

Szent István University
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Effects of environmental variables on populations and
communities of land snails (Gastropoda, Mollusca)

Brief Summary of Doctoral Thesis

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2015

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1. Introduction and aims

Exploration and understanding the relationship between distribution of habitats and species are the common goal of ecology and conservation biology. From a conservation aspect, it is important to recognize of the scale dependent role of habitats and their structures in the life of the associated species. This knowledge allows one to assess habitats based on local fauna within a larger (regional) scale context, and to find critical habitats for nature conservation. While on smaller (local) scale the recognition of important habitat structures could ensure the appropriate management for the sheltered rare and/or protected species of the habitat. The data of geographical distribution and habitat choice of land snails mainly available through opportunistic field surveys (Kerney et al 1983, Fehér and Gubányi 2001, Pintér and Suara 2004). Empty and broken shells are often included in faunistic studies (Rundell and Cowie 2003, Emberton et al. 1996), as indicator of species occurrence. However the results of investigations based on living specimens are more reliable, the empty shell could indicate the presence of elusive, small sized or inactive species (Thurman et al. 2008, Cameron and Pokryszko 2005, Rundell and Cowie 2003) and provide solution for challenges of seasonal activity or rarity of species. Furthermore broken shells can be used in comparison of habitats on regional scale (Pearce 2008). Although larger scale studies aimed to compare the habitats found no significant difference in the rate of shell accumulation within similar habitats (Rundell and Cowie 2003, Pearce 2008), on smaller scale the accumulation rate were biased among taxonomic groups and microhabitats (Millar and Waite 1999, Pearce 2008, Černohorská et al. 2010). Numerous studies aimed to examine the distribution of land snails showed that the local distribution of snail are affected by micro-scale variation in microclimate (Baur and Baur, 1993, Sólymos and Nagy 1997), soil pH and calcium (Juříčková et al. 2008, Gärdenfors et al. 1995, Martin and Sommer 2004), humidity and availability of structural elements such as coarse woody debris (Kappes 2005, Kappes et al. 2006) or rock outcrops (Nekola 1999, Nekola and Smith 1999). Despite of the land snails, as other invertebrates, choose their habitats on fine local scale, the microhabitat scale surveys gained momentum only in recent years (Kappes et al. 2006, Sólymos és Páll-Gergely 2007, Sólymos et. al. 2009b), but there are still knowledge gaps in term of distribution of species and the relation among species, habitat structures and vegetation (Kappes et al. 2006, Sólymos and Páll-Gergely 2007, Sólymos et. al. 2009b).

As the man disturb and alter the self-regulating processes even of natural forests, designation of such forest area became necessary, where the natural processes are undisturbed. Parallel the designation of Hungarian forest reserves their reasearch-oriented hierarchical classification were carried out based on uniform methodology (Standovár 2002, Horváth and Bölöni 2002, Czájlik 2002). The assessment of habitats is considering

properties of several species by using community indices. The simplest index is the number of species, which is not sensitive to species rarity. However in most cases the habitat shelter higher number of rare species were regarded more valuable. The number of species could be weighted by the regional or local rarity of the species. This measure were quantified by Mollusc Rarity Index (Sólymos 2004, Sólymos and Fehér 2005). But we can measure the rarity or commonness of species on local scale by using an index like Shannon entropy, which taking into consideration the relative local commonnes of the species. Furthermore the local commonness or rarity could be linked to regional commonness or rarity. The well known positive correlation between local and regional frequency / rarity could be used that locally common species are regionally common as more individual is required larger living space (habitat) (Gaston 1994). Knowing this correlation we expect that regionally rare species tend to be rare on local scale. The bias from the general estimate could be important, thus during habitat assessment it is worth to consider of rarity at different spatial scales. If a species is regionally rare but locally abundant the chance of population to survive is higher. But if a regionally common species tend to be locally rare is not as important from a conservation aspect. Each areas could be designated because of their high biodiversity, others could be important for shelter few rare species, but with relatively high abundance. The number of species and diversity indices were widely used for assessing habitats. Dévai and Miskolczi (1987) used the large scale rarity for working out a local-scale assessment method, while Szabó (1994) linked the local abundace with the regional rarity. But we do not know example of using the rarity of different scale in an integrated methodology.

In the first part of this study we investigated the impact of environmental factors on snail species richness and abundance in a micro-scale analysis with well replicated natural experiment using a balanced sampling design. We studied four distinct microhabitat types (litter, live trees, dead wood, rock) in 16 dolinas of the Alsó-hegy (Gömör-Tornai Karst, Aggtelek National Park) considering also topographic factors. This complex dataset allowed simultaneous analysis of habitat requirements of individual species and investigation of the effect of biases of the applied sampling methods (time restricted direct search and soil plus litter sampling) on the results of ecological inferences. In the second part, we assessed the performance of different measures of habitat quality based on data of land snails. We would answering the following questions:

- A1) Does the proportion of broken shells differ among the different sampling methods?
- A2) Does the proportion of broken shells differ among the taxonomic groups?
- A3) Does the proportion of broken shells differ among the different microhabitat types?

A4) How do the diversity of Gastropod fauna and abundance of populations change with topographic factors?

A5) Do the diversity of Gastropod fauna and the abundance of populations differ among the different microhabitat types?

A6) Is there any relation among the microhabitat types and their Gastropod communities?

B1) What correlation is among the distribution and abundance of snail fauna of forest reserves and the MRI value of each species?

B2) How do the order of forest reserves change according to the habitat quality measurements of different?

2. Materials and methods

2.1. Effects of microhabitats and environmental variables on quantitative relations of living and dead snail communities

Samples were taken from 16 dolinas of Alsó-hegy Plateau belongs to Gömör-Torna Karst (Aggtelek National Park, Fig. 1.) between 16 and 18 of August, 2007 (for detailed data of dolinas see Vilisics et al. 2008). Vegetation within most of the dolinas was deciduous forest: scree forest (*Mercuriali-Tilietum*), alternating with oak-hornbeam forest (*Carici pilosae-Carpinetum*), beech forest (*Melittio-Fagetum*) and gorge forest (*Phyllitidi-Aceretum*). The dimension of each dolina was 0.5-2 ha in extent and 10-30 m depth. Four type of microhabitats (litter, live trees, dead wood and rock) were investigated. In the case of litter microhabitat seven samples were collected along a north-south transect (outside rim, inside edge and middle in both aspects and the bottom) in each dolina, while three random locations were taken in each of the other three microhabitat types. The aspect, vertical zone (bottom, middle or edge of the dolinas) of sample location and the litter depth and moisture were recorded in each sample location. We applied two complementary sampling methods: litter sampling (selective for small sized shell) and time-restricted direct search („timed search” for short, effective for collect bigger shells) (Cameron és Pokryszko 2005; Sólymos et al. 2007a) 1 liter soil and litter sample were collected in each sampling location. Concerning of live tree (T), dead wood (D) and rock (R), litter samples were collected adjacent to the sampled trees or rocks, and not from the structure themselves. While samples of litter microhabitat were taken from at least 2 m from other sampled microhabitats. Timed search took five minutes per replicate in a 1 m radius of litter sampling, but no more than 25 cm from the sampled live tree, dead wood or rock. Altogether we collected 256

samples. If the sample of a sampling location did not contain individuals, it was registered as „empty-sample”. During the taxonomic identification we categorized the shells according to extent of their deterioration (Domokos 1995).

