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DIETARY ANALYSIS FROM FECAL SAMPLES: HOW MANY SCATS ARE ENOUGH?

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Diets of mammals are increasingly being inferred from identification of hard parts from prey eaten and recovered in fecal remains (scats). Frequencies with which particular prey species occur among collections of scats are easily compiled to describe the average diet, and can be used to compare diets between and within geographic regions, and across years and seasons. Important to these analyses is the question of statistical power. In other words, how many scats should be collected to compare the diet among and between species? We addressed this problem by using Monte Carlo simulations and frequency of occurrence methods to analytically determine the consequence of sample size on the dietary analysis of scats. We considered 2 questions. First, how is the statistical power affected by sample size? Second, what is the likelihood of not identifying a prey species? We randomly sampled predetermined numbers of scats ($n = 10$–200) from computer-generated populations of scats containing prey of known species and frequencies of occurrences. We also randomly sampled a large database of field-collected scats from Steller sea lions (*Eumetopias jubatus*). We then used standard contingency table tests such as chi-square and Fisher's exact test to determine whether differences between our samples and populations were statistically significant. We found that a minimum size of 59 scats is necessary to identify principal prey remains occurring in >5% of scats. However, 94 samples are required when comparing diets to distinguish moderate effect sizes over time or between areas. These findings have significant implications for the interpretation of published dietary data, as well as for the design of future scat-based dietary studies for pinnipeds and other species.

Key words: diet, fecal samples, frequencies of occurrence, pinnipeds, sample size, simulations

Historically, dietary studies of a number of species relied on identifying the stomach contents of individuals that had been shot (e.g., Murie and Lavigne 1986; Perez and Bigg 1986; Spalding 1964). More recently, greater emphasis has been placed on developing nondestructive methods to determine diet (Iverson et al. 2004; Korschegen 1980; Litvaitis 2000; Pierce and Boyle 1991; Putman 1984). At the forefront of these alternative techniques has been scat analysis, namely the identification and quantification of identifiable parts that have passed through the digestive systems of mammals (e.g., Arim and Naya 2003; Bowen 2000; Ciucci et al. 1996; Corbett 1989; Dellinger and Trillmich 1988; Harvey 1989; Katona and Altbacker 2002; McInnis et al. 1983; Orr and Harvey 2001; Reynolds and Aebischer 1991; Storr 1961; Tollit et al. 2004; Zabala and Zuberogoitia 2003). Scat analysis is increasingly being used to determine the diets of pinnipeds (seals and sea lions), canids (wolves, dogs, coyotes, and foxes), ursids (bears), felids (cats), viverrids (civets and genets), and mustelids (otters and badgers—e.g., Bartoszewicz and Zalewski 2003; Bull 2000; Ferreras and Macdonald 1999; Hewitt and Robbins 1996; Hutchings 2003; Krueger et al. 1999; Malo et al. 2004; Moleón and Gil-Sánchez 2003; Mukherjee et al. 2004; Nùñez et al. 2000; Pardini 1998; Patterson et al. 1998; Silva and Talamoni 2003; Virgós et al. 1999).

The remains of most species that are consumed can be identified by using reference collections of potential food items. For example, fish can be identified from uniquely shaped structures such as ear bones (otoliths) and jaw bones (dentaries), and small mammals can be identified from cranial structures and other bones that survived the digestive process. Similarly, insects can be identified from exoskeletons, and consumed plants can be identified macroscopically from seeds and fruits, or from cellular characteristics of plant fragments. Thus, scats can provide a snapshot of the types of prey that were consumed by an individual animal, and have an advantage over stomach contents because of the relative ease

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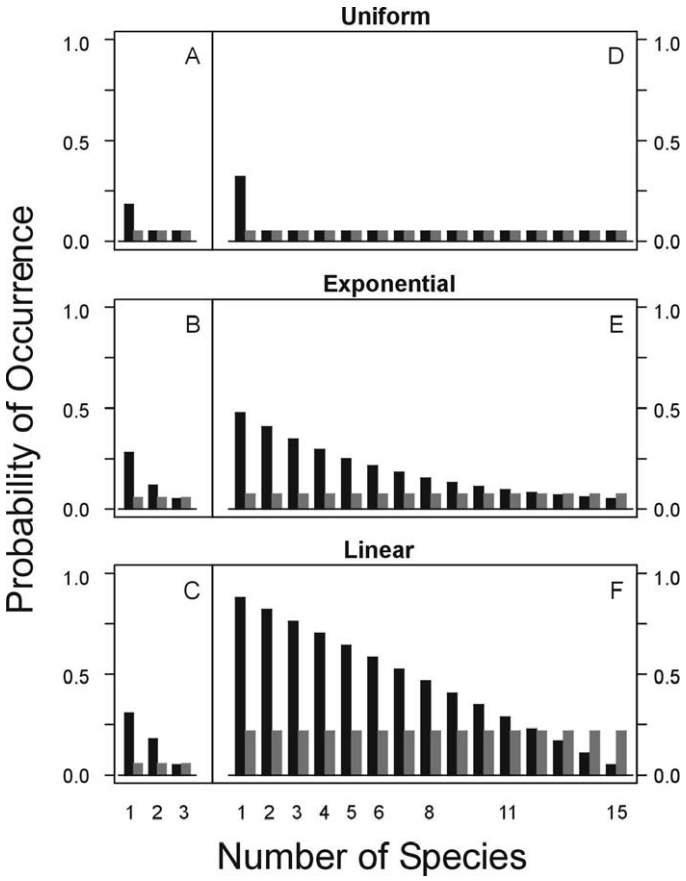

