A NEW APPROACH TO STUDYING AND SAMPLING LAND MOLLUSCS: HABITAT STRUCTURE AND THE EFFECTS OF SCALE ON LAND MOLLUSCS

ALEX MENEZ

The University of Wales, Cardiff and the Gibraltar Museum, Gibraltar

Abstract *Many studies have demonstrated associations between the distributions and diversities of land molluscs at large scales. We know very little, however, about the importance of factors at small scales and almost nothing about the effects of scale variance. Quantitative sampling of land molluscs and measurement of environmental variables at fine scales allows these effects to be studied. Using this approach it is possible to: (1) assess the effects of environmental variables such as components of habitat structure on diversity across scales and, (2) determine sampling efficacy. Data from locations in southern Iberia show that increased heterogeneity is associated with a higher number of species, but not with higher abundances of species, and that there are significant changes across scales for number of species, abundances and diversity.*

Key words *Land molluscs, sampling, diversity, heterogeneity, complexity, habitat structure, species estimators, southern Iberia*

A large number of studies have demonstrated associations between the distributions and diversities of land molluscs and large-scale factors such as climate, altitude, geology and habitat type (Cain, 1983; Solem, 1984; Cook, 2001; Aubry *et al*., 2005). We know very little, however, about how important factors are at scales of about 50x50m and almost nothing at all about the effects of varying scales, in the same location, on these relationships. Cameron (2002a) has reported that individual quadrats may contain substantial proportions of a regional fauna, and Nekola and Smith (1999) found that the maximal number of species in 0.04m^2 quadrats in Winsconsin Cliff communities accounted for up to 91 percent of maximal number of species in 1m^2 quadrats.

Although there are recently published studies on land mollusc diversity from delimited sampling areas (Waldén, 1981; Emberton, 1995, 1997; Tattersfield, 1996; Winter and Gittenberger, 1998; Cameron, 2000b) and using subsampling (e.g. subsampling a 25m^2 plot using 0.0625m^2 plots (Labaune and Magnin, 2001), and using different points for substratum collection at sites (Nekola, 1999) these have not specifically considered the data from the same locations at varying scales. There are now several studies that have been conducted at sites of 1x1km, often using smaller plots within these (typically 20x20m or 40x40m) to assess land mollusc species diversities (Winter and Gittenberger, 1998; Schilthuizen and Rutjes, 2001; Cameron, 2002). The first studies of this kind were from rainforests and were inspired by the comment from Solem (1984) that in rainforests: 'snails may be abundant on ecotonal fringes, but generally are neither diverse nor abundant'. More recently, Cameron and colleagues (2003) applied a similar protocol in Cretan maquis, that represents the first published study of this type from a Mediterranean area. We are now beginning to accumulate quantitatively collected data from different regions, some quite distant from one another. These data may provide the rudiments of a quantitative model to explain the distributions and diversities of land molluscs on a large scale (Solem, 1984; Gardner, 1998).

The effects of area and of habitat structure have been extensively studied for many taxa and numerous models have been proposed to explain these (Hart and Horwitz, 1991; Brown, 1995; Lomolino, 2000). There are very few studies that have specifically looked at these effects with land molluscs and almost no quantitative data exist (Nilsson *et al*., 1988). A problem with assessments of this kind has been that different elements of structure (e.g. vegetation, soil and rocks) and amounts of these, are often confounded making it difficult to compare results between studies (McCoy and Bell, 1991; Beck, 2000). A habitat structure model proposed by

Contact author : alexmenez@gibtelecom.net

McCoy and Bell (1991) has three axes that take account of ecological relationships that are affected by structure. These are heterogeneity (types of structure, such as rocks and vegetation), complexity (amount of structure), and scale (size of area used to measure heterogeneity and complexity). This model allows the effects of habitat structure to be teased out and quantified across various scales at the same locality, the results of which can then be compared to other localities.

