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Statistical precision of diet diversity from scat and pellet analysis

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Abstract

Knowledge of trophic interactions is of vital importance for understanding ecological community dynamics. While techniques such as direct observation of prey consumption and stomach content analysis are suitable for some species; for wide ranging carnivores, especially those of conservation concern, analysis of matter in faecal scats or regurgitated pellets is still common practice. This study investigates sample sizes needed to predict changes in the diversity of the diet of three carnivore species (grey seals, Mexican wolves and long horned owls). Using a bootstrapping process, estimations of precision of diet diversity (i.e. the number and evenness of prey species, as measured using Simpson's index) were made with increasing numbers of scats sampled. Precision of diversity of diet was much greater for grey seals than owls or wolves, largely because the number of prey items in a scat was much higher. The results show that changes in seal diet diversity between different areas of the North Sea could be elucidated with analysis of as few as three scats from each region. However, demonstrating differences in diet diversity between two closely related owl species would not be possible even if the contents of >> 500 pellets were analysed. The results provide guidelines for scat or pellet sample size for future studies, as well as indicating that in some cases – for example in grey seals – scat samples may be an efficient method of sampling for changes diet, and hence prey availability as caused by anthropogenic pressures such as climate change and fishing.

Key words: Mexican wolf, gray seal, long horned owl, bootstrap, confidence interval, power analysis

1. Introduction

Effective conservation or environmental management requires knowledge of ecosystem interactions, and these interactions are often elucidated by the study of the diet of a species (e.g. Stokes, 1992). Such trophic interactions are especially important for many of the flagship conservation species, such as carnivorous mammals, predatory fish and birds of prey, which frequently occupy the upper trophic levels of food chains (Norrdahl and Korpimaki, 1995; Reid et al., 2005; Smith, 2005).

While many methods to determine diet are possible, some, such as stable isotope analysis, are relatively costly and give only indicative results of trophic level interactions (e.g. Jennings et al., 2002). For example, marine organisms have higher carbon and nitrogen isotopic signatures compared with terrestrial foods, so researchers were able to differentiate between salmon and deer consumption by wolves by analysing stable isotopes in their hair (Darimontet al., 2008). Other methods, such as stomach contents analysis, are common for fish species (Hyslop, 2006) but are generally not used for most birds and mammals unless the individuals are found dead (e.g. Beatson, 2007), while direct observation is usually prohibited by time or logistical constraints. Scat or pellet analysis is a particularly useful inexpensive and non-invasive method of studying the feeding ecology of elusive carnivores (Ciucciet al., 1996; Maruccoet al., 2008). Hair, feathers, bones (or bone fragments) and other remains may pass through the digestive system relatively unaltered (Kelly and Garton, 1997), or may be regurgitated by birds such as owls (Yom-Tov and Wool, 1997) and corvids (LaudetandSelva, 2005).

While studies have examined the procedure for accurate collection of scats, for example, to determine how best to sample to avoid pseudo-replication caused by the collection of multiple scats from the same animal deposited consecutively (Maruccoet al., 2008), no studies appear to have accurately examined the number of scats or pellets that need to be sampled to obtain an accurate estimation of diet, nor how many scats or pellets may need to be sampled to detect significant differences in diet between populations.

Given the time-consuming nature of scat analysis, limiting the number of samples required to test for changes in diet or accurately test hypotheses should be a key consideration of diet studies, essentially analogous to power analysis in most survey or experimental designs. In this study, we investigate the accuracy of determining diet diversity from wolf scats, seal scats and owl pellets. Furthermore, we estimate how many scats would need to be collected to determine differences in diets between populations.

