Assessment of the point-frame method to quantify wolf Canis lupus diet by scat analysis

Authors: Ciucci, Paolo, Tosoni, Elisabetta, and Boitani, Luigi

Source: Wildlife Biology, 10(2) : 149-153

Published By: Nordic Board for Wildlife Research

URL: https://doi.org/10.2981/wlb.2004.020

Assessment of the point-frame method to quantify wolf *Canis lupus* diet by scat analysis

Paolo Ciucci, Elisabetta Tosoni & Luigi Boitani

Ciucci, P., Tosoni, E. & Boitani, L. 2004: Assessment of the point-frame method to quantify wolf *Canis lupus* diet by scat analysis. - Wildl. Biol. 10: 149-153.

Scat analysis is a widely used technique to assess food habits of wolves *Canis lupus*, but complete dissection and thorough hand separation of the undigested remains for their individual identification is laborious and time consuming. In addition, this technique is susceptible to inter-observer sources of error. Alternatively, the point-frame method allows systematic sampling of undigested remains of faecal samples and greatly reduces the processing time. Based on a sample of 200 wolf scats, we compared hand separation and point-frame methods using four widely used scat analysis quantification methods (frequency, volume and biomass models). Qualitative and quantitative estimates of the wolf diet showed close agreement between hand separation and point-frame procedures, but point-frame sampling allowed for an 85% reduction of the processing time. Given that the method is properly applied and its assumptions are met, we conclude that application of the point-frame method is reliable and more time effective than hand separation of wolf scat. The point-frame method could also provide a more rigorous sampling approach to reduce observer subjectivity as to what constitutes an accurate hand separation of undigested remains in scat.

Key words: Canis lupus, food habits, scat analysis, wolf

Paolo Ciucci, Elisabetta Tosoni & Luigi Boitani, Università di Roma 'La Sapienza', Dipartimento di Biologia Animale e dell'Uomo, Viale dell'Università, 32-00185 Roma, Italy - e-mail addresses: paolo.ciucci@uniroma1.it (Paolo Ciucci); etosoni@libero.it (Elisabetta Tosoni); luigi.boitani@uniroma1.it (Luigi Boitani)

Corresponding author: Paolo Ciucci

Received 15 September 2002, accepted 14 May 2003

Associate Editor: Henryk Okarma

Wolf scat analyses form an integral part of wolf ecology studies, and may provide critical or complementary information on wolf food habits otherwise difficult to obtain (Peterson & Ciucci 2003). However, due to several potential problems (e.g. Reynolds & Aebisher 1991), reliable scat analyses depend on correct choice of quantification methods (Ciucci, Boitani, Raganella Pelliccioni, Rocco & Guj 1996), acknowledgement of their interpretational limits (Frenzel 1974, Kelly 1991, Reynolds & Aebisher 1991) and training, dedication and performance of the observers (Spaulding, Krausman & Ballard 2000).

Complete dissection of scat and careful separation by hand of the undigested remains for identification is a laborious and time consuming procedure that competes with accuracy (Spaulding et al. 2000), sample size (*cf.* Reynolds & Aebisher 1991) and other research activities. The point-frame method, originally used by plant ecologists to systematically sample plants in the field, can alternatively be adapted to quantify diet composition from different sources of data (i.e. stomachs and scats). Using scats, the method involves systematic sampling of the undigested remains of faecal samples, thereby allowing a reduction in time and effort if compared to hand separation. Point-frame sampling was originally adapted to quantify rumen contents (Chamrad & Box 1964), and more recently it has been applied to estimate bear *Ursus arctos* diets from stomach contents (Sato, Mano & Takatsuki 2000). The point-frame method has also been used to assess coyote *Canis latrans* diets from scat (Meinzer, Ueckert & Flinders 1975, Johnson & Hansen 1977). Based on only eight coyote scats, Johnson & Hansen (1977) compared point-frame sampling with hand separation, and they reported comparable dry weight estimates of undigested remains, whereas the former method allowed an eight-fold reduction in time spent per faecal sample.

