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ASSESSMENT AND IMPROVEMENT OF SCAT ANALYSIS METHODOLOGY FOR DETERMINING RED FOX DIET

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ОЦІНКА ТА ВДОСКОНАЛЕННЯ МЕТОДОЛОГІЇ АНАЛІЗУ ПОСЛІДУ ДЛЯ ВИЗНАЧЕННЯ РАЦІОНУ РУДОЇ ЛИСИЦІ

ABSTRACT

Purpose of the work. The aim of the study was to develop a standardized and reproducible method for scat analysis to obtain comparable results on species' feeding habits.

Methodology. Fresh red fox scats (less than 24 hours old) were collected, georeferenced by GPS, dried, and disinfected in alcohol. Sample size was calculated using power analysis. Diet composition was analyzed micro- and macroscopically after washing and sieving (2 mm and 1 mm). Food remnants were identified under a microscope, and diet proportions were calculated as percentages of total fragments. The reliability of volumetric estimations was tested by comparing estimated and measured volumes of nine food categories. Inter- and intraobserver reliability were assessed by regression analysis. The independence of samples was verified through similarity index analysis based on distances between sampling points.

Scientific novelty. A standardized and statistically validated methodology for red fox scat analysis was developed. The improved volumetric method allows the application of two-way multivariate ANOVA, minimizing required sample size and ensuring reproducible results. The study defined the minimal distance between independent samples, the optimal number of scats and food remains to be analyzed, and demonstrated high intra- and interobserver reliability. The method enables accurate, comparable estimation of diet composition and temporal or spatial differences in feeding habits.

Conclusions. The study provides a reliable method for analyzing red fox scats to estimate diet composition. The improved volumetric approach allows the use of two-way multivariate ANOVA with fewer samples. Ensuring sample independence and sufficient numbers is important. Intra- and interobserver consistency was high. Collecting at least seven independent samples per site, spaced by 40 m and repeated three times, and analyzing enough food remains provides accurate and comparable results. This method can help monitor feeding habits in mammals for conservation purposes.

Key words: diet composition, faecal analysis, sample size, volumetric method, red fox

АНОТАЦІЯ

Мета роботи. Метою дослідження було розробити стандартизований і відтворюваний метод аналізу екскрементів для отримання порівняльних результатів щодо харчових звичок виду.

Методологія. Зібрано свіжі екскременти лисиці звичайної (не старші 24 годин), зафіксовано координати GPS, висушено та продезінфіковано в спирті. Розмір вибірки визначали методом аналізу потужності. Склад раціону досліджували мікро- та макроскопічно після промивання і просіювання (2 мм і 1 мм). Харчові рештки ідентифікували під мікроскопом, а частку кожного компонента розраховували у відсотках від загальної кількості фрагментів. Надійність об'ємних оцінок перевіряли шляхом порівняння розрахованих і виміряних об'ємів дев'яти категорій харчових ресурсів. Схожість результатів між різними спостерігачами, а також узгодженість повторних вимірювань одного спостерігача оцінювали за допомогою регресійного аналізу. Незалежність проб перевіряли за індексом подібності залежно від відстані між точками відбору.

Наукова новизна. Розроблено стандартизовану та статистично перевірену методику аналізу екскрементів лисиці звичайної. Удосконалений об'ємний метод дає змогу застосовувати двофакторний багатовимірний дисперсійний аналіз (ANOVA), зменшуючи необхідний обсяг вибірки та забезпечуючи відтворюваність результатів. Визначено мінімальну відстань між незалежними пробами, оптимальну кількість зразків і решток для аналізу, а також підтверджено схожість результатів між різними спостерігачами та узгодженість повторних вимірювань одного спостерігача. Метод дозволяє точно оцінювати склад раціону та його просторово-часові відмінності.

Висновки. Дослідження пропонує надійну методику аналізу екскрементів лисиці звичайної для оцінки складу раціону. Удосконалений об'ємний підхід дозволяє застосовувати двофакторний багатовимірний дисперсійний аналіз (ANOVA) з меншою кількістю зразків. Важливо забезпечити незалежність проб та достатню їх кількість. Схожість результатів між різними спостерігачами та повторні вимірювання одного спостерігача була високою. Збір щонайменше семи незалежних зразків на ділянці з відстанню 40 м і трьома повтореннями, а також аналіз достатньої кількості решток дозволяє отримати точні та порівнянні результати. Методика може бути корисною для моніторингу харчових звичок ссавців у програмах охорони природи.