FIG. 1.—Six of 21 combinations of population comparisons made between declining (black bars) and uniform (gray bars) distributions of prey. Each panel contains 2 populations of scats that differ by an effect size of 0.30 (see ''Statistical considerations''). Panels A–C show populations containing 3 prey species, whereas panels D–F show populations with 15 species. Our simulations compared 3 scenarios. The 1st compared a uniform population with a 2nd population that was uniform except for a single dominant prey species (A and D). The 2nd and 3rd scenarios compared a uniform population with a population of prey that declined in an exponential (B and E) or linear manner (C and F). Random samples of 10, 20, . . . , 200 scats were drawn from these populations to determine the minimum sample size needed to differentiate the populations with 80% power in a contingency table analysis. Note that effect size is the same in all 6 panels.

of obtaining samples and the nondestructive nature of the sampling procedure.

The most basic analysis of scat data starts by identifying the prey species that are present in a single scat. The frequency of occurrence for any given prey species in a sample of scats is in turn calculated as the proportion of all scats collected that contained that particular species. The frequencies of occurrence of all prey species from 2 populations are usually organized in contingency tables and statistically compared by using chi-square or Fisher's exact tests to determine whether diets differ between locations, years, seasons, or species (e.g., Bull 2000; Malo et al. 2004; Nùñez et al. 2000; Patterson et al. 1998).

Numbers of species identified in individual scats varies by predator. For example, among pinnipeds, it is common for more than 35 different species of prey to be identified (e.g., Olesiuk et al. 1990; Sinclair and Zeppelin 2002; Tollit and Thompson 1996). However, only about 3–15 species are typically considered common (i.e., they occur in $\geq$5% of scats). The other prey species are generally thought not to form a regular part of the diet and are either consumed opportunistically or were perhaps contained within the stomach of the primary prey (i.e., secondary prey—Pierce et al. 2004). Many dietary studies tend to pool prey remains into categories such as flatfish, gadids, and squids for pinnipeds; earthworms, insects, vertebrates, and grass for badgers; or fish, passerine birds, and crustaceans for mink. In general, the large numbers of prey species consumed are often reduced to 6–12 diet groups for the purpose of simplifying descriptions and comparisons of diets (e.g., Bartoszewicz and Zalewski 2003; Ferreras and Macdonald 1999; Hewitt and Robbins 1996; Hutchings 2003; Malo et al. 2004; Merrick et al. 1997; Mukherjee et al. 2004; Nùñez et al. 2000; Patterson et al. 1998; Silva and Talamoni 2003; Virgós et al. 1999).

Descriptions of diets have relied on as few as 10 scats, to more than 1,000 scats (e.g., Bartoszewicz and Zalewski 2003; Olesiuk et al. 1990; Patterson et al. 1998; Pontier et al. 2002; Riemer and Brown 1997; Sinclair and Zeppelin 2002; Zabala and Zuberogoitia 2003). The question of how many scats are sufficient to detect differences in diet over time or between sites is often not considered, yet it has implications for the interpretation of results. At the low end of the sample-size scale, inaccurate conclusions about what mammals eat might be made if too few scats are collected, whereas at the upper end, financial resources might be wasted if too many samples are collected.

We used Monte Carlo simulations to address the question of how many scats should be collected to compare diets. Our simulations incorporated the restrictions and characteristics of pinniped scat studies, but the results are broadly applicable to other mammals with similar limitations. The simulations compared 2 populations, which can be envisioned as any number of possible combinations of dietary comparisons (e.g., interspecific, intraspecific, intersexual, intrasexual, inter–age class, intersite, and so on), and yielded results that have bearing on the interpretation of dietary data and for the design of future dietary studies for a wide range of species that rely on identifying parts recovered in fecal remains.

MATERIALS AND METHODS

Simulation methods.—Our basic approach was to randomly sample two populations of computer-generated scats that contained known numbers (n) of prey species at various probabilities or frequencies of occurrence (Fig. 1). Frequency of occurrence (f_{ij}) for prey species j in population i was defined as:

$$f_{ij} = \sum_{k=1}^{s_i} O_{ijk},$$

where s_i is the total number of scats simulated for population i, and O_{ij} is the outcome of a Bernoulli trial where the prey species is either

present ($O_{ij} = 1$) or absent ($O_{ij} = 0$). Probability of occurrence (P_{ij}) was calculated as:

$$P_{ij} = \frac{f_{ij}}{s_i}.$$

Presence of any prey species within a scat was independent of the presence of any other species. The frequency of occurrence of prey species j in the 1st population was proportional to the frequency of occurrence of prey species j consumed by the 2nd population, such that the proportionality constant was independent of population. Thus, we were interested in determining the sample size for comparing 2 populations rather than for reconstructing diet.

