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ORIGINAL ARTICLE

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A simplified method for conducting ecological studies of land snail communities in urban landscapes

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Abstract Although land snail communities could be a good indicator of soil quality and landscape structure impact, they are seldom analysed in an urban context, probably because of the lack of interest in this ecosystem and because no fast and reliable method of inter-site comparison seems to exist. The classical method of removing large quantities of soil leads to degradations unsuitable in urban gardens and involves time-consuming sorting. For the purpose of ecological comparisons, we analysed different methods to assess land snail communities in urban parks in order to set up a simplified strategy. Snail communities were sampled in three parks within the city of Paris (France) using (1) quadrat method (litter and soil removed over a given area, snails sorted later in the laboratory), (2) visual search in situ (hand-picking snails in the leaf litter), (3) wooden boards placed on the ground and regularly checked, and (4) pitfall traps, usually used for insect sampling. Our results suggest that the wooden board and pitfall trap did not yield enough data to determine community structure and that visual search was not sufficient to sample all dominant species, especially the smallest ones. In order to allow for replication of samples, we suggest a mixed strategy suitable for ecological comparisons, combining visual searches of five 0.5 m^2 areas (15 min for each area) and litter and soil sampling on two 0.0625 m^2 quadrats.

Keywords Gastropods · Land snails · Community · Urban ecosystem · Capture methods

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Introduction

Terrestrial gastropods have recently been increasingly studied as ecological models, for instance to understand the effect of landscape fragmentation (Gotmark et al. 2008; Kappes et al. 2009) or human exploitation of habitats (Dedov and Penev 2004). Snails could be good ecological indicators because of their restricted mobility, small body size and role in the food chain (Kerney and Cameron 1979; Baur and Baur 1993). Although inventories are available for many areas, ecological studies are still rare and terrestrial molluscs are generally poorly studied within the context of biodiversity conservation or habitat typology (Triantis et al. 2009). The snail community is rarely studied in urban areas (but see Horsák et al. 2009) although it could provide valuable information on soil functioning and quality in this perturbed ecosystem and on degree of isolation of natural areas within the urban matrix.

The study of land snail communities is probably hampered by the lack of an easy method that would allow ecological comparisons. In fact, land snail inventories have involved various methods that were primarily aimed at establishing exhaustive lists of the species present in a given habitat, and more rarely at a comparison of snail communities in different sites (but see Suominen 1999; Gotmark et al. 2008; Raheem et al. 2008; Liew et al. 2010). In ecology, most methods are not exhaustive, but the repetition of the same method in different places provides elements of comparison for analysing species responses and environmental effects. For example, the ‘‘punctual abundance index'' used for bird communities consists of observing all birds seen or heard during a given time (usually 5–20 min; Bibby et al. 2000). Although the method is repeated several times during a season, these repetitions are not sufficient to obtain a comprehensive species list. However, several studies have clearly shown that between 70 and 95% of the species are detected, which allows for comparisons. These partial lists give good community typologies based on the commonest species (Bibby et al. 2000).

The most widely used method for studying land snail communities is the extraction of gastropods from defined quadrats from which litter and soil are removed over a depth of 3–5 cm (Valovirta 1996; Cameron and Pokryszko 2005; Cucherat and Demuynck 2008). Quadrat size varies according to the heterogeneity and area of the site, but it is usually 20 × 20 or 25 × 25 cm (see a review in Cucherat and Demuynck 2008). The quadrats are randomly placed and repeated to cover an area of between 0.5 and 4 m^2. Samples are air-dried in the laboratory, then passed through a sieve column, and sorted for snails under a dissecting microscope (Hylander et al. 2005; Watters et al. 2005; Kappes et al. 2009). Another method of soil analyses is based on volume (usually 10 or 20 l of soil is processed), samples being selected non-randomly within a defined area (e.g. Waldén 1981); in this case, samples can be washed (Horsák 2003). This method can give different results from quadrats but is less commonly used (Cameron and Pokryszko 2005). These soil methods are time-consuming, since 1 l of collected leaf litter can represent several hours of processing, depending on its richness (Cucherat and Demuynck 2008; unpublished data). In addition, they are not always feasible, for example in urban contexts, where removing a large number of soil samples in parks or gardens is not always possible.

Several authors have completed their studies by using visual search to look for larger and more sparsely distributed species (see a review in Cucherat and Demuynck 2008). Visual search implies picking snails by hand from tree trunks, fallen wood, stones and crevices, generally over a given area or along a transect (Gotmark et al. 2008) and during a specific period of time (between 15 and 60 min).

