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DETERMINATION OF MINIMUM SAMPLE SIZE TO ESTIMATE DIET DIVERSITY IN ANURAN SPECIES

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Rarefaction analysis was applied to determine the minimum sample size required for any desired degree of accuracy of diet diversity of six anuran species in a particular habitat. This method is suggested for the design of sampling strategies in long-term amphibian studies. In the present study the minimum sample sizes were 28, 36, 32, 13, 24 and 8 for *Rana esculenta*, *R. arvalis*, *Hyla arborea*, *Bombina bombina*, *Pelobates fuscus* and *Bufo bufo*, respectively. The greater the diet diversity of a species, the larger the minimum sample size required, and the smaller the similarity between individuals within species.

INTRODUCTION

Since 1984 we have studied the spatial and temporal variation of food diversity of anuran species in a protected marshland area. One methodological problem that has arisen is the comparison of diet diversity when different numbers of each species are sampled. When using common diversity indices to quantify the degree of diet selectivity of anurans it is clear that sample size (number of individuals) affects the calculated value. To determine the sample size required for any desired degree of accuracy, rarefaction analysis was chosen (Simberloff, 1972; Heck *et al.*, 1975; James & Rathbun, 1981), as it is in no way specific for one type of sampling method when applied to the measurement of diet composition of anurans. From the rarefaction curves we estimated the number of sampled individuals needed to reach at least 95% of the whole-sample diversity.

An additional advantage of applying the required minimum sample size is that the disturbance of anuran communities can be minimized.

METHODS

The study was conducted in Diás Island, located in a protected wetland area of south-west Hungary. Anurans were collected in an alder swamp forest and meadow covering the central part of the island, in 1992 and 1993 (Török & Csörgö, 1992). This island is no larger than 1 ha, and according to our observations, specimens of the studied species use both habitats for feeding. To avoid the influence of seasonal differences in diet, we collected three samples of equal size in spring, summer and autumn. A total of 641 stomach contents of anuran specimens belonging to six species were analysed (*Bombina bombina* 42% [juveniles]; *Pelobates fuscus*, *Bufo bufo*, *Hyla arborea* 30%; *Rana esculenta* complex 24% in 1992, 45% in 1993; *R. arvalis* 49% in 1992, 33% in 1993). To obtain the food, a stomach flush method was used (Fraser, 1976; Legler & Sullivan, 1979; Opatrny, 1980; Griffiths, 1986). A 10 cm long plastic cannula 1 mm in diameter was attached to a 10 ml medical syringe. The cannula was gently inserted through the mouth and into the stomach. We used 5-50 ml water depending on the size of the frogs or toads.

Prey was preserved in 70% methanol. A total of 9117 prey items were identified to class, order or family level. In the present analysis a total of 38 taxa were represented as the specimens could not all be identified to species level.

DATA ANALYSIS

Several indices have been used to estimate the diet diversity (niche breadth) and diet similarity (niche overlap) of different animal species (Colwell & Futuyma, 1971; Hurlbert, 1978, 1982; Abrams, 1980; Feinsinger *et al.*, 1981; Griffiths, 1986). We used the Shannon-Weaver index to estimate the diversity of the diet (Shannon & Weaver, 1949):

$$H=-\sum_{i=1}^{s} p_i \ln p_i$$

where p_i is the proportion of prey category i (by number). S is the number of categories.

Rarefaction analysis is a method designed to determine how diversity changes with increasing sample size. Subsets of x samples were used, where x varies from one to the actual number of samples (N) used to calculate diversity. If possible, 200 subsets of size x were randomly chosen and the average diversity estimated. In general, rarefaction curves asymptote when the sample size is large enough to show that the estimation is no longer dependent on sample size. We accepted the sample size which gave at least 95% of the diversity calculated for the total sample size of a species. Two types of function (exponential and hyperbolic) can be applied to compute the minimum sample size. We used the hyperbolic as this function converges to a limit (H_{lim}), which is the maximal diet diversity for a given species in a given habitat

$$H=H_{lim}-a(1/x)$$

where x is the number of the anuran specimens and a is the slope.