Statistical considerations.—Sample size depends on 3 statistical parameters, alpha (α), beta (β), and effect size. Alpha, the probability of rejecting the null hypothesis when it is true (type I error rate) is typically set at 0.05 in ecological studies (Sokal and Rohlf 1995; Zar 1996). Beta, the probability that a difference is not detected when it does exist (type II error rate) is rarely considered in ecological studies. It relates to correctly rejecting the null hypothesis and defines the statistical power of the test (i.e., power = 1 − β). A reasonable value for power in ecological studies such as scat analysis is 0.80 (Cohen 1988:56).

The 3rd parameter, effect size, is a measure of how different 2 samples are. Effect size captures information about differences between 2 populations that is independent of sample size. It is a relative measure that takes a value of zero when the null hypothesis is true and a value greater than zero when the null hypothesis is false. Effect size increases as the difference between 2 populations increases, thus serving as an index of the degree of departure from the null hypothesis (Sheppard 1999). For a contingency table analysis, which is the most commonly employed statistical technique for comparing 2 sets of diet frequencies, the maximum effect size is 1.0, and effect sizes of 0.10, 0.30, and 0.50 are considered to be small, moderate, and large, respectively (Cohen 1977).

Our simulations were designed to determine the minimum sample size required to detect a difference between 2 populations with an effect size of 0.30 and a power of 80% at an α level of 5%.

Details of simulations.—We began by randomly drawing a sample of 10 scats from each of 2 populations and calculated the observed frequency of occurrence for all n prey species by counting the number of times each species occurred in the sample of 10 scats. Next, we tested if a significant difference could be detected between the 2 samples (see section on ''Statistical considerations''). We then drew another 2 samples of 10 scats each, tested for a significant difference, and repeated this procedure 50,000 times, noting the P-value outcome of the statistical test each time.

After comparing samples of 10 scats, we increased the sample size to 2 samples of 20 scats and carried out another 50,000 comparisons. Sample sizes were then increased incrementally by 10 until we had compared 2 samples consisting of 200 scats each.

Species richness in scat samples.—The number of prey species consumed will influence the number of scats that need to be collected. Although any number of species might be consumed, most diets in the wild appear to consist of 3–15 primary prey species, with many species consumed secondarily or incidentally (e.g., they may have been in the stomachs of prey—Olesiuk et al. 1990; Pierce et al. 2004). We therefore chose to examine scenarios where at least 3 species and at most 15 species were eaten with probabilities of occurrences of at least 5%. The 5% level was chosen as a significant cutoff below which we believed prey species were either too sparse to be reliably observed or were too rare to be considered an important prey type.

Patterns of frequency of occurrence and effect size.—Given that there are many ways to obtain an effect size of 0.30 with n species per scat, we chose the most conservative scenario (i.e., the one that would yield the largest number of samples needed to significantly distinguish a 0.30 effect size with at least 80% power). We numerically created populations of scats that contained 3, 4, 5, 6, 8, 11, and 15 prey species with different probability distributions and frequencies of occurrence >5%. We selected 3 distributional forms (i.e., linear, exponential, and uniform except for 1 species) and compared them to uniform distributions. Hence, we generated 21 sets of scat populations that had effect sizes of 0.30 and yielded the smallest exact chi-square statistics for each set (see Fig. 1 for examples of 6 of the 21 computer-generated populations).

Table 1.—Probabilities of observing prey species 1, 2, . . . , n in 2 populations of scats.

	Species 1	Species 2	. . .	Species n	Population marginals
Population 1	$P_{\text{obs}_{11}}$	$P_{\text{obs}_{12}}$	. . .	$P_{\text{obs}_{1n}}$	$\sum_{j=1}^{n} P_{\text{obs}_{1j}}$
Population 2	$P_{\text{obs}_{21}}$	$P_{\text{obs}_{22}}$	. . .	$P_{\text{obs}_{2n}}$	$\sum_{j=1}^{n} P_{\text{obs}_{2j}}$
Species marginals	$\sum_{i=1}^{2} P_{\text{obs}_{i1}}$	$\sum_{i=1}^{2} P_{\text{obs}_{i2}}$	. . .	$\sum_{i=1}^{2} P_{\text{obs}_{in}}$	1.0

