Tropical leaf litter inhabiting invertebrates, patterns of abundance and sampling methods

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Abstract:
Two studies of leaf-litter inhabiting invertebrates in Seychelles are reviewed. It is concluded that the most useful method of ecological investigation for this group is to use a Winkler apparatus and Tullgren funnel to sample litter inhabiting animals. This is the methodology used in studies undertaken by the Nature Protection Trust of Seychelles. The data do not demonstrate significant seasonality in numbers but do indicate seasonal distribution patterns. This should be taken into account during future studies.

Introduction

Large numbers of terrestrial invertebrates have been collected from Seychelles over the last 150 years. Many of these have been subject to taxonomic study but prior to 1988 no quantitative estimates of abundance had been made. In 1988 Birdlife International started a long-term study of invertebrate abundance on Fregate island as part of the Seychelles Magpie Robin Recovery Plan (Komdeur 1988). These studies were extended to cover Aride island in 1989 (Castle & Mileto 1991), sampling continues on Fregate but was terminated on Aride in 1994. In 1990 an ecological survey of the *Pisonia sechellarum* Friedmann, 1987 forest on Silhouette was undertaken (Oxford University Silhouette Expedition 1990). This included a extensive leaf litter sampling for invertebrates. Since then there has been an ongoing project to collect comparative data from all habitat types in the islands (Nature Protection Trust of Seychelles 1993). An important aspect of both projects has been the comparison of samples from different times of year to determine if there is any seasonal pattern of abundance. The results of these comparisons and the value of data arising from the different methods of the two projects is discussed below. In this discussion the studies on Fregate are considered in detail (those from Aride being restricted in number and being inconsistent in their methodology), those undertaken by the Oxford University Silhouette Expedition 1990 and the Nature Protection Trust of Seychelles are referred to as NPTS studies.

Methods employed by the projects

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Fregate study - The method used has been to take a 12.5cm diameter sample of soil and a 30×30cm sample of leaf-litter. Within each sample the number of invertebrates measuring over 1cm long (0.5cm for beetles and spiders) was determined by sorting through the sample by hand. 7 samples were taken at each site, this has been repeated quarterly (Komdeur 1988; Gretton 1991; McCulloch 1993). This method was employed on both Fregate and Aride until 1993 when the Aride method was altered to reduce the soil sample diameter to 10cm and to count all animals longer than 0.5cm for all groups (Lucking & Ayrton 1993).

NPTS studies - During the Oxford University Silhouette Expedition 1990 fifteen $4m^2$ samples were taken, covering a total area of $60m^2$. Only leaf litter was sampled. This was sieved using a 2.5mm mesh to remove the larger leaves and twigs which were then sorted by hand to remove the larger animals. The residue was placed into net bags and hung in a Winkler apparatus, which gradually dried the litter over a period of 3-4 days. As the litter dries the animals move to the surface and fall through the net bag into a container of alcohol below. After 3-4 days the residue was further sorted by hand to ensure that all animals had been extracted. All animals collected were then recorded, irrespective of size.

This method was employed at the sites studied subsequently using at least 10 samples, with two modifications: area per sample was reduced to $1m^2$ to speed up the drying process and two forms of litter container were used within the Winklers. In some samples net bags were used as in the original method, in others wire mesh trays were used. The net bags hold small quantities of litter in a vertical alignment wheras the mesh trays hold larger quantities horizontally. The efficinecy of the two containers are compared below. Samples were taken in June/July and December/January to compare dry and wet seasons respectively.

As these methods appear to be unreliable for collecting microscopic invertebrates (primarily collembola and mites) (Oxford University Silhouette Expedition 1990) additional data on these groups were collected by the use of a standard Tullgren funned with a funnel containing $0.01m^2$ of leaf litter above a container of alcohol and below a light bulb, providing a source of heat. The data collected by this means were compared with the Winkler data from equivalent sites to assess the efficiency of Winklers in mite and collembolan collection.

Comparisons

Litter containers in the NPTS studies were compared using a comparison of percentages of different taxa extracted from *Casuarina equisetifolia* leaf litter in the Roche Caiman Bird Sanctuary (Table 1.). As can be seen from this comparison the efficiency of net bags (indicated by the percentage of each taxon extracted) significantly exceeds that of mesh trays only for isopods and arachnids. No Diptera, Mallophaga or Coleoptera were found in mesh tray samples or Symphyla in net bags so these cannot be compared. This indicates that the use of net bags is preferable to trays as it reduces the need for hand sorting for isopods and arachnids.