Most land mollusc studies do not include an assessment of the time needed to find most species at a site, nor of the volume of substratum needed to find most of the species contained in the substratum (Menez, 2001). In addition analyses of diversity and habitat relationships have sometimes been based on, or have included, qualitative methods (Barker and Mayhill, 1999; Craw, 2001). The standardization of sampling effort and sample size are important considerations in the design of ecological studies (Southwood, 1978; Schneider, 1994; Bart *et al*., 1998; Magurran, 2004). Bishop (1977), and more recently Menez (2001, 2002), discussed the use of quantitative sampling techniques for land molluscs and for the recording of environmental variables in land mollusc studies. A main obstacle in comparing land mollusc studies is that samples may not be collected in a way that permits estimates of sampling error to be made. A key paper is that by Cameron and Pokryszko (2005) which reviews the sampling literature, highlights problems with methods, and provides advice on sampling issues.

A part of my research in southern Iberia over the last few years has focused on applying the McCoy and Bell (1991) habitat structure model to environmental and land mollusc data that have been collected quantitatively. My aim is to determine relationships between the number of species and their abundances and heterogeneity (types of habitat structure) and complexity (amounts of habitat structure), and how these vary at different scales. The sampling layout is conceptually based on Scheiner *et al.* (2000) and is a hierarchical nested sample design (Boyero, 2003; Fleishman *et al*., 2003) that allows the effects of scale to be analyzed.

A stratified design (Southwood, 1978; Bart *et al*., 1998) is used at each of the 40x40m sites to select the positions of four plots each of 5x5m ($25m^2$). Stratification is used to ensure maximal spread of the plots at the sites by randomly assigning one plot in each of four blocks, this being the stratum of the stratified design. In each plot five quadrats of 1x1m are randomly selected. For each site a sequence is generated such that the positions of the plots, and the quadrats in the plots, are established before arriving at the site. This procedure provides a 'sampling map' for the site and a different map is constructed for each site. Because random number-generating algorithms in computer programs are often not strictly random (Sokal and Rohlf, 1995), all random numbers are obtained using a die and random number tables (Bart *et al*., 1998; Kirkwood, 1988; Greenwood, 1996), the tables used are those from Bliss (1967).

A randomly selected point is marked at the site with a wooden post (termed the origin point). The linear distance horizontally to the right, and the perpendicular distance below this point correspond to x and y axes from which any coordinate at the site may be found. The positions of the plots are located using a metre ruler along these axes. The same technique, but now applied to the distances corresponding to the axes of each of the plots in turn, is used to locate the positions of the quadrats in each plot.

An aluminium quadrat frame, that can be dismantled, is placed as near to the surface of the ground as possible. If shrubs cover an area beyond that of the quadrat, which makes placement difficult because of the shape of the shrub, the quadrat can be partially disassembled and then placed on the ground surface (to encompass the shrub, or parts of it) and then reassembled. A standardized procedure is followed to sample each quadrat:

1. Vegetational cover is measured by placing a 1-metre ruler vertically at the top left corner and bottom right corner of the quadrat, holding a pole across the two rulers corresponding to the height levels of each layer, and then visually assessing the vegetation cover at 10cm intervals (Kent and Coker, 1992; Bullock, 1996; Lepš and Šmilauer, 2003). In cases where the vegetation is higher than one metre, an additional 1-metre ruler is placed on top of the first ruler to provide a measureable distance of 2 metres.
2. A thorough search of all vegetation from, but not including, vegetation layer 2 (i.e. beginning 20cm from ground level) upwards for all land molluscs is then done. The locations of the molluscs in the vegetation (e.g. on annual