Data analysis were carried out by R program packages (R Development Core Team 2012). We used the `lm` function for linear modeling (R Core Team 2012), the `glmer` function in the `lme4` package for mixed effect models (Bates et al. 2012), and the `dispindmorisita` function in the `vegan` package (Oksanen 2012) for quantifying interspecific aggregation.

Since we observed the bias of the amount of broken shells among microhabitat types from the beginning of our work, we carried out the analysis of shell accumulation using the data of the first two fully processed dolinas. The examined 64 samples were classified by sampling methods and microhabitat types, Species were grouped according to family-group (Clausiliidae, Helicoidea, Zonitidae s. lat. [including Oxychilidae, Pristilomatidae and Daudebardiidae] and other families), than they were categorized according to adult shell size (major shell dimension is smaller or greater than 5 mm). We used logistic regression to determine the relation among the proportion of broken shells and sample type (microhabitat type, sampling method) or species specific category (taxonomy, adult size).

To identify the microhabitat associations for each of the 39 species *starta* (collections of microhabitat types) were created by binary classification in a way that the abundance should be significantly higher in the stratum than among them. Then we used Poisson generalized mixed effect models with log link. We did not combine counts from litter samples and timed searches, but we used a fixed effect describing methodologies as covariate (0 for litter samples, 1 for timed search samples). Random effects were introduced to deal with the non-independence of the samples taken at the same locations for the two methods. The mixed model consisting of only the method variable and the random effects was considered the null model (abbreviated as LTDR, this refers to the microhabitat types associated with high abundance, in this case all four of them). We introduced dummy variables describing high and low abundance strata based on different combinations of the microhabitat types. This provided us 14 different combinations besides the null model to depict the high abundance stratum. We used these dummy variables to fit all possible models and calculated model weights based on Akaike's information criteria (AIC) values. We selected the model with the highest model weight as the best supported microhabitat association for that species. We used additional fixed effects accounting for environmental variation within strata (litter moisture, litter thickness, exposure and vertical zone). The concentration of model support with respect to the total of 15 models was quantified using the Simpson index calculated from the model weights (Burnham and Anderson 2002) as probabilities.

We used linear model to exploring the relation among the species richness and the environmental predictor variables. Species richness as response variable was calculated

based on detection/non-detection data from all species found in the litter and timed search data combined. The response variable was $\log(x + 1)$ transformed to ensure valid support in a general linear model. Predictor variables consisted of microhabitat as a categorical variable, aspect (0=north and flat, 1=south, 0.5=east and west), vertical zone (ordered categories, 0=bottom, 1=middle, 2=edges and outside of dolinas), litter moisture (ordinal, 1-3), and litter depth (in cm). The full model included all predictors as main effects and all second order interactions. The model best supported by the data was selected using backward stepwise model selection based on AIC values.

We calculated the Morisita index of aggregation for each species to compare the aggregation patterns within the low vs. high abundance strata. The Morisita index assumes a Poisson count distribution; the expected index value is 1 for random distribution and higher for aggregated distribution. Counts were based on either litter samples or timed search, whichever had higher total count for the respective species. Morisita index was calculated for species with at least 10 total counts from each abundance stratum are shown (based on litter samples or timed searches, whichever had higher total count).

With detections from both litter and timed search samples at each sampling location, we calculated pairwise Jaccard's similarity index (Podani 2000) based on non-empty samples with total number of detections higher than zero ($n = 244$). We plotted summary statistics (median, interquartile range, extreme values) of similarities within and between microhabitat types to draw conclusions about average species turnover within and among microhabitats. We used boxplot with notches to test if medians of similarity differ among groups (Chambers et al. 1983).

2.2. Habitat assessment

We collected land snails in the three forest reserve: Alsó-hegy (Aggtelek National Park), Haragistya–Lófej (Aggtelek National Park) and Ropoly (Duna–Dráva National Park). Timed restricted direct search was used in the core area of all the three reserves. Sampling took eight minutes per sample in a 50*50 cm quadrat. We investigate three-three, 1 ha in extent part of coreareas of each forest reserve, 30 samples were taken per hectare. Analysis contained both fresh and broken shells (Rundell and Cowie 2003, Pearce 2008). However species with shells less than 5 mm were excluded from the assessment, because the selectivity of timed restricted direct search method (Sólymos et al. 2007a). Thus data of 611 individuals of 21 land snail species were used in this part of our work (Alsó-hegy 14 species, 235 individuals; Haragistya–Lófej: 10 species, 205 individuals; Ropoly 13 species, 171 individuals). We assessed the performance of five different measures of habitat quality frequently used in practical nature conservation works. We used species richness (not

sensitive to species rarity), Shannon's diversity index (sensitive to local scale rarity), mean rarity index (mean of regional rarity scores of species), and two indices that combine the local and regional commonness/rarity of a species [regional rarity scores weighted by relative frequency (N) or by its reciprocal value (1/N)]. Local rarity was based on the relative frequencies of species calculated from the sample counts. For regional rarity of species, we followed the conservation prioritization scheme developed for the Hungarian mollusc fauna (Mollusca Rarity Index, MRI for short) (Sólymos 2004). The geographical distribution of land snails were into three groups: the distribution of species cover 1) whole Europe or larger area, 2) Central-Europe or 3) the Alpin-Carpathian region (Bertrand 2006). The faunas of each forest reserve were compared by Jaccard index.