To our knowledge, point-frame sampling has never been applied to wolf *Canis lupus* scat analyses, nor has it been evaluated in terms of accuracy and processing time. Based on a large sample of wolf scats (N = 200), we compared point-frame and hand separation as alternative procedures to sample and quantify undigested remains in scats. In doing so, we compare results according to the most frequently used diet quantification methods and provide relative measures of laboratory time required by the two procedures.

Methods

Wolf scats (N = 1162) were collected all year round from June 1999 to June 2001 in the Pollino National Park (39°57'N, 16°01'E), Italy, along trails and travel routes used by wolves in five different pack territories. Afterwards, the faecal samples were autoclaved and processed according to laboratory procedures (Reynold & Aebisher 1991); the details of these are provided elsewhere (Ciucci et al. 1996). To evaluate the reliability of the point-frame method (Chamrad & Box 1964), we applied both point-frame and hand separation methods to a random subsample of 200 scats. For both methods, each faecal sample was soaked in water for 24-48 hours, completely fragmented and then thoroughly filtrated (0.7-0.5 mesh size) in running water several times. Then, the hand separation method involved: a) complete dissection of the scat and individual separation by hand of washed undigested remains (macro-components) according to major categories (e.g. hair, bones, nails, soft tissues, feathers, seeds and other plant material, chitinous parts of invertebrates and non-food items) and putative systematic groups based on macroscopic characters (mostly colour, length and texture of hairs); b) oven drying macro-components for storage prior to identification; c) sampling of a quite large number of hairs for microscopic identification within each group of macro-components, and d) with the aid of a reference grid, visual estimation of the volumetric proportions of the identified macro-components within the faecal sample. Following the description of Chamrad & Box (1964), the point-frame method did not require hand separation of the undigested remains. Instead, because the method assumes a random distribution of each item in the sampling population, macro-components were thoroughly mixed and were evenly spread in a high-sided, enamelled lab tray. At the top of the tray a sliding frame holding 10 pins equally spaced at a 45° inclination allowed for a systematic sampling of the undigested remains of each faecal sample. We used 50 pin drops per faecal sample (10 drops x 5 fixed positions). Individual macro-components thus selected were then microscopically identified, and both frequency and volumetric data were taken directly from the hits recorded (Chamrad & Box 1964, Johnson & Hansen 1977). To eliminate inter-observer bias, we used a single, trained observer (ET), whose reliability in microscopic identification of hairs had been tested prior to the analysis with a blind test based on 120 hairs of local mammal species (Ciucci et al. 1996).

Results by the hand separation and point-frame methods were then compared in terms of qualitative description of the diet, descriptive statistics of the overall sample (i.e. type and number of occurrences) and quantitative estimates of diet composition. Quantitative estimates were based on most commonly used scat analysis methods for wolf, namely frequency of occurrence (i.e. number of occurrences of a given item/total number of occurrences), cumulative volumetric proportions and average volume proportions when present. The overall diet was also compared in terms of number of equivalents (*sensu* Floyd, Mech & Jordan 1978) on which biomass prediction models are generally applied (Floyd et al. 1978, Weaver 1993). Non-food items were excluded from the quantitative assessment of the

Table 1. Item occurrences detected by hand separation and point-frame methods based on a subsample of 200 wolf scats collected in Pollino National Park, Italy, during June 1999 - June 2001. The items per scat are given as means ± SD, and the means did not differ between the hand separation and point-frame methods (t-test: $0.29 \leq P \leq 0.55$). Non-food items included Graminae, leaves, dirt, dung beetles, twigs and bark.

