



Quantitative extraction of macro-invertebrates from temperate and tropical leaf litter and soil: efficiency and time-dependent taxonomic biases of the Winkler extraction

Frank-Thorsten Krell^{a,*}, Arthur Y.C. Chung^b, Emma DeBoise^a, Paul Eggleton^a, Alessandro Giusti^a, Kelly Inward^a, Sylvia Krell-Westerwalbesloh^a

^a*Soil Biodiversity Programme, Department of Entomology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK*

^b*Forestry Department, Forest Research Centre, Sandakan, Sabah, Malaysia*

Received 24 January 2004; accepted 25 October 2004

KEYWORDS

Soil macro-invertebrates;
Extraction;
Winkler extractor;
Coleoptera;
Chilopoda

Summary

Winkler extractors, a simple device presumed to extract macro-invertebrates efficiently from soil and litter samples, is being used increasingly in ecological surveys and functional studies of soil macro-invertebrate communities. In this study the extraction efficiency and taxonomic bias of the Winkler extraction are evaluated for extraction periods of 3 h up to 7 weeks, calibrated by hand-sorting after 7 weeks. The method extracts most macro-invertebrates completely or to a proportion of over 90% except Isopoda, Diplopoda and Mollusca. However, for an exhaustive result, a long extraction period of several weeks is necessary. For the most speciose group (adult beetles) and for the commonly most abundant group (ants), a short extraction of 3 days was sufficient to get 70% of the individuals and nearly all species. Three days was also sufficient to recover the rank abundance order of beetle families, while for 'higher taxa' and for Chilopoda species, 4 and 3 weeks were necessary, respectively. Optimum extraction times for the abundant macro-invertebrate groups and possible adjustment factors for the soil macro-invertebrates of temperate woodlands are proposed to compensate the taxonomic bias caused by short extraction periods. However, for recording an accurate snapshot of the soil and litter fauna at a particular time, shorter extraction periods are advisable because of the short life cycle of many

*Corresponding author. Tel.: +44 20 7942 5886; fax: +44 20 7942 5229.
E-mail address: f.krell@nhm.ac.uk (F.-T. Krell).

soil invertebrates causing emergence of later stages or a second generation during longer extraction periods. The problem of contamination of samples is also discussed. © 2005 Elsevier GmbH. All rights reserved.

Introduction

Soils are biodiversity hotspots (Ghilarov, 1977; Giller, 1996). The overwhelming majority of terrestrial organisms depend on soils at least during some part of their life cycle. Soil ecosystems are increasingly a major focus of ecology and ecosystem studies (Lavelle and Fragoso, 2000; André et al., 2002; Bardgett, 2002; Goede and Brussaard, 2002). Soil, however, is like a black box: optically impenetrable, and moreover, generally visible from only one side, the surface. If we want to study organisms in natural soil ecosystems quantitatively, we have to extract them from the soil.

Since most of the soil and litter animals are tiny and numerous, not easy to see with the naked eye, quantitative extraction of soil invertebrates requires special methods. Most of them rely on prerequisites which are not always available at remote study sites, such as costly and unwieldy apparatuses or electricity supply (e.g. Berlese–Tullgren funnel, Macfayden air-conditioned funnel, etc., cf. Lasebikan et al., 1978). The simple and cheap method of pitfall trapping is strongly biased towards those groups which actively move on the surface and does not quantitatively record litter and soil dwellers which stay in the substratum or disseminate by flying.

A simple and handy device which is being used increasingly in ecological surveys and functional studies of soil macro-invertebrate communities is the Winkler extractor (Ward, 1987; Hammond, 1990; Olson, 1991; Belshaw and Bolton, 1994; Fisher, 1998; Chung et al., 2000). It was first invented in 1907 by Emil Moczarski (an expert on Pselaphinae) in Vienna (Austria) and put on the market by the Winkler & Wagner company, Vienna (Holdhaus, 1910), where it acquired its present name. Recent descriptions of the Winkler extractor have been published by Kühnelt and Walker (1976), Besuchet et al. (1987), Owen (1987), Fisher (1998), New (1998) and Bestelmeyer et al. (2000). The procedure is simple: leaf litter and top soil is sifted through a wire sieve with a mesh width of about 10 mm to exclude large particles and reduce the volume of the material. The sifted material is suspended in mesh bags within a closed sack of cloth. A bottle with alcohol is fixed at the bottom of this sack, so that the organisms fall into the bottle upon leaving the substratum in the mesh bag.

Fisher (1999) and Kalif and Moutinho (2000) found Winkler extraction to be the most efficient method for ant sampling in terms of species numbers obtained, compared with pitfall trapping and Berlese extraction. In Winkler extractors, the substratum dries out much more slowly than in other extraction apparatuses (e.g. in Berlese–Tullgren funnels). Organisms sensitive to desiccation (which may therefore routinely die in Berlese–Tullgren funnels if the substratum is dried rapidly) survive in Winkler extractors for long enough to be extracted, collected and recorded (Scheerpeltz, 1968).

The Winkler extraction works through two mechanisms: (a) random locomotive activity of the organisms – by moving through the substratum in the mesh bag, the organisms accidentally fall out of the bag if they reach the edge of the substratum; (b) desiccation of the substratum – when the microclimate in the substratum becomes unfavourable, organisms leave the substratum intentionally. The method has the advantage of very low methodical and technical requirements and is, therefore, easily and effectively applicable all over the world, even in remote regions where no electricity or other infrastructure is available.

Different authors have used and proposed very different time periods for the Winkler extraction process, mostly without giving a rationale for their suggestion. The extraction time ranges from 24 h (Leponce et al., 2004), 2 days (Olson, 1991; Fisher, 1999; Bestelmeyer et al., 2000; Kalif and Moutinho, 2000; [followed by hand-sorting for 45 min]), 3 days (Ward, 1987; Belshaw and Bolton, 1994; Chung et al., 2000; Longino et al., 2002; Brühl et al., 2003), 4 days (Majer and Delabie, 1999), at least 6 days (Brühl et al., 1999 [followed by hand-sorting]) to 10 days (Ratsirarson et al., 2002), or a vague ‘several days’ (Freude et al., 1965; Hammond, 1990).

In this study, we evaluate the extraction efficiency of the Winkler method for extraction periods of 3 h up to 7 weeks, calibrated by hand-sorting after 7 weeks. We compare the extraction rates and taxonomic bias at order level for all soil macro-invertebrates, at family level for beetles and at species level for beetles and centipedes. To check whether similar patterns are present in tropical environments, beetle samples of a 6-day extraction experiment carried out in Sabah (Malaysia) were

included. We will propose the optimum extraction time for the abundant macro-invertebrate groups and possible adjustment factors for the soil macro-invertebrates of temperate woodlands to compensate the taxonomic bias caused by short extraction periods.