2. Methods

Data were collected from previous research on wolf scats, seal scats and owl pellets (Table 1). From these data, the mean number of prey items per scat was calculated (Table 1). Using an R script (R Core Development Team, 2011), prey items were sampled at random (with replacement after sampling), with every prey item having a probability of being chosen equal to the proportional occurrence of that prey item in the diet. The number of items per scat/pellet was determined randomly, to the nearest whole positive number, from a normal distribution with the mean value equating to that calculated in Table 1. Samples were taken to obtain data representative of analysing between 2 to 500 scats or pellets. The value of Simpson's Index of diversity (S.I.) was calculated for each number of scats using the equation given in Simpson (1949):

$$S.I. = \sum[(n^2 - n) / (N^2 - N)]$$

where n is the number of a given prey species in a sample and N is the total number of all prey species consumed over all samples. S.I. was used since it is sample size independent, and will not increase with the number of scats sampled, as would most other diversity indices

(Rosenzweig,1995). This means that only the precision of the diversity should alter, rather than there being any systematic sample-size bias (note that the value of S.I. decreases with increasing diversity, with 0 being the most diverse and 1 being the least). This process of sampling from 2 to 500 scats was bootstrapped 10,000 times and mean value of S.I. was calculated for each scat number. 95% confidence intervals were calculated using the simple process of removing the highest and lowest 2.5 % of values (Crawley, 2005).

3. Results

For all of the test cases considered, the mean value of Simpson's Index (S.I.) was constant regardless of the number of scats sampled, indicating that it was truly sample size independent. However, there was considerable variability around the mean, as indicated by the 95% confidence limits (Figure 1). The variability decreased for all of the cases considered as the number of scats increased, but decreased considerably faster for those animals that had more prey items per scat – for example, the confidence limits of seal diet diversity (with a mean of 36.2 prey items per scat) decreased much more rapidly than those of the wolf diet diversity (with a mean of 1.04 prey items per scat - Figure 1 a and b).

Using a general indication of ecological statistical precision, that the standard error of the mean (S.E.) is < 5 % of the mean value (e.g. Southwood, 1978), these results suggest that the number of seal scats required is 12 scats. For owl diet analysis, a total of 200 pellets need to be sampled; and for wolf diet, > 500 scat samples would be needed for the S.E. to be < 5% of the mean.

To detect differences in seal diets between different sites in the North Sea does not require excessive sampling of scats. To illustrate this, the diet of grey seals at different sites, as well as the calculation of S.I. for each site is given in Table 2. Assuming the same confidence intervals will apply to different populations of seals (essentially an assumption of any parametric and most non-parametric statistical tests – Underwood, 1996) then it can be seen that differences between Donna Nook and Shetland can be obtained with just three representative scat samples (confidence limits do not overlap – Figure 2a). However, eight scats would be required from each site to identify differences in diet diversity between Orkney and Shetland (Figure 2 b). In reality, recommended sample sizes would need to be bigger than this to ensure random and representative sampling, but, once this was achieved, the analysis would be suitably powerful to detect meaningful differences.

Data from Reed (2004) on the temporal change in diet of Mexican Wolves (Table 3) shows that S.I. varies from year to year. The largest difference in diversity in diet occurs between 1999 and 2000, a difference in Simpson's Index of 0.249. To determine significant differences in the temporal change in diet diversity a total of 19 scats would need to be representatively sampled (n = 19, upper confidence interval = 0.110, lower confidence interval = 0.136, combined confidence interval = 0.246, which is lower than difference between S.I. values). However, to detect the difference between diet diversity between 1998 and 2001, a total of 195 representatively sampled scats would be required (difference in S.I. = 0.0730, at n = 195, upper confidence interval = 0.0351, lower confidence interval = 0.0378, combined confidence interval = 0.0729). However, differences in diet diversity between barn owls and great horned owls (from Maser and Brodie, 1966 – Table 4), indicate a difference in S.I. of only 0.02. This means that in order to detect difference in diet diversity >> 500 representatively sampled pellets would be needed.

