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Efficiency of sampling methods and effort to assess arthropod diversity in Azorean native forests

CLARA GASPAR, P. CARDOSO, P.A.V. BORGES & K.J. GASTON

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The BALA project (Biodiversity of Arthropods of Laurisilva of the Azores) is a research initiative to quantify the spatial distribution of arthropod biodiversity in native forests of the Azores archipelago. Arthropods were collected using a combination of two techniques, targeting epigean (ground dwelling) and canopy (arboreal) arthropods: pitfall traps (with Turquin and Ethylene solutions) and beating samples (using the three most dominant plant species). A total of 109 transects distributed amongst 18 forest fragments in seven of the nine Azorean islands were used in this study. The performance of alternative sampling methods and effort were tested. No significant differences were found in the accumulated number of species captured whether an alternative method was used or whether another transect with similar effort was established in another location within the same fragment. A combination of Ethylene and Turquin traps captured more species per individual, Turquin and beating captured more species per sample, and Turquin captured more species per unit time. An optimization exercise was performed and we found that the protocol applied during recent years is very close to optimal, allowing its future replication with confidence. The minimum combinations of sampling effort and methods, in order to monitor or to inventory diversity, taking into account different proportions of sample completeness are discussed.

Key words: Beating, Pitfall, Sample completeness, Sampling effort, Species accumulation curves

Clara Gaspar[1,2] (email: cgaspar@ennor.org), Pedro Cardoso[1,3], Paulo A.V. Borges[1,4] [1]Azorean Biodiversity Group (CITA-A) and Portuguese Network for Ecological and Environmental Research, Departamento de Ciências Agrárias, Universidade dos Açores, Pico da Urze, 9700-042 Angra do Heroísmo, Portugal; Kevin J. Gaston[2,5], [2]Biodiversity and Macroecology Group, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK; [3]Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland; [4]Azorean Biodiversity Group, Centre for Ecology, Evolution and Environmental Changes (Ce3C), Departamento de Ciências Agrárias, Universidade dos Açores, Angra do Heroísmo, Portugal; [5]Environment and Sustainability Institute, University of Exeter, Penryn, Cornwall TR10 9EZ, UK.

INTRODUCTION

It is widely recognised that the species diversity recorded in a given site will greatly depend on the sampling effort (i.e., number of samples) and on the sampling methods applied in the field (Hortal et al. 2001; Moreno & Halffter 2001; Longino et al. 2002; Romo et al. 2006; Ellison et al. 2007; Ferrer-Paris et al. 2013). Efficient effort and methods can guarantee that the least time, money

and number of persons are needed to attain the highest fraction of species possible (Cardoso 2009; Cardoso et al. 2009a). Both parameters, effort and methods, should be optimized, otherwise, the overall sampling efficiency may be compromised. However, statistical techniques to evaluate sampling efficiency are scarce and have seldom been tested, particularly for methods. In fact, whilst some attempts have been made to explore sampling effort efficiency using species rarefaction techniques (DeVries et al. 1997; Standen 2000; Sackmann et al. 2006; Solow & Roberts 2006), studies focusing on the evaluation of the efficiency of combining different sampling methods and effort per method combinations have been poorly developed (but see Jiménez-Valverde & Lobo 2006; Cardoso 2009; Cardoso et al. 2009a; Tista & Fiedler 2011).

Proposals of sampling programmes to inventory and monitor diversity should be included in every long-term ecological management plan, but they have been neglected until the present (Stork et al. 1996; Rohr et al. 2007). Commonly, each collector uses sampling methods and efforts that are selected based on his/her own experience and preference. Besides potentially making for a generally more costly and less effective choice, this also has the disadvantage of not allowing comparability among places sampled by different people and may lead to errors in the selection of priority areas or in the definition of management priorities at larger spatial scales.

Different sampling effort and method efficiencies may be needed depending on whether the aim is to inventory or to monitor diversity (Stork et al. 1996; Longino & Cowell 1997). An inventory of diversity is desirable when poor information is available for the target group and area. As the aim is to maximize diversity, a high level of sample completeness (i.e., number of observed species in relation to expected species) is required. In contrast, monitoring of diversity is periodic, to determine any signs of change in the diversity itself or a change in the habitat that may affect diversity (Noss 1990; Stork et al. 1996; Bawa & Menon 1997; Hilty & Merenlender 2000; Yoccoz et al. 2001; Danielsen et al. 2005). In this case, minimization of time and resources should be favoured, thus, a lower level of sample completeness may be acceptable. Usually, surrogates of diversity or of habitat quality also prove particularly useful to monitor diversity (see Gaspar et al. 2010 for arthropods in the Azores).