Materials and methods

Study sites

A temperate and a tropical forest site were sampled:

(a) England, Hants, New Forest (Goriup, 1999; Tubbs, 2001), Whitley Wood, between Brockenhurst and Lyndhurst (50°51.0'N, 1°34.5'W), mature oak-beech pasture woodland (Peterken et al., 1999), National Vegetation Classification W10; sampling date: 16.vii.2002.

(b) Malaysia, Sabah (Borneo), Sepilok Arboretum (Lee and Berhaman (1992); situated adjacent to the Kabili-Sepilok Forest Reserve; 5°52.6'N, 117°56.7'E), logged-over lowland dipterocarp forest that includes good regenerating stands of naturally occurring forest species; mean annual rainfall 3144 mm; sampling date: 03. x .2001 (transitional period between drier and wetter months).

Collecting and extracting protocol

Fifteen (England) and ten (Malaysia) 1 m² samples of leaf litter and soil were taken every 7 m along a 100 m transect in the New Forest and from random spots in the Sepilok Arboretum, respectively. The litter was collected by hand and the soil was scraped up to a depth of about 3 cm with a trowel. The samples were sifted through a wire sieve with a mesh width of about 10 mm to exclude larger particles (e.g. twigs, stones). After sifting, the samples were transferred to cloth (Malaysia) or plastic bags (England) in the field for transport to the laboratory where they were emptied into the mesh bags. A maximum of three mesh bags were suspended in each Winkler extractor so as to not overload its metal frame. During filling and suspending, a plastic tray was placed below to catch falling debris, which was returned to the mesh bags using a funnel. The Winkler extractors were left suspended at room temperature over a 6-day period (Malaysia) or over 7 weeks (England). For the latter ones, we used a roof store at The Natural History Museum, London, where the temperature was around 5 °C higher than room temperature on hot days. The collecting bottles, filled

with 80% ethanol, were emptied after 3 h, 6 h, 12 h, 1 day, 2 d, 3 d, 4 d, 5 d, 6 d, 9 d, 12 d, 15 d, 18 d, 3 weeks, 4 w, 5 w, 6 w, and 7 w. After 7 weeks, we finished the Winkler extraction of the English samples because at that time, any appreciable emergence of most of the invertebrate groups had stopped for 2 weeks (except for Hymenoptera and Oligochaeta, which emerged in numbers up to 7 weeks; detailed information might be obtained from the senior author). The samples were then hand-sorted to record any remaining macro-invertebrates.

Since the sifted soil and litter samples dry out more slowly in the core than outside, some organisms may move inside (instead of leaving) the mesh bag and die there. Therefore, Freude et al. (1965) suggest to mix the samples daily. However, this introduces another methodical parameter that is difficult to standardize and may lead to a loss of fleeing arthropods. Therefore, we left the samples untouched. Scheerpeltz (1968) found that alcohol in the collecting bottles makes beetles stay in the substratum rather than falling into the bottles. However, he gave no quantitative or qualitative data. On the other hand, in Renner's (1982) pitfall experiments, alcohol was an attractant for most Coleoptera, Diptera and Hymenoptera. Since our extraction time was very long and we checked the substratum afterwards by hand-sorting, we consider a possible attractive or repellent effect of alcohol irrelevant for the present study.

Winkler extractors, mesh bags and sifters were supplied by Firma Hildegard Winkler, Dittesgasse 11, A-1180 Wien, Austria (e-mail: winkler@ento-winkler.at). A collapsible Winkler extractor is available from RN Dr. Ondrej Šauša, Entomological Instruments and Literature, Meličkovej 6/71, SK-841 05 Bratislava, Slovakia (e-mail: fyzisau@savba.sk).

Considered groups

In this study, ants, beetles (adults and larvae), earthworms and Isopoda were considered because together (in the tropics including termites), these groups make up 93% of both mean biomass density and mean abundance of macro-invertebrates obtained from hand-sorted tropical soil-monoliths (Lavelle and Fragoso, 2000). Additionally, we considered macro-invertebrate groups which emerge from soil-litter samples in significant numbers, such as non-formicid Hymenoptera (adults and larvae), Diptera (adults and larvae),

Lepidoptera (larvae), Hemiptera, Arachnida, Chilopoda, Diplopoda, and Mollusca (snails and slugs).

Collembola and Acari were not considered, because their high abundance would have required subsampling of the soil/litter quadrates to allow counting in a reasonable time. Moreover, despite their abundance in our samples, active extraction methods seem to be not very efficient for micro- and meso-arthropods (André et al., 2002). Other abundant groups of soil micro-invertebrates, e.g. Nematodes, are generally extracted by more suitable methods (McSorley and Walter, 1991) than the Winkler method.

Identification

All specimens were identified to order. Two groups efficiently extracted by the Winkler method (99.8%, 98.6%) but with different emergence patterns were identified to species (Coleoptera, FTK; Chilopoda, ED; detailed species lists might be obtained from the senior author). Five specimens of exotic species (see 'Discussion': 'Contamination') were excluded from the analysis being possible contaminants. The beetles from Borneo were sorted to parataxonomic units ("morphospecies") (FTK). The authors are aware of the limited reliability and usefulness of sorting to parataxonomic units (Krell, 2004). However, checking of the sorting by P.M. Hammond (The Natural History Museum, London) probably reduces the error rates to an extent that is negligible for purposes of the present study. The specimens are deposited in The Natural History Museum, London, and (a part of the Sabah samples) in the Forest Research Centre, Sepilok, Malaysia.

Ordination

To compare the species composition of beetles and centipedes in the samples with increasing extraction times, we used ordination. Since the number of specimens of most orders and species are high at the beginning of the Winkler extraction and low at the end, we assume that they follow a linear response model rather than a unimodal one. Hence, we use Principal Components Analysis (PCA), a method of direct gradient analysis (Canoco 4.51, Biometris – Plant Research International, Wageningen, The Netherlands; ter Braak and Šmilauer, 2002; Lepš and Šmilauer, 2003) with the options: species scores divided by standard deviation; species data log transformed; samples standardized by norm to exclude the trivial effect of

increasing number by accumulation of extraction samples over time).

