classes. However, one dimension alone was not sufficient to clearly differentiate among age classes which is in agreement with many similar studies (Chapman 2004, Southgate 2005, Delibes-Mateos et al. 2009), and it seems that the accuracy of using pellet size data to determine age class is highly dependent on the species under investigation. In the greater bilby *Macrotis lagotis*, Southgate (2005) was able to differentiate immature-independent individuals (< 500 g) from larger females and males (> 500 g) by using the faecal pellet diameter. Indeed, using a second year of data, we were also able to validate various models and found that the best model to predict age class most accurately, specifically in differentiating female calves from other age categories, was a combination of length, width and depth of the pellets with an accuracy of 91%. Overall, we found that adult females could be more clearly separated from the other two age classes based on pellet dimensions as calf and yearling pellet sizes were more variable, similar to what was found in moose (MacCracken & Van Ballenberghe 1987).

The model that was the most accurate for data grouping, as determined by the discriminant analysis, was the combination of length and width measurements. However, using the validation accuracy from the second year of data, only 86% of pellet groups were correctly identified compared to an 91% accuracy found with the length, width and depth model. The discriminant analysis suggested that either length and width together or the length, width and depth model could be used to most accurately separate the calf age class (81% correct identification) from yearling or adult reindeer (see Table 2). Therefore, we believe that the combination of all three measurements (length, width and depth of the pellet groups) will provide a good basis on which to separate age classes in Svalbard reindeer and perhaps other species of ungulates if the gender of the animal which produced the faecal pellets is known.

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Table 3. Results of the linear mixed models assessing the relationship between live weight of females and pellets length, width, depth and volume of Svalbard reindeer. The effect of month (entered as a class variable with two levels, February and April, the latter being the reference), year (entered as class variables with two levels, 2008 and 2009, the latter being the reference level), and age class (entered as a class variable with three levels, adults (A), yearlings (Y) and calves (C) with yearling as the reference level). Individual ID was entered as a random factor. Significant terms are shown in italics.

	b	SE	t (df = 55)	P-value
Length (cm)				
Intercept	34.289	2.550	13.44	*< 0.001*
Length	1.698	1.639	1.04	0.305
Age class: A - Y	13.956	1.188	11.75	*< 0.001*
C - Y	-6.078	1.479	-4.11	*< 0.001*
Year: 2008 - 2009	-9.435	0.660	-14.29	*< 0.001*
Month: February - April	10.115	0.418	24.22	*< 0.001*
Width (cm)				
Intercept	19.947	2.736	7.29	*< 0.001*
Width	22.063	3.285	6.72	*< 0.001*
Age class: A - Y	12.219	1.149	10.64	*< 0.001*
C - Y	-5.821	1.375	-4.23	*< 0.001*
Year: 2008 - 2009	-8.269	0.645	-12.81	*< 0.001*
Month: February - April	8.898	0.427	20.84	*< 0.001*
Depth (cm)				
Intercept	14.450	2.959	4.88	*< 0.001*
Depth	31.107	3.846	8.09	*< 0.001*
Age class: A - Y	11.546	1.125	10.26	*< 0.001*
C - Y	-5.759	1.334	-4.32	*< 0.001*
Year: 2008 - 2009	-7.972	0.629	-12.67	*< 0.001*
Month: February - April	8.967	0.405	22.17	*< 0.001*
Volume (cm^3)				
Intercept	31.140	1.482	21.01	*< 0.001*
Live weight	7.368	1.248	5.90	*< 0.001*
Age class: A - Y	12.615	1.143	11.04	*< 0.001*
C - Y	-5.670	1.382	-4.10	*< 0.001*
Year: 2008 - 2009	-8.625	0.638	-13.52	*< 0.001*
Month: February - April	9.264	0.431	21.48	*< 0.001*