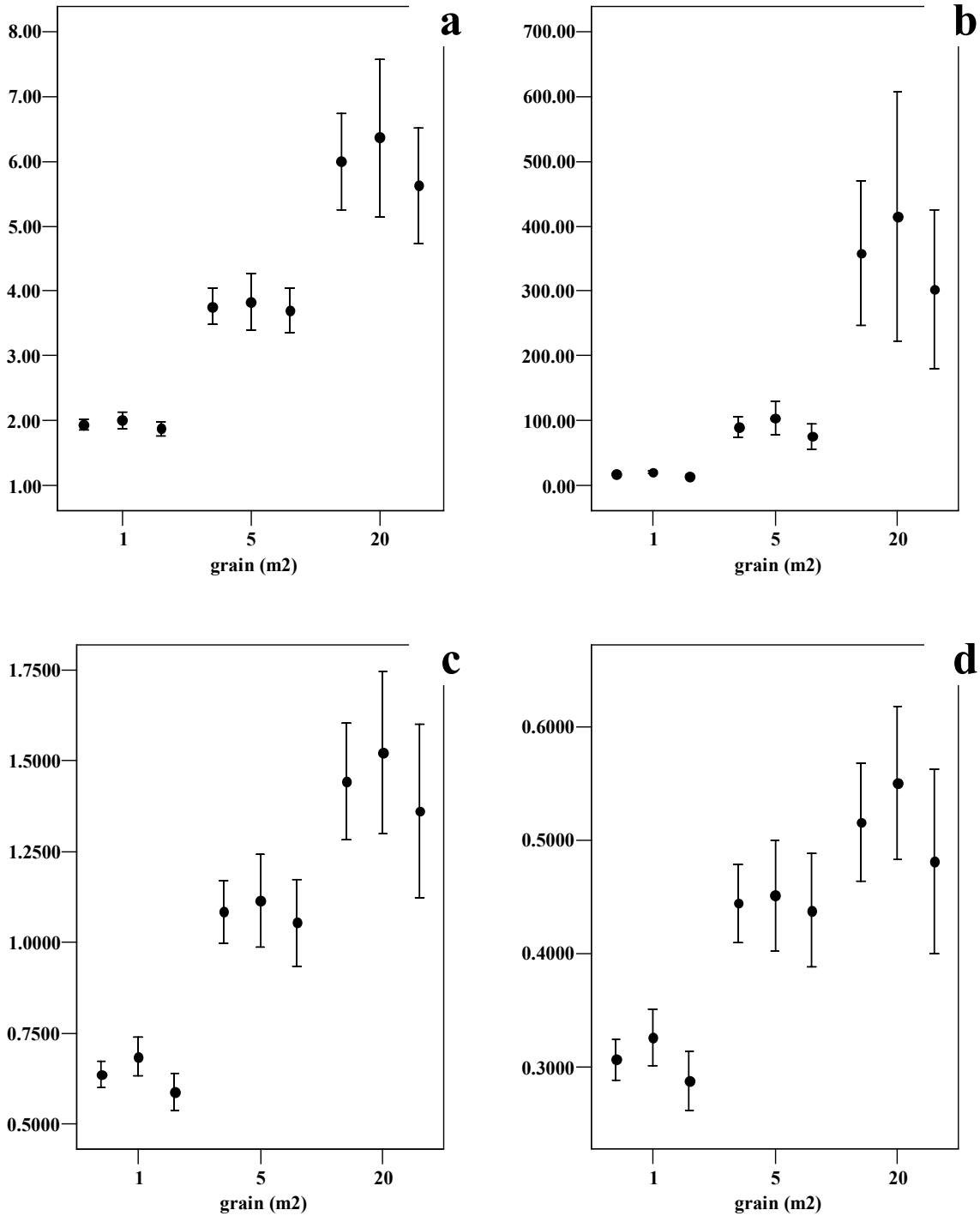

Figure 1 **a** Number of species **b** Abundances **c** Shannon ($\log_2$) diversity **d** Simpson diversity at three levels of grain for 20 sand, 20 steppe and 20 garigue sites from southern Iberia (pooled data). For each level of grain in each of the plots, the left hand bar shows results for wet and dry period sites combined, the central bar for wet period sites, and the right hand bar for dry period sites. Markers denote mean, crossbars denote lower and upper 95% confidence intervals of the mean. (Data points for levels of grain are:1m^2: wet and dry periods: n=1200, wet period: n=600, dry period: n=600; 5m^2: wet and dry periods: n=240, wet period: n=120, dry period: n=120; 20m^2: wet and dry periods: n=60, wet period: n=30, dry period: n=30).

