

A RELIABLE METHOD OF ANALYZING DIETARIES OF MYCOPHAGOUS SMALL MAMMALS

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ABSTRACT—Two methods of analyzing the dietaries of mycophagous small mammals were compared. Fecal pellets were collected from 11 northern flying squirrels and 12 Townsend's chipmunks, all caught live. In 1 method, pellets from each individual were examined microscopically; in the other, samples from 3 or 4 individuals from each species were pooled and the number of slides and the fields of view used for each slide were reduced, resulting in less time and effort to collect data. Diet analysis by the two methods did not differ significantly for either mammal species.

A comprehensive understanding of interactions and interdependencies in forest ecosystems is becoming essential as goals of forest management change. Interactions among populations of animals comprising various trophic levels are some of the least understood in Pacific Northwest forests. One food-web linkage now becoming clearer is that between the northern spotted owl (*Strix occidentalis caurina*) and the northern flying squirrel (*Glaucomys sabrinus*). *Strix occidentalis caurina* feeds primarily on *G. sabrinus* over much of its range (Carey and others 1992). In the Pacific Northwest, *G. sabrinus* feed principally on fungi, primarily truffles and false-truffles (hereafter referred to as "truffles") (Maser and others 1985; Carey 1995). Most truffle species form ectomycorrhizae with conifers. The conifers provide the fungi with a source of carbon from photosynthesis. The fungi aid the tree in absorption of water and nutrients from the soil. *Glaucomys sabrinus* and other small mammals eat fungal sporocarps, but spores of the fungi pass through the gut intact and are egested to form new fungal colonies in new locations (Trappe and Maser 1976; Claridge and others 1992).

The dietaries of small mammals that feed on fungi (mycophagy) have been studied extensively and others 1978; Malajczuk and others 1987; Claridge and May 1994; Carey 1995). Stomach contents provide an accurate record of the animal's last meal, and fecal samples from the upper and lower intestine reflect

previous meals. However, a nonlethal method to assess dietaries of small mammals is necessary for long-term studies. Analysis of the components of fecal pellets allows for repeated capture of individuals in order to measure temporal variation in dietaries.

Several techniques have been used to analyze the dietaries of live-captured individuals. McIntire and Carey (1989) developed the most comprehensive techniques for analyzing fecal collections of mycophagous mammals. They used a compound microscope to identify spores of truffle species and distinguish plant and insect parts and lichen fragments. They suggested 3 microscopic mounts per individual and 50 fields of view at 400X magnification per mount to estimate the relative abundance and diversity of components in the dietary accurately. However, their technique is time-consuming and impractical for large data sets. We conducted this study to compare their method with a modified version designed to assess the dietaries of mycophagous small mammals reliably but in less time. Discussion of the implications of the differences in the dietaries of the 2 mammal species will be presented elsewhere.

METHODS

We live-trapped small mammals at Fort Lewis Military Reservation, Thurston County, Washington, as part of a large, integrated ecosystem management experiment. The

TABLE 1. Number of samples, mounts, and fields examined for each species of mammal by method 1 and method 2.

Species	Method	Samples	Mounts/ sample	Fields/ mount	Total fields
<i>Glaucomys sabrinus</i>	1	11	3	50	1650
	2	3	5	25	425
<i>Tamias townsendii</i>	1	12	3	50	1800
	2	3	5	25	425

forests were 55 to 65 yr old and composed primarily of Douglas-fir (*Pseudotsuga menziesii*). Trapping procedure was according to Carey and others (1991). Fecal samples (2 to 3 fecal pellets) from each of 11 *G. sabrinus* were collected from a single trapping grid in January 1993. Fecal pellets from 12 Townsend's chipmunks (*Tamias townsendii*) were collected from a single trapping grid in October 1992.

Pellets from *T. townsendii* were preserved for microscopic analysis with a few drops of formalin; *G. sabrinus* pellets were air-dried. Use of formalin was discontinued after trapping in fall of 1992 because of problems with toxicity and waste disposal. Air-drying preserves samples adequately, but increases risk of disease transmission (for example, Hanta virus). We therefore changed to preserving pellets with a few drops of 70% EtOH to reduce risks of disease transmission to researchers. None of the above methods changes the composition of the fecal pellets.