Results

Extraction efficiency at higher taxonomic level

Specimen accumulation curves for the major insect groups are given in Fig. 1a, for the remaining macro-invertebrate groups in Fig. 1b. The Winkler extraction is generally an efficient method for all insect groups, spiders, centipedes and those earthworm species that live in litter or topsoil, because more than 90% of the specimens of these groups left the substratum over a 7 weeks period. The extraction efficiency was lower in Diplopoda (85%) and rather insufficient in Mollusca (65%) and Isopoda (57%). A high proportion of the Isopoda were dead at 7 weeks, while most Diplopoda and Mollusca were still alive.

If we want to record more than 70% (50%) of the specimens present in the soil/litter samples, we have to choose the following extraction times for the different groups: Formicidae 2 d (1 d), adult Coleoptera 3 d (2 d), Coleoptera larvae 12 d (3 d), Lepidoptera larvae 6 d (3 d), Diptera 12 d (5 d), Hemiptera 9 d (5 d), Hymenoptera (excl. ants) 3 w (12 d), Arachnida 9 d (3 d), Diplopoda 18 d (4 d), Chilopoda 4 w (3 w), Oligochaeta 3 w (15 d). More than 50% of Mollusca and Isopoda are extracted after 12 d and 3 w, respectively.

Taxonomic bias at higher taxonomic level

At the level of major soil macro-invertebrate groups, the composition of the samples changes with increasing extraction periods (Fig. 2). The last column in Fig. 2 should serve as a reference of the 'true' composition since it includes the hand-sorted material after the Winkler extraction. The final rank-abundance order of the most abundant 10 groups, comprising 96% of the individuals, was reached only after 5 weeks (Diptera–adult Coleoptera–Hymenoptera excl. ants–Coleoptera larvae–Arachnida–Oligochaeta–Lepidoptera–Chilopoda–Formicidae–Mollusca). The definitive rank-abundance order of the first six of them is reached after 4 weeks. On day four and day six, only the two and three leading positions are established, respectively. With extraction periods of a week or less, we greatly overestimate the proportion of adult beetles and ants, underestimating the proportion of Diptera, earthworms and molluscs.

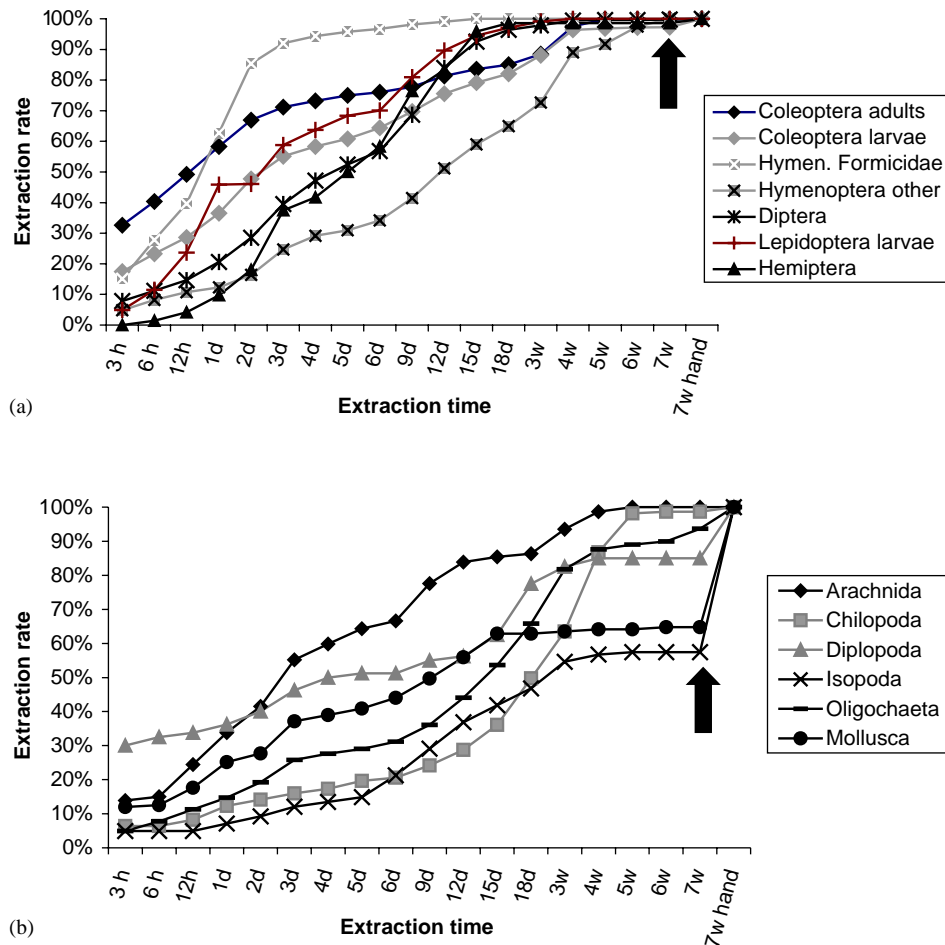


Figure 1. Efficiency of the Winkler extraction: specimen accumulation curves for soil and litter macro-invertebrate groups. Arrow: percentage of specimens extracted after 7 weeks. 7 w hand: substratum hand-sorted after 7 weeks. Note: the stronger ascent of some curves after 6 days and 3 weeks is caused by the increased period covered by one interval. (a) Insects; (b) other macro-invertebrates.

Taxonomic bias: Coleoptera species and families

Coleoptera are the most speciose group of soil macro-arthropods, they are abundant ($n = 1226$ in our English samples, $n = 431$ in the Sabah samples), and are extracted very efficiently by the Winkler method (99.8% after 7 weeks). The species accumulation curve for the English samples shows an intermediate saturation from 3 to 6 days extraction time (Fig. 3). Afterwards, although 355 additional specimens emerged, these included only 12 individuals (1%) belonged to nine additional species (15%). In the Sabah samples a possible saturation occurs from 5 days extraction time (Fig. 3). However, because of the limited extraction period and no hand-sorting of the substratum at the end of the experiment, we cannot be sure that saturation is final. After day three, 43 additional species (10%) in 44 individuals (10%) emerged.

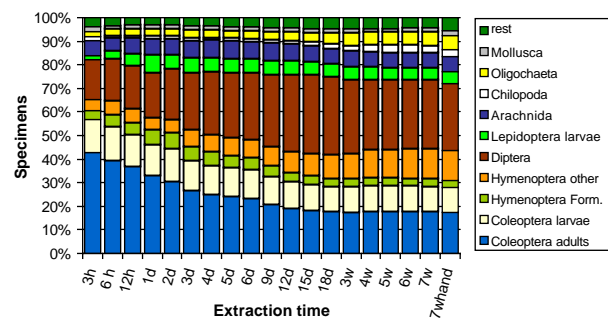


Figure 2. Proportions of soil macro-invertebrate groups among the extracted specimens from New Forest (England) samples after certain periods of time (accumulated). 7 w hand: including hand-sorted specimens after 7 weeks.