TABLE 1. Slope (a) and asymptote (H_{lim}) of hyperbolic curves fitted to data of rarefaction analysis, and the minimum number of anurans (n_s) required to estimate the 95% limit of diet diversity (ANOVA for goodness of fit).

	1992					1993				
	H_{lim}	a	F	P	n_s	H_{lim}	a	F	P	n_s
R. esculenta c.	2.65	-3.34	595.1	0.001	22	2.65	-3.70	205.2	0.001	28
R. arvalis	2.50	-2.48	1169.0	0.001	20	2.59	-4.71	142.9	0.001	36
H. arborea	/	/	/	/	/	2.35	-3.79	458.6	0.001	32
B. bombina	/	/	/	/	/	1.81	-1.15	322.1	0.001	13
P. fuscus	2.19	-1.80	876.1	0.001	16	2.21	-2.67	283.4	0.001	24
B. bufo	1.48	-0.42	187.8	0.001	6	1.14	-0.46	124.8	0.001	8

Species for which more stomachs are required to reach a diversity plateau have higher diet diversity than other species. To quantify this we calculated the similarity between individuals using the proportional similarity index (Renkonen, 1938):

$$S_{is}=\Sigma\min(p_{1,i}\,;\,p_{2,i})$$

where $p_{1,i}$ is the proportion of prey category i in one individual, $p_{2,i}$ in the other individual.

To calculate both diversity and similarity indices we used software by Dolph Schluter (Schluter, 1988) which was available from the author upon request.

RESULTS AND DISCUSSION

Rarefaction analysis was used to produce curves where the different asymptotes reflected the differences in diet diversity among the anuran species (Fig.1). The slopes of the curves increased steeply with increasing asymptotes (Fig. 2). The observed values fitted each curve closely (Table 1).

The most common anurans were the two *Rana* species in the study area. Both of them usually eat a variety of prey species showing a high diversity (Kovács & Török, 1992; Török & Csörgö, 1992). Similarly to these results, Medvedev (1974), Zimka (1974), Loman (1979) and Löw *et al* (1990) found high diet diversity in *Rana arvalis*. In our study the mean number of prey items per stomach ranged between 6.2-12.5 (Table 2). Zimka (1971) found similar values (5.6-7.2) in *R. arvalis* stomachs. Külhorn (1960) studied the diet of *R. esculenta* and also showed diverse food composition where the most common prey groups were Coleoptera, Gastropoda, Diptera, Homoptera and Araneidea. This pattern was similar to the present findings, except for Gastropoda and Homoptera, which were underrepresented in the diet of our *R. esculenta* population. Both *Rana* species were numerous in the study area which made it possible to collect large data sets for an accurate estimate of the rarefaction curves. For the *Rana esculenta* complex, 95% of diet diversity can be estimated from 22 and 28 individuals in the two years.