We compared 2 methods of diet analysis. Method 1 was similar to the procedure of McIntire and Carey (1989). Pellets were macerated in small vials with 15 drops of deionized water (approximately 4 to 5 times the volume of the pellets) and shaken thoroughly. A pair of parallel-sided forceps was then plunged into the suspension, closed, and withdrawn with a drop of suspension, which was transferred to a microscope slide; the procedure was repeated 3 times per sample. One drop of Melzer's reagent (iodine, potassium iodide, and chloral hydrate in aqueous solution) and 3 drops of PVA (polyvinyl alcohol, Omar and others 1979) were added and the solution was covered with a 22 X 22-mm cover slip. Thus, 3 slides were prepared for each individual (Table 1). The slides were left on a warming tray for 12 to 24 hr at 40°C. For each slide, 50 randomly selected fields of view (a total of 150 fields per sample) were examined at 400X magnification with a compound microscope. Fungal spores were identified to genus according to Castellano and others (1989). Plant fragments and insect parts were identified to lowest possible taxonomic level. Miscellaneous and unidentifiable items occurring in <5% of the fields were grouped into the category, "other."

In method 2, the remaining suspensions of fecal samples from the 11 *G. sabrinus* were divided into 3

groups of 3 or 4 individuals; the 12 *T. townsendii* suspensions were divided into 3 groups of 4 individuals. Five slides from each of the pooled samples were then prepared as above (Table 1). Twenty-five fields on each slide were examined for the same items as in method 1, for a total of 125 fields per sample pool. We believe this combination of the number of slides and fields examined to be an adequate compromise by which to capture the abundance and diversity of diet items while substantially reducing the person-hr required to collect the data. The time required to perform each of the above analyses was recorded. Multivariate analysis of variance (MANOVA) and Kruskal-Wallis nonparametric tests were used to analyze the mean frequency of occurrence and relative frequency for method 1 or method 2 for each diet item (fungal genus, plant part, insect part, etc.). A univariate *F* test ($\alpha = 0.05$) was then performed to test for significant differences between estimates of diet items by the 2 methods. We used Spearman rank order correlation to determine how well the rank order of diet items was preserved by pooling.

RESULTS AND DISCUSSION

Mean percent frequency of occurrence (Table 2) and percent relative frequency (Table 3) as estimated by method 2 did not differ statistically from those estimated from method 1 for either species. No differences were found for diet components of *T. townsendii* between the sampling methods, except for the single *Leucogaster* spore ($p = 0.046$, 1 df) found in 1 field of view in 1 of the pooled samples (probabilities ranged from 0.864 to 0.103, Kruskal-Wallis 1way ANOVA for each component). This single spore is of little biological relevance, as it was rare (< 0.5%) and its origin cannot be determined. It may have been present in the soil and accidentally consumed while attached to the peridium of another truffle, or, alternatively, it may have been left over from a meal eaten several days or weeks before (Cork and Kenagy 1989a). Coefficients of variation (CV) were high (>100%) for many dietary items, especially rare items, as would be expected with a Poisson distribution. Variances based on individual

TABLE 2. Mean percent frequency of occurrence and coefficient of variation of fungal spores and other food items in the fecal pellets of the population by sampling method: individual animals (method 1), pooled samples of 3 to 4 animals (method 2).