A PCA of all species data for both the English and the Sabah samples shows that the composition of the resulting species changes with extraction time

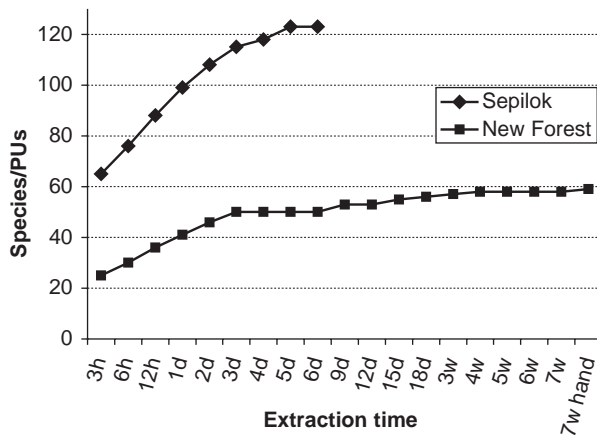


Figure 3. Species accumulation curve of soil and litter beetles from the New Forest (England) (species) and Sepilok (Sabah, Malaysia) (parataxonomic units). The Sabah experiment was stopped after 6 days. 7 w hand: substratum hand-sorted after 7 weeks.

(Figs. 4a and b). Since the extracted species are arranged on a trend line according to the exposition time, and are not distributed randomly, a taxonomic bias according to extraction time is present. To illustrate this bias, we used the most abundant families as a proxy (Fig. 5), because the species are too numerous and mostly represented by too few individuals to produce a clear graph. The family composition in the English samples changes up to 5 weeks extraction time (Fig. 5a). Before that Ptiliidae were overestimated, Staphylinidae underestimated. The relative abundance of Scydmaenidae was highest between 12 days and 3 weeks extraction time. However, the abundance rank of the six most abundant families does not change after day three in the English samples (Ptiliidae–Staphylinidae–Scydmaenidae–Curculionidae–Carabidae–Lathridiidae).

In the Sabah samples, the most obvious pattern in the change of species composition with extraction time is the increasing proportions of Pselaphinae and Silvanidae and the decreasing proportion of the remaining Staphylinidae with the length of extraction (Fig. 5b). However, the rank abundance of the six most abundant families does not change from day two. We get the same result when we treat the subfamily (and former family) Pselaphinae separately (Staphylinidae–Pselaphinae–Scydmaenidae–Curculionidae–Ptiliidae–Silvanidae–Dryopidae).

Taxonomic bias: Chilopoda species

The centipede species composition keeps changing over 5 weeks, but approximates the final

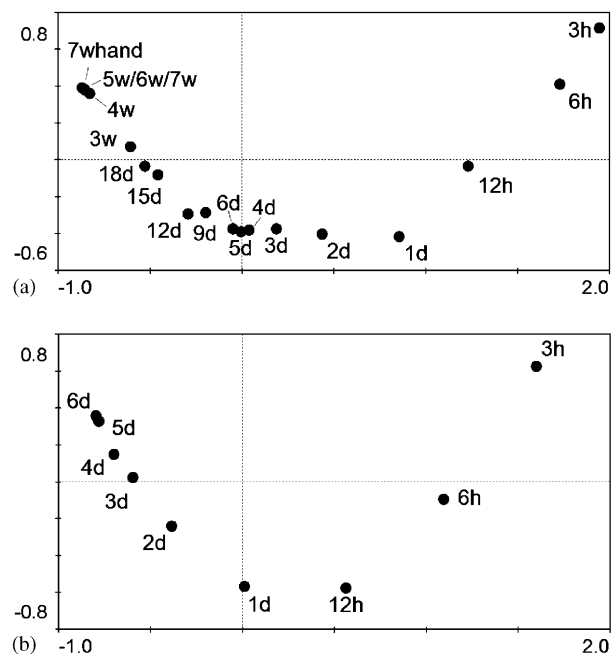


Figure 4. Principal Components Analysis of beetle species data from litter and soil from the extracted by the Winkler method after certain periods of time (data accumulated; samples standardized by norm). (a) New Forest (England) (15 m²; 3 h to 7 weeks, eigenvalues: axis 1 = 0.730; axis 2 = 0.131). (b) Sepilok (Sabah, Malaysia) (10 m²; 3 h to 6 days; eigenvalues: axis 1 = 0.702; axis 2 = 0.153).

pattern at day 15 (Fig. 6). The main variation in the species composition (of these samples) is caused by *Schendyla nemorensis* (Koch) (Schendylidae) and *Geophilus easoni* Arthur et al. (Geophilidae) which emerged in numbers only from day 15 onwards. The final rank-abundance structure was recorded with an extraction time of at least 3 weeks.

Discussion

The Winkler extraction is a simple method which does not need any human involvement in the extraction process itself. It almost completely extracts all but one of the most important soil macro-invertebrate groups (Lavelle and Fragoso, 2000) from temperate soils, including leaf litter earthworms. However, it is not an adequate method for the extraction of Isopoda and molluscs. Shell-bearing snails, the overwhelming majority of soil molluscs, are relatively tolerant to desiccation. Isopoda show a particular behaviour to regulate the water content