3. Results

3.1. Effects of microhabitats and environmental variables on quantitative relations of living and dead snail communities

During the investigation of shell accumulation, we found differences among the samples of the two sampling method in the proportion of broken shells and proportion of small- and large shell sized taxa. The samples of litter sampling contained more broken shells (805) and more small (less than 5 mm) sized shell (1159) than the samples of timed search (broken shell: 198, small sized shells: 22). In the case of microhabitats, most number of broken shells were found in rock (370) and fewest in deadwood microhabitats (118). The proportion of broken shells were also highest in rock microhabitat, while lowest in dead wood. According to the comparison of sampling methods the samples of litter sampling contained higher proportion of broken shells than those of timed search. However, the interaction of sampling method and microhabitat showed that the timed search resulted higher proportion of broken shells in the case of the rock microhabitat, whereas there was no significant interaction in the case of litter as microhabitat and live trees. The proportion of broken shells biased significantly among the taxonomic groups and shell size categories. We found higher proportion of large sized species, most of the specimens belonged to Helicoids, but we also showed differences among the smaller (*Daudebardia*, *Vitrea*) and larger (*Oxychilus*, *Aegopinella*) sized species of Zonitids, member of latter group tend to accumulate more.

Fourteen out of the 39 examined species were too rare (less than 20 individuals altogether) in our samples to reliably determine habitat associations. The model showed only *Cochlodina orthostoma* as a generalist, with no distinct microhabitat associations. A generalist classification for *Acanthinula aculeata* and *Cochlodina laminata* was also supported by model weights. Low Simpson index for these two species indicated ambiguous model identification by highest model weight. Two species (*Aegopinella minor* and *Punctum pygmaeum*) were most abundant in samples of litter and rock microhabitats, and five species (*Alinda biplicata*, *Discus perspectivus*, *Helicodonta obvoluta*, *Monachoides vicinus* and *Vitrea diaphana*) were associated with dead wood and rock microhabitats. The remaining 17 species were associated with single microhabitat type, mostly with rock (12 species). Two species (*Bulgarica cana* and *Cochlodina cerata*) were associated with live trees, and also two species showed higher abundance in coarse woody debris (*Macrogaster borealis bielzi*, *Macrogaster ventricosa*). No species was found to be exclusively litter specialist (Fig. 1.).

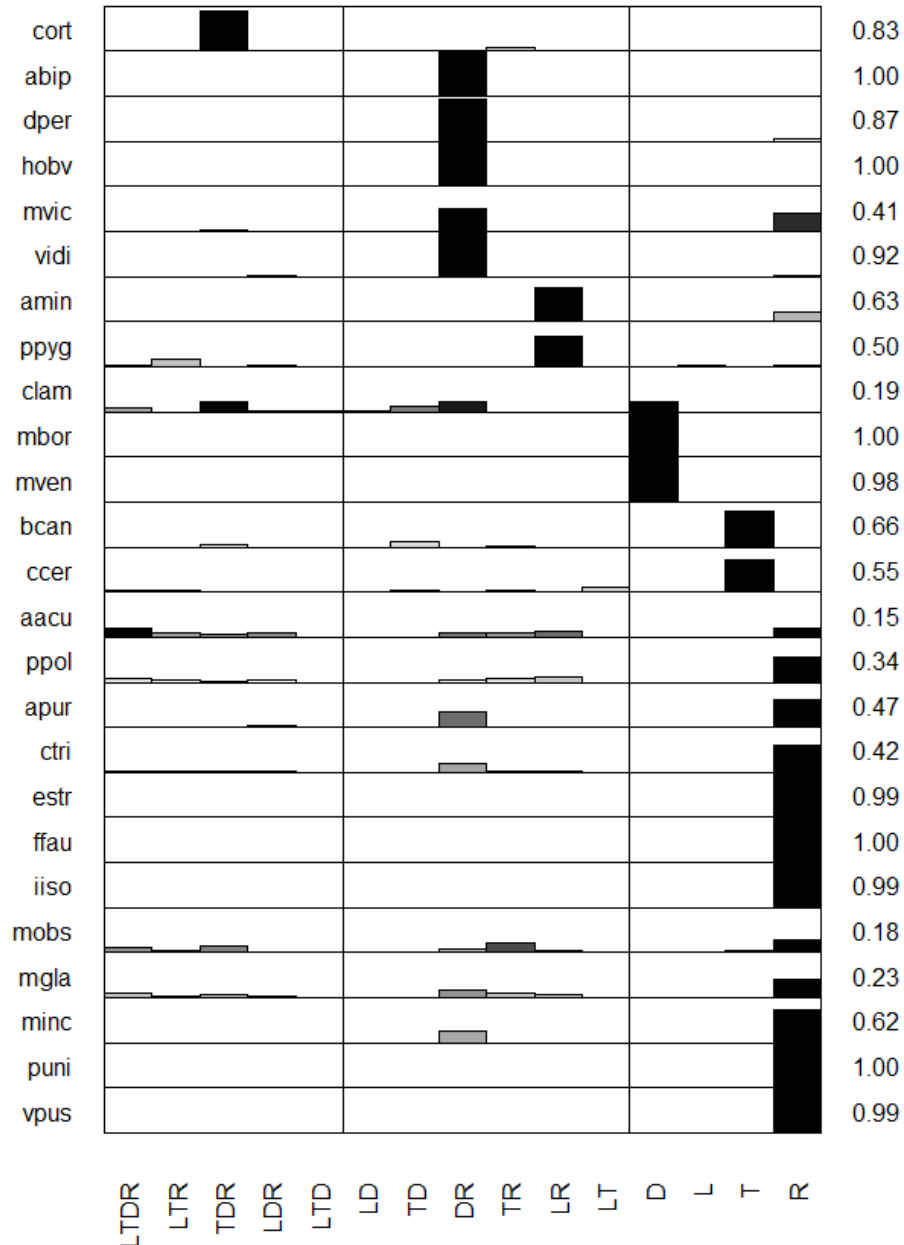


Fig. 1.: Microhabitat associations for 25 land snail species based on model weights from mixed effects count models, 14 species with total count less than 20 individuals were omitted from the figure. Rows represent species.