Ключові слова: склад раціону, аналіз екскрементів, розмір вибірки, об'ємний метод, лисиця звичайна

Introduction

Trophic relations can influence activity, social and spatial organization of animals; additionally, predators have a central role in structuring of ecosystems (Dell'Arte & Leonardi, 2005; Zabala & Zuberogoitia, 2003). Our subject, the red fox (*Vulpes vulpes* Linnaeus, 1758), is a widespread predator living on all the continents except South America.

Detailed knowledge of feeding habits is important in order to understand the ecology of the species'. Historically, a wide range of methods was used. The diet of herbivores was studied mostly by volumetric methods, while that of carnivores by frequency of occurrence. Scat analysis may have difficulties that complicate their interpretation (Ciucci et al., 2004; Reynolds & Aebisher, 1991), besides, it was commonly used in the literature because scat collection is non-invasive and cost-effective. The number of collected faeces, the number of identified fragments and all the sampling procedures are different in the surveyed studies.

A few studies found it necessary to define the minimal sample size and analyse the spatial distribution that will guarantee the independence of scats (Katona & Altbäcker, 2002; Trites & Joy, 2005). Marucco et al. (2008) suggested using an additive method for collecting fecal samples of wolves living in groups. Independence is important because it can give information about the actual food choice of individuals.

For frequency of occurrence 94 scats will ensure that existing differences will be statistically detected, whereas 59 scats will ensure that at least one scat contains a species that has a 5% probability of occurring in a scat (Trites & Joy, 2005). Martin et al. (1995) collected scats from badgers latrines; the number of samples in each season was different. Hovens & Tungalaktuja (2005) collected samples of wolves in each month and made a conclusion that 5-7 are insufficient for their method. Homolka (1982) used 10-20 samples for the different studies. Katona & Altbäcker (2002) (volumetric method) suggested collecting 10 independent samples from one site in a season, which is many times lower than suggested by Trites & Joy (2005) for frequency of occurrence.

The aim of the present work was to improve the various existing scat analysing methodologies by establishing a standardized methodology which gives comparable and reproducible results on the feeding habits of species. For this, it is necessary to determine the number of required faecal samples; the minimal distance between sampling points, which would guarantee the independence of samples; a suitable statistical data processing procedure; and the number of analysed food remnants within a faecal sample for a correct estimation.

Materials and methods

Scats (n=383) were collected in Bócsa, Bugac and Orgovány at Kiskunsági National Park and in Nagykovácsi at Duna-Ipoly National Park. Our suggested volumetric methodology consists of the following steps:

Sampling – Only fresh (not older than 24 hours) faecal samples of red foxes were collected. The time and location of samples were recorded on the collecting boxes. The exact coordinates of the sampling points were determined by GPS and were recorded on a data sheet and on a map (1:10000) in order to calculate the distance between them. The samples were dried for 2 days. After that the samples were separately soaked in alcohol over 24 hours for disinfection.

Sample size determination – Minimum sample size was determined with the "Statistica" programme from StatSoft with power analysis sample size calculation.

Microhistological analysis – The diet composition of foxes was determined by microscopic and macroscopic analysis of remnants in faeces. The scats were considered as sample units and treated separately. We loosened each sample by pincers and thoroughly washed them for 20 minutes in flowing warm water through two sieves with mesh sizes of 2 mm and 1 mm.

The 2 mm sieve captured large amorphous items such as plants and insects, while the finer sieve captured mostly hairs. After drying of washed samples, the contents of sieves were put in separate bags with identification codes on them. We sampled the food remnants from both sieves with pincers and with plotting paper under the Petri dish. From the 2 mm sieve 50 pieces of remnants were chosen for identification from 50 fixed points, while from the 1 mm sieve 100. The remains were examined by microscope using magnification of 4x-200x. The cuticular and medullar preparations of hairs were made by the method of Teerink (1991). Proportion of diet components was estimated in each scat separately by:

Ni / Nt x 100, where Ni – the number of fragments of item i; Nt – the total number of fragments. These percentages were used for statistical calculations.

The reliability of estimation – First we estimated the volume of 10 scats by our suggested volumetric method without separation of components and they were analysed as mentioned in the microhistological analysis section. Then we separated the components into 9 categories. After this, the exact volume of each item category was measured by a plastic hypodermic syringe (20 ml). The air was pressed out of the syringe. After, we compared the exact volume of each item category with the volumes estimated by our suggested volumetric method.

Interobserver tests – Tests were performed by two authors using the same methodology. The observers made all the consecutive steps of the microhistological analysis separately. The relationship between results was estimated by regression analysis.