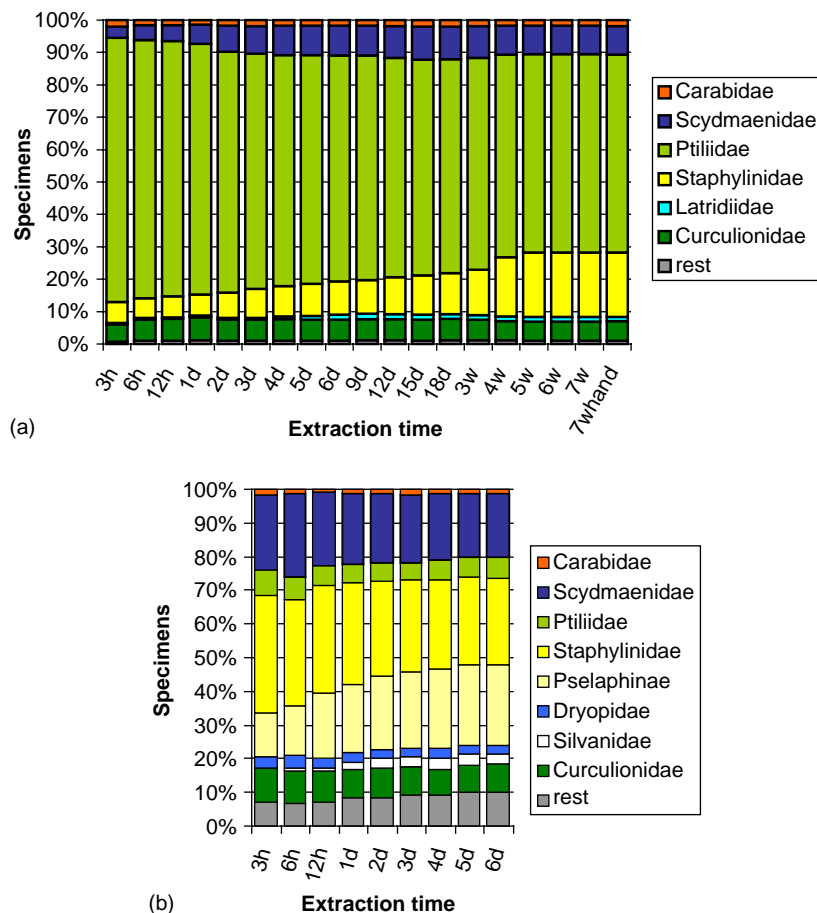


Figure 5. Proportions of beetle families among the extracted specimens from samples after certain periods of time (accumulated). (a) New Forest (England); the Staphylinidae include 11 specimens of Pselaphinae. 7 w hand: including hand-sorted specimens after 7 weeks. (b) Sepilok (Sabah, Malaysia).

of their body by moving to drier localities when coming from a moist atmosphere (Wieser, 1984). The slow drying of the litter in the mesh bags probably does not prompt the Isopoda to leave the substratum.

In the Winkler extraction, however, most groups need a long period to display the maximum possible proportion extracted, from 15 days for ants and 18 days for Hemiptera up to probably more than 7 weeks for Coleoptera larvae, Diptera and earthworms (Fig. 1a,b). The optimum extraction time depends on the aims of the study. If species richness only is the required outcome, the extraction time has to be as long as possible. If a snapshot of the soil and litter fauna at a particular time has to be recorded (point diversity), the extraction period should be shorter than the life cycle or better shorter than half the life cycle of the more abundant species (to avoid recording adults where eggs or larvae were originally present in the samples).

Bias of short extraction periods and necessary compensating factors

Traditionally short extraction times of 2 or 3 days (Ward, 1987; Olson, 1991; Belshaw and Bolton, 1994; Fisher, 1999; Chung et al., 2000; Kalif and Moutinho, 2000; Longino et al., 2002; Brühl et al., 2003) are not sufficient to get a picture of the absolute composition of the soil macro-invertebrate fauna (see Fig. 2). However, a shorter extraction time is practicable when focusing on particular groups. An extraction time of 3 days is certainly sufficient for ants since our results from the temperate woodland (92% extraction) correspond to previously published results (85% in the Afrotropics, Belshaw and Bolton, 1994; 88% in North America, Ward, 1987). Even 2 days might be acceptable for ants. Three days is probably also sufficient for adult beetles (if we do not aim at inventories or presence-absence studies) because in both our temperate and tropical samples, mainly

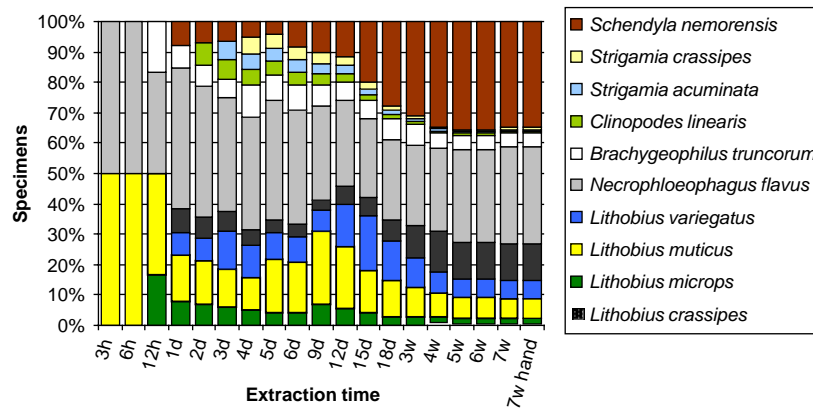


Figure 6. Proportions of centipede species among the extracted specimens ($n = 169$) from New Forest (England) samples after certain periods of time. 7w hand: including hand-sorting after 7 weeks.

singletons of previously unextracted species emerged after day three. Only two singletons of species that we have never found in the sampling area using an extraction period of 3 days (over 3 years, and 239 m² of sampling; unpubl. results) emerged in this experiment using an extraction period longer than 3 days (*Atomaria fuscipes* (Gyll.), Cryptophagidae, and *Cryptocephalus pusillus* F., Chrysomelidae) which indicates that no group of slowly emerging beetles is present in the samples. Thus, a 3 day extraction period is suitable for the most speciose group (beetles) and the often most abundant group (ants) in forest leaf litter and soil.

Because of pervasive time constraints in field-work schedules, extraction periods of many weeks or up to a point when no more individuals come out of the substratum are mostly impracticable. Extraction periods of several days up to a week should, however, be a realistic target. Based on the present study, we calculated compensating factors to estimate the real number of individuals for the various soil macro-invertebrate groups extracted after 1 day to 7 weeks (Table 1). We suggest that these compensating factors might be roughly applicable at least for temperate woodlands in Europe, probably in a wider range (Holarctic?), because we see no reason why soil macro-invertebrates should have fundamentally different emergence patterns in other temperate woodlands (probably except for very dry habitats). However, this has to be tested and recalculated, particularly for other climatic regions and different types of woodlands.

Advantages and disadvantages of long extraction

For inventories and all studies focusing on the maximum number of species recorded a long

extraction period is preferable. A long period of operation of the Winkler extractors, however, raises three possibilities for biased results. A more important bias is caused by the short life cycle of many litter arthropods leading to recording of adults which were pupae, larvae or even eggs in the original samples. The eggs might have been laid by individuals leaving the substratum so that two generations are recorded. Quantitatively less important but nevertheless of possible relevance for inventories is secondary infestation of the substratum suspended in the Winkler extractors. The influence of predation during the extraction period cannot be quantified on the basis of the available data. However, since the extraction of predators such as Araneae and particularly Chilopoda is relatively slow. Therefore, the influence of predation might increase with longer extraction time.

