MALACOLOGIA, 2009, 51(2): 389–393

DOES SHELL ACCUMULATION MATTER IN MICRO-SCALE LAND SNAIL SURVEYS?

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INTRODUCTION

Land snails are most often surveyed to generate inventories with sites often less than 1 ha in extent and sampling taking place in microhabitats within the site that researchers consider likely to harbor snails (Cameron & Pokryszko, 2005; Menez, 2001, 2007). The most cost effective approach to site-level sampling has been suggested as a combination of bulk sampling of litter/soil and time restricted search (Emberton et al., 1996). This requires a single visit to each site, and the combination of methods enables the investigator to maximize the likelihood of finding both micro (< 5 mm) and macro (≥ 5 mm) snails (Sólymos et al., 2007).

This approach often relies on dead shells as a probabilistic indicator that a given species is present (Thurman et al., 2008) because several constraints (remote locations, investigator availability, weather conditions) may not enable surveys to be undertaken during the species' activity periods. Assessing species presence on the basis of dead shells can be realistic, given the rather sedentary and cryptic behavior of land snails, and in most cases gives comparable results at the site scale (Rundell & Cowie, 2003).

Recently, there is an increase in surveys that are addressing microhabitat-scale ecological differences regarding the relative abundances and species compositions of communities (e.g., under logs vs. leaf litter) within a site (Kappes et al., 2006; Sólymos & Páll-Gergely, 2007). Taphonomic issues (e.g., differential preservation of shells in different microhabitats) and biases introduced by sampling methods with different selectivity to dead specimens might become increasingly important as spatial scale decreases.

We carried out a field experiment in dolines (sinkholes, large karstic depressions) of the Aggtelek karst area, Hungary, to investigate the interacting effects of aspect (heat load) and microhabitat on land snail assemblages. Here we focus on the methodological issues raised by a stratified sampling design applied at the microhabitat scale. We aimed to determine the most straightforward strategy for comparisons among microhabitat types, that is, leaf litter, live trees, dead trees (here, equivalent to coarse woody debris) and rocks. We investigated how rates of shell disintegration and sampling method influence our interpretations of microhabitat-land snail community relationships.

MATERIALS AND METHODS

We surveyed 16 dolines at the Alsó-hegy plateau of the Aggtelek National Park, northern Hungary, August 16–18, 2007. The plateau and the dolines were forested and mainly covered by hornbeam (*Carpinus betulus*) and beech (*Fagus sylvatica*). The base rock was Triassic (Wetterstein) limestone. Soils of the dolines were rendzinas on the rims and plateaus in-between them, and brown forest soils on the slopes and in the bottoms of the dolines; soil pH (H_2O) in these soils is close to neutral, slightly basic (6–7.5) (Tanács & Barta, 2006). The dolines were 0.5–2 ha in extent. We collected land snails from four microhabitat types: litter, live wood, dead wood (i.e., coarse woody debris), and rock. In each doline, seven litter microhabitat samples were taken along a north-south transect, and three samples were taken in each of the other microhabitat types. We collected 1 L of litter per sample ("litter sampling" for short) and employed a time-restricted search (five minutes per replicate) in a 1 m radius around the litter sample location. Litter samples were collected adjacent to live wood, dead wood and rocks, and not from the wood or rocks themselves. Litter samples in the litter microhabitat were not collected near wood or rocks but that

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TABLE 1. Total number of individuals (fresh + broken, percentages for rows are given in parentheses) partitioned according to sampling method, microhabitat type and adult body size categories.

Method and microhabitat	Adult body size	
	Large	Small (< 5 mm)
Timed search		
Dead wood	51 + 14 (67.1 + 18.4)	11 + 0 (14.5 + 0.0)
Litter	33 + 28 (50.8 + 43.1)	3 + 1 (4.6 + 1.5)
Live wood	18 + 16 (48.6 + 43.2)	2 + 1 (5.4 + 2.7)
Rock	49 + 136 (25.9 + 72.0)	2 + 2 (1.1 + 1.1)
Soil-plus-litter sampling		
Dead wood	26 + 29 (11.7 + 13.1)	92 + 75 (41.4 + 33.8)
Litter	52 + 62 (8.7 + 10.3)	219 + 267 (36.5 + 44.5)
Live wood	63 + 56 (22.7 + 20.2)	74 + 84 (26.7 + 30.3)
Rock	108 + 98 (19.6 + 17.8)	211 + 134 (38.3 + 24.3)

the live wood, dead wood, and rock samples. Altogether, 32 samples (16 litter samples + 16 timed samples) were taken per doline.