Table 1. Litter container efficiency in the Roche Caiman Bird Sanctuary
significant differences marked with an asterisk (using a comparison of two Poisson distributions for 10 samples)

Taxon	Mean number extracted		Mean number in residue		% extracted	
	net bag	mesh tray	net bag	mesh tray	net bag	mesh tray
Hymenoptera	4	2	4	1	50	67
Dictyoptera	1.5	1	1	1.5	60	40
Diptera	1	0	1.5	0	20	-
Mallophaga	5	0	2	0	71	-
Coleoptera	1	0	0	0	100	-
Isopoda	4.5	20	1	17	82*	54
Symphyla	0	26.5	0	1.5	-	95
Arachnida	2.5	2	2	7	56*	22

Table 2. Microinvertebrate numbers in leaf litter samples on Mahé

Taxon	Site	Mean density (m^{-2})		t	P
		Winkler	Tullgren		
Mites	Bird Sanctuary	158.8	590.0	3.51	0.007 **
	Le Niol	54.8	708.0	2.23	0.089
	Congo Rouge	20.7	10.0	0.83	0.468
Collembola	Bird Sanctuary	161.8	910.0	2.32	0.077
	Le Niol	20.7	40.6	0.68	0.534
	Congo Rouge	3.7	3.50	0.14	0.898
Mallophaga	Bird Sanctuary	6.0	50.0	1.39	0.237
	Le Niol	0.9	50.0	1.79	0.148
	Congo Rouge	0.3	0.0	0.77	0.495
Pseudoscorpiones	Bird Sanctuary	0.0	0.0	-	-
	Le Niol	0.3	0.0	1.94	0.082
	Congo Rouge	0.3	0.0	0.77	0.495

A comparison of the numbers of microinvertebrates collected by Winklers and Tullgren funnels is given in Table 2. The only significant difference between the
Winkler and Tullgren funnel samples is that of mites at the Bird Sanctuary. Of the three sites compared this has the highest mite density, although mites are abundant at the other sites their distribution is sufficiently patchy to mean that the variance is sufficiently high to prevent statistical significance. The significance level achieved decreases in accordance with altitude because variance also increases with altitude (probably due to climatic effects). A similar pattern is demonstrated by the collembola data, although this time without significance in the Bird Sanctuary. Further samples from a range of sites are required to validate these comparisons.

Seasonality was investigated in NPTS samples by a t-test of the numbers of each taxon collected at three sites in two seasons. 10 samples were available from each site and season. The three sites selected are at different altitudes allowing

Table 3. Seasonal changes in invertebrate abundance on Mahé

Site	Taxon	Mean number		t	P
		July	January		
Bird Sanctuary	Amphipoda	12.5	3.8	2.4	0.042 *
Le Niol	Isopoda	4.9	0.0	2.38	0.039 *
Le Niol	Lepidoptera	0.7	0.0	2.33	0.045 *
Le Niol	Hymenoptera	1.9	47.0	38.48	0.000 ***

identification of any altitude effects on seasonality. The sites were Roche Caiman Bird Sanctuary (0m above sea level), Le Niol (250m), Congo Rouge (700m). Significant results are shown in Table 3. and all data summarised in Appendix I. Differences in abundance between the two seasons are significant for Bird Sanctuary amphipods, and Le Niol ants, lepidoptera and isopoda. No seasonal effects are detectable in the stable mist forest climate of Congo Rouge.

The lack of widespread seasonality makes the four groups that do appear to show some effects worth further consideration. It should be noted that there is no consistent pattern in the significant taxa; amphipods, Lepidoptera and woodlice are abundant in June whereas ants are most abundant in December.

The Bird Sanctuary amphipods are present in the leaf litter throughout the year and appear to be randomly distributed in the December samples (present in 70 % of samples). In June they are found in fewer samples (10 %) but in very high numbers. This can be explained by the observation that in all the high density samples in June amphipods were congregated under lumps of coral. These were probably sheltering in the relatively humid microclimate under the coral as, in this season, most of the leaf litter is considerably drier than in January. Thus the apparent seasonality in numbers may be an artefact of seasonality of distribution.

Lepidoptera and woodlice abundance in June at Le Niol goes against the expected pattern of low dry season numbers and is a misinterpretation of the data. Rather than abundance in June, these should be seen as being uncommon in December. In all samples where numbers are below the mean for that season ants are present at high densities. This indicates that seasonality does not influence numbers directly, rather ant numbers are the primary factor influencing their abundance. This is not surprising as both groups are flightless (Lepidoptera represented by larvae and pupae only) and subject to high levels of predation by ants ('Hymenoptera' in tables). Ant abundance follows the expected seasonal pattern: present in low numbers in most samples but in very high numbers in a single sample. Unlike amphipods this does not appear to be a seasonal microclimate restriction but results from colonial behaviour causing a single sample to increase the mean to such a level that a significant difference is unavoidable, despite a lack of any real seasonal influence. None of these differences is significantly correlated with rainfall (Pearson's rank correlation coefficient $P>0.05$ for all cases), thus the NPTS data do not identify any effect of season on animal abundance at any altitude.