	Total occurrences		Items per scat	
Category	Hand separation	Point-frame	Hand separation	Point-frame
Food items	219	227	1.09 ± 0.38	1.13 ± 0.37
Non-food items	262	243	1.31 ± 0.76	1.21 ± 0.85
All items	481	470	2.40 ± 0.88	2.35 ± 0.97

diet. When a faecal sample contained more than one mammal species, unclassified fragments of bone and soft tissue were assigned to the species with the highest share based on hair identification.

We compared estimates of the overall diet (i.e. distributions of food items by proportions) by hand separation and point-frame methods using the Kolmogorov-Smirnov two-sample test, and tested the concordance between the relative rankings by non-parametric correlation (r_s). We further assessed the reliability of the point-frame method by testing the hypothesis that the slope of the regression coefficient of point-frame vs hand separation estimates did not differ from 1, as expected, if the two methods produced equal results (Sato et al. 2000). The Mann-Whitney U-test was used to compare mean volumes of each food item as estimated by the hand separation and point-frame methods.

We also compared the time required by hand separation and point-frame methods, measuring to the nearest minute the time needed for each laboratory phase which the two methods do not have in common. Estimates of the overall time needed to entirely process a faecal sample were obtained including washing and filtration time which both procedures have in common. Time needed for autoclaving, soaking and oven-drying of the samples was not included, as these steps can be considered complementary to other lab phases.

Results

The qualitative description of the diet was not affected by the sampling method, as most of the 200 scat-sample components revealed by hand separation (N = 17) were also detected by the point-frame method (N = 16). A single non-food component (dung beetles), detected by hand separation in a single faecal sample in trace amounts (i.e. 3% by volume), was not revealed by the point-frame sampling. In terms of food items, qualitative description of the diet was the same according to the two methods, as both detected the same 10 dietary components (nine mammal and one fruit species). Also in terms of total occurrences (including food and non-food items), hand separation and point-frame methods produced comparable results (Table 1). Considering the different categories revealed in the diet, the average number of items per scat did not differ between the two methods (t-test: $-1.06 \leq t_{398} \leq 1.17$, $0.24 \leq P \leq 0.55$; see Table 1).

The quantitative assessment of the diet also showed close agreement between the hand separation and point-frame methods according to frequency and volumetric data (including the number of equivalents; Table 2), both in terms of relative importance of the food items (Kolmogorov-Smirnov two-sample tests: $0.09 \leq D_{11} \leq 0.18$, $P > 0.1$) and concordance between final rankings

Table 2. Comparison of wolf diet estimates obtained by hand separation and point-frame methods according to four different quantification methods, based on a subsample of 200 wolf scats collected in Pollino National Park, Italy, during June 1999 - June 2001. The food items are ranked in accordance to frequency data obtained by hand separation, and the mean % volume expresses mean ± SD, when the item is present (i.e. N ≥ 4 occurrences). The means did not differ between the hand separation and point-frame methods (Mann-Whitney test: $0.31 \leq P \leq 0.87$)

	Frequency (%)		Cumulative volume (%)		No. equivalents (%)		Mean % volume	
Food item	Hand separation	Point-frame	Hand separation	Point-frame	Hand separation	Point-frame	Hand separation	Point-frame
Wild boar	58.7	58.6	60.42	60.46	116.89	117.50	92 ± 22.8	88.3 ± 28.5
Cattle	21.6	20.7	23.10	23.39	41.79	43.85	89.8 ± 24.4	91.5 ± 21.7
Goat	5.6	5.3	7.33	7.60	12.98	12.78	92.8 ± 18.3	98.8 ± 3.9
Sheep	5.6	5.3	2.70	2.17	7.92	7.28	70.8 ± 31.0	61.9 ± 35.2
Horse	2.8	3.1	3.39	3.36	5.51	5.97	89.8 ± 23.6	95.5 ± 9.8
Roe deer	1.9	1.8	0.38	0.30	3.33	3.18	83.3 ± 33.3	79.5 ± 32.5
Hare	0.9	1.3	1.31	1.19	1.92	2.42	-	-
Wolf	0.9	1.8	0.57	0.83	2.00	2.42	-	-
Undef. mammals	0.9	1.3	0.07	0.10	2.00	3.00	-	-
Small mammals	0.5	0.4	0.01	0.01	0.03	0.03	-	-
Fruit	0.5	0.4	0.71	0.59	-	-	-	-