Intraobserver tests – All the consecutive steps of the microhistological analysis were performed two times by one of the authors with the same methodology. The relationship between the results was estimated by regression analysis.

Independency of scats – Fresh scats of red foxes were collected (n=15) in Nagykovácsi. On the first day the scats were marked and on the next day we collected all the unmarked ones. By similarity index we investigated the diet overlap of each sample. The distances between sampling points were determined. The relationship between the distance and similarities of samples was tested by regression analysis. The similarity of scats was calculated by Renkonen's proportional similarity index:

$Sis=\sum\min(P1,i; P2,i)$, where P1,i is the proportion of prey category in one individual, P2,i in the other individual.

Comparison of the estimated volumes obtained by our suggested volumetric methodology with the exact volumes and with the following frequency of occurrence results:

Frequency of occurrence 1: frequency of occurrence expressed as a percentage of the total number of scats.

Frequency of occurrence 2: frequency of occurrence expressed as a percentage of the total number of occurrences of all food items.

Results

The power analysis for sample size calculation indicates that different statistical procedures need different sample sizes (Table 1; Fig 1). The group sample size for one-way ANOVA depends on the number of groups, while the Chi square test – on the differences in population variance. A minimum of seven faecal samples is sufficient from the same sampling site within the same sampling period (within a grouping variable) for the two-way multivariate ANOVA. The number of rows depends on the number of independent variables (e.g. number of sites and seasons) and the number of samples within groups (within independent variables); the number of columns depends on the number of item categories (according to unpublished data, in the case of long-term research this number will be more than eight).

The proportions of main food types in two sieves (n=336) were highly correlated rs=0.63-0.82, p=0.00.

Intra- (n=7) and interobserver (n=7) reliabilities were measured to be high (Regression analysis: r=0.91-1.00, b=0.73-1.11, $p<0.05$), except for fox hair (r=0.37, b=0.22, p=0.41). The proposed microhistological faeces analysis does not depend on the observer.

The results of contents analysis made by the proposed method are similar to the percentage of volume (rs=0.83-1, $p<0.05$) (Fig. 2).

There was no negative correlation between the distance of sampling places of scats and their diet similarity (n(distances)=105, rs=0.18, p=0.23). The similarity between two samples at a distance of 41 m was Sis=0.25. Larger distance alone cannot guarantee the independence because in some cases samples were found to be similar within a distance of several hundred meteres.

The independent samples had individual patterns with high variability.

Table 1

Required group sample size for different statistics

Statistics	Required sample size	
One mean, t-Test	2-tailed	265
	1-tailed	216
2 mean, t-Test, ind. samples	2-tailed	527
	1-tailed	429
2 mean, t-Test, dep. samples	2-tailed	32
	1-tailed	26
1-way ANOVA	2 groups	170
	3 groups	103
	4 groups	77
	5 groups	63
	6 groups	54
	7 groups	48
	8 groups	43
	9 groups	40
	10 groups	37
Chi square test, when var1 is 1.25 times higher than var0	2-tailed	417
	1-tailed	342
Chi square test, when var1 is 1.5 times higher than var0	2-tailed	126
	1-tailed	104
Chi square test, when var1 is 2 times higher than var0	2-tailed	43
	1-tailed	36
F-Test	2-tailed	90
	1-tailed	74