4. Discussion

This is seemingly the first study to provide empirical evidence on the number of scats or pellets that need to be sampled to provide meaningful ecological results. It is clear that the precision of the diet diversity estimate increases, as expected, with the number of scats sampled, but also crucially that it increases with the number of prey items contained in each scat. Opportunistic predators that feed on other large animals (such as wolves in this study), therefore, require far more scat samples to accurately determine their diet. However, although many studies on wolf diet do not contain > 500 scat samples per 'treatment' group, as calculated here, the number of scats needed to determine large differences or changes in diet can be far lower. Equally, this study set to measure diet diversity, which should be a good measure of overall species consumed (i.e. identification of all prey species in scat or pellet samples once a suitable level of precision of diversity has been obtained should be representative of typical species consumed). However, by using a sample size independent index, such as Simpson's index, the rarest species may not be found, even if a precise and accurate estimate of S.I. is made (Rosenzweig, 1995; Attrill et al., 2001), and more samples may be required to detect these in scats. Equally, to detect only the most common prey species fewer scats would need to be sampled, lowering the effort needed.

It is important to note that this study makes use of a major assumption of many parametric and non-parametric statistical tests - that variance between treatment groups is constant (reviewed by Underwood, 1996). In reality, this may not be the case, and the confidence intervals (related to variance and number of scats sampled) may vary between different treatment groups (for example, the confidence intervals for diet of Donna Nook seals and Shetland seals may not be the same). However, given the nature of this assumption in many statistical tests, it is not unrealistic to apply it here. Indeed, most power calculations used to estimate sample size for surveys and experiments require a 'best guess' approach to variance across all treatments. Since (even small) differences in variance are likely to occur between groups, it is best to use any figures on minimum number of scats conservatively, and to take more than the recommended number if time, money and logistics allow further sampling.

Nevertheless, the current approach does give an indication of the number of scats/pellets required to accurately determine diet diversity. Furthermore, the relatively low number of seal scats needed to determine differences in diet diversity may allow this to be a monitoring tool to detect changes in diet, likely to represent differences in prey availability which could be used to track changes in fish populations caused by factors such as climate change or fishing.

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Table 1. Sources and summary statistics of the data used in this study.

Species	Source	Number of scats / pellets	Number of prey items	Prey items per scat
Grey Seal (*Halichoerus grypus*)	Hammond and Grellier (2005)	356	12,900*	36.2
Mexican Wolf (*Canis lupus baileyi*)	Reed (2004)	251	265	1.06
Great Horned Owl (*Bubo virginianus*)	Maser and Brodie (1966)	621	1,931	3.11

* Data for seals is based on otoliths with two per fish: here, the number of otoliths is divided by two to determine the number of prey items

Table 2. Food items found in grey seal scats in the North Sea, UK. Modified from Hammond and Grellier (2005).

Species	Donna Nook	East Coast	Orkney	Shetland	Total
Cod (*Gadus morhua*)	153	218	398	49	818
Whiting (*Merlangius merlangus*)	1,432	529	378	59	2,398
Haddock (*Melanogrammus aeglefinus)*	43	479	577	16	1,115
Saithe (*Pollachius virens*)	0	3	119	48	170
Norway pout (*Trisopterus esmarkii*)	0	76	646	36	758
Sandeel (*Ammodytes tobianus*)	4,459	23,049	40,989	23,743	92,240
Sole (*Solea solea*)	289	0	1	0	290
Plaice *(Pleuronectes platessa)*	246	196	204	2	648
Herring *(Clupea harengus)*	75	10	20	24	129
Sprat (*Sprattus* spp.)	0	6	1	0	7
Dragonet (*Callionymus lyra*)	1,417	69	101	3	1,590
Garfish (*Belone belone*)	0	2	32	68	102
Short-spined seascorpion (*Myoxocephalus scorpius*)	1,032	126	273	25	1,456
Long-spined seascorpion (*Taurulus bubalis*)	403	1	157	4	565
Simpson's Index	0.278	0.867	0.872	0.972	0.815

Table 3. Food items found in Mexican grey wolf scats. Comparison values are expressed as percent frequency of occurrence. Modified from Reed (2004).