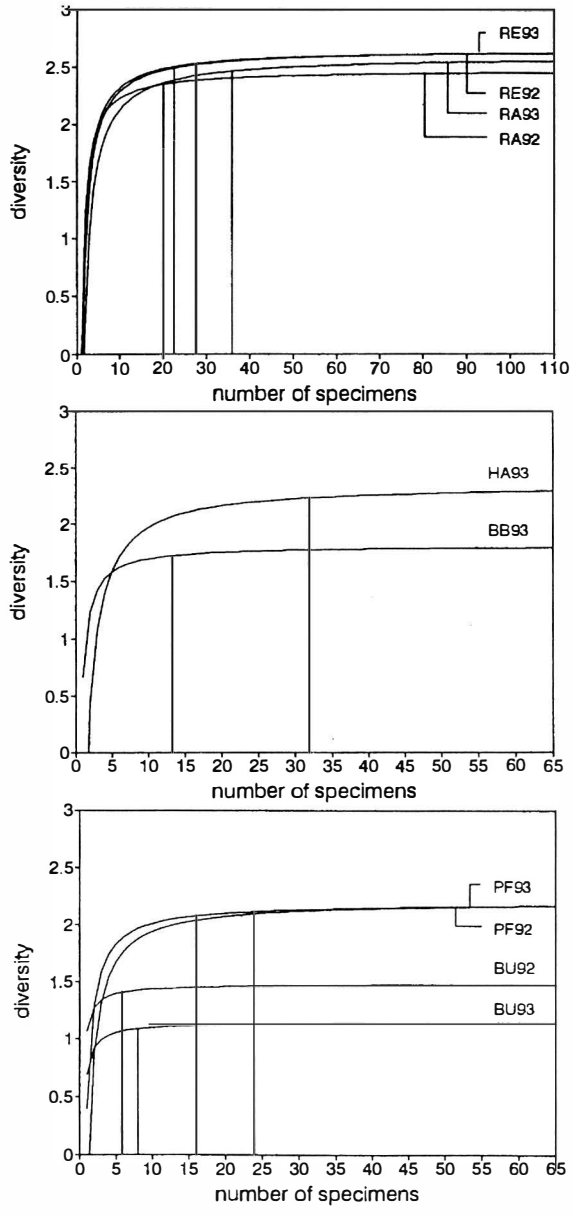

FIG. 1. Hyperbolic curves fitted to data of rarefaction analysis. Vertical lines show the number of anurans needed to estimate the 95% of their diet diversity (RE=*Rana esculenta* complex, RA=*R. arvalis*, HA=*Hyla arborea*, BB=*Bombina bombina*, PF=*Pelobates fuscus*, BU=*Bufo bufo*, 92=1992, 93=1993).

TABLE 2. Intraspecific similarities, S_{is} (mean, SD) and number of items per stomach (mean, SD, n) in six anuran species in Diás Island, Hungary.

	1992					1993				
	S_{is}		prey/stomach			S_{is}		prey/stomach		
	mean	SD	mean	SD	n	mean	SD	mean	SD	n
R. esculenta c.	0.18	0.20	12.5	4.56	76	0.15	0.18	8.7	4.56	110
R. arvalis	0.21	0.23	6.2	4.56	72	0.20	0.20	8.8	4.56	139
H. arborea	/	/	/	/	/	0.16	0.25	2.7	4.56	58
B. bombina	/	/	/	/	/	0.30	0.30	47.4	4.56	35
P. fuscus	0.16	0.24	7.0	4.56	43	0.26	0.27	5.2	4.56	62
B. bufo	0.53	0.22	73.4	4.56	24	0.60	0.25	58.0	4.56	23

For *Rana arvalis* the minimum sample sizes were 20 and 36 in the two years.

Hyla arborea occupies different microhabits from the ground to the top of vegetation implying that its diet is variable. However its diet was less diverse than that of *Rana* species (Kovács & Török, 1992). The smallest number of items per stomach was found in this species. As with the *Rana* species, a relatively large number of individuals (32) was needed to estimate the 95% limits of diet diversity in *Hyla arborea.*

Bombina bombina, the most aquatic species, fed mainly on collembolans living on the surface of the water and, to a lesser extent, on amphipods and beetles. The average number of prey items per stomach was quite high. Studying the sibling species, *B. variegata*, Kuzmin (1990) found a much smaller prey number per specimen than we did, although the most abundant prey types showed a great similarity to the prsent data. Studies conducted in Ukraine and South Russia reported a rather different diet composition (Medvedev, 1974), Goncharenko *et al.*, 1978; Tertyshnikov & Gorovaya, 1982). Coleoptera, Lepidoptera larvae and Diptera were the dominant prey species, but collembolans were absent. In Diás Island the diversity was 1.79, which is relatively low, and the 95% limit was reached at 13 specimens. The high number of prey per stomach and the high similarity in the diet between individuals meant that diversity could be estimated from a small sample size in this species.

We studied two nocturnal species, *Pelobates fuscus* and *Bufo bufo*. The diet of the former was much more diverse than that of the latter (Kovács & Török, 1992). The number of prey items per stomach was also comparatively low. The minimum sample size was 24 individuals for *Pelobates fuscus* but only 8 for *Bufo bufo* (Fig. 1.). The latter species foraged mainly for patchily distributed prey (more than 60% of its diet consisted of ants); therefore the number of prey items per stomach was extremely high (Table 2). As in *Bombina bombina,* too few specimens were obtained to compute the diversity for this species. Medvedev (1974) reported a high preference for Coleoptera in *Pelobates fuscus* implying a very low diet diversity. Other studies also showed small and highly aggregated