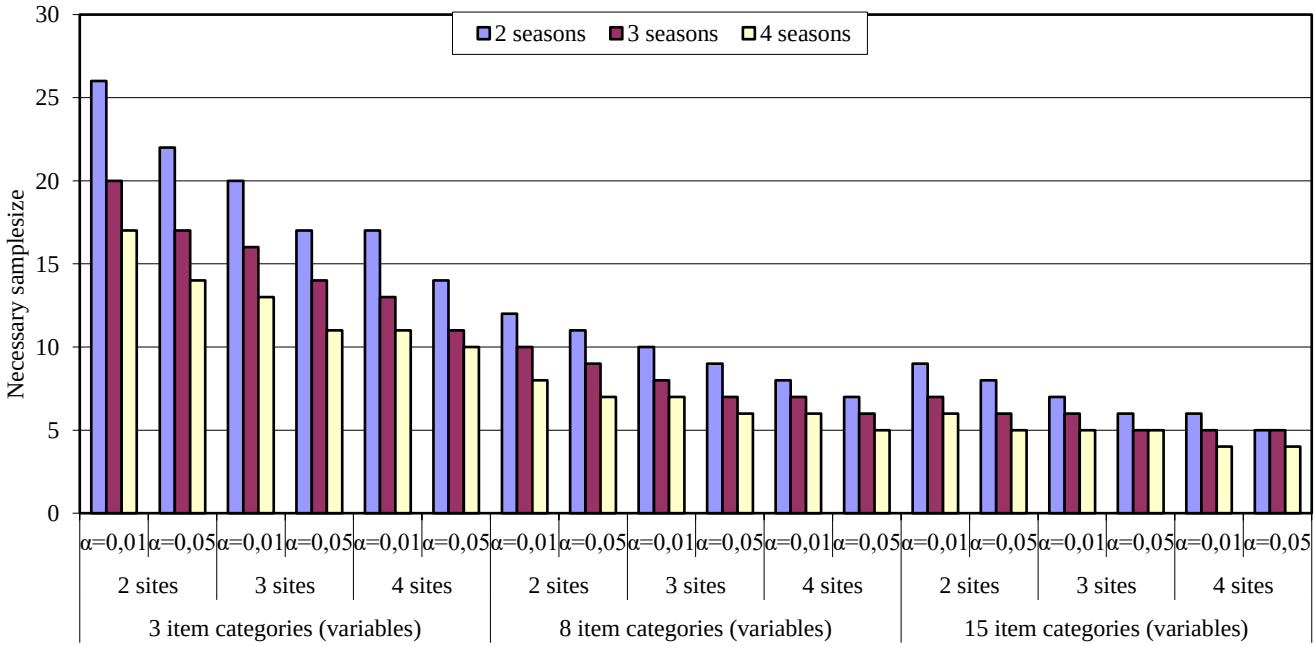

Fig. 1. Required sample size for 2-way ANOVA. The number of rows depends on the number of independent variables (like number of sites and seasons) and the number of samples within groups (within independent variables); the number of columns depends on the number of item categories (according to unpublished data, in the case of long-term research this number will be more than eight)

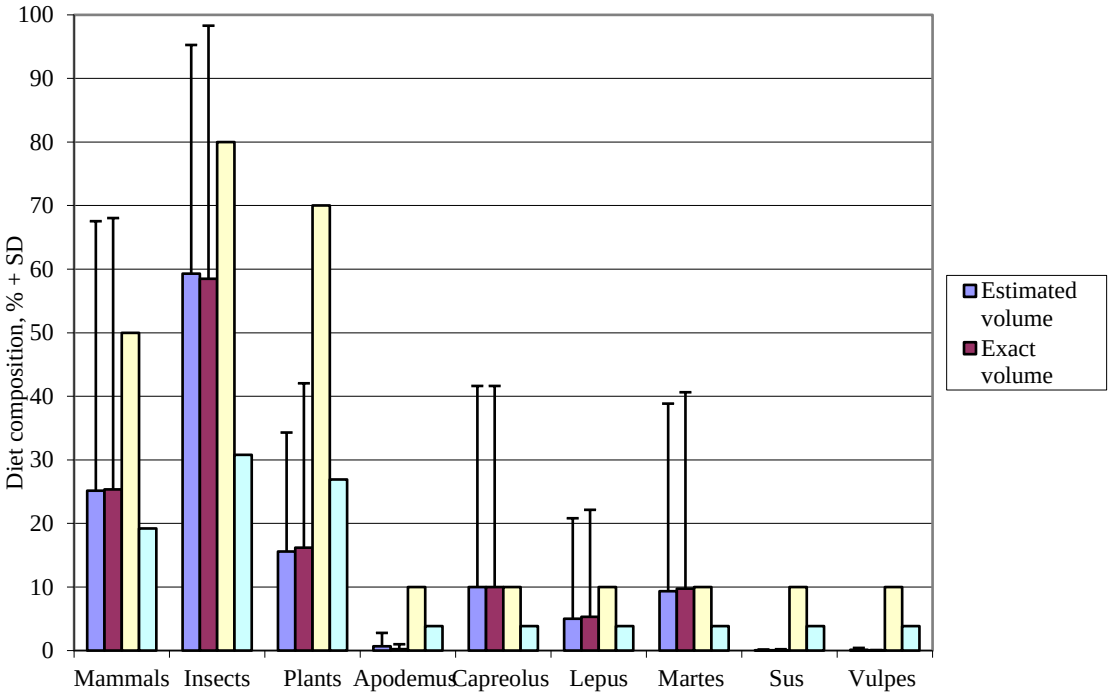

Fig. 2. Similarity between percentages. Comparison of the exact volume of categories of 10 samples with their estimated volume made by the proposed volumetric method and by two types of frequency of occurrence methods