Columns represent microhabitat association types, labels refer to the high abundance stratum (combination of microhabitat types: L=litter, T=live tree, D=dead wood, R=rock). Bars and shading in each cell represent model weights, higher and darker bars stand for more concentrated model support for particular model(s). Concentration of model support is measured by the Simpson coefficients placed at the right sides of the panels.

cort = *Cochlodina orthostoma*, abip= *Alinda biplicata*, dper= *Discus perspectivus*, hobv= *Helicodonta obvolvata*, mvic= *Monachoides vicinus*, vidi= *Vitrea diaphana*, amin= *Aegopinella minor*, ppyg= *Punctum pygmaeum*, clam= *Cochlodina laminata*, mbor= *Macrogastrea borealis*, mven= *Macrogastrea ventricosa*, bcan= *Bulgarica cana*, ccer= *Cochlodina cerata*, aacu= *Acanthinula aculeata*, ppol= *Platyla polita*, apur= *Aegopinella pura*, ctri= *Carychium tridentatum*, estr= *Euomphalia strigella*, ffau= *Faustina faustina*, iiso= *Isognomostoma isognomostomos*, mobs= *Merdigera obscura*, mgla= *Morlina glabra striaria*, minc= *Monachoides incarnata*, puni= *Petasina unidentata*, vpus= *Vertigo pusilla*

We calculated the value of Morisita index for 19 species in low and high abundance strata. The values of the two strata were similar in the case of two species (*Aegopinella minor* and *Punctum pygmaeum*) associated to litter and rock microhabitats, while for the rest of the species the value of the index was on average 3.93 times higher in the low abundance stratum than in the high abundance stratum. Median of the Jaccard's similarity among locations was lowest within live tree microhabitat (0.20), higher in litter microhabitat (0.25), and highest in coarse woody debris (0.35) and rock (0.39). The analysis among microhabitats showed that the median of similarity index was lowest among litter – coarse woody debris (0.18) and litter – rock (0.18) pairs, and was highest between coarse woody debris and rock microhabitats (0.31). Median values within and among the groups differed quite substantially according to the non-overlapping notches in Fig. 2. Quantiles showed the similar pattern, however interquartile ranges overlapped mostly for between microhabitat comparisons.

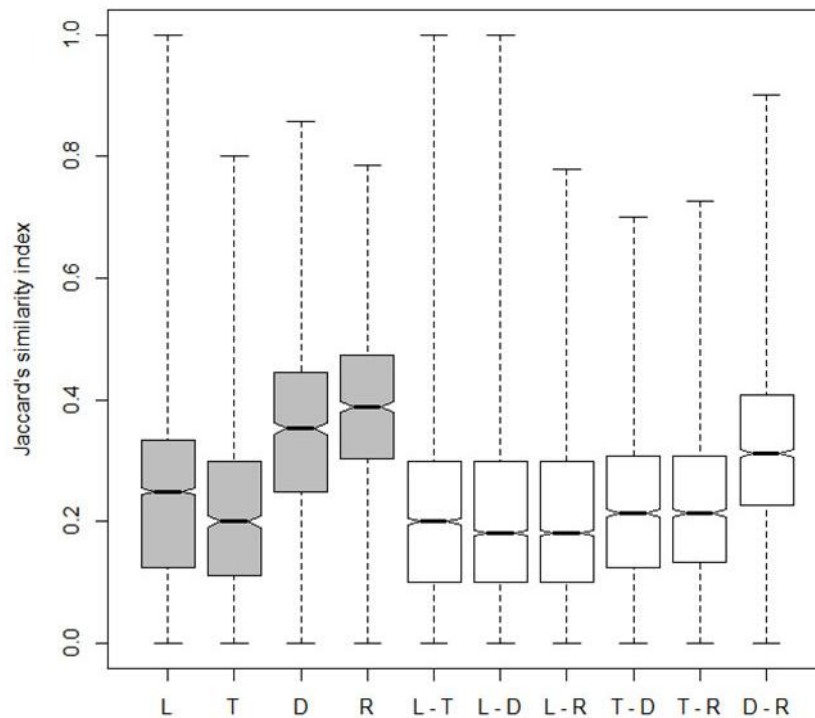


Fig. 2.: Summary statistics for pairwise Jaccard's similarity indices among locations (n = 244) within (grey boxes) and between (white boxes) microhabitat types (L=litter, T=live tree, D=dead wood, R=rock). If the notches of two plots do not overlap this is 'strong evidence' that the two medians (midline) differ. Boxes represent the interquartile range, whiskers represent extreme values.

The species richness of land snails was mainly affected by microhabitat type, vertical zone and litter moisture. Since litter depth was correlated with microhabitat type, while aspect was correlated with litter moisture, these variables were not part of the best supported final model. Significant interaction were found between vertical zone and microhabitat indicating

that zone effect differently on the species richness of each microhabitats. We showed high number of species regardless of zone in dead wood microhabitat, while in the case of other three microhabitat types the species richness declined from the bottoms of dolinas to the edges (Fig. 3.). The decline was significant in litter microhabitat.

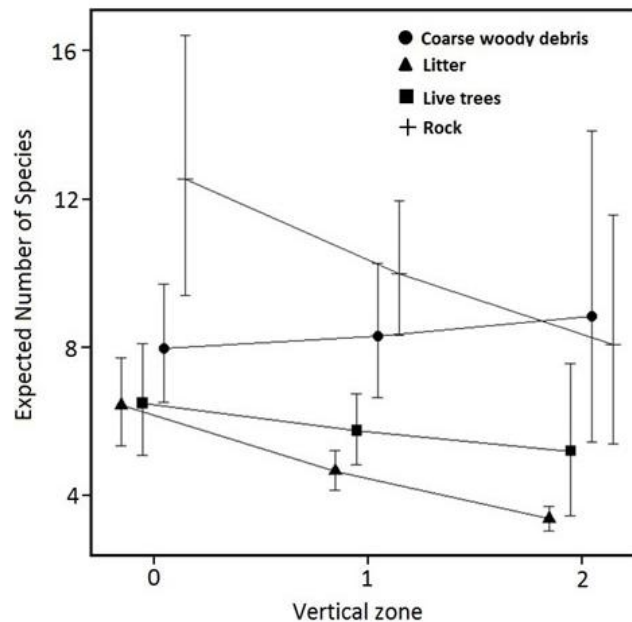


Fig. 3.: Expected number of species as a function of microhabitat type and vertical zones within dolinas based on the general linear model after controlling for other variables in the model. Whiskers represent 95% confidence intervals around point estimates.