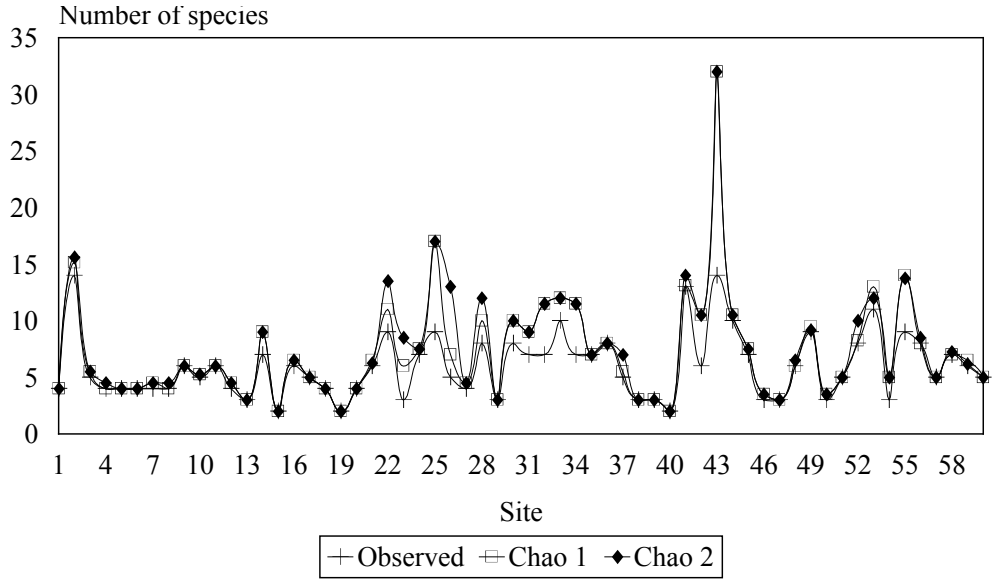

Figure 2 Observed number of species at 60 sites in southern Iberia (sites were sampled using the method described in text) compared to values of the Chao 1 and Chao 2 non-parametric species estimators (calculated using EstimateS software[vv] with 1000 randomizations of data per site).

stem, herbaceous leaf etc.), as well as the height of these locations (measured from ground level with a 1-metre ruler, to the nearest 0.5cm), are recorded. This is done before assessing cover of rocks and other variables on the ground, and before the search for species on the ground (and under rocks) to allow the vegetation to be assessed prior to any disturbance, which results from searching the vegetation for the molluscs. Once this is done, the ground level features, such as rock cover and soil cover are assessed (Menez, 2002).

3. Next, the ground level and vegetation layer 2 are thoroughly searched for molluscs. The procedures described here often result in the upper layers of vegetation being disturbed and rocks and other ground level features being disrupted. In all cases, however, rocks, plants etc. should be returned to their original positions as far as possible to reduce any damage that the sampling may cause.

This quadrat sampling protocol involves the systematic and thorough search for molluscs on the vegetation and under rocks and other ground-level features, as well as the recording of the environmental variables. This type of sampling is termed here *dissection sampling* to describe the detailed examination and analysis of the quadrat components and to highlight the differences between this method and less thorough search and data recording techniques sometimes used in studies. The thoroughness of the method is reflected in the time required to sample a quadrat: of 200 randomly selected sampled quadrats from sand, steppe and garigue habitat types the mean is 13.31 minutes (minimum 6.5; maximum 41.0; standard deviation 4.31).

The protocol allows quadrats to be sampled to the same level of detail, regardless of habitat heterogeneity and complexity, because the steps involved are systematic and standardized throughout: in a quadrat all vegetation is searched, and all rocks over-turned, for example.

My work has involved the sampling of 20 sites in each of three habitat types (identified based on criteria in Rivas Goday (1968), Rivas-Martinez (1973, 1981, 1987) and Arroyo and Marañón (1990). These habitat types are sand, steppe and garigue, which are fairly widespread in southern Iberia and in the Mediterranean region in general. I also analyze the effects of the dry and wet periods of the year (Blondel and Aronson, 1999; Font Tullot, 2000) by sampling 10 sites during each of these periods.

I apply the model at three spatial scales (i.e. levels of grain) by sampling at $1m^2$, $5m^2$ and $20m^2$. I then analyze the data (mostly using correlation and analysis of variance methods) collected at the three scales and determine if there are changes across them. By using partial correlation analyses I am able to see if some

types of heterogeneity (e.g. rock, logs etc.) affect abundances and number of species independently of the total complexity. I call these *principal structures* because they play an important role in determining abundances and number of species (unpublished data). Other workers have made similar findings, and Kappes and colleagues (2006) reported that coarse woody debris (which they defined as logs greater than 20cm in diameter) significantly increased both snail abundance and number of species in forests in Central Slovakia. Principal structures are detectable at different scales (Tews *et al.*, 2004) and the random allocation of quadrats for their sampling can miss them, especially if the structures are aggregated and/or present in low numbers.