We calculated the effect size by translating observed counts to observed probabilities standardized such that the sum of all observed probabilities equaled 1 according to

$$P_{\text{obs}_{ij}} = \frac{P_{ij}}{\sum_{i=1}^{2}\sum_{j=1}^{n} P_{ij}}$$

(Table 1). The product of the marginal proportions (from Table 1) gives the expected cells ($\hat{P}_{\text{exp}_{ij}}$) of the null hypothesis. Effect size (w) between observed ($P_{\text{obs}_{ij}}$) and expected ($\hat{P}_{\text{exp}_{ij}}$) frequencies of occurrence was then calculated according to Cohen (1977:221) as

$$w = \sqrt{\sum_{i=1}^{2}\sum_{j=1}^{n} \frac{(\hat{P}_{\text{exp}_{ij}} - P_{\text{obs}_{ij}})^2}{\hat{P}_{\text{exp}_{ij}}}}.$$

Within the constraint of effect size equaling 0.30, we began by setting the probability of occurrence at 5% for all species except 1 (from 1 of the 2 populations). For the population that declined exponentially, numbers of prey species j, denoted by f_{ij}, for $j = 1, \ldots, n$ were calculated as

$$f_{ij} = f_{i1} e^{c(j-1)}$$

where a decay constant c determined the ''rate of decline'' or the distribution of the sorted species frequencies according to

$$c = \frac{\ln(f_{in}/f_{i1})}{n-1}.$$

The final comparison was between a linearly declining population and a uniform population.

Based on the considerations about the desired accuracy (i.e., the minimum probability of occurrence of 5%) and the numbers of species to be identified (n), we created 420 sampling regimes. This included 3 declining prey distributions (uniform except 1 species, an exponential decline, and a linear decline), 7 levels of species richness ($n = 3, 4, 5, 6, 8, 11$, and 15 species), and 20 different sample sizes ($s = 10, 20, 30, \ldots, 200$). These combinations of prey distributions and numbers of prey species ensured that we could determine the most conservative sample sizes needed to detect moderate differences in diet.

Contingency table analysis.—We used standard contingency table tests to test for significant differences, and ran Fisher's exact tests in the