	1998		1999		2000		2001		Total	
Food items	No.	%	No.	%	No.	%	No.	%	No.	%
Elk (*Cervus elaphus canadensis*)										
Adult	55	36.9	5	25.0	21	38.2	16	39.0	97	36.6
Calf	54	36.2	7	35.0	23	41.8	12	29.3	96	36.2
Deer (*Odocoileus virginianus* and *O. hemionus*)										
Adult	5	3.4	1	5.0	1	1.8	-	-	7	2.6
Fawn	-	-	2	10.0	2	3.6	2	4.9	6	2.3
Unknown native ungulate	21	14.1	-	-	4	7.3	4	9.8	29	10.9
Domestic cattle (*Bos taurus*)	-	-	4	20.0	2	3.6	5	12.2	11	4.2
Porcupine (*Erethizon dorsatum*)	1	0.7	-	-	-	-	-	-	1	0.4
Nuttalls's cottontail (*Sylvilagus nuttallii*)	-	-	-	-	-	-	1	2.4	1	0.4
Red squirrel (*Tamiasciurus hudsonicus*)	2	1.3	1	5.0	-	-	-	-	3	1.1
Golden-mantled ground squirrel (*Spermophilus	3	2.0	-	-	1	1.8	-	-	4	1.5

lateralis)										
Mouse (*Peromyscus* spp.)	3	2.0	-	-	-	-	-	-	3	1.1
Unknown rodent	1	0.7	-	-	1	1.8	-	-	2	0.8
Aves	1	0.7	-	-	-	-	-	-	1	0.4
Insecta	1	0.7	-	-	-	-	1	2.4	2	0.8
Planta	2	1.3	-	-	-	-	-	-	2	0.8
Total number of food items	149		20		55		41		265	
Total number of scats	139		19		52		41		251	
Number of food items per scat	1.07		1.05		1.06		1.00		1.06	

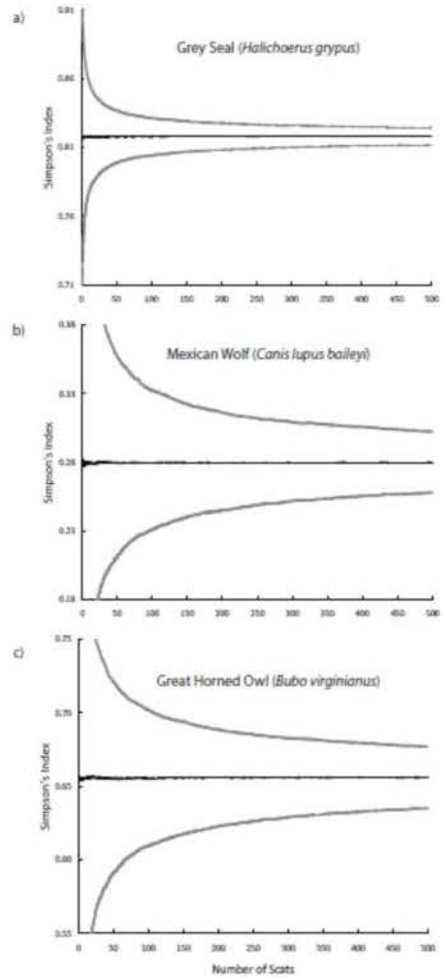
Table 4. Food items found in great-horned owl pellets, Oregon, USA. Modified from Maser and Brodie (1966).