The data so far are showing that increased heterogeneity is associated with a higher number of species, but not with higher abundances of species. The effects of heterogeneity, independent of total complexity, are more marked on the numbers of species than on their abundances. There are also significant changes across the scales for measures including number of species, abundances and diversity, with differences also for wet and dry periods (Figure 1).

This study is the first to assess the effects of habitat heterogeneity and complexity on the number of species and species abundances of land molluscs at varying scales ($1m^2$, $5m^2$ and $20m^2$) at the same locations. The use of the model and associated sampling methods described here allow the testing of specific hypotheses, such as: 'The number of species increases as the spatial scale is increased', 'There is more variation in habitat complexity in some habitat types than in others' and, 'There is more variation in habitat heterogeneity in some habitats, in comparison to others, in the dry and wet periods of the year'.

Because the quadrats are sampled exhaustively they can be considered to be 'known sampled universes' for statistical purposes (or as near as it is possible to get in field conditions). This feature allows the testing of the performance of species estimators, such as Chao1 and Chao2 (Magurran, 2004) at small scales. Preliminary data show that the estimators perform quite well even at the scales used here (Figure 2), supporting the finding of Hortal and colleagues (2006) that estimators are precise in spite of variations in grain, and substantiating their claim that estimators may be used to compare numbers of species from different sampling strategies.

There are caveats that have to be taken into account with the methods, however. These include the inability of quadrat sampling to provide adequate species inventories in some cases, and the possibility that aggregation of individuals may change over time making it difficult to detect some species at small scales of measurement (Loscasciulli and Boag, 1987). This highlights a divide in the objectives of sampling: sampling to determine complete inventories (or as complete as possible), or sampling to determine species-environmental relationships (Cameron and Pokryszko, 2005). The first requires the inclusion of sampling soil and leaf litter to pick up species not found in the vegetation or surface layers. What we need is a revision of datasets from different regions and habitat types that differentiate between species (and their abundances) sampled from soil and leaf litter, and those not collected from these components of the habitat. An objective analysis would provide an indication of the number of 'missed' species when soil and leaf litter are not sampled, and if there are general trends across regions and habitat types. The author's unpublished data from $1km^2$ southern Iberian sites, for example, indicate that up to 20% of species may be missed if soil and leaf litter sampling are not included for determining species inventories.

These, and other, problems are comprehensively treated by Cameron and Pokryszko (2005), and this paper is essential reading for anyone designing a sampling protocol. Some of the deficiencies of the method presented in the present paper can be ameliorated by increasing the number of quadrats and sites sampled (Anne Chao, *pers. com.*). However, the nature of the sampling method itself, and its use at varying scales, can serve to highlight the optimal numbers of quadrats (and area) required to answer some of the topical questions about land molluscs and their relationships to habitat structure.

REFERENCES

AUBRY S, MAGNIN F, BONNET V, PREECE RC 2005 Multi-scale altitudinal patterns in species richness of land snail communities in south-eastern France. *Journal of Biogeography* **32**: 985-998.

ARROYO J & MARAÑÓN T 1990 Community ecology and distribution spectra of Mediterranean shrub-lands and heathlands in Southern Spain. *Journal of Biogeography* **17**: 163-176.

BARKER GM & MAYHILL PC 1999 Patterns of diversity and habitat relationships in terrestrial mollusc communities of the Pukeamaru Ecological District, northeastern New Zealand. *Journal of Biogeography* **26**: 215-238.

BART J, FLIGNER MA & NOTZ WI 1998 *Sampling and Statistical Methods for Behavioral Ecologists.* Cambridge: Cambridge University Press.

BECK MW 2000 Separating the elements of habitat structure: independent effects of habitat complexity and structural components on rocky intertidal gastropods. *Journal of Experimental Marine Biology and Ecology* **249**: 29-49.

BISHOP MJ 1977 Approaches to the quantitative description of terrestrial mollusc populations and habitats. *Malacologia* **16**: 61-66.

BLISS CI 1967 *Statistics in Biology*, Vol.1. McGraw-Hill, New York.

BLONDEL J & ARONSON J 1999 *Biology and Wildlife of the Mediterranean Region.* Oxford University Press, Oxford.