($0.998 \leq r_s \leq 0.999$, $P < 0.0001$). Only a single, minor item (hares; < 1% in frequency) was assigned an inferior rank by the point-frame method both in frequency and cumulative volume data, all other ranks being equal (see Table 2). As from regression analyses based on frequency, cumulative volume and number of equivalents, slopes of point-frame vs hand separation estimates did not differ from the expected value of 1 for total agreement ($0.997 \leq$ slopes ≤ 1.006, $-1.71 \leq t_{10} \leq 0.89$, $0.48 \leq P \leq 0.88$). Also in terms of mean volumes (%) of each category in the faecal samples when present, the hand separation and the point-frame methods yielded comparable estimates (Mann-Whitney U-test: $4.5 \leq U \leq 989$, $0.31 \leq P \leq 0.87$).

As from frequency data, age ratios of domestic and wild ungulates as estimated from the hair detected in faecal samples, did not differ between hand separation and point-frame methods ($0.07 \leq \chi^2 \leq 0.75$, $13 \leq N \leq 120$, $0.39 \leq P \leq 0.87$; Fig. 1). However, the point-frame method detected a higher proportion (13%; N = 115) of undetermined (with respect to age) wild boar hair than by hand separation (4%; N = 120).

Excluding washing and filtration, the overall time spent on processing a faecal sample by hand separation averaged (± DS) 70 ± 48 minutes (N = 168), including 58 ± 44 minutes (N = 170) for actual separation, and an additional 11 ± 14 minutes (N = 197) for microscopic identification. Contrasting this, the time needed per faecal sample by the point-frame method (50-pins) averaged (± DS) 6 ± 5 minutes (N = 200), i.e. 9% of the time required by hand separation. These estimates, by adding 10 minutes for washing and filtering each faecal sample for both methods, predict that to process our entire sample of 1,162 wolf scats, a trained technician would need about 8.5 months by hand separation and about 52 days using the point-frame method.

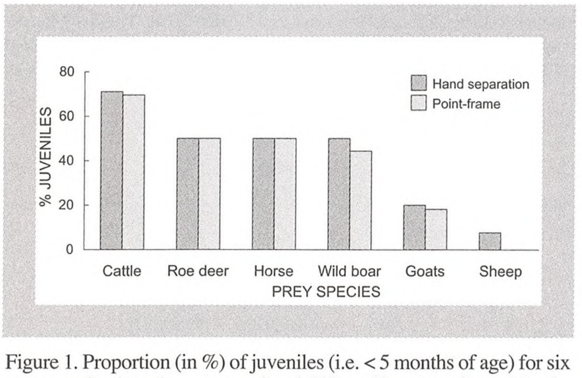

Figure 1. Proportion (in %) of juveniles (i.e. < 5 months of age) for six prey species based on a sample of 200 wolf scats collected in the Pollino National Park, Italy, during June 1999 - June 2001 according to hand separation and the point-frame methods.

Discussion

In line with previous findings (Chamrad & Box 1964, Johnson & Hansen 1977, Sato et al. 2000), our results confirm that point-frame is a reliable and efficient method for wolf diet studies based on scat analysis, and that its estimates are in close agreement with those obtained in hand separation. The minor differences we detected between the two methods (especially in the number of non-food item occurrences), and which could be expected on the basis of the sampling procedure and the structure of the sampled material (*cf.* Chamrad & Box 1964, Sato et al. 2000), reveal that these potential sources of bias play a negligible role with respect to wolf scat content. We therefore conclude that the point-frame method is a reliable alternative to hand separation of wolf scat, and allows for a significant reduction in time and effort to process faecal samples. Although our results are based on a relatively simple diet (mostly wild boar and cattle) the reduction in time we report by point-frame largely allows for wide margins of efficiency also under different conditions.