Two other sampling methods are sometimes used in snail inventories: the pitfall trap, classically used to capture insects (especially carabid beetles), can be used for land snails (Suominen 1999); the board method, in which wooden boards are placed on the ground (for example, 40 × 50 cm boards; Oggier et al. 1998; Suominen et al. 2002) for an extended period (generally 1 month) and then checked for snails underneath. These refuge traps are more frequently used for slug studies (Grimm and Paill 2001).

While investigating variations in land snail communities in urban parks according to management history and quality, we realised that the widely used method is very time-consuming and difficult to apply in gardens. In this context, we looked for a faster sampling method that would enable comparisons in sites with a low density of terrestrial snails. The aim was not to get a comprehensive species list. We present here the methodologies we used, compare their results and propose a sampling strategy for inter-site comparisons of the presence and abundance of common land snails.

Sites and methods

Study sites

We selected three public parks with restricted-access areas, i.e. without major trampling, within the city of Paris, completely disconnected from natural or semi-natural areas. These gardens were wooded, had leaf litter and humus, and had comparable soil humidity. The first one was the Jardin Ecologique (JE) (0.5 ha), part of the very old garden of the National Museum of Natural History, which was enclosed in the 1950s (no public access) and managed with minimal human intervention ever since. It has old trees (many native trees such as *Quercus* spp.) and the soil is mostly covered by ivy (pH 7.5). The second site was the garden of the Bibliothèque Nationale François Mitterrand (BN) (1.06 ha) created in 1994 and featuring old pine trees, *Pinus sylvestris*, and some *Betula pendula* and *Quercus robur*. The soil is covered by ivy, grasses and nettles (pH 7.0). It is completely enclosed between buildings, and all of the soil was brought from outside when the complex was built. The third site was another part of the garden of the National Museum of Natural History known as the Labyrinthe (LA) (0.2 ha), which was closed to the public in 1995, and which features numerous exotic trees (conifers, evergreens and deciduous; pH 7.4).

Sampling methods

Since our goal was to find a sampling strategy to obtain enough data to allow inter-site comparisons within the shortest possible time and with the least amount of soil removed, we tested both the minimum number of sample replicates needed and the advantages of complementary methods. All our park samples involved areas under deciduous trees.

We first applied the quadrat method over an area of 25 × 25 cm (Q = 0.0625 m^2), in what was considered to be the best habitat. Quadrats were always separated by more than 10 m. We removed leaf litter and 3 cm of soil in the three parks in April–May 2009. Samples were sifted with a Winckler sieve (10-mm mesh) and then dried in the laboratory for 3 days. The leaf litter was passed through 3-, 2-, and 0.6-mm sieves. The two largest fractions were thoroughly searched with the naked eye and the third was sorted under a dissecting microscope. Since it is considered that no adult terrestrial molluscs are smaller than 0.6 mm, the smallest fraction was discarded (Tattersfield 1998; De Winter and Gittenberger 1998; Fontaine et al. 2007a). Shells of juveniles (i.e. protoconch with or without the first teleoconch whorl) were not collected and are therefore not included in the analysis since their identification can be problematic. In BN, we collected the soil in each quadrat separately (n = 4). In JE, two samples were lumped together (JE1, n = 6). We also analysed the quadrat method using a single collection of four quadrats in JE (JE2) and in LA (LA2). This last choice was in accordance with previous works that suggested an area of 0.25 m^2 as optimal (Watters et al. 2005; Hausdorf 2007). All sorting and identification were done by the same person.

In May–June 2009, the visual search method was applied to the sites sampled for leaf litter. This integrated a search not only on the surface of the soil but also in the upper soil layer. An area of 0.5 m^2 was defined, and the observer first hand-picked snails on the vegetation, under stones, in the leaf litter and on fallen wood, then within the litter and the soil to a depth of 3 cm. The search was limited to 15 min per 0.5 m^2 and per observer. Observer effect was tested with two or three observers who sampled sites a few meters from each other. Nine samples were collected in BN, eight in JE and four in LA.

In June–July, wooden boards measuring 30 × 50 cm were placed on the ground (below vegetation), 20 m apart. Four wooden boards in JE were checked after 1 month and then 2 months ($n = 8$); three in BN were also checked after 2 months ($n = 6$), and three in LA were checked after 1 month ($n = 3$). Six pitfall traps (10 m between each) were installed during June and July in JE and BN and were checked each month.

Since the most comprehensive method (leaf litter sorting) does not allow sampling of slugs, this group was excluded from our analyses.

