The Effect of Slide and Frequency Observation Numbers on the Precision of Microhistological Analysis

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Abstract

The number of slides and frequency observations per slide required for microhistological analysis was determined. Slides should be prepared so that at least 20 frequency observations are recorded per slide. When five slides were prepared per sample, reasonable estimations were obtained for species comprising 20% or more of the diet. Minor and trace species in the diet were poorly estimated indicating a large number of slides are needed for adequate precision.

The microhistological technique has become one of the most favored techniques to determine the botanical composition of livestock, big game, small mammal, and insect diets. The basic procedure for the method was outlined by Sparks and Malechek (1968). Considerable time is involved in both slide preparation and plant fragment identification. The objectives of this study were to determine the number of slides and the total number of frequency observations required per slide to achieve different precision levels for major, minor, and trace species in the diet. A major species would comprise 20% or more of the diet; minor, 5 to 19%; and trace, less than 5%.

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Methods

Esophageally fistulated heifers were used to collect diet samples on forest and grassland range at the Starkey Experimental Range in northeastern Oregon. Diet samples were analyzed using the procedures of Sparks and Malechek (1968). Twenty systematically located fields were examined per slide for particle frequency. A field was considered to be the area of the slide delineated by a microscope using 125-power magnification. Each species found in each location was recorded. Only fragements of epidermal tissue (other than hairs) were used as positive evidence of the presence of a particular species at a particular location. The frequency percentages for each species (number of fields that the species occurred in out of 20 fields multiplied by 5) were recorded for each slide. The table developed by Fracker and Brischle (1944) was used to convert frequency to particle density. Particle density was then expressed as the percentage of each particular species found on a slide in

Table 1. Correlation coefficients between slide pairs which fall into five categories based on the total number of frequency observations recorded per slide.

	Total frequency observations recorded per slide				
	<10	10–14	15–19	20–24	>24
Correlation[1] Coefficient	.55	.83*	.89**	.97**	.98**
N[2]	86	115	134	139	141

*Significant at $P<.05$.
**Significant at $P<.01$.
[1]Percentages by weight of individual species found on a slide were used as variables for correlation between slide pairs within a diet sample. Variables from all 20 samples were used in calculating the correlation coefficient for each category.
[2]The sume of the number of individual species encountered on all slide pairs.

Table 2. The number of slides per diet sample required to achieve confidence intervals of ± 10%, ± 20%, ± 30% of the sample mean at 80%, 90%, and 95% probability levels.

Percent by weight of[1] species in sample	80% ± 10%	80% ± 20%	80% ± 30%	90% ± 10%	90% ± 20%	90% ± 30%	95% ± 10%	95% ± 20%	95% ± 30%
Major species									
30 >	1	1	1	3	1	4	1	1	
20–29	3	1	1	6	1	1	9	2	1
Minor species									
10–19	9	2	1	19	5	2	30	8	4
5–9	20	6	3	41	10	4	60	15	7
Trace species									
0–4	49	12	5	100	25	11	156	39	17

[1]Each value represents the average of 30 separate estimations.

relation to the total density of all species. Percent dry weight composition of each species was assumed to be the same as its calculated relative density (Sparks and Malechek 1968).

In order to study the influence of the total number of frequency observations recorded per slide on precision, slides were prepared so that the total number of frequency observations marked per slide fell into five categories. These categories included less than 10, 10 to 14, 15 to 19, 20 to 24, and over 24. Two slides from each of 20 diet samples were examined for each frequency category. This resulted in the examination of 200 slides (20 samples × 5 categories × 2 slides per category per sample). Correlation was used to compare diet similarity between slide pairs from the 20 diet samples within each category (Cooley and Lohnes 1971). The percent by weight of each species found on an individual slide was determined, and these values were used as correlation variables for each slide pair within a diet sample. Variables from slide pairs from all 20 diet samples were used in calculating the correlation coefficient for each category.

The number of slides required per diet sample for estimating the percent by weight of major, minor, and trace species was calculated using the formula given by Stein (1945). The 80, 90, and 95% probability levels were used for calculating sample sizes required to be within 10, 20, and 30% of the population mean. Slide number estimations for major, minor, and trace species were based on the variance associated with five slides prepared so that at least 20 total frequency observations were recorded per slide. The average of 30 separate estimations was used to determine the number of slides required per sample for each level of precision.

Results

The correlation coefficients between slide pairs falling into the five frequency categories are given in Table 1. Slides prepared so that more than 24 frequency observations were recorded gave little improvement in correlation coefficients over slides with 20 to 24 frequency observations. However, when fewer than 21 frequency observations were marked, the correlation coefficients were sharply reduced compared to when 21 or more were marked. These data show that slides should be prepared so that at least 20 frequency observations are recorded per slide.

Data presented in Table 2 show the number of slides needed per diet sample for major, minor, and trace species to achieve different precision levels. Nine slides per sample would estimate all major species in the diet within 10% of the mean at the 95% confidence level. However, 60 slides per sample would be required for minor species and 156 slides for trace species to achieve this level of precision. These data show that the precision of estimates for individual species depends on the importance of the species in the diet. In most studies involving microhistological analysis, five or fewer slides were read per sample. Data from these studies concerning minor or trace species are highly imprecise based on the results from our study. Therefore, conclusions should not be drawn concerning the relative proportions of these species in the diet.

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