Diet item	<i>Tamias townsendii</i>				<i>Glaucomys sabrinus</i>			
	Frequency (%)		CV (%)		Frequency (%)		CV (%)	
	Method 1 (n = 12)	Method 2 (n = 3)	Method 1 (n = 12)	Method 2 (n = 3)	Method 1 (n = 11)	Method 2 (n = 3)	Method 1 (n = 11)	Method 2 (n = 3)
<i>Elaphomyces</i>	0.1	0.3	346.4	173.2	4.3	3.7	149.1	128.9
<i>Gautieria</i>	—	—	—	—	55.9	96.7	87.8	1.6
<i>Hymenogaster</i>	0.3	0.0	248.6	—	0.2	0.0	3.3	—
<i>Hysterangium</i>	9.2	27.7	313.1	170.7	33.3	56.0	131.2	15.9
<i>Leucogaster</i>	0.0	0.5	—	173.2	76.5	96.3	42.0	4.2
<i>Leucophleps</i>	—	—	—	—	7.2	5.7	316.5	106.4
<i>Melanogaster</i>	8.1	16.8	204.0	82.6	82.5	92.0	34.8	14.1
<i>Picoa</i>	—	—	—	—	0.2	0.3	331.7	173.2
<i>Rhizopogon</i> ^a	85.0	97.9	36.7	3.8	37.3	50.3	102.2	56.1
<i>Scleroderma</i>	—	—	—	—	2.4	0.7	331.7	173.2
<i>Tuber</i>	0.6	1.1	346.4	173.2	0.1	0.0	208.8	—
Plant	35.2	44.0	101.5	34.5	19.4	12.8	67.2	61.6
Insect	8.1	13.3	106.6	64.2	0.2	0.7	237.1	173.2
Epigeous	0.2	0.3	346.4	173.2	—	—	—	—
Other	1.4	0.0	237.3	—	2.2	0.4	121.5	0.0
Unknown spore	—	—	—	—	1.3	0.0	208.8	—

^a May contain related genera not distinguishable by spores alone.

TABLE 3. Percent relative frequency of fungal spores and other food items in the fecal pellets of the population by sampling method: individual animals (method 1), pooled samples of 3 to 4 animals (method 2).

Diet item	<i>Tamias townsendii</i> (% relative frequency)		<i>Glaucomys sabrinus</i> (% relative frequency)	
	Method 1 (n = 12)	Method 2 (n = 3)	Method 1 (n = 11)	Method 2 (n = 3)
<i>Elaphomyces</i>	0.07	0.15	1.33	0.89
<i>Gautieria</i>	—	—	17.31	23.25
<i>Hymenogaster</i>	0.20	0.00	0.06	0.00
<i>Hysterangium</i>	6.21	13.71	10.31	13.46
<i>Leucogaster</i>	0.00	0.25	23.68	23.15
<i>Leucophleps</i>	—	—	2.23	1.37
<i>Melanogaster</i>	5.47	8.32	25.54	22.12
<i>Picoa</i>	—	—	0.06	0.07
<i>Rhizopogon</i> ^a	57.35	48.47	11.55	12.09
<i>Scleroderma</i>	—	—	0.74	0.17
<i>Tuber</i>	0.40	0.54	0.03	0.00
Plant	23.75	21.78	6.01	3.08
Insect	5.47	6.58	0.06	0.17
Epigeous	0.13	0.15	—	—
Other	0.94	0.00	0.68	0.10
Unknown spore	—	—	0.40	0.00

^a May contain related genera not distinguishable by spores alone.

samples, thus, would be expected to be higher than variances based on pooled samples (in the sense of the Central Limit Theorem), as pooling is similar to averaging; CV for method 2 (pooled samples) were less than CV for method 1 (Table 2). Consequently, the lack of significant differences may be due in part or in total to large variances in the data. Any 1 fecal sample cannot contain a representative sample of an individual's or a population's diet; many commonly consumed food items will be absent. In the Oregon Coast Range, Carey (unpubl. data) found through bootstrapping analysis that ≥ 7 samples are necessary to estimate taxonomic richness in dietaries of both *T. townsendii* and *G. sabrinus*. Many more than 12 samples would be necessary to obtain small variances for major items; one would not expect to obtain small variances for rare items. Pooling fecal samples before analysis (method 2), however, reduces the occurrence of zero values for many diet items. A Spearman rank order correlation analysis confirmed that pooling maintained the relative importance of dietary items as measured by averaging individual samples ($\rho = 0.789$, $p = 0.0126$). Thus, we believe that the lower variances observed with method 2 can improve the ability to detect differences among populations.