3.2. Habitat assessment

Five out of 21 species were found in all of investigated forest reserve (*Cochlodina laminata*, *Aegopinella minor*, *Euomphalia strigella*, *Monachoides incarnatus*, *Helicodonta obvoluta*). All these are common or very common european species (MRI: 2–5 points). Six species were showed out from two forest reserves. Three of them come from Alsó-hegy – Haragistya-Lófej and three from Alsó-hegy – Ropoly forest reserves. Species found in the pair of Haragistya-Lófej - Ropoly were not found. Two out of three species found only in Alsó-hegy and Ropoly forest reserves were Central-European (*Alinda biplicata*, *Laciniaria plicata*), while one species (*Discus perspectivus*) was Alpine-Carpathian. All species were common (MRI: 4–5 point). One out of three species occurring in Alsó-hegy és a Haragistya-Lófej forest reserves was a very common European species (*Euconulus fulvus*, MRI=3) and two were rare Alpin-Carpathian (*Cochlodina cerata*, *Monachoides vicinus*; MRI=7). 11 species were found in only one forest reserve.

Table 1.: List of collected species with their MRI values, number of individuals in each forest reserve and the habitat quality measures. (P: protected species)

distribution	Species	MRI	Alsó-hegy	Haragistya-Lófej	Ropoly
alpin-carpathian	<i>Cochlodina cerata</i> (Rossmäsler, 1836)	7 (P)	9	7	
	<i>Macrogastra borealis</i> (O. Boettger, 1880)	7	1		
	<i>Monachoides vicinus</i> (Rossmäsler, 1842)	7 (P)	7	11	
	<i>Petasina unidentata</i> (Draparnaud, 1805)	6 (P)	6		
	<i>Discus perspectivus</i> (Megerle von Mühlfeld, 1816)	5	40		9
central-european	<i>Bulgarica cana</i> (Held, 1836)	7	2		
	<i>Cochlodina orthostoma</i> (Menke, 1830)	6 (P)		1	
	<i>Aegopinella ressmanni</i> (Westerlund, 1883)	6			22
	<i>Macrogastra plicatula</i> (Draparnaud, 1801)	6			72
	<i>Helicodonta obvoluta</i> (O. F. Müller, 1774)	5	39	39	12
	<i>Macrogastra ventricosa</i> (Draparnaud, 1801)	5			2
	<i>Alinda biplicata</i> (Montagu, 1803)	4	8		3
	<i>Laciniaria plicata</i> (Draparnaud, 1801)	4	1		14
	<i>Aegopinella minor</i> (Stabile, 1864)	3	92	81	6
	<i>Helix pomatia</i> Linnaeus, 1758	3 (P)			1
european	<i>Monachoides incarnatus</i> (O. F. Müller, 1774)	3	6	52	24
	<i>Aegopinella pura</i> (Alder, 1830)	5		3	
	<i>Euconulus fulvus</i> (O. F. Müller, 1774)	3	1	4	
	<i>Euomphalia strigella</i> (Draparnaud, 1801)	3	9	2	1
	<i>Cochlodina laminata</i> (Montagu, 1803)	2	14	5	4
	<i>Zonitoides nitidus</i> (O. F. Müller, 1774)	2			1
	Total number of individuals		235	205	171
Number of species		14	10	13	
Shannon index		1.90	1.60	1.87	
Mean of MRI		4.71	4.40	3.92	
Lokális gyakorisággal (N) súlyozott MRI átlag		4.05	3.75	4.99	
Lokális ritkasággal (1/N) súlyozott MRI átlag		4.95	4.73	3.16	

We found three rare species in Alsó-hegy (two Alpine-Carpathian: the protected *Petasina unidentata*, and *Macrogastra borealis* and one Central-European: *Bulgarica cana*; MRI: 6–7). Among the two species of Haragistya–Lófej forest reserve there is a common European species (*Aegopinella pura*) and a rare Central-European (*Cochlodina orthostoma*). One out of five species collected exclusively in Ropoly was very common European (*Zonitoides nitidus*) and four were Central-European. Two of the latter mentioned species were rare (*Aegopinella ressmanni*, *Macrogastra plicatula*; MRI: 6), one was common (*Macrogastra ventricosa*) and one very common (*Helix pomatia*) (Fig.4.).

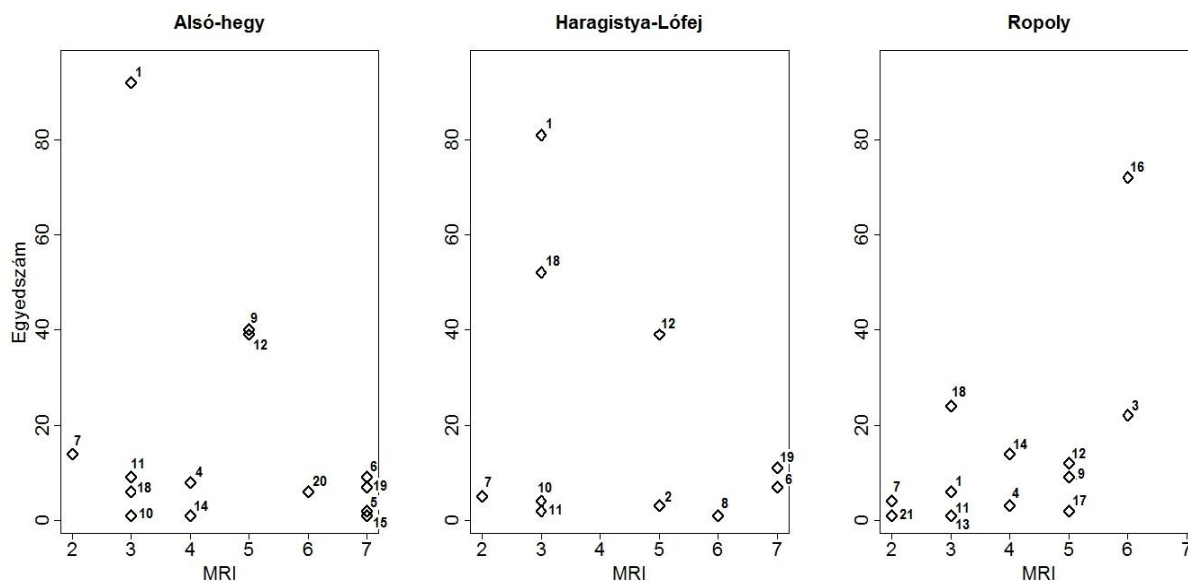


Fig 4.: The number of individuals according to their MRI values, in each forest reserve..