BOYERO L 2003 Multiscale patterns of spatial variation in stream macroinvertebrate communities. *Ecological Research* **18**: 365-379.

BROWN JH 1995 *Macroecology.* University of Chicago Press, Chicago.

BULLOCK J 1996 Plants. *In* Sutherland WJ (ed.) *Ecological Census Techniques.* Cambridge University Press, Cambridge, 111-138.

CAIN AJ 1983 Ecology and Ecogenetics of Terrestrial Molluscan Populations. *In* Wilbur KM (ed.) *The Mollusca*, Vol. 6. Academic Press, New York, 597-647.

CAMERON RAD & POKRYSZKO BM 2005 Estimating the species richness and composition of land mollusc communities: problems, consequences and pratical advice. *Journal of Conchology* **38**: 529-547.

CAMERON RAD 2002a Some species/area relationships in the Bitish land mollusc fauna and their implications. *Journal of Conchology* **37**: 337-348.

CAMERON RAD 2002b The land molluscs of North Ronaldsay, Orkney: human intervention and island faunal diversity. *Journal of Conchology* **37**: 445-453.

Cameron RAD, Pokryszko BM. 2005. Estimating the species richness and composition of land mollusc communities: problems, consequences and practical advice. *Journal of Conchology* 38: 529-547.

CAMERON RAD, MYLONAS M, TRIANTIS K, PARMAKELIS A &VARDINOYANNIS K 2003 Land-snail diversity in a square kilometre of Cretan maquis: modest species richness, high density and local homogeneity. *Journal of Molluscan Studies* **69**: 93-99.

COOK A 2001 Behavioural ecology: on doing the right thing, in the right place at the right time. *In* Barker GM, ed. *The Biology of Terrestrial Molluscs.* CABI, Hamilton, 447-487.

CRAW R 2001 A cladistic analysis of New Zealand land mollusca: where is the evidence? *Journal of Biogeography* **28**: 543-545.

EMBERTON KC 1995 On the endangered biodiversity of Madagascan land snails. *In* Bruggen AC Van, Wells SM, Kemperman Th CM (eds.) *Biodiversity and Conservation of the Mollusca*. Backhuys, Leiden, 69-89.

EMBERTON KC 1997 Diversities and distributions of 80 land-snail species in southeastern-most Madagascan rainforests, with a report that low-lands are richer than highlands in endemic and rare species. *Biodiversity and Conservation* **6**: 1137-1154.

FLEISHMAN E, BETRUS CJ & BLAIR RB 2003 Effects of spatial scale and taxonomic group on partitioning of butterfly and bird diversity in the Great Basin, USA. *Landscape Ecology* **18**: 675-685.

FONT TULLOT I 2000 *Climatología de España y Portugal*, 2nd ed. Ediciones Universidad de Salamanca, Salamanca.

GARDNER RH 1998 Pattern, process, and the spatial analysis of spatial scales. *In* Peterson DL, Parker VT (eds.) *Ecological Scale. Theory and Applications.* Columbia University Press, New York.

GREENWOOD JJD 1996 Basic techniques. In: Sutherland WJ (ed.) *Ecological Census Techniques.* Cambridge University Press, Cambridge, 11-110.

HART DD & HORWITZ RJ 1991 Habitat diversity and the species-area relationship: alternative models and tests. *In* Bell SS, McCoy ED, Mushinsky HR (eds.) *Habitat Structure, the Physical Arrangement of Objects in Space.* Chapman and Hall, London 46-68.

HORTAL J, BORGES PAV & GASPAR C 2006 Evaluating the performance of species richness estimators: sensitivity to sample grain size. *Journal of Animal Ecology* **75**: 274-287.

KAPPES H, TOPP W, ZACH P & KULFAN J 2006 Coarse woody debris, soil properties and snails (Mollusca: Gastropoda) in European primeval forests of different environmental conditions. *European Journal of Soil Biology* **42**: 139-146.

KENT M & COKER P 1992 *Vegetation Description and Analysis. A Practical Approach.* Chichester: John Wiley.

KIRKWOOD BR 1988 *Essentials of Medical Statistics.* Blackwell Scientific Publications, Oxford.

LABAUNE C & MAGNIN F 2001 Land snail communities in Mediterranean upland grasslands: the relative importance of four sets of environmental and spatial variables. *Journal of Molluscan Studies* **67**: 463-474.