Life cycle bias

Three abundant beetle species show a bimodal or trimodal emergence pattern (Fig. 7). The super-abundant *Acrotrichis intermedia* Gillm. (Ptiliidae) ($n = 749$) and *Geostiba circellaris* (Grav.) (Staphylinidae) ($n = 136$) have a second smaller peak between day six and day 12, and a third larger one between day 18 and week four (in *Geostiba* week five). *A. intermedia* develops from egg to adult in 22.4 days at 20 °C (De Conninck and Coessens, 1981), and possibly in a few days less at higher temperatures. The complete development of *G. circellaris* takes 43–57 days at 16 °C (Schminke, 1978). At higher temperatures, this period decreases, too. The third peak is therefore likely to be caused by a second generation, emerged from eggs present in the original samples or laid by the adults extracted during the first few days. The longer lasting second peak of

Table 1. Factors to estimate the real number of specimens from Winkler extraction samples from temperate woodlands after different extraction times

	Formicidae	Coleoptera adults	Coleoptera larvae	Other Hymenoptera	Diptera	Lepidoptera	Hemiptera	Arachnida	Chilopoda	Diplopoda	Isopoda	Oligochaeta	Mollusca
1d	1.6	1.7	2.7	8.1	4.8	2.2	10.3	3.0	8.1	2.8	14.1	6.8	4.0
2d	1.2	1.5	2.1	6.1	3.5	2.2	5.5	2.4	7.1	2.5	10.8	5.2	3.6
3d	1.1	1.4	1.8	4.0	2.5	1.7	2.7	1.8	6.3	2.2	8.3	3.9	2.7
4d	1.1	1.4	1.7	3.4	2.1	1.6	2.4	1.7	5.8	2.0	7.4	3.6	2.6
5d	—	1.3	1.6	3.2	1.9	1.5	2.0	1.6	5.1	2.0	6.7	3.4	2.4
6d	—	1.3	1.6	2.9	1.8	1.4	1.7	1.5	4.9	2.0	4.7	3.2	2.3
9d	—	1.3	1.4	2.4	1.5	1.2	1.3	1.3	4.1	1.8	3.4	2.8	2.0
12d	—	1.2	1.3	2.0	1.2	1.1	1.2	1.2	3.5	1.8	2.7	2.3	1.8
15d	—	1.2	1.2	1.7	1.1	1.1	—	1.2	2.8	1.6	2.4	1.9	1.6
18d	—	1.2	1.2	1.5	—	—	—	1.2	2.0	1.3	2.1	1.5	1.6
3w	—	1.1	1.1	1.4	—	—	—	1.1	1.6	1.2	1.8	1.2	1.6
4w	—	—	—	1.1	—	—	—	—	1.2	1.2	1.8	1.1	1.6
5w	—	—	—	1.1	—	—	—	—	—	1.2	1.7	1.1	1.6
6w	—	—	—	—	—	—	—	—	—	1.2	1.7	1.1	1.5
7w	—	—	—	—	—	—	—	—	—	1.2	1.7	1.1	1.5

G. circellaris coincides with the longer life cycle of this species. The second peak is probably caused by individuals hatched from pupae or developed from more resilient late third instars present in the original samples. Most of the individuals of *Othius subuliformis* Steph. (syn. *O. myrmecophilus* Kies.; Staphylinidae) ($n = 64$) emerged during a second peak between 18 days and 4 weeks. The development from egg to adult in this species takes more than 200 days and the second instar needs low temperatures (Schminke, 1978). Hence, life cycle patterns do not explain the second peak in this species. Their late main emergence can either be explained by a more resilient ecological valency of the adults, or most of the specimens were pupae in the samples (phenological reasons). If the first of these explanations is true, then this species would be the only one in the present study for which a short extraction period leads to an underestimation of relative adult abundance.

Two of the slowly emerging insect groups, Diptera and (mainly parasitic) Hymenoptera (Fig. 1a) also have a short life cycle (Blackburn, 1990). The late emerging individuals were probably present in an earlier developmental stage in the original samples.

Contamination

From our English samples, five exotic specimens emerged (which were removed from the dataset for analysis). During the first 3 h and on day three, one specimen each of the powder-post beetle *Minthea rugicollis* (Walker) (Lyctidae) emerged from two different Winkler extractors. It is a pantropical species, occurring in northern Australia, south-east Asia, tropical Africa and South America (Geis, 2002), which has been imported to Britain several times (Aitken, 1975, p. 97). However, some of our Winkler extractors were previously used in the southern Côte d'Ivoire from where this species is recorded (Lesne, 1924, p. 98). A Winkler extractor contamination is, therefore, the most likely explanation for this record. On day one, an exotic *Euconnus* sp. (Scydmaenidae) emerged, possibly of the same origin as the *Minthea* specimens. We found two specimens of *Adistemia watsoni* (Wollaston) (Lathridiidae) in two Winkler samples after the fifth week. In the UK, this species of a Chilean genus has mostly been found in houses, rarely urban gardens and never in natural woodlands (Welch, 1984). Since it seems to be well established in the London area and has been recorded from the Natural History Museum for nearly a century, we

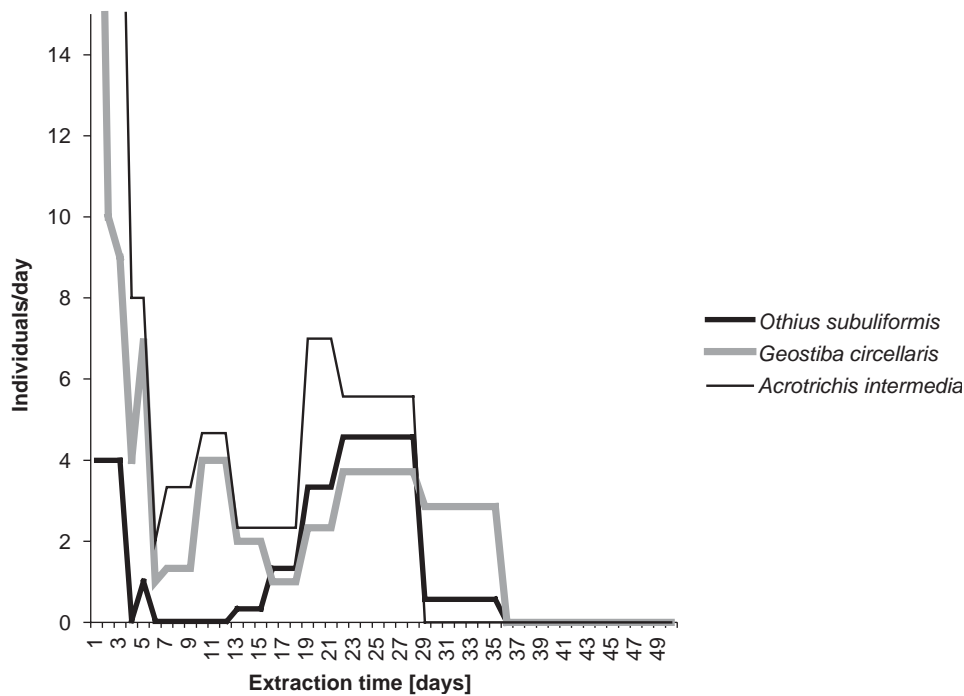


Figure 7. Winkler extracted individuals per day of three species of abundant beetles over 7 weeks.