1: *Aegopinella minor*; 2: *A. pura*; 3: *A. ressmanni*; 4: *Alinda biplicata*; 5: *Bulgarica cana*; 6: *Cochlodina cerata*; 7: *C. laminata*; 8: *C. orthostoma*; 9: *Discus perspectivus*; 10: *Euconulus fulvus*; 11: *Euomphalia strigella*; 12: *Helicodonta obvolvata*; 13: *Helix pomatia*; 14: *Laciniaria plicata*; 15: *Macrogastra borealis*; 16: *M. plicatula*; 17: *M. ventricosa*; 18: *Monachoides incarnata*; 19: *M. vicinus*; 20: *Petasina unidentata*; 21: *Zonitoides nitidus*.

We assessed the similarity of forest reserves by using Jaccard index. We found the faunas of Alsó-hegy and Haragistya–Lófej reserves similar to each other (0.5), while the Ropoly differed from both of them (Alsó-hegy – Ropoly: 0.421; Haragistya – Ropoly: 0.278).

Investigating the habitat quality measures we examined firstly the two widely used diversity indices: the number of species (S) and the Shannon-index (H'). In this case the values of Alsó-hegy and Ropoly were similar (S= 14 ill. 13; H'=1.90 ill. 1.87, in order), while the Haragistya–Lófej showed the lowest values (S= 10; H'= 1.60) (Table 1.). Than MRI values, which taking into consideration of regional and local rarity of the species, were ordered to each species (Fig. 4.) and the mean of MRI was calculated for each forest reserve by using the number of species. Thus we got the following, descending order Alsó-hegy (4.71) > Haragistya–Lófej (4.40) > Ropoly (3.92) (Table 5.). We found similar result if the MRI was weighted by local rarity (1/N), due to the species with high MRI value living in Alsó-hegy and Haragistya–Lófej forest reserves. The values are declining from Alsó-hegy to Ropoly [Alsó-hegy (4.95) > Haragistya–Lófej (4.73) > Ropoly (3.16)]. But if we weighted the MRI with local abundance the order were changed: Ropoly (4.99) > Alsó-hegy (4.05) > Haragistya–Lófej (3.75), due to the regionally rare but locally abundant species from Ropoly forest reserve (Table 1.).

4. Discussion

In the first part of present study I aimed to examine the impact of environmental factors on snail species richness and abundance in a micro-scale analysis with well replicated natural experiment using a balanced sampling design. We studied four distinct microhabitat types (litter, live trees, dead wood, rock) in 16 dolinas of the Alsó-hegy (Gömör-Tornai Karst, Aggtelek National Park) considering also topographic factors. Two complementary sampling methods (litter sampling and timed restricted direct search) were applied. This complex dataset allowed simultaneous analysis of habitat requirements of individual species and investigation of the effect of biases of the applied sampling methods on the results of ecological inferences. While in the second part we assessed the habitats based on their land snail fauna.

The results underlined that the main bias of the two widely used, above mentioned sampling methods derives from the body- and shell sizes of the molluscs effectively collected by them (Cameron és Pokryszko 2005; Sólymos et al. 2007a). In addition, present study found further differences significantly affecting the results. The proportion of broken shells was significantly higher in the samples collected by soil plus litter sampling than by time-restricted direct search. The proportions of broken shells also varied according to their taxonomic group (Menez 2002). Helicoids tended to accumulate more than other groups. This can be attributed to their relatively large, thick shells compared to Zonitids or other smaller sized and thinner shelled taxa. In the case of Clausiliids, although their shells are also durable, we could not find significant shell accumulation. The reason might be that this species group is associated with the coarse woody debris, where intensive microbial (decaying) processes are taking place (Jönsson et al. 2008). Opposite processes might run in rock microhabitat, where several species live in the crevices or under the stones and their shells were not exposed to the chemical and physical effects, thus the shell degradation will be slower (Thurman et al. 2008). In accordance with these findings, the analysis of the proportion of broken shells among microhabitats showed that the rate of shell accumulation was less in the case of dead wood and highest in the rock microhabitat. Thus the observed difference of shell accumulation rate in relation to shell size and taxa also might account for microhabitat preference. Although broken shells could be used as indicators in comparisons similar habitats (Rundell és Cowie 2003), according to our results the examination of shell accumulation is necessary in the case of microhabitat scale surveys. If significant bias were found in the proportion of fresh and broken shells, it is appropriate to use only fresh specimens, thus the results would be more comparable.

We assessed microhabitat associations for 39 land snail species based on multimodel inference and generalised linear mixed models. Patterns of microhabitat

associations were highly nested among microhabitat types with high number of specialist species in dead wood and in rock microhabitats. We did not find generalist species associated similarly to all four microhabitats. Species (*Cochlodina orthostoma*, *Acanthinula aculeata*, *Cochlodina laminata*) showed generalist by the model, were also less abundant in litter. Most species associated only one microhabitat preferred rock, somewhat fewer dead wood. High proportion of these species are considered as "hygrophilous" species (Kerney és mtsai 1983, Welter-Schultes 2012). These findings are comparable with former results that species richness of land snails increase near lime rock outcrops (Nekola 1999) while on smaller scale dead wood provide favourable microclimate for them (Kappes et al. 2006). We found that species richness in dead wood and rock microhabitats remained high irrespective of the topographic effects, as opposed to litter and live tree microhabitats, where richness decreased with drier microhabitat conditions due to topography. Thus the litter microhabitat provide favourable environmental conditions for specialist (hygrophilous) species only in short temporary periods otherwise impede the colonization of new habitat patches (Müller et al. 2005).

According to Morisita index the micro-scale environmental factors have strong effect on the abundance of local snail fauna. The abundance varied greatly within optimal microhabitat while reached evenly low values in suboptimal patches. Predictability of community composition was more reliable within favourable habitats (high abundance strata, dead wood and rock), because of the high number of coexisting species than in the more available, but unfavourable patches (low abundance strata, litter live tree). This could be interpreted as a "crowding effect", that the saturation of favourable, but due to the spatial distance, less available patches is higher both in term of species richness and abundance. The spatial distribution and availability also affect on population dynamics. High quality habitats (such as dead wood or rock) have uneven spatial distribution. Furthermore dead wood has relatively small spatial extent and characterized by intensive microbial and fungal decaying processes which might affect on colonization- extinction dynamic of land snails in a way that we were not able to recognize in this study. The nested pattern of microhabitat association showed that rock and dead wood microhabitats, which may shelter resource populations, have key role in the maintenance of local snail fauna due to their optimal microclimate and humidity, and the long term stability in the case of rock. But even these favourable microhabitats could not provide optimal environment for all the species and ensure their long term survival. The high number of species of the dolinas' bottom and north facing slopes drive from the provided optimal local moisture conditions (Vilisics et al. 2011, Sólymos et al. 2009c). The topographical factors might create "microrefugia" (Dobrowski 2011), which can shelter against unfavourable environmental changes. Such microrefugia or microhabitats providing favourable condition, would locally shelter the invertebrate population

against the current climatic changes (Parmesan 2006). Regional habitat protection strategy which taking into consideration the topographic heterogeneity and availability of microhabitats as well as ensure the large scale ecological processes could contribute the protection and maintenance of soil invertebrates (Noss 1990, Haufler et al. 1996). This might preserve the natural ecosystem and forest dynamic with the supporting local fauna.