Snails were identified to species and categorized according to extent of shell deterioration. Distinction of live animals and fresh shells was not feasible due to the delay in sample processing of litter samples, so these were combined and constituted the “fresh” group. Whitened, disintegrating and broken shells constituted the “broken” group.

Here we use the data from two dolines for which the samples have been fully processed. The 64 samples were grouped according to sampling method and microhabitat, and the species were grouped according to adult size – major shell dimension: smaller or greater than 5 mm; Kerney et al., 1983; semislugs were grouped together with small species – and family-group – Clausiliidae, Helicoidea, Zonitidae s. lat. (including Oxychilidae, Pristilomatidae, Daudebardiidae), and others.

We used the proportion of broken shells per sample in a logistic regression (binomial GLM) to determine its relation to sample type (method, microhabitat) and species specific category (adult size, taxonomy). The state of snails in a sample being either “fresh” or “broken” is a random binomial process with number of broken shells over total number of individuals as the probability of being broken and total number of individuals as the number of independent trials. We used the R software (R Development Core Team, 2008) for the computations. Data processing was done using the mefa 2.0 (Sólymos, 2008) R package. The full data set and the data analytic code are provided by Sólymos & Kemencei (2008).

RESULTS

The total number of fresh and broken shells differed both among microhabitat types and sampling methods. Most broken shells were found in rock and fewest in dead wood microhabitats. The numbers of small species were consistently lower in timed searches than in litter samples (Table 1).

The proportion of broken shells per sample was significantly associated with sampling method, microhabitat type and their interaction (null deviance: 88.9, df = 61; residual deviance: 55.8, df = 54; AIC: 287.4; Table 2). The proportion of broken shells was highest in the rock and lowest in the dead wood microhabitats. The litter sampling resulted in higher proportions of broken shells than the timed searches. However, the interaction showed that the proportion of broken shells in the dead wood microhabitat was lower in timed searches than in the litter sampling, whereas it was higher in rock microhabitats. There was no significant interaction with sampling method in the litter and live wood microhabitats (Table 2).

The proportion of broken shells per species was significantly associated with taxonomy and body size (null deviance: 26.8, df = 30; residual

TABLE 2. Logistic regression coefficients for the proportion of broken shells per sample. Effect sizes of the reference categories of factors (dead wood for microhabitat, and timed search for method) are included in the intercept.

Covariates	Estimate	Std. Error	p-value
Intercept	-1.6917	0.2908	< 0.001
Microhabitat			
Litter	0.8846	0.3667	< 0.05
Live wood	0.9140	0.4128	< 0.05
Rock	1.3772	0.3116	< 0.001
Method			
Litter sampling	0.9334	0.3142	< 0.01
Litter microhab. × Litter sampling	-0.7272	0.3915	0.063
Live wood × Litter sampling	-0.8381	0.4419	0.058
Rock × Litter sampling	-1.4839	0.3426	< 0.001

deviance: 14.2, df = 25; AIC: 134.9; Table 3). The proportion was high in the large bodied families, and highest among helicoids. Small sized zonitids (s. lat., *Daudebardia*, *Vitrea*) exhibited lower proportions of broken shells than larger sized zonitids (s. lat., *Oxychilus*, *Aegopinella*).

DISCUSSION

Our study reconfirmed that there is no universally appropriate and unbiased method for sampling land snails. We showed again that timed search and soil-plus-litter sampling is biased towards opposite ranges of the body size continuum (Cameron & Pokryszko, 2005; Sólymos et al., 2007). Besides selectivity for different size classes, shell accumulation rate also differed according to sampling method. We found that proportions of dead shells were higher for litter sampling than for timed searches, and higher proportions of small species are correlated with higher proportions of dead shells as opposed to live snails.