suppose that our two specimens infested the Winkler extractors in the Museum.

Contamination is an often overlooked problem with methods that extract specimens distant from the collection area. Theoretically, the risk increases with time. However, in our case the samples were contaminated with two exotic species very early during the extraction time. Overall, contamination seems to be a minor and easily identifiable problem if the extraction is performed in enclosed rooms, but it has to be taken into consideration for inventories, presence-absence studies and in studies relying on parataxonomic sorting (cf. Krell, 2004). Contamination might be a more severe problem if the extraction is performed in an open place, say under a roof at a field station.

Conclusion

Winkler extractors are simple to use, easy to transport and do not require complex infrastructure. They tend to be used when time is limited, hence an efficient rapid extraction method is required. Generally, Winkler extractors have been used to characterize point diversity at the sampling time for ecological surveys. We have shown that for such ecological survey work extraction times of 3–7 days are probably optimal. Any larger time periods risk the appearance of secondarily emerging adults

that were not present at that stage in the original samples (e.g., adult beetles, Diptera, Hymenoptera). For inventory work, however, extraction for 6–7 weeks gives more complete results, but may not give an accurate picture of the original samples. Giving this optimal extraction time (3–7 days), the Winkler extraction is good for ants, beetles and spiders, but poor for centipedes, isopods, leaf litter earthworms, and molluscs. For inventory work, the Winkler method enables an exhaustive extraction of all soil macro-invertebrates except for isopods, millipedes, and molluscs.

Acknowledgements

We are grateful to Peter Hammond, Roger Booth and Maxwell Barclay, NHM, for help in checking and identifying difficult groups. Fieldwork in Sabah was part of the project “Tools for Monitoring Soil Biodiversity in the ASEAN Region”, funded by the Darwin Initiative, UK (project leader: David Jones). Momin Binti, Richard L. Ansis and John L. Yukang of the Entomology Section, FRC helped with field and laboratory work in Sepilok. We thank Chey Yun Khen (Forestry Research Centre, Sepilok) for support, Jonathan Spencer, Forestry Commission, for permission to undertake our studies in the New Forest, and Dorothy Newman, NHM, for helpful comments.

References

- Aitken, A.D., 1975. Insect Travellers. Vol. 1. Coleoptera. Technical Bulletin, Ministry of Agriculture, Fisheries and Food, Agricultural Development and Advisory Service, Pest Infestation Control Laboratory 31, xvi+191pp., 12 pls.
- André, H.M., Ducarme, X., Lebrun, P., 2002. Soil biodiversity: myth, reality or conning? *Oikos* 96, 3–24.
- Bardgett, R.D., 2002. Causes and consequences of biological diversity in soil. *Zoology* 105, 367–374.
- Belshaw, R., Bolton, B., 1994. A survey of the leaf litter ant fauna in Ghana, West Africa (Hymenoptera: Formicidae). *J. Hymen. Res.* 3, 5–16.
- Bestelmeyer, B.T., Agosti, D., Alonso, L.E., Brandão, C.R.F., Brown, W.L., Delabie, J.H.C., Silvestre, R., 2000. Field techniques for the study of ground-dwelling ants. An overview, description, and evaluation. In: Agosti, D., Majer, J.D., Alonso, L.E., Schultz, T.R. (Eds.), *Ants. Standard methods for measuring and monitoring biodiversity*. Smithsonian Institution Press, Washington, pp. 122–144.
- Besuchet, C., Burckhardt, D.H., Löbl, I., 1987. The “Winkler/Moczarski” elector as an efficient extractor for fungus and litter Coleoptera. *Coleopt. Bull.* 41, 392–394.
- Blackburn, T.M., 1990. Comparative and experimental studies of animal life history variation. D.Phil. Thesis, University of Oxford.
- Brühl, C.A., Eltz, T., Linsenmair, K.E., 2003. Size does matter—effects of tropical rainforest fragmentation on the leaf litter ant community in Sabah, Malaysia. *Biodivers. Conserv.* 12, 1371–1389.
- Brühl, C.A., Mohamed, M., Linsenmair, K.E., 1999. Altitudinal distribution of leaf litter ants along a transect in primary forest on Mount Kinabalu, Sabah, Malaysia. *J. Trop. Ecol.* 15, 265–277.
- Chung, A.Y.C., Eggleton, P., Speight, M.R., Hammond, P.M., Chey, V.K., 2000. The diversity of beetle assemblages in different habitat types in Sabah, Malaysia. *Bull. Entomol. Res.* 90, 475–496.
- De Conninck, E., Coessens, E., 1981. Life cycle and reproductive pattern of *Acrotrichis intermedia* (Coleoptera: Ptiliidae) in experimental conditions. *J. Nat. Hist.* 15, 1047–1055.
- Fisher, B.L., 1998. Ant diversity patterns along an elevational gradient in the Réserve Spéciale d’Anjanaharibe-Sud and on the Western Masoala Peninsula, Madagascar. *Fieldiana Zool. N.S.* 90, 39–67.
- Fisher, B.L., 1999. Improving inventory efficiency: a case study of leaf-litter ant diversity in Madagascar. *Ecol. Appl.* 9, 714–731.
- Freude, H., Harde, K.W., Lohse, G.A., 1965. Die Käfer Mitteleuropas, 1: Einführung in die Käferkunde. Goecke & Evers, Krefeld, 214pp., pls.
- Geis, K.-U., 2002. Gebietsfremde Splintholz- und Bohrkäfer, nach Mitteleuropa mit Importholz und anderen Gütern eingeschleppt. Eine Bestandsaufnahme (Coleoptera: Lyctidae, Bostrichidae). *Mitt. Int. Ent. Ver. Suppl.* 10, 1–100.
- Ghilarov, M.S., 1977. Why so many species and so many individuals can coexist in the soil. *Ecol. Bull. (Stockholm)* 25, 593–597.
- Giller, P.S., 1996. The diversity of soil communities, the ‘poor man’s tropical rainforest’. *Biodivers. Conserv.* 5, 135–168.
- Goede, R.G.M.de, Brussaard, L., 2002. Soil zoology: an indispensable component of integrated ecosystem studies. *Eur. J. Soil Biol.* 38, 1–6.
- Goriup, P. (Ed.), 1999. *The New Forest Woodlands. A Management History*. Pisces Publications, Newbury.
- Hammond, P., 1990. Insect abundance and diversity in the Dumoga-Bone National Park, N. Sulawesi, with special reference to the beetle fauna of lowland rain forest in the Toraut region. In: Knight, W.J., Holloway, J.D. (Eds.), *Insects and the Rain Forests of South East Asia (Wallacea)*. Royal Entomological Society of London, London, pp. 197–254.
- Holdhaus, K., 1910. Die Siebetechnik zum Aufsammeln der Terricolfauna (nebst Bemerkungen über die Oekologie der im Erdboden lebenden Tierwelt). *Z. Wiss. Insektenbiol.* 6, 1–4, 44–57.
- Kalif, K.A.B., Moutinho, P., 2000. Comparison of three ant-sampling methods in a tropical forest in eastern Amazonia. *Bol. Mus. Para. Emílio Goeldi Nova Sér. Zool.* 16, 75–81.
- Krell, F.-T., 2004. Parataxonomy vs. taxonomy in biodiversity studies—pitfalls and applicability of “morphospecies” sorting. *Biodivers. Conserv.* 13, 795–812.
- Kühnelt, W., Walker, N., 1976. *Soil biology with special reference to the animal kingdom*, second ed. Faber & Faber, London.
- Lasebikan, B.A., Belfield, W., Gibson, N.H.E., 1978. Comparison of relative efficiency of methods for the extraction of soil microarthropods. *Rev. Écol. Biol. Sol* 15, 39–65.
- Lavelle, P., Fragoso, C. (Eds.), 2000. I.B.O.Y. 2000 (International Biodiversity Observation Year). Soil macrofauna: an endangered resource in a changing world. Report of an International Workshop held at Bondy (France) 19–23 June 2000. IRD, Laboratoire d’Ecologie des Sols Tropicaux, Bondy (pdf at: <http://www.bondy.ird.fr/lest/iboy/workshop-report.pdf>).
- Lee, Y.F., Berhaman, A., 1992. The Sabah Forestry Department’s Arboretum at Sepilok, Sandakan. *Sandakanian* 1, 23–36.
- Leponce, M., Theunis, L., Delabie, J.H.C., Roisin, Y., 2004. Scale dependence of diversity measures in a leaf-litter ant assemblage. *Ecography* 27, 253–267.
- Lepš, J., Šmilauer, P., 2003. *Multivariate Analysis of Ecological Data using CANOCO*. Cambridge University Press, Cambridge.
- Lesne, P., 1924. *Les coléoptères bostrychides de l’Afrique tropicale française*. Encyclopédie Entomologique, 3. Lechevalier, Paris.
- Longino, J.T., Coddington, J., Colwell, R.K., 2002. The ant fauna of a tropical rain forest: estimating species richness three different ways. *Ecology* 83, 689–702. Appendix A. Methods used in the inventory of the ants