In the second study, we assessed the performance of five different measures of habitat quality based on the fauna of three Hungarian forest reserves [Alsó-hegy, Haragistya–Lófej (both in the Aggtelek National Park) and Ropoly (Duna–Dráva National Park)]. The number of species were average in all the three reserves (Farkas 2005, 2008, Uherkovich and Varga 2005) due to the applied sampling method (Cameron és Pokryszko 2005; Sólymos et al. 2007a) and the design of sampling (species associated to rock surface were not include by samples). We found that the local land snail fauna was mainly affected by biogeographical distance of the reserves and habitat naturalness. This phenomena appeared through the abundance and distribution of rare species. The comparison of the fauna of forest reserves showed that the local fauna was highly affected by regional fauna, thus the geographically closer reserves have similar fauna. Species occurring in all or in the two distant reserves have wider range (and lower MRI value) than most of the species found only in one or in the two close reserves. These effects could be followed through the species composition of investigated areas. Due to the vicinity of the Carpathians several Alpine-Carpathian species (e.g. *Cochlodina cerata*, *Petasina unidentata*) occurring in Alsó-hegy and Haragistya-Lófej forest reserves (both situated in North-Hungarian Mountains), while these species are missing from Ropoly which is isolated from mountains. However Ropoly has other valuable species (*Aegopinella ressmanni*) not occurring in the other investigated reserves. This shows that the snail faunas mainly reflect the naturalness and the differences could be explain by biogeographical reasons and not by former or recent human activity. (However the recorded low number of species and individuals in Haragistya-Lófej reserve might caused by intensive forest management). The number of species and Shannon index as rough estimates, reflect the research-oriented hierarchical classification of reserves (Alsó-hegy > Ropoly > Haragistya-Lófej; Horváth és Bölöni 2002). While the indices created by the different weighted MRI showed more variable ordering. If we weighted MRI with local rarity the two northern reserves Alsó-hegy and Haragistya-Lófej reached high values due to their both locally and regionally rare Alpine-Carpathian species. But when the MRI were weighted by local abundance the Ropoly got the highest value to its regionally rare but locally frequent species (*Macrogastera plicatula* and *Aegopinella ressmanni*). These results suggest that habitat quality assessments should rely on different and complementary indices. Incongruences of multiple indices can help in identifying the conservation value of habitats.

New scientific results

- 1) This study showed that the selectivity of used sampling methods (litter sampling and timed search) biased according to shell size and the proportion of broken shells. **The proportion of broken shells differed significantly among the samples of each sampling methods. In the case of three microhabitats, the samples of litter sampling contained more broken shells than the samples of timed search**, while the samples of rock microhabitat contained more broken shells with timed search.
- 2) According to our results, **the collected samples significantly differ by the amount of contained broken shells** depending on features and running biological processes of microhabitat types. Thus the accumulation rate of broken shells might affect the results of small-scale ecological investigations.
- 3) **The investigation of broken shells based on taxonomic groups showed the bias of shell accumulation rate and this could affect the results of ecological studies.** According to our results the shells of Helicoids tended to accumulate more in a microhabitat, than the shells of Zonitids or Clausilids.
- 4) **The results on microhabitat association of 25 species showed that most species rely on one or two, well defined microhabitat type.** Most specialist species (12) were found in rock microhabitat. *Macrogaster borealis bielzi* and *Macrogaster ventricosa* reached their highest number of individuals in dead wood microhabitat, while *Bulgarica cana* and *Cochlodina cerata* showed highest abundance near live trees. Two (*Aegopinella minor* and *Punctum pygmaeum*) out of seven species associated to two microhabitat types preferred litter and rock microhabitats, while five species (*Alinda biplicata*, *Discus perspectivus*, *Helicodonta obvoluta*, *Monachoides vicinus*, *Vitrea diaphana*) tend to be more abundant in deadwood and rock. Our results showed only few generalist species (*Cochlodina orthostoma*, *Acanthinula aculeata*, *Cochlodina laminata*).
- 5) **The results of intraspecific aggregation showed even distribution of specimens in favourable microhabitats (high abundance strata) than in suboptimal (low abundance strata) ones.**
- 6) **Comparison of patches within and among microhabitats showed that rock and dead wood are more consistent microhabitats.**
- 7) **Species richness was influenced by microhabitat types, topographic factors and local moisture conditions.** According to the interaction among microhabitat types and vertical zone the sample position within a dolina affect differently the species richness of different microhabitat types.

- 8) **We found that the local land snail fauna was mainly affected by biogeographical distance of the reserves and the habitat naturalness. This phenomena appeared through the abundance and distrubution of rare species.** The fauna of the geographically closer reserves were similar. Species occuring in all or in the two distant reserves have wider range (and lower MRI value) than most of the species found only in one or in the two close reserves.
- 9) **The number of species, the Shannon index and the measures created by different weighted of MRI showed variable order of priority among forest reserves.** The number of snail species and Shannon index as rough estimates, reflec to the reasearch-oriented hierarchical classification of forest reserves (Alsó-hegy > Ropoly > Haragistya-Lófej; Horváth és Bölöni 2002). While the indices created by the different weighted MRI showed more variable ordering. If we weighted MRI with local rarity the two northern reserves Alsó-hegy and Haragistya-Lófej reached hight values due to their both locally and regionally rare Alpine-Carpathian species. But when the MRI were weighted by local abundance the Ropoly got the hihgest value to to its regionally rare but locally frequent species (*Macrogastrea plicatula* and *Aegopinella ressmanni*).

Publications related to the dissertation

Publications in peer-reviewed journals with impact factor

Kemencei Z., Farkas R., Páll-Gergely B., Vilisics F., Nagy A., Hornung E., Sólymos P.