Discussion

We analysed the variance components of the consecutive steps of the non-invasive scat analysis of the red fox diet estimates. We improved a volumetric method in a way what would allow the usage of two-way multivariate ANOVA, which requires the lowest sample size (Fig. 1). According to Zabala and Zuberogoitia (2003) the volumetric method shows the relative importance of ingested food items. First we investigated how many faecal samples need to be collected on a site in a season, then the number of hairs and other remains that need to be identified. We determined the minimal distance between independent scat samples. Our results suggest that it is necessary to analyse the samples separately in order to accurately estimate diet components and their variance. Some techniques are susceptible to interobserver sources of error (Ciucci et al., 2004). The intra- and interobserver reliabilities of the suggested methodology were measured to be high. The exact volume of faecal components is very close to the estimated volume (Fig. 2). So, this is a reliable method, which is able to investigate the percentage of volume of scats contents, and it is able to estimate site-dependent differences in the feeding habits of foxes and their change in time.

In the literature there are problems with samples size, which can be related to the methods used (Hovens & Tungalaktuja, 2005; Martin et al., 1995). Trites & Joy (2005) concluded that for the Chi square test it is necessary to use 94 samples. Scat analysis with the volumetric method, which we propose allows using ANOVA, which will prevent the problem of sample size. We suggest using a minimum of 63 samples for comparison, because for the two-way multivariate ANOVA three times three series minimum of seven independent samples are necessary at $\alpha=0.05$ when the power goal is 0.90.

Marucco et al. (2008) suggested performing scat contents analysis for diet estimation of wolves and combining these results with data on kills, which would allow a better representation of the missed kills not documented by other techniques. They concluded that small carnivores produce fewer scats per individual prey item; therefore the independence of samples will not be problematic. Our results suggest that the diet overlap estimated by Renkonen's proportional similarity index can be used as an additive method. Besides, it can only be used as a verification of the independence after the microhistological faeces analysis. The studied sites need to be large enough for seven fox individuals and the samples should be

collected along permanent tracks. The minimum distance between sampling points needs to be 40 m. The minimum distance was 100 m in the case of the European hare (Katona & Altbäcker, 2002) having the same home range size as the fox. The highest variance observed was between the individual compositions of samples; this possibly originated from the actual food choices of the individuals (Katona & Altbäcker, 2002). In the literature, there are cases when during the analysis the researchers made a mixture of samples (Mátrai et al., 2004; Ramirez et al., 1997; Szemethy et al., 2003) and subsamples were taken out from this mixture. Individuality of diet composition makes the validity of diet analysis from a faecal sample mixture doubtful.

We propose the described methodology because it gives reliable and reproducible results, which do not depend on the observers. This way the researchers can more precisely investigate the feeding habits of mammals, which is important in monitoring and managing restoration of threatened species. Without the correct knowledge of a particular species' diet and of the food availability in the restoration sites positive results will not be guaranteed. So, we propose collecting seven independent samples at least 40 m from each other at a time from each site, then repeating this process two more times with a set period of time between repetitions, thus collecting 3×7×3=63 samples altogether. Ciucci et al. (2004) used 50 remains with point-frame method but suggested 100. Our results suggest using 150 remains, choosing one remnant from 50 different points of 2 mm sieve, one from 100 different points of 1 mm sieve and use their summarised results when calculating the percentages of volume.

Conclusions

The study provides a standardized and reliable methodology for analyzing red fox scats to estimate diet composition.

The improved volumetric method allows the use of two-way multivariate ANOVA with a smaller sample size, while ensuring reproducible results.

Individual variability in diet was identified as the main source of variation, emphasizing the need to analyze samples separately.

High intra- and interobserver consistency confirms the method's reliability.

Collecting at least seven independent samples per site, spaced by 40 m and repeated in three series, and analyzing 150 food remains per sample, ensures accurate and comparable dietary assessments.

This approach can be applied to monitor feeding habits and support conservation and management programs for mammals.

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Автор заявляє про відсутність конфлікту інтересів / The author declares no conflict of interest.

Декларація про генеративний штучний інтелект і технології на основі штучного інтелекту в процесі написання / Declaration on Generative Artificial Intelligence and AI-enabled Technologies in the Writing Process

Під час написання статті та збору даних не використовувалися генеративні технології штучного інтелекту або інші технології на основі ШІ / No generative artificial intelligence or AI-enabled technologies were used in the writing or data collection for this article.

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