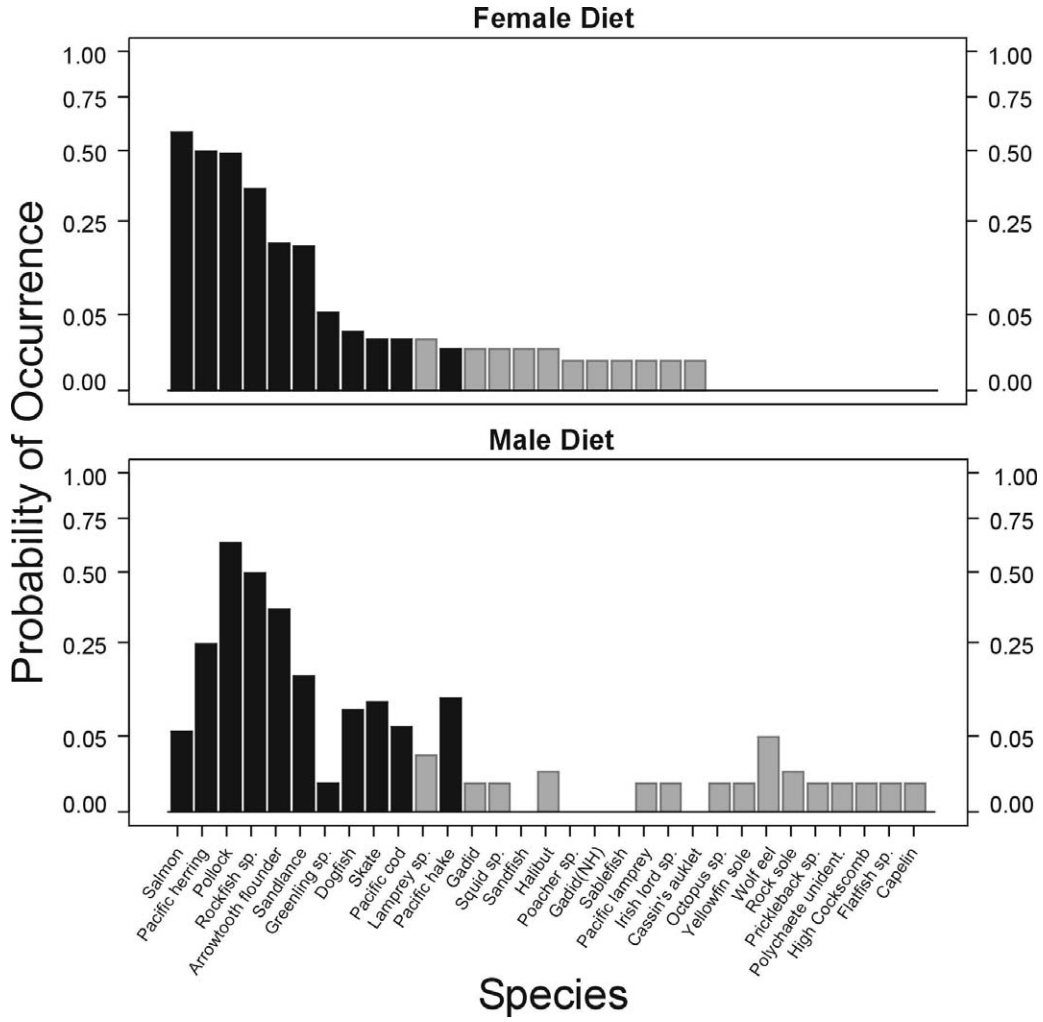

FIG. 2.—An example of pinniped dietary data showing the frequency of occurrences of 31 species or categories of prey identified in the 143 scats of female and 133 scats of male Steller sea lions collected during the summer of 1997 and 1998 in Southeast Alaska (from A. W. Trites, in litt.). Note that frequencies of occurrence when arranged from highest to lowest have an exponential declining distribution, and that only 7 species consumed by females and 10 by males occurred in $\geq$5% of the scats. Overall, 11 species of the 31 identified had frequencies of occurrences $\geq$5% (black bars).

statistical software program R (version 1.8.1, www.r-project.org) with certain constraints introduced to maximize computing power. If the sum of all contingency table cells was greater than 200, or there were $\geq$10 prey species in the diet, we used a chi-square approximation to the Fisher's exact test instead.

The number of tests out of 50,000 in which we could not reject the null hypothesis gave the type II error rate and an estimate of power implying that there was not enough power to confirm a difference, not that the difference was not there. Thus, to obtain statistical power of at least 0.80, fewer than 10,000 of our 50,000 tests had to accept the null hypothesis of no difference. If more than 10,000 tests were positive, we concluded that the power was inadequate to reliably reject the null hypothesis when differences existed between the 2 populations.

An assumption of contingency table analyses is that the species in the scats are independently consumed (Zar 1996). This assumption of independence of prey occurrence is violated in community ecology (Gotelli 2000), and is likely violated to some extent for most mammalian scats as well. However, demonstrating dependence is difficult, and we are unaware of studies that have quantified this for different mammals. We therefore chose not to incorporate dependence into our simulations given that it is not well understood and would not affect the minimum sample size estimates predicted by our simulations. Our simulation scenarios compared the most conservative prey distributions in scat, and yielded results that refer to the minimum sample size needed to always detect an effect size of at least 0.30 within the constraints we specified. They are therefore applicable whether or not there is independence among prey species.

Scat samples from the wild.—In addition to assessing the sample size needed to distinguish computer-generated populations of scats, we applied our methods to scats collected from Steller sea lions (*Eumetopias jubatus*) that use the Forrester Island complex of haulouts in southeastern Alaska (Fig. 2). Scats were collected from 3 adjacent breeding areas dominated by mature females ($s_1 = 133$ scats) and at a haulout used by males ($s_2 = 143$) during the summers of 1997 and 1998. We combined data from 2 consecutive years to attain sufficient sample sizes to assess type I and type II error rates. We randomly selected 10, 20, . . . , 130 scats from each of the 2 populations of scats to see how many wild-collected scats were required to reliably detect the difference between the males and females with the desired level of power (80%). We assessed type I error rate by selecting 2 samples of

10, 20, 30, . . . , 70 scats from the haulout used by males, and assessed whether a difference could be found. After 70 scats, we randomly assigned 70 scats to each of the 2 populations and resampled within for samples of 80, 90, . . . , 200. As before, we used the cutoff for occurrence at 5% for at least 1 haulout, below which prey species were not included in the comparative analysis. These 2 resampling procedures (for assessing type I and type II error rates) were repeated 1,000 times for each sample of size 10, 20, . . . , 200 scats.

By using these same data, we combined the species into 8 categories of prey (e.g., gadids, flatfish, and so on) similar to the method used in Merrick et al. (1997). We then repeated the type II error simulation, and assessed the effect on statistical significance and effect size.

Results

Data points plotted in the 3 left panels of Fig. 3 each represent 7 million simulations from 140 combinations of diet richness ($n = 7$) and sample sizes ($s_i = 10, 20, \ldots, 200$). The lines represent the probability of committing a type II error when testing whether 2 sets of scat samples were different if drawn from populations with moderate effect sizes of 0.30. The most conservative scenario (i.e., with uniform frequencies for all species except 1; Fig. 3A; Table 2) indicates that relatively large sample sizes are required to distinguish the 2 populations compared to distinguishing an exponentially declining or linearly declining frequency of occurrence from uniform frequencies (Table 2; Figs. 3B and 3C).

Type II error rates of <20% indicate the sample sizes that are required to confirm statistical differences with 80% power. Values above this line indicate the sample sizes that had too little power to reliably reject the null hypothesis when real differences existed between the populations. In general, larger sample sizes were required to detect differences between populations that consumed fewer species (e.g., $n = 3$) compared to those that had higher dietary diversity (e.g., $n =$ 15 species; Table 2; Figs. 3D–F). The most conservative scenario found that sample sizes increased from 107 scats to compare the diets of mammals that consumed an average of 15 principal prey species, to more than 200 scats if the diet consisted of just 3 species (Fig. 3A). However, this is likely too conservative a sampling scheme and unrepresentative of real scat data. Scat samples collected in the wild suggest that frequency of occurrence of prey species tends to decline exponentially (e.g., Ferreras and Macdonald 1999; Hutchings 2003; Malo et al. 2004; Moleón and Gil-Sánchez 2003; Silva and Talamoni 2003; Sinclair and Zeppelin 2002). Under this scenario, 51 scats are needed to distinguish populations consuming 15 principal prey species, and 179 scats would be needed when there are only 3 species. If the frequency of occurrence fell linearly with numbers of species consumed, the number of scats required would have been 23 for 15 species, and 168 for 3 species.