Results of the 2 sampling methods for items in the diet of *G. sabrinus* did not differ significantly ($p = 0.871$ to 0.174). *Gautieria*, *Melanogaster* and *Leucogaster* were the dominant food items in the dietary for both methods. The increase of 6.7% in mean relative frequency of *Gautieria* (Table 3) with method 2 was not statistically significant and is unlikely to be biologically meaningful, given the high variance of the data. Again, the Spearman rank order correlation test confirmed that pooling maintained the relative dominance of food items in the dietary ($\rho = 0.937$, $p = 0.0005$).

The average time to analyze pellets for each individual animal when using method 1 was 55 min. This figure includes maceration of the sample, staining, mounting, and recording the data for 50 fields on each of 3 slides. The time to macerate and mount is not affected by pooling. Time savings come from fewer fields per slide and fewer total preparations. The average time to analyze pellets for 3 or 4 animals when using method 2 was 70 min for 5 slides, with 25 fields per slide. Approximate total time to analyze a sample of 12 individual small mammals would be 11 person-hr; pooling 3 or 4 animals in a single sample reduces this to 3.5 hr. The people who performed these analyses had formal training in mycology, particularly identification of fungi and their spores. A considerable learning curve should be expected if persons

performing the analysis do not have a background in mycology.

Readers should be aware of limitations of this type of analysis. The relative abundance of the common (dominant) food items can be assessed with confidence. Variances around the frequencies of rare items prevent precise assessment of the proportionality and, therefore, importance of these items in the dietary. The primary limitation of fecal analysis is that only indigestible portions of the items consumed are present. This is generally not a problem, as most truffles are composed of sterile tissue with spore-bearing cells directly embedded or contained in small spore-lined chambers (locules). Special concern is necessary, however, as some species in the truffle genera *Elaphomyces* and *Radiigera* consist of a thick peridium (outer skin) and a powdery gleba (spore mass). Small mammals eat only the peridium and discard the spores; thus, only the spores consumed accidentally can be accounted for in the fecal pellets (Trappe and Maser 1977; Cork and Kenagy 1989a; J. Trappe and W Colgan, pers. obs.). The same is probably true for the gastromycete species in the genera *Lycoperdon* and *Scleroderma*. Any analysis done only on fecal pellets runs a strong risk of underestimating the relative quantity (and potential importance) of these fungi in the diet, as the soft tissue comprising the peridium would be digested, leaving almost no trace in the fecal pellets. This may be of special concern because these truffles can account for a substantial resource for small mammals in some ecosystems. North (1993) showed that *Elaphomyces granulatus* accounted for 92% of the standing crop of truffles in some sites. On these same sites in a different year, Carey (1995) found *Elaphomyces* in 10% of fields of fecal samples from *G. sabrinus* in old-growth forests and 3% of fields in second-growth forest. Carey also detected *Elaphomyces* in *G. sabrinus*, but not in *T. townsendii*, in natural and managed forests in the Coast Range, again at low mean frequencies (12 to 22%). Thus, *Elaphomyces* is routinely detected in fecal samples. Whether its frequency is proportionate to its relative abundance or nutritional importance in the dietary is unknown, but Cork and Kenagy (1989b) found the peridium of *Elaphomyces* to be of marginal nutritional value to another Pacific Northwest squirrel, the Cascade golden-mantled ground squirrel (*Spermophilus saturatus*). Like *G. sabrinus* and *T. townsendii*, *S. saturatus* in the wild feed preferentially on *Rhizopogon* (Cork and Kenagy 1989a, 1989b). Similarly, the relative importance of large seeds (for example, bigleaf maple, *Acer macrophyllum*) in the dietary may be underestimated, because the seed itself is highly digestible and large in mass relative to the seed

coat, which may or may not be consumed intact.

Estimating the diet of mycophagous mammals by analyzing fecal pellets is problematic and time-consuming. The pooling procedure illustrated above is an adequate alternative when large numbers of samples are to be processed. We believe that the ability to process large numbers of samples in a rapid and economical way offsets any loss in resolution, precision, or both. Indeed, pooling may confer an advantage by lowering variances.

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