Statistical analyses

Species accumulation curves were calculated for each park and each observer using EstimateS version 8.2.0 software (Colwell 2009). We obtained the Mao Tau estimator and 95% confidence intervals with 50 runs. We used the t test from Statview 4.0 software.

Results

Snail communities

Combining all methods, 22 species were found throughout our study areas. JE appeared to be the richest site with 20 species, whereas BN and LA had 13 species each (Table 1). Quadrat method provided between 92 and 100% of the species, visual search between 55 and 61.5% and wooden boards between 7.6 and 69%, the latter giving the maximum variability. Pitfall traps yielded four and seven species only, or less than 33% in both cases. For this reason, this method was not included in the following analyses.

The abundance varied greatly according to species: *Carychium tridentatum, Punctum pygmaeum, Discus rotundatus, Lauria cylindracea* and *Vallonia costata* were usually represented by more than 100 individuals per quadrat in JE, whereas most of the other species were represented by a few individuals only. In JE and LA, the most common species were *Lauria cylindracea* and *Vallonia costata*, whereas *Cochlicopa lubricella* and *Trochulus* spp. (*T. sericeus* or *T. hispidus*) were the most abundant species in BN.

Sample sizes

The first step was to define the best sample size for each method, i.e. the minimum size providing more than 80–85% of the species, obtained by adding samples. The shape of the curve allows this minimum sample to be determined (Fig. 1). For the quadrat method, we observed a plateau after two quadrats (2 Q). We obtained 78% of the species at the 2 Q stage in BN and 83% of the species with 2 Q in JE1 (in comparison with 4 Q in JE1). Four Q obviously seems better, but in the second study, JE2 gave the same results as JE1 with two or four samples.

For visual search, the three curves appear to be relatively parallel and four or five 0.5 m^2 searches were effective to obtain more than 80% of the species found by this method. However, only about 50–60% of all the species were detected by this method and very small species (under 1 mm) such as *Carychium tridentatum, Punctum pygmaeum,* and *Vitrea contracta* abundant in JE using the quadrat method, were not observed.

The three curves are very different for the wooden board method (with only one species in BN). It shows that this method is not reliable for comparative studies of species richness, especially if the wooden boards are only left in place for 2 months.

Observer effect

We supposed that in the visual search method, a method that wanted to promote, the observer effect could be important. In fact, analyses of the cumulative number of species obtained by successive replication of 0.5 m^2 searches (Fig. 2) showed that differences between observers differed by one species only. The difference in the detection of the number of species was not significant: for the 13 potential species of BN, we obtained all $t > -0.488$ and all $P > 0.412$ (three observers analysing three 0.5 m^2 each), and for the 22 potential species of JE, $t = 1.323$ and $P = 0.199$ (two observers analysing four 0.5 m^2 each).

Discussion

Species identified with the different methods were among the most common land snails of northern France. No checklist of the mollusc fauna in the Paris area currently exists. However, according to the available documentation

Table 1 List of land snail species obtained with different methods in three parks in the Paris area (JE, BN and LA)

	JE					BN				LA		
	Quadrats JE1 (6 Q)	Quadrats JE2 (4 Q)	JE visual search (8 units)	Wooden board (8 units)	Pitfall trap (12 units)	Quadrats (4 Q)	Visual search (9 units)	Wooden board (6 units)	Pitfall trap (12 units)	Quadrats (4 Q)	LA visual search (4 units)	Wooden board (3 units)
Carychium tridentatum	+ + +	+ + +										
Cecilioides acicula	+ +	+	+							+		
Cepaea sp.	+						+		+			
Cernuella neglecta						+	+					
Clausilia rugosa						+						
Cochlicopa lubricella	+	+ +	+			+ + +	+ +		+	+		
Cornu aspersum	+	+	+		+						+	
Discus rotundatus	+ + +	+ + +	+ + +	+ + +	+ +	+				+ + +	+ +	+ +
Helix pomatia	+	+	+									
Lauria cylindracea	+ + +	+ + +	+ + +	+ +	+					+ + +	+ + +	+ +
Merdigera obscura	+	+										
Oxychilidae spp.	+ +	+ +	+ +		+ +	+ +	+ + +	+	+ +	+	+	+
Paralaoma servilis	+ +	+ + +	+	+		+ +	+			+ + +	+ +	+ +
Pomatias elegans	+	+	+		+	+				+ +	+	+
Punctum pygmaeum	+ + +	+ +				+						
Pupilla muscorum	+	+				+ +	+			+		
Trochulus sp.	+ +	+ +	+ +	+	+	+ +	+ + +			+ +	+ +	+
Truncatellina callicratis	+					+				+ + +	+	+ +
Vallonia costata	+ + +	+ + +	+ +	+	+					+ + +	+ + +	+ + +
Vallonia pulchella	+	+				+ +	+			+	+	+
Vertigo pygmaea	+											
Vitrea contracta	+ +	+										
Number of species	20	17	11	5	7	12	8	1	4	12	10	9