Collecting too few scats increases the likelihood of not finding a species in a scat that is consumed in low numbers. There is also the problem of dietary preferences of a single individual becoming a larger part of the sampling error. Even by restricting dietary analyses to species that occur at frequencies of ≥5%, zeros will likely occur in the observed

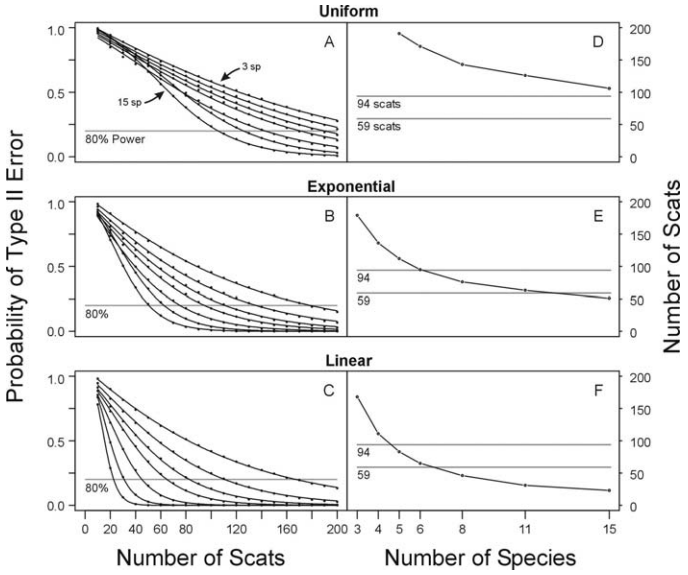

Fig. 3.—Probabilities of committing a type II error (panels A–C) when testing whether the diets from 2 sets of scat samples are statistically different ($\alpha = 0.05$, effect size = 0.30), and the minimum numbers of scats required to detect dietary differences (panels D–F) for varying combinations of species richness and frequencies of occurrences. The top curve of panels A–C represents 3 prey species, followed by 4, 5, 6, 8, 11, and 15 species (with 15 species being the bottom curve in all 3 panels). Each data point represents the mean proportion of 50,000 simulations that failed to reject the null hypothesis that diets did not differ. Numbers of samples drawn from each population ranged from 10 to 200 scats. Frequencies of occurrence of prey in the population of scats had 3 assumed distributions: uniform except for 1 species (panel A), exponential (panel B), and linear (panel C). Combinations of sample sizes and species richness that fell below 80% power had insufficient power to confirm a difference. Panels D–F show the minimum numbers of scats needed to detect dietary differences among populations by numbers of species in the diet (with an effect size of 0.30, an α level of 0.05, and $1 - \beta$ of 0.80). Curves were fit with a least-squares fitting procedure for a logistic decline (Splus 6.1, Insightful Inc., Seattle, WA). Sample sizes of 59 scats ensured that species with >5% frequency of occurrence could be identified, whereas 94 scats ensured that diets containing 6 or more species with linearly or exponentially declining frequencies of occurrence could be distinguished statistically.

frequency table for small sample sizes. Based on binomial probabilities, at least 59 scats should be collected to be 95% confident of collecting at least 1 scat containing a species with $P_{ij} = 5\%$ probability of occurrence ($P(f_{ij} \geq 1 | s_i = 59) = 0.9515$, where presence of species within scats are independent). Collecting 59 scats would suffice for comparing populations of scats containing 12 or more exponentially distributed species of prey (Fig. 3E), or for comparing those containing 7 or more that are linearly distributed (Fig. 3F). However, increasing the sample size to 94 scats (based on our simulations; Fig. 3B) ensures that diets containing 6 or more species with linearly or exponentially declining frequencies of occurrence can be statistically distinguished (Figs. 3E and 3F).

The Steller sea lion scats collected in Alaska (Fig. 2) contained 22 species of prey in the scats of females, and 26

TABLE 2.—Minimum numbers of scats required to detect differences between 2 populations containing 3–15 prey species (with an effect size of 0.30, an α level of 0.05, and $1 - \beta$ of 0.80) and assuming that frequencies of occurrence of prey decline linearly or exponentially, or are uniform for all but 1 species. Sample sizes were derived from computer simulations.