Proportions of dead shells also varied according to taxonomic group. Helicoids tended to accumulate more (i.e., disintegrate less) than other groups. This can be attributed to their relatively large, thick shells compared to zonitids or other smaller sized and thinner shelled taxa (Menez, 2002). Clausiliids, however, showed lower proportions of broken shells relative to helicoids, although their shells are also

TABLE 3. Logistic regression coefficients for the proportion of broken shells per species. Effect sizes of the reference categories of factors (other for family and large for size) are included in the intercept.

Covariates	Estimate	Std. Error	p-value
Intercept	-0.9454	0.1509	< 0.001
Family			
Clausiliidae	0.2886	0.2233	0.196
Helicoidea	0.4715	0.1765	< 0.01
Zonitidae s. lat.	0.2292	0.1852	0.216
Size			
Small	0.2406	0.1602	0.133
Zonitidae s. lat. × Small	-0.5343	0.2529	< 0.05

durable and relatively large. Their association with coarse woody debris might result in more rapid decay due to high fungal and microbial activity in woody debris. Thus, the observed species and size specific differences in shell accumulation may be linked to the species' microhabitat preferences.

When surveying at a fine, that is, microhabitat scale, different shell accumulation rates make among-microhabitat comparisons problematic. We found that shell accumulation rate was lowest in dead wood and highest in rock microhabitats. This may be related to the longevity and relative extent of these structural elements, with coarse woody debris decaying completely in up to 110 years (Holeksa et al., 2008; Lombardi et al., 2008; Rock et al., 2008), whereas rocks are an almost permanent and large feature in these forest habitats. Shell accumulation was intermediate in the live wood and litter microhabitats. Live trees might stand for a similar length of time as coarse woody debris, but dead wood provides good shelter and a food source during the relatively short time frame of intermediate decay stages when snails are able to retreat beneath the bark. Thus, woody debris can be considered as a more dynamic component than live trees (Jönsson et al., 2008).

Although we performed our study in a karstic area, where soil pH is neutral, slightly basic (6–7.5; Tanács & Barta, 2006), the effects of soil pH on shell disintegration in different microhabitats might add another level of complexity to our findings. Coarse woody debris has slightly positive effect on pH (Kappes et al., 2007), while on acidic soils, leaf litter can considerably buffer soil pH (Wäreborn, 1970). The increase of soil pH can also be expected adjacent to limestone outcrops. At the same time, high proportions of empty shells might also influence soil pH. These may result in differential shell disintegration, too.

Although dead shells might be good indicators of the occurrence and abundance of single species (Thurman et al., 2008), or when comparing species richness across sites (Rundell & Cowie, 2003), they pose considerable problems in microhabitat-scale comparisons. We suggest that the effect of shell accumulation should be assessed prior to any analysis at micro-scales. If the proportion of dead to live shells differs significantly among microhabitats, the most appropriate method is to use fresh (living) specimens only, and thus make the results more comparable. Further, because of the complex interaction between sampling method, microhabitat and taxonomic group, we suggest the use of timed search over litter sampling. Timed search is more cost effective, and can result in higher number of replicates. Living specimens can also be identified more easily, and released in the field, although vouchering is essential in order to permit future verification especially for difficult to identify species. In addition to microhabitat scale timed searches, it is advisable to collect soil-plus litter samples at the whole habitat scale to gain information on small sized species. Based on these complementary approaches, specific size classes (i.e., < 5 mm) could be excluded from timed search results to enhance comparability at the microhabitat level. This approach would result in both a maximized species list at the habitat scale and detailed abundance data for micro-scale comparisons. The methodology adopted should be geared to the research question being addressed.

ACKNOWLEDGEMENTS

We thank Robert Cowie for comments and improving the English. Robert Cameron and an anonymous reviewer made valuable suggestions on an earlier version of the manuscript. Field work was supported by the ÁOTK-NKB 15714 grant. PS was supported by a NSERC postdoctoral fellowship and the Alberta Biodiversity Monitoring Institute. This paper is No. 2 of the "Dolina 2007 Project".

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Revised ms. accepted 24 January 2009