Q Quadrat unit measuring 0.0625 m^2, *visual search unit* area of 0.5 m^2, *wooden board unit* area of 50 × 30 cm over 1 month, *pitfall trap unit* one trap for 1 month
Abundances: + <2 individuals per species and per unit, + + between 2 and 10 individuals, + + + >10 individuals

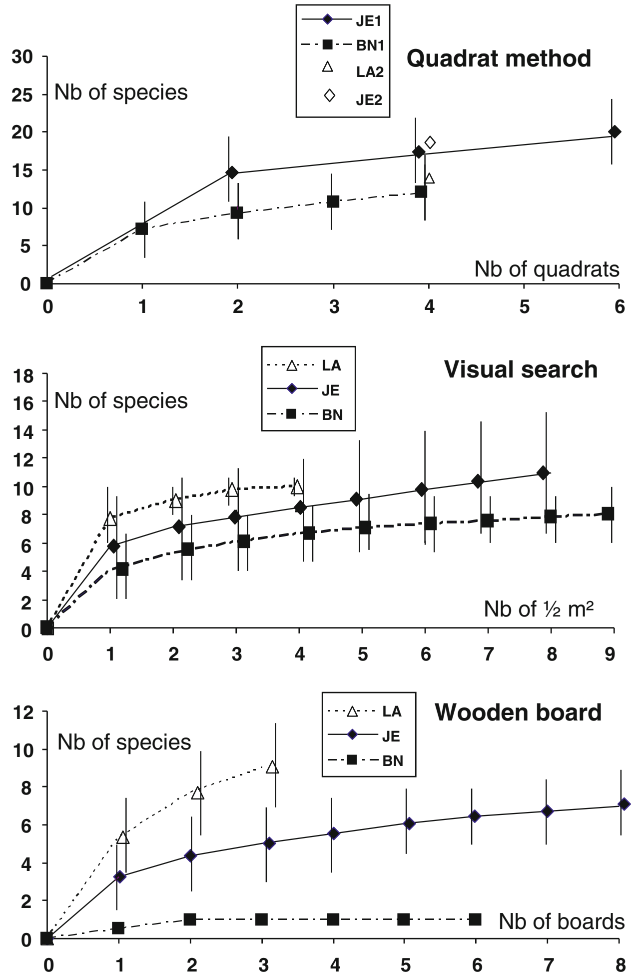

Fig. 1 Number (*Nb*) of land snail species obtained in three parks (JE, BN, LA) according to replication of the samples; the randomisation curves were obtained with EstimateS software, and 95% confidence intervals are given

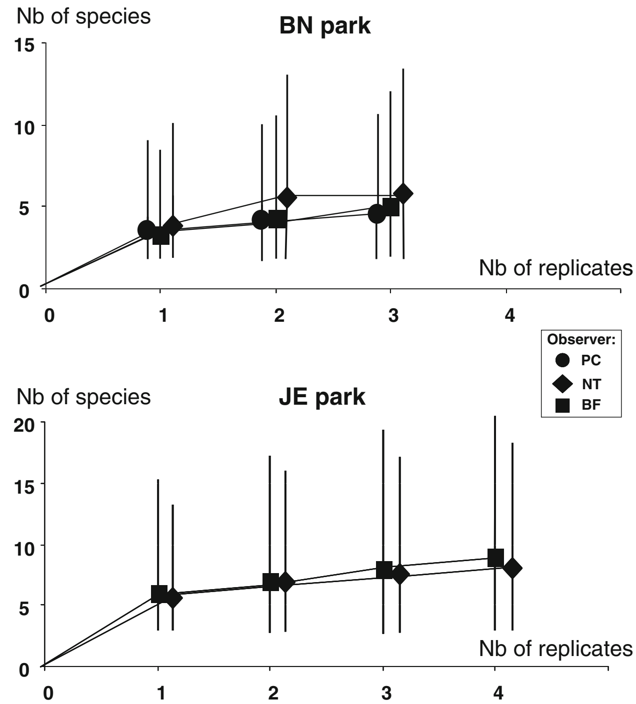

Fig. 2 The effect of observers (PC, NT and BF) tested on snail communities in two parks (BN and JE). Each replicate involved 0.5 m^2 of soil (3 cm under the surface and 30 cm above) analysed over 15 min. The randomisation curves were obtained with EstimateS software and 95% confidence intervals are given

(Kerney et al. 2006; INPN 2009) and to the more complete and recent inventories in a neighbouring department, Loir-et-Cher (Brault and Gervais 2004), the fauna of the sampled urban sites is representative of the common species found in semi-natural environments in this area and accounts for approximately one-third of the global fauna of the area.