Number of prey species in diet	Minimum sample sizes associated with 3 prey distributions		
	Linear	Exponential	Uniform
3	168	179	>200
4	111	136	>200
5	83	112	191
6	65	94	171
8	46	76	143
11	31	63	126
15	23	51	106

species in the scats of males. Considering only those 11 species with occurrences >5%, the post hoc effect size was 0.42. At this level, a sample size of only 19 scats was sufficient to conclude that diets differed between males and females. Combining species into 8 prey categories and rerunning the type II error simulation resulted in similar conclusions (i.e., 18 scats were sufficient to detect the difference). However, had the effect size been smaller (i.e., 0.30 instead of 0.42), our simulations indicate that 85 scats would have been required to detect the difference between diets of males and females for an exponential decline in frequency of occurrence of prey species. Sample size did not affect type I error rates for diet of males given that fewer than 5% of the simulations (i.e., <50 of 1,000 tests for all sample sizes) rejected the null hypothesis.

DISCUSSION

Knowing how many scats should be collected to compare differences in diet requires a certain level of understanding about the data that might be obtained. Variables that need to be considered include the number of prey species consumed; the effect size, power, and alpha level; as well as information about the likely frequencies of prey occurrence and their distributional form. Existing methods to determine sample sizes (e.g., Cohen 1988; Erdfelder et al. 1996) are useful when there are no constraints limiting the distributional form, and where these variables can be quantified a priori, but they are not particularly instructive when such information is unavailable. Our simulations addressed the problem of not having a priori information by fixing certain statistical variables (i.e., alpha level, power, and effect size) and setting some realistic limits on biological variables (e.g., on occurrence frequencies, numbers of principal prey species, and so on). We took a precautionary approach to estimate the number of required scats. Thus, the number we recommend be collected is the minimum needed to ensure that a real difference between 2 populations of scats (i.e., a 0.30 effect size) is found to be statistically significant with 80% power at the 5% level. This recommendation is based on our simulation results that incorporated the described assumptions about prey occurrence. It is also premised on the commonalities shared by many published dietary studies of mammals, as well as the insights we have obtained about prey species richness from our own collections of pinniped scats.

The most extreme scenario we considered for comparing the dietary frequencies of 2 populations with a moderate effect size indicated that more than 200 scats would be needed to ensure that a difference is statistically detected with any number of prey species in the diet. However, this pattern of frequency of occurrence is unlikely to ever occur in the wild. Frequency patterns of prey species recovered from scats of a wide range of mammals suggest that exponential declines are more representative of our simulations (e.g., Ferreras and Macdonald 1999; Hutchings 2003; Malo et al. 2004; Moleón and Gil-Sánchez 2003; Silva and Talamoni 2003; Sinclair and Zeppelin 2002). Thus, in terms of species with similar dietary distributions, the most conservative exponential frequency pattern should be used to guide the choice of required sample sizes (Fig. 3E).

Assuming an exponentially declining frequency of occurrence, the number of scats that should be collected is 179 when there are only 3 prey species (or prey categories) in the diet, and 51 when 15 species or categories are present (Table 2; Fig. 3E). Although diets with only 2 principal prey species have been reported (e.g., Riemer and Brown 1997), it is more common to find at least 5 species consumed by a single population (e.g., McInnis et al. 1983; Mukherjee et al. 2004; Nùñez et al. 2000; Perez and Bigg 1986; Pontier et al. 2002; Sinclair and Zeppelin 2002). Because additional species are likely to be present in 1 of the populations being compared, we considered scenarios with 6 or more principal prey species. Our simulations showed that 94 scats were required as a conservative minimum to ensure that diets containing at least 6 prey species could be distinguished (Figs. 3B and 3E). This number is consistent with the sample size of 100 that Hammond and Rothery (1996) suggested for reconstructing seal diets, and is higher than the minimum of 70 fecal samples that Corbett (1989) suggested for assessing diets of dingoes. Number of required samples would drop should the effect size be larger, or should the frequency distribution tend to decline in a more linear fashion.

It may seem counterintuitive that larger numbers of samples are required to distinguish diets made up of only a few species compared to distinguishing diets that contain a greater diversity of prey. The expectation based on traditional contingency table power formulae is that larger sample sizes will ensure that greater numbers of categories or species are identified, and that they can presumably be distinguished from one another (e.g., Erdfelder et al. 1996). Our results show that fewer scats are required to compare diets containing a greater diversity of prey remains for a fixed effect size (e.g., 0.30) and having frequency of occurrence ≥5% and distributional assumptions of pinniped scat analysis. Under our simulation scenarios, more power was associated with greater numbers of prey species consumed because the discrepancy between populations with higher numbers of species was larger than for fewer species under our distributional assumptions. This is probably best understood by comparing the left (3 species) and right (15 species) panels of Fig. 1. The effect size in all panels is 0.30, but the difference

between the declining and uniform distributions is smaller for the 3-species examples (left panels of Fig. 1). Thus, a greater number of samples would have to be taken to ensure that this smaller difference between the 2 populations was real and not an artifact of sampling bias.