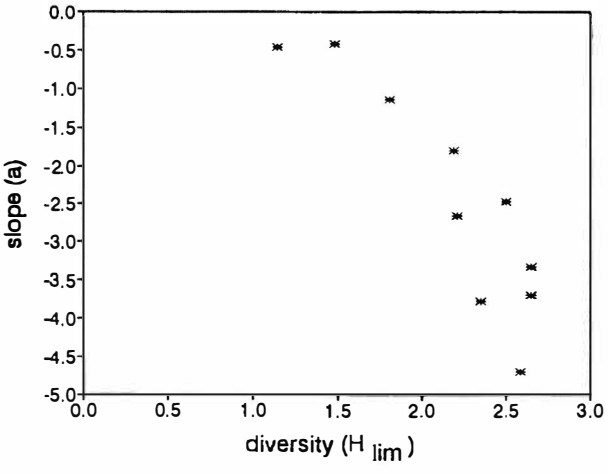

FIG. 2. Relationship between slope (a) and asymptotic diversity (H_{lim}) of the hyperbolic curves fitted to data of rarefaction analysis (four and six species in 1992 and 1993, respectively are pooled).

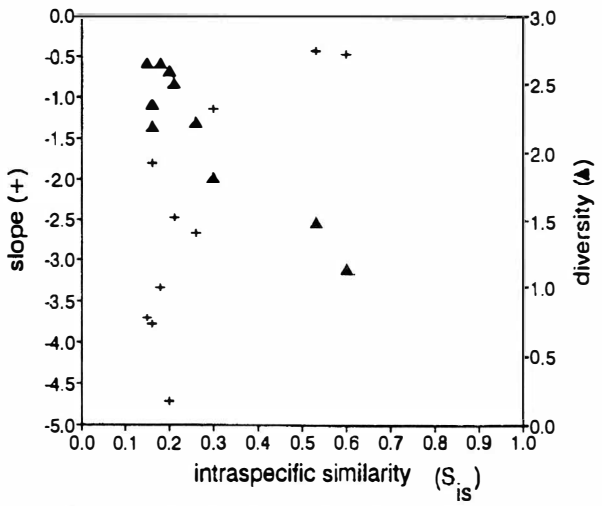

FIG. 3. Slope (crosses) and asymptotic diversity (triangles) of the hyperbolic curves fitted to data of rarefaction analysis in relation to intraspecific similarity (S_{is}) (four and six species in 1992 and 1993, respectively are pooled)

prey types in *Bufo bufo* (Medvedev, 1974; Wheater, 1986; Kuzmin, 1990).

The intraspecific variance of diet diversity can influence the shape of the individual diversity curve. One of the extremes is when all specimens eat different food types, the other is when all individuals eat the same types. In the first case the curve increases steeply, while in the other case the diversity is independent of the sample size and shows a horizontal line.

Intraspecific diet similarity was also computed among individuals. It ranged between 0.15 and 0.30, except for *Bufo bufo* where similarity reached 0.60 (Table 2). The negative correlation (r=-0.93, t=7.21, P=0.0001, n=10) between similarity and the asymptotic value in the rarefaction curves reflects the fact that diversity can be estimated from small sample sizes in species with a low asymptotic value (Fig. 3.).

The correlation between similarity and slope of rarefaction curve was positive and significant (r=0.80, t=3.70, P=0.0060, n=10).

In conclusion, this study has demonstrated how diet diversity depends on sample size in six anuran species. Rarefaction analysis allows the estimation of a minimum sample size in all species. The higher the diversity, the steeper the slope of the saturation curve. The diet diversity negatively depended on intraspecific diet similarity.

This analysis is applicable in preparing long-term field studies on the diet of any group of animals. The first step is to collect a large sample of each species - in the case of amphibians, approximately 60-80 individuals. After identifying the food items the diversity of the diet is calculated. These values are then used to compute the rarefaction curve and the asymptote of hyperbolic function fitted on the rarefaction curve. Where diversity reaches 95% of the asymptote value the minimum sample size can be determined. Sample sizes around this minimum value can be obtained for subsequent sampling periods.

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