- of La Selva Biological Station. Ecol. Arch. E083-011-A1 (<http://www.esapubs.org/archive/ecol/E083/011/appendix-A.htm>).
- Majer, J.D., Delabie, J.H.C., 1999. Impact of tree isolation on arboreal and ground ant communities in cleared pasture in the Atlantic rain forest region of Bahia, Brazil. *Insectes Soc.* 46, 281–290.
- McSorley, R., Walter, D.E., 1991. Comparison of soil extraction methods for nematodes and microarthropods. *Agric. Ecosyst. Environ.* 34, 201–207.
- New, T.R., 1998. *Invertebrate Surveys for Conservation*. Oxford University Press, Oxford.
- Olson, D.M., 1991. A comparison of the efficacy of litter sifting and pitfall traps for sampling leaf litter ants (Hymenoptera, Formicidae) in a tropical wet forest, Costa Rica. *Biotropica* 23, 166–172.
- Owen, J.A., 1987. The 'Winkler extractor'. *Proc. Trans. Br. Ent. Nat. Hist. Soc.* 20, 129–132.
- Peterken, G.F., Spencer, J.W., Field, A.B., 1999. Plan for the Ancient & Ornamental Woodlands of the New Forest. Forestry Commission.
- Ratsirarson, H., Robertson, H.G., Picker, M.D., Noort, S.van, 2002. Indigenous forests versus exotic eucalypt and pine plantations: a comparison of leaf-litter invertebrate communities. *Afr. Entomol.* 10, 93–99.
- Renner, K., 1982. Coleopterenfänge mit Bodenfallen am Sandstrand der Ostseeküste, ein Beitrag zum Problem der Lockwirkung von Konservierungsmitteln. *Faun.-Ökol. Mitt.* 5, 137–146.
- Scheerpeltz, O., 1968. Irrwege in den Versuchen zur Erfassung von Zoonosen. *Nachrichtenbl. Bayer. Ent.* 17, 86–96.
- Schminke, G., 1978. Einfluß von Temperatur und Photoperiode auf Entwicklung und Diapause einiger Staphylinidae. *Pedobiologia* 18, 1–21.
- ter Braak, C.J.F., Šmilauer, P., 2002. *CANOCO Reference Manual and CanoDraw for Windows User's Guide. Software for Canonical Community Ordination (version 4.5)*. Biometris, Wageningen and České Budějovice.
- Tubbs, C.R., 2001. *The New Forest. History, ecology & conservation*. New Forest Ninth Centenary Trust, Lyndhurst.
- Ward, P.S., 1987. Distribution of the introduced Argentine ant (*Iridomyrmex humilis*) in natural habitats of the lower Sacramento Valley and its effects on the indigenous ant fauna. *Hilgardia* 55 (2), 1–16.
- Welch, R.C., 1984. *Adistemia watsoni* (Woll.) (Col., Lathridiidae) in Cambridgeshire. *Entomol. Mon. Mag.* 120, 206.
- Wieser, W., 1984. Ecophysiological adaptations of terrestrial isopods: a brief review. *Symp. Zool. Soc. London* 53, 247–265.