In the old JE garden, we observed the presence of large species (up to 30 × 50 mm) such as *Helix pomatia,* and of small ones (0.9 × 1.8 mm) such as *Carychium tridentatum*. This first approach to the assessment of land snail communities in a large city shows the potential complexity of the fauna and the feasibility of studies in this context.

On the basis of our results, the wooden board method (with a high degree of variability and capturing only a few species) and the pitfall trap method (very few species) should be avoided for our purposes. These two methods seem to be interesting for studies in population ecology or for slugs (Grimm and Paill 2001), but did not appear to be adapted to our aim of comparing community ecology. Although Suominen (1999) used pitfall trapping intensively, he noted that this method is not really effective for sampling terrestrial gastropods. The wooden board method could be useful for comparing the abundances of some common species at different sites, but it would probably require leaving them on site for several months. This result corroborates the previous conclusion of Oggier et al. (1998) that cardboard traps might be best suited for examining biological population issues on selected species over relatively large areas.

The visual search method is rapid and entails neither degradation nor soil removal. It appears to be a good ecological method for investigations of snail communities in an urban context. The visual search method we applied was more thorough than the one used by Gotmark et al. (2008) and similar to the one used by Raheem et al. (2008): we not only searched for snails on tree trunks, fallen wood, stones and crevices, but in the leaf litter and soil as well. We thus combined searches of snails in and above the litter and obtained good general results, both in terms of the number of species and the homogeneity of the community. It should be stressed that it is important to have several replicates of visual searches per site since land snails are very dependent on microhabitats. For example, the species composition may be different if sampling is done near a boulder, a tree, in a depression or on flat ground, all within the same macrohabitat (Fontaine et al. 2007b; Cucherat and Demuynck 2008).

However, this method is not sufficient, even with a good number of replications, since some species have never been detected with it. It creates a bias when these species are abundant. This was especially the case for very small species such as *Carychium tridentatum, Punctum pygmaeum* and *Vitrea contracta,* small species with very high densities in JE and present in each quadrat sample (several hundred shells found). In the two other gardens, the visual search method gave results for the most common species that were similar to those of the quadrat method (Table 1). Accordingly, it appears indispensable to analyse soil using a dissecting microscope and to supplement visual searches with the quadrat method. Since abundant small species were present in each of the 25 × 25 cm areas, and since we found that two and four samples gave similar results in several cases, the choice of two samples for the quadrat method appears to be sufficient to include small species that may be present and to limit sorting time as well.

Consequently, in order to easily and rapidly study snail communities in urban areas, we suggest a sampling strategy based on a mixed method. We retained the visual search as the basis of our analysis: five units of 0.5 m^2 each were searched for 15 min. We supplemented these data with a quadrat analysis: two quadrats of 0.0625 m^2 each, with a depth of 3 cm of soil removed and sifted in the laboratory. If we apply this strategy to our present data (selecting the first samples analysed in each case), we obtain 17 and 9 (number of species) and 2.07 and 1.67 (Shannon index), respectively, for JE and BN. The difference between the number of individuals (for all species) found by all of the methods tested in a park, and the number of individuals identified using our selected strategy for the same park, was only 0.2 and 0.5%, respectively, for JE and BN. The four or five species overlooked by our strategy represent only the rare ones.

This light sampling methodology is designed for inter-site comparisons of the presence and abundance of common species. It is not reliable for comprehensive species inventories, which can only be done with a careful sampling based mainly on litter sieving. With an ecological goal, our results can be used to compare, for example, the impact of garden management on biodiversity at a local scale: we hypothesize that JE had a greater degree of richness and diversity than BN and LA because of its age and the fact that it had not been subject to human disturbance for a long time. Raheem et al. (2008) also used this kind of mixed strategy in tropical forests and gardens, but litter and soil samples were searched in the field. Consequently, it was difficult to observe the smallest species.

Gastropods are an important component of the ecological soil functioning and need to be taken into account in biodiversity conservation, as well as in the definition of urban biodiversity. According to our analyses, a simpler monitoring strategy could be applied to litter and wood on the ground, but further testing needs to be done on other habitat structures such as grasses, and on other systems such as forests.

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