Seasonal effects have been reported from Fregate (Komdeur 1988) but not Aride (Lucking & Ayrton 1993). Statistical analysis of the published data do not support these reports as animal numbers are not significantly correlated with either the date or the monthly rainfall total. The reported changes concern percentage changes in total fauna in each habitat type between consecutive samples. The demonstrated abundance changes do not conform to a seasonal pattern and may be due to other changes within the habitat, such as tree planting and livestock movement. Seasonal changes are further obscured by the very high variance figures recorded as a result of the very small sample sizes.

Summary

The data presented above demonstrate that the most useful means of collecting leaf-litter inhabiting invertebrates is to use use a Winkler apparatus for the extraction of most taxa. This should be supplemented by the use of a Tullgren funnel to collect micro-invertebrates. In the Winkler either mesh bags or wire trays can be used, the former are the most efficient for most groups.

Seasonality was not detected in the numbers of different invertebrates from three different altitudes. There was a seasonal pattern of distribution in some groups, most obviously amphipods. This will result in sampling errors from small scale studies and should be taken into account. The lack of seasonality in numbers means that samples can be compared irrespective of season of collection as long as the area covered is sufficient to overcome the distributional problem (over $10m^2$).

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Appendix I. Mean numbers of invertebrates at each site

Site	Taxon	Mean number		t	P
		July	January		
Bird Sanctuary	Annelida	0.0	0.3	0.42	1.000
	Mollusca	5.3	5.0	0.30	0.725
	Amphipoda	12.5	3.8	2.43	0.042
	Isopoda	58.8	112.0	0.89	0.405
	Myriapoda	0.2	11.7	0.91	0.455
	Araneae	5.2	8.3	0.51	0.631
	Thysanura	0.2	0.0	0.68	0.516
	Psocoptera	1.2	1.3	0.13	0.897
	Dictyoptera	0.8	1.7	0.67	0.520
	Dermaptera	1.2	4.7	1.44	0.281
	Mallophaga	6.0	5.3	0.20	0.847
	Homoptera	0.2	0.0	0.68	0.516
	Diptera	4.0	1.7	0.73	0.491
	Lepidoptera	3.7	0.3	1.20	0.370
	Hymenoptera	13.5	8.3	0.53	0.609
	Coleoptera	6.8	6.3	0.58	0.621
Le Niol	Nemertea	0.0	1.0	0.43	0.667
	Annelida	2.0	0.0	0.87	0.478
	Mollusca	12.2	15.0	0.67	0.510
	Myriapoda	2.2	0.5	1.10	0.298
	Araneae	5.9	0.0	1.92	0.090
	Opilionida	2.0	1.0	0.43	0.707
	Schizomida	0.3	3.5	5.89	0.060
	Pseudoscorpiones	0.3	0.0	1.96	0.081
	Isopoda	4.9	0.0	2.38	0.039 *
	Amphipoda	0.0	1.0	0.67	0.500
	Psocoptera	2.5	0.5	1.25	0.241
	Dictyoptera	1.6	3.5	1.13	0.284
	Dermaptera	0.0	16.5	33.00	0.190
	Mallophaga	0.4	0.0	0.78	0.454
	Hemiptera	1.0	0.0	1.81	0.204
	Diptera	0.0	1.0	0.42	0.667
	Lepidoptera	0.7	0.0	2.33	0.045 *
	Hymenoptera	1.9	47.0	38.48	0.000 ***
	Coleoptera	9.1	6.0	1.11	0.293
Congo Rouge	Mollusca	5.7	9.0	0.50	0.667
	Isopoda	1.7	0.0	0.94	0.444
	Myriapoda	1.6	9.0	1.00	0.557
	Thysanura	0.3	0.0	0.50	0.667
	Psocoptera	0.3	0.0	0.50	0.667
	Dermaptera	0.3	0.0	0.50	0.667
	Orthoptera	0.0	1.0	0.52	0.720
	Mallophaga	0.3	0.0	0.50	0.667

	Diptera	4.3	2.0	0.63	0.594
	Hemiptera	0.3	0.0	0.50	0.667
	Hymenoptera	32.3	6.0	0.51	0.663
	Coleoptera	7.7	11.0	0.61	0.604