LEPŠ J & ŠMILAUER P 2003 *Multivariate Analysis of Ecological Data Using CANOCO*. Cambridge University Press, Cambridge.

LOMOLINO MV 2000 Ecology's most general, yet protean pattern: the species-area relationship. *Journal of Biogeography* **27**: 17-26.

LOSCASCIULLI O & BOAG DA 1987 Microdistribution of Terrestrial Snails (Stylommatophora) in Forest Litter. *Canadian Field Naturalist* **101**: 76-81.

MAGURRAN AE 2004 *Measuring Biological Diversity.*

Blackwell Science, Oxford.
MCCOY ED & BELL SS 1991 Habitat structure: the evolution and diversification of a complex topic. *In* Bell SS, McCoy ED, Mushinsky HR (eds.) *Habitat Structure, the Physical Arrangement of Objects in Space*. Chapman and Hall, London, 3-27.
MENEZ A 2001 Assessment of land snail sampling efficacy in three Mediterranean habitat types. *Journal of Conchology* **37**: 171-175.
MENEZ A 2002 The standardization of abiotic variable data collection in land mollusc research. *Journal of Conchology* **37**: 581-583.
NEKOLA JC & SMITH TM 1999 Terrestrial gastropod richness patterns in Winconsin carbonate cliff communities. *Malacologia* **41**: 253-269.
NEKOLA JC 1999 Terrestrial gastropod richness of carbonate cliff and associated habitats in the Great Lakes Region of North America. *Malacologia* **41**: 231-252.
NILSSON SG, BENGTSSON J & ÅS S 1988 Habitat diversity or area *per se*? Species richness of woody plants, carabid beetles and land snails on islands. *Journal of Animal Ecology* **57**: 685-704.
RIVAS GODAY S 1968 Algunas novedades fitosociológicas de España meridional. *Collectanea Botanica* **7**: 998-1031.
RIVAS-MARTINEZ S 1973 Avance sobre una sintesis corologica de la peninsula Iberica, Baleares y Canarias. *Anales del Instituto Botanico AJ Cavanilles* **30**: 69-87.
RIVAS-MARTINEZ S 1981 Les étages bioclimatiques de la végétation de la Péninsule Ibérique. *Anales Jardin Botanico de Madrid* **37**: 251-268.
RIVAS-MARTINEZ S 1987 *Memoria del Mapa de Series de Vegetacion de España*. ICONA.
SCHEINER SM, COX SB, WILLIG M, MITTELBACH GG, OSENBERG C & KASPARI M 2000 Species richness, species-area curves and Simpson's paradox. *Evolutionary Ecology Research* **2**: 791-802.
SCHILTHUIZEN M & RUTJES HA 2001 Land snail diversity in a square kilometre of tropical rainforest in Sabah, Malaysian Borneo. *Journal of Molluscan Studies* **67**: 417-423.
SCHNEIDER DC 1994 *Quantitative Ecology. Spatial and Temporal Scaling.* Academic Press, San Diego .
SOKAL RR & ROHLF FJ 1995 *Biometry.* 3rd ed. WH Freeman, New York.
SOLEM A 1984 Introduction. *In* Solem A, Bruggen AC Van (eds.) *World-Wide Snails. Biogeographical studies on non-marine Mollusca*. Brill/Backhuys,Leiden, 1-5.
SOUTHWOOD TRE 1978 *Ecological Methods With Particular Reference to the Study of Insect Populations*, 2nd ed. Chapman and Hall, London.
TATTERSFIELD P 1996 Local patterns of land snail diversity in a Kenyan rain forest. *Malacologia* **38**: 161-180.
TEWS J, BROWSE U, GRIMM V, TIELBÖRGER K, WICHMAN MC, SCHWAGER M JELTSCH F 2004 Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. *Journal of Biogeography* **31**: 79-92.
WALDÉN HW 1981 Communities and diversity of land molluscs in Scandinavian woodlands. I. High diversity communities in taluses and boulder slopes in SW Sweden. *Journal of Conchology* **30**: 351-372.
WINTER AJ DE & GITTENBERGER E 1998 The land snail fauna of a square kilometer patch of rainforest in southwestern Cameroon: high species richness, low abundance and seasonal fluctuations. *Malacologia* **40**: 231-250.