Body mass vs pellet size

As we hypothesized, pellet size and body mass differed between calves, yearlings and adult; being larger for adults and smaller for calves. We also found a positive relationship between female body mass and pellet width, depth and volume. Calves were on average nearly 20 kg lighter than adult reindeer. Many other studies have suggested that as the study species ages or grows, faecal pellet size and mass will also increase (Simonetti & Fuentes 1982, Chapman 2004, Sanchez-Rojas et al. 2004). Coe & Carr (1983) found a highly significant linear relationship between ungulate body mass and dry weight of faecal pellets. However, Southgate (2005) only found a weak relationship between pellet diameter and animal weight in the greater bilby. There was also strong evidence that width, depth and volume of faecal pellets are well predicted by body weight, and therefore may be able to help predict age class in the Leporidae (Zahratka & Buskirk 2007).

Although we only used female data from one population of Svalbard reindeer, we believe it would be possible to apply these same procedures and models to other *Rangifer* populations, other ungulate species, and most likely other wildlife that produce faecal pellets (Ball 2010). Given that body mass is strongly correlated to faecal pellet size, we may either scale up our dimension ranges using, for example, the ratio of body mass or use their allometric relationships.

During our analysis, we often found that the month of sampling influenced variation in our dimensions (see Table 1). It may, therefore, be important in the future to examine the influence of seasonal changes in diet, insect activity and weather conditions on faecal pellet size, problems which have been raised by several authors (Neff 1968, MacCracken & Van Ballenberghe 1987, Khan & Goyal 1993, Alvarez 1994, Sanchez-Rojas et al. 2004, Delibes-Mateos et al. 2009). On Svalbard, the reindeer are free-ranging and feed on natural vegetation. Therefore, we would not expect a significant difference in diet within the species, especially between two winter months since weather conditions are relatively similar during this period. During our collection period, the climate was quite harsh, thus the incidence of insects is negligible and the chance of melting and heavy rains was extremely low, therefore, the decay in size of the pellets between the two months was minor. Another direction that could be explored is to examine the effect of age and moisture content of the pellet on the accuracy of age class estimation. In several studies, the pellets were dried prior to measurement; however, we would expect that if the animals were free-ranging, the water content of the pellets would not vary to a large extent as the individual animals have similar diets (Coe & Carr 1983, MacCracken & Van Ballenberghe 1987, Khan & Goyal 1993, Reilly 2002, Sanchez-Rojas et al. 2004, Delibes-Mateos et al. 2009). However, Reilly (2002) found that drying the dung was not necessary and that there was not a great enough effect of decay on the dung size of Sumatran elephants *Elaphas maximus sumatranus* to make it worthwhile, but perhaps in our case, there

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may be some effect of the freezing process on the samples, this topic should be explored further.

We here show that a combination of pellet measurements can be used to differentiate between calf, yearling and adult female Svalbard reindeer. The dimensions we found most important to measure were a combination of maximum length, maximum width and depth. We measured only pellets known to be produced by females, but studies employing pellet measurements to gain insight into population status would normally pick up pellets in the field, and thus not know the gender of the animal which produced them. Reindeer are sexually dimorphic in size (e.g. Weladji & Holand 2003) so the pellet measurements and analysis we suggest would normally have to be combined with a technique (like DNA analysis) to separate the pellets made by each gender. If male and female pellets were not identified, it is possible that, for example, pellets produced by a yearling male might be classified as being produced by an adult female and thus confound any insights into population status. We hope that pellet size-age class data, in combination with DNA and hormone techniques, may improve the current non-invasive techniques that are used to monitor wild populations of reindeer and perhaps other species of ungulates (Morden et al. in press). Reilly (2002) found that dung data were more reliable to estimate age and population size than direct observations of elephants, which we hope will also prove to be the case for rare or secretive wildlife species. Faecal pellet data have several benefits in wildlife management, mainly lowering the cost of monitoring programs. It is a non-invasive procedure causing no harm to the animals, and it may provide us with valuable information on population dynamics, such as recruitment rate, to help offer glimpses into the future (Reilly 2002, Sanchez-Rojas et al. 2004).

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