Combining species into a fixed set of meaningful species groups (e.g., Malo et al. 2004; Merrick et al. 1997; Moleón and Gil-Sánchez 2003; Silva and Talamoni 2003) could require a larger sample size to detect the same effect size. This cautionary note may be further complicated if prey species that occur in <5% of the scat can collectively represent >5% when combined with other scarce species. In our case, however, we found that combining species into 8 species groups (as we did with the sea lion data set) had a minimal consequence for effect size (from 0.42 to 0.39), and that the sample sizes required for 80% power were comparable (19 and 18 scats, respectively).

One of the considerations in dietary analysis is the financial cost associated with collecting and analyzing scats. Another is the relative ease or difficulty of obtaining sufficient sample sizes. Scats from some species, such as sea lions, may accumulate above the high water line, whereas those of other species, such as harbor seals, are more often tidally washed away each day (Bigg et al. 1990). Some species bury their feces, whereas others leave them in predictable locations. Therefore, it will not always be possible to collect 94 scats, but it may be possible to pool samples from adjacent areas to increase the statistical power of the test. In this case, the probability of detecting different prey species is assumed to remain constant such that a stratified sampling scheme would not be needed (McArdle 1990). In general, larger sample sizes reduce the amount of total variability that is attributable to sampling error. Collecting only a few scats will introduce sampling error associated with such factors as differences in scat volume (Arim and Naya 2003) and differences in dietary preferences of individual animals.

Collecting 94 scats will ensure that existing differences will be statistically detected, whereas 59 scats will ensure that at least 1 scat contains a species that has a 5% probability of occurring in a scat. However, this lower estimate assumes that any species consumed is recovered in the scat. The reality is that most prey remains are digested to some degree and only a fraction of what is ingested is retained in scat (Ciucci et al. 1996; Korschegen 1980; Litvaitis 2000; McInnis et al. 1983; Pierce and Boyle 1991; Putman 1984; Wijnsma et al. 1999). The efficiency of digestion will thus affect the ability to accurately determine frequency of occurrence (Arim and Naya 2003). However, problems associated with complete digestion of bones can be reduced by using all-structure identification techniques to determine the presence of prey species (Browne et al. 2002; Olesiuk et al. 1990). This has been demonstrated for some species through captive studies that have detected all experimentally fed prey in scats, despite varying widely in their susceptibility to digestion (e.g., Cottrell and Trites 2002; Tollit et al. 2003). However, small (<2-fold) differences in passage time and in the number of scats across which meals are distributed can influence the interpretation of diet, but are not considered to be significant sources of bias (Tollit et al. 2003). Thus, collecting about 60 scats should be a reasonable target to determine the presence of prey in the diet assuming that the prey items are independently and identically distributed.

Our simulations have ignored issues of digestion rates, size of scat, and relative prey sizes (see Arim and Naya 2003) and have operated under the assumption that constant proportions of species pass into the scat of all populations. We did not attempt to imply the sample size required to correct for such biases, but only to detect differences in frequencies at different times and locations, or between different populations or species. Controlled feeding experiments with captive individuals are needed to properly interpret what the remains recovered from scats actually represent. In the meantime, dietary analyses from scats collected in the wild simply document whether or not any given species of prey is present in a single scat and are restricted to the simple interpretation of identifiable bones and other hard parts. Further refinement of the interpretation of prey items recovered in scats through captive feeding studies (e.g., Cottrell et al. 1996; Marcus et al. 1998; McInnis et al. 1983; Staniland 2002) and the development of analytical techniques to reconstruct diets (e.g., Laake et al. 2002; Olesiuk 1993) may change our estimates of the number of scats that need to be collected.

The target number of scats to be collected will depend on the number of principal species being tracked and on the distribution of expected frequencies (i.e., uniform, exponential, or linear). The sample sizes suggested by Fig. 3 are much larger than some might have expected. This is largely because we chose the most conservative scenarios to ensure that existing differences in diet are not overlooked. A general rule of thumb should be to collect approximately 60 scats based on the binomial probability of detecting species that occur with frequencies >5%. This number should be appropriate for detecting large effect sizes, but may not be adequate for medium effects sizes of 0.30. About 100 scats (either at a single site or pooled across sites) is a more appropriate number if the primary goal is to detect and track differences across time or geographic area.

The number of scats is an important consideration in describing and comparing diets, but it is not the only one. Consideration must also be given to such things as the diversity of individuals sampled (sexes and age classes), the size of the geographic area, and the times of year when scats are collected. Characterizing diet from a larger number of scats collected from a few individuals, or from 1 sex, or from 1 site, or over only a few days may not be particularly informative and may be hopelessly biased. Thus, a thoughtfully collected smaller sample of scats may be more representative of a population's diet than a larger sample that has been haphazardly collected.

Our results apply to the comparison of fecal samples, and can be extended to other sets of data such as stomach contents that document the frequency with which different species or categories of diet types occur. They also provide a framework and general guideline for the number of scats that should be collected to ensure that proper conclusions are drawn about diet by using frequency of occurrence and whether differences exist in space or time.

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