Microhabitat associations of land snails in forested dolinas: implications for coarse filter conservation. *Communtty Ecology*, 15: 180-186, 2014

IF₂₀₁₃: 1,200

Sólymos P., **Kemencei Z.**, Páll-Gergely B., Farkas R., Vilisics F., Hornung E.: **Does shell accumulation matter in micro scale land snail surveys?** *Malacologia*, 51:(2) pp. 389-393, 2009

IF₂₀₀₉: 1.440

Publications in peer-reviewed journals without impact factor

Kemencei Z., Hornung E., Vilisics F., Sólymos P.: **Élőhelyek természetvédelmi értékelése szárazföldi csigák segítségével erdőrezervátumok példáján bemutatva – [Habitat quality assessment based on species rarity: case study of land snails in Hungarian forest reserves]** *Állattani Közlemények* 96(1-2): 91-100, 2011

Sólymos P., Farkas F., **Kemencei Z.**, Páll-Gergely B., Vilisics F., Nagy A., Kisfali M., Hornung E.: **Micro-habitat scale survey of land snails in dolines of the Alsó-hegy, Aggtelek National Park, Hungary.** *Mollusca* 27:(2) pp. 167-171, 2009

Sólymos P., Vilisics F., **Kemencei Z.**, Páll-Gergely B., Farkas R., Nagy A., Kisfali M., Hornung E.: **Globális változás, lokális túlélés: kitettség és nedvességi grádiens hatása avarlakó gerinctelenekre.** – [Global change, local survival: effects of aspect and moisture on epigeic invertebrate communities] *Természetvédelmi Közlemények* 15: pp. 396-411, 2009

Datasets

Sólymos P., **Kemencei Z.**: **Methodological study data set of land snails from the Dolina 2007 project.** Dataverse Network Study, 2008
<http://thedata.harvard.edu/dvn/dv/psolymos>

Sólymos P., **Kemencei Z.**, Farkas R., Páll-Gergely B.: **Complete data set of land snails from the Dolina 2007 project collected by time restricted search.** Dataverse Network Study, 2009 Study, 2009 <http://thedata.harvard.edu/dvn/dv/psolymos>

Conference presentations

Presentations with abstract:

Kemencei Z., Hornung E., Sólymos P., Vilisics F.: **Szárazföldi csigák ritkaságán alapuló minősítési módszerek tesztelése erdőrezervátumok példáján.** — 4. Szünzoológiai Szimpózium, Magyar Természettudományi Múzeum, Budapest, 9. April 2010.

Kemencei Z., Sólymos P., Farkas R., Páll-Gergely B., Vilisics F., Hornung E.: **Land snails on micro scales: interesting patterns and methodological challenges** — Molluscan Forum, London (Egyesült Királyság), 2008

Kemencei Z., Sólymos P., Páll-Gergely B., Farkas R., Vilisics F., Hornung E.: **Kulcs élőhelyek szerepe a környezeti szélsőségek mérséklésében: csigák fajgazdagsága az Aggteleki-karszt töbreiben** — 3. Kvantitatív Ökológiai Szimpózium, Budapest, 18–19. March 2008.

Presentations without abstract:

Kemencei Z., Hornung E., Sólymos P.: **Esettanulmány erdőrezervátumok molluscafaunájának természetvédelmi értékelésére.** — Magyar Biológiai Társaság Állattani Szakosztályának 980. előadóülése, 2. December 2009.

Kemencei Z., Sólymos P., Páll-Gergely B., Farkas R., Vilisics F., Hornung E.: **Szárazföldi csigák mikroskálán: kulcs élőhelyek és módszertani kihívások** — Magyar Malakológus találkozó, 19. September 2009.

Posters

Kemencei Z., Hornung E., Sólymos P., Vilisics F.: **Habitat quality assessment based on species rarity: case study of land snails in Hungarian forest reserves.** — 24th International Congress for Conservation Biology (ICCB 2010), Edmonton, Canada, 3-7. July 2010.

Kemencei Z., Sólymos P., Farkas R., Páll-Gergely B., Vilisics F., Hornung E.: **Key habitats on micro-scale: where rare species are common** — 2nd European Congress of Conservation Biology, Prague, Czech Republic: 1-5. September 2009.

Kemencei Z., Sólymos P., Páll-Gergely B., Farkas R., Vilisics F., Hornung E. (2009): **Öröm az ürömben: csigafajok élő egyedszámának modellezése üres csigahéjak figyelembe vételével** — 8th. Hungarian Congress of Ecology, Szeged: 28-26. August 2009.

Kemencei Z., Sólymos P., Farkas R., Páll-Gergely B., Vilisics F., Hornung E.: **Effects of shell accumulation in micro-scale land snail surveys** – 10th central European workshop on soil zoology, České Budějovice, Czech Republic: 21-24. April 2009.

Kemencei Z., Sólymos P., Farkas R., Páll-Gergely B., Vilisics F., Hornung E.: **Kulcs élőhelyek mikroskálán: ahol a ritka csiga fajok gyakoriak** — V. Hungarian Conference on Conservation Biology, Nyíregyháza, 6-9 November 2008.

Kemencei Z., Sólymos P., Farkas R., Páll-Gergely B., Vilisics F., Hornung E.: **Key habitat structures shelter land snail assemblages against unfavourable environmental** — EURECO - GFOE 2008 "Biodiversity in an Ecosystem Context" – Lipcse, Germany, 15.-19. September 2008.

Acknowledgement

I would like to express my gratitude:

To my supervisor Dr. Erzsébet Hornung and to Dr. Péter Sólymos for their endless patience and personal and professional support through the long years of my PhD work.

To Dr. Gyula Huszenicza and Dr. Miklós Rusvai, the former and the current head of the Postgraduate School of Veterinary Science of Szent István University for the opportunity to fulfil my PhD.

To Dr. Barna Páll-Gergely, Roland Farkas, Dr. Ferenc Vilisics, Máté Kisfali, Dr. Antal Nagy and Anna Kulcsár for their valuable help in fieldwork.

The colleagues of National Institute of Environment and Nature Conservation Department (Ministry of Agriculture) for their personal support.

To Éva Kapcsos and Zoltán Mezei Zoltán for their assistance on the first steps of my scientific endeavour.

To Dr. Zoltán Fehér and Dr. Róbert Gallé for reviewing my work and their comments to improve the final form of this dissertation.

Last, but not least to my Mother, my family and my friends for their patience and encouragement.

This work was supported by a grant (17422) from Postgraduate School of Veterinary Science of Szent István University

Collecting permit numbers for my work: 10944–4/2009 in the case of Alsó-hegy and Haragistya–Lófej forest reserves and 5410–4/2009 in the case of Ropoly forest reserves.