As some methodological inconsistencies in lab processing of wolf scat samples are still reported (e.g. inter-observer bias; Spaulding et al. 2000), they underline that some subjectivity is still inherent in standardised procedures (see also Sato et al. 2000). One basic assumption of the hand separation method is that all items in a single faecal sample are found and identified, and this is generally accomplished by complete dissection and thorough separation of undigested remains (Reynolds & Aebisher 1991, Ciucci et al. 1996, Spaulding et al. 2000). However, there is uncertainty as to what different observers perceive as a complete and thorough hand separation (*cf.* Spaulding et al. 2000). In this perspective, the point-frame method provides a more objective way to tally and sample undigested remains in faecal samples and therefore represents a more robust procedure if more than one observer is involved in the analysis.

In comparing hand separation and point-frame procedures, we used 50 drops per faecal sample in point-frame sampling. However, even if we doubled the sampling intensity (i.e. 100 drops/faecal sample) this would still have caused a significant reduction in time by providing estimates more robust to sampling variability. Therefore, in areas where wolves are expected to have a more diversified diet than the one we hereby report, point-frame sampling with 100-drops/faecal sample could be advisable.

Acknowledgements - we are grateful to N. Boldrini, C. Braschi, M. Caporioni, F. Crispino, S. d'Alessio, I. Gatto and G. Gervasio for their field assistance. Funding was provided by the Ente Parco Nazionale del Pollino and the University of Rome 'La Sapienza'.

References

Chamrad, A.D. & Box, T.W. 1964: A point frame for sampling rumen contents. - Journal of Wildlife Management 28: 473-477.

Ciucci, P., Boitani, L., Raganella Pelliccioni, E., Rocco, M. & Guj, I. 1996: A comparison of scat-analysis methods to assess the diet of the wolf Canis lupus. - Wildlife Biology 2: 37-48.

Floyd, T.J., Mech, L.D. & Jordan, P.A. 1978: Relating wolf scat content to prey consumed. - Journal of Wildlife Management 43: 528-532.

Frenzel, L.D. 1974: Occurrence of moose in food of wolves as revealed by scat analysis: a review of North American studies. - Le Naturaliste Canadien 101: 467-479.

Johnson, M.K. & Hansen, R.M. 1977: Comparison of point frame and hand separation of coyote scats. - Journal of Wildlife Management 41: 319-320.

Kelly, B.T. 1991: Carnivore scat analysis: an evaluation of existing techniques and the development of predictive models of prey consumed. - M.Sc. thesis, University of Idaho, Moscow, 200 pp.

Meinzer, W.P., Ueckert, D.N. & Flinders, J.T. 1975: Food niche of coyotes in the rolling plains of Texas. - Journal of Range Management 28: 22-27.

Peterson, R.O. & Ciucci, P. 2003: The wolf as a carnivore. - In: Mech, L.D. & Boitani, L. (Eds.); Wolves: behaviour, ecology and conservation. University of Chicago Press, pp. 104-130.

Reynolds, J.C. & Aebisher, N.J. 1991: Comparison and quantification of carnivore diet by faecal analysis: a critique, with recommendations, based on a study of the fox Vulpes vulpes. - Mammal Review 21: 97-122.

Sato, Y., Mano, T. & Takatsuki, S. 2000: Applicability of the point-frame method for quantitative evaluation of bear diet. - Wildlife Society Bulletin 28: 311-316.

Spaulding, R., Krausman, P.R. & Ballard, W.B. 2000: Observer bias and analysis of gray wolf diets from scats. - Wildlife Society Bulletin 28: 947-950.

Weaver, J.L. 1993: Refining the equation for interpreting prey occurrences in gray wolf scats. - Journal of Wildlife Management 57: 534-538.