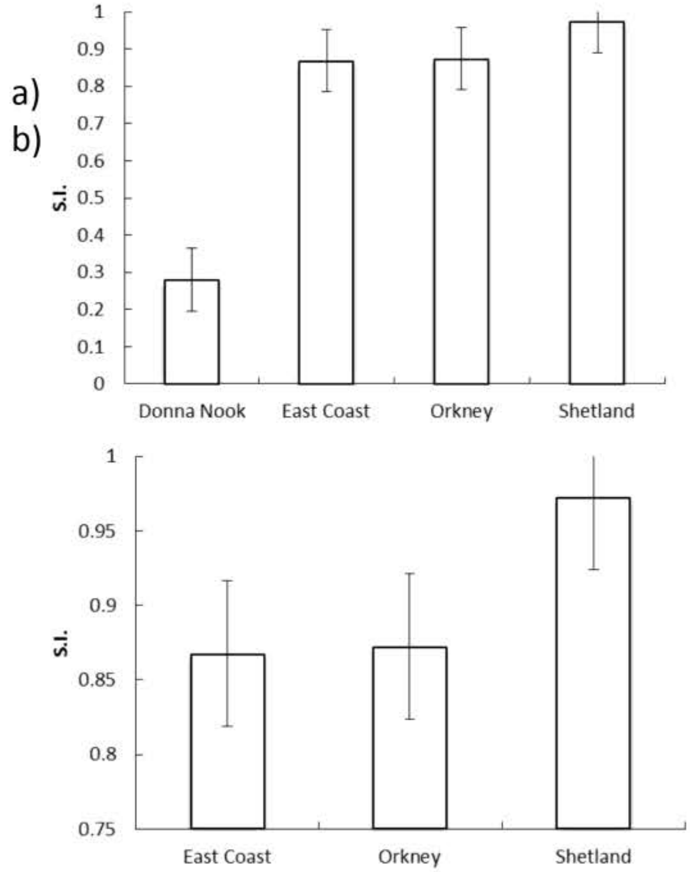
Prey animals	No. in pellets	No. In loose remains	Total of each	Number per pellet	% of diet
Vole (*Microtus* spp.)	483	1035	1518	2.444	78.61
Shrew (*Sorex* spp.)	103	93	196	0.316	10.15
Deer mouse (*Peromyscus maniculatus*)	59	50	109	0.175	5.64
Shrew mole (*Neurotrichus gibbsii*)	30	15	45	0.072	2.33
Aves	3	32	25	0.056	1.81
House mouse (*Mus musculus*)	4	5	9	0.014	0.47
Brown rat (*Rattus norvegicus*)	1	5	6	0.010	0.31
Camas pocket gopher (*Thomomys bulbivorus*)	1	3	4	0.006	0.21
Jumping mouse (*Zapus trinotatus*)	2	2	4	0.006	0.21
Townsend mole (*Scapanus townsendii*)	0	1	1	0.002	0.05
Dusky-footed woodrat (*Neotoma fuscipes*)	0	1	1	0.002	0.05
Townsend chipmunk (*Eutamias townsendii*)	0	1	1	0.002	0.05
Northern flying squirrel (*Glaucomys sabrinus*)	1	0	1	0.002	0.05
Western skink (*Eumeces*	1	0	1	0.002	0.05

skiltonianus)					
Total	688	1243	1931	3.109	99.99

Figure 1. Mean (black line) and 95% CI (grey lines) of Simpson's index of diversity from samples sizes of 1 to 500 scats or pellets. Note, scales on all figures are identical in magnitude, but positions on scale vary between figures. S.I. decreases with increasing diversity.

Figure 2. Simpson's Index (± 95% C.I.) of diversity applied to seal scat samples from Donna Nook (Lincolnshire, UK) and three sites in Scotland. (a) Confidence intervals calculated where scat number is 3. (b) Confidence intervals calculated where scat number is 8,note change in scale and that Donna Nook is therefore excluded.


a)
Grey Seal (*Halichoerus grypus*)
Simpson's Index
b)
Mexican Wolf (*Canis lupus baileyi*)
Simpson's Index
c)
Great Horned Owl (*Bubo virginianus*)
Simpson's Index
Number of Scats



a)
b)
S.I.
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Donna Nook
East Coast
Orkney
Shetland
S.I.
1
0.95
0.9
0.85
0.8
0.75
East Coast
Orkney
Shetland