An extensive standardised sampling programme to assess epigean (ground dwelling) and canopy (arboreal) arthropods diversity has been established in the native forests of the Azores archipelago in recent years within BALA project - "Biodiversity of Arthropods of Laurisilva of the Azores" (Borges et al. 2005; Ribeiro et al. 2005; Gaspar et al. 2008). However, the efficiency of the sampling effort and methods applied has never been explored. In this study, the arthropod data from the sampling program are used to: a) compare the efficiency of different sampling methods and efforts, b) evaluate the performance of alternative sampling methods and efforts, c) determine minimum combinations of methods and numbers of samples needed to attain different proportions of sample completeness, d) evaluate if the base protocol is optimized, and e) discuss distinct sampling protocols to inventory and to monitor arthropod diversity in Azorean native forests.

MATERIAL AND METHODS

Study area

This study was developed in the remote islands of the Azores archipelago, located in the North Atlantic Ocean (37-40ºN, 25-31ºW). The archipelago has a recent volcanic origin (0.30 - 8.12 million years old), with frequent volcanic and seismic activities. The climate is temperate humid at sea level, and cold oceanic at higher altitudes. The atmospheric humidity is high with small temperature fluctuations throughout the year.

The Azorean islands have a particular type of forest, Laurisilva, composed of mainly endemic evergreen tree and shrub species, the most dominant being *Juniperus brevifolia* (Seub.) Antoine, *Laurus azorica* (Seub.) Franco, *Ilex perado* Aiton subsp. *azorica* (Loes.) Tutin, *Vaccinium cylindraceum* Sm. and *Erica azorica* Hochst. ex Seub. The native forest in the Azores is characterised by a dense tree and shrub cover of small stature, closed canopy, extensive overlay of bryophytes, high levels of humidity and low understorey light.

At present, due to human intervention, Laurisilva is mainly restricted to high and steep areas, where there are almost no economic interests. Amongst the nine islands of the archipelago, Terceira preserves one of the largest and least disturbed native forest covers of the Azores, with more than 2300 ha, which corresponds to about 40 % of the total native forest in the archipelago (Gaspar et al. 2008, 2011).

Sampling protocol

During the summers of 1999 to 2004, transects were established in 18 native forest fragments distributed across seven Azorean islands. The fragments selected represent most of the native forest cover of the archipelago, as the remaining patches are highly fragmented, small (less than five hectares), located at low altitudes and/or strongly disturbed by exotic plants or cattle. At least four transects were set per fragment. Sites were chosen in a stratified random design (as long as they were accessible) to capture the overall diversity of vegetation subtypes occurring in each fragment.

Along each transect of 150 m length and 5 m width, thirty pitfall traps were placed and ten beating samples per dominant plant species (commonly three species) were made and will be hereafter referred to as base transects. Pitfall traps to sample epigean (ground-dwelling) arthropods consisted of plastic cups with 4.2 cm diameter and 7.8 cm deep. Fifteen traps were half-filled with a non-attractive solution (anti-freeze, containing a small proportion of Ethylene glycol as a preservative) and will be hereafter named Ethylene traps. The other fifteen traps were half-filled with a general attractive solution prepared mainly with dark beer and some preservatives and referred to as Turquin traps (for further details see Turquin 1973 and Borges 1992). A few drops of liquid detergent were added to both solutions to reduce surface tension. The traps were sunk in the soil (with their rims at surface level) every 5 m, starting with a Turquin trap and alternating with the Ethylene traps. They were protected from rain using a plastic plate, about 5 cm above surface level and fixed to the ground by two pieces of wire. The traps remained in the field for two weeks. Canopy sampling for arboreal arthropods was conducted during the period that pitfall traps remained in the field, when the vegetation was dry. A 5 m wide square was established every 15 m (10 squares in total per transect). In each square, a replicate of the three most abundant woody plant species was sampled. The evaluation of the most dominant plants in a given transect was made visually by each member of the team and then discussed. In most of the studied transects, three species clearly dominated over the remaining plants and the choice was unanimous. In some transects, fewer than three were present and only those were considered. For each selected plant, a branch was chosen at random and a beating tray placed beneath. Five beatings were made using a stick. The tray consisted of a cloth inverted pyramid 1 m wide and 60 cm deep (adapted from Basset 1999) with a plastic bag at the end.

A more intensive sampling effort was applied to selected base transects on Terceira Island to test particular sampling additions: 1) transect extension, 2) non-dominant plants and 3) resampling in a different year. The three additions of sampling effort were tested independently for distinct transects located in different forest fragments of Terceira Island. A limited amount of resources prevented the implementation of this intensive sampling protocol on a large number of sites.

(1) A transect was extended by adding another 150 m long transect at the end of the base transect, following a similar direction. The same sampling methods and effort were applied to the extended transect, that is, 30 pitfall traps and 30 beating samples were made. Transect extension was carried out in three sites of the forest fragment of Sta. Bárbara and Mistérios Negros (TESB).

(2) In the same 10 squares defined to sample three dominant plant species along a base transect, three less dominant (not rare) plant species were also sampled. In total, 30 samples of non-dominants were available to be compared with 30 samples of the three most dominant species. This additional sampling was performed in four sites of the Biscoito da Ferraria (TEBF) fragment.

(3) Some base transects were re-sampled in a different year. Removable marks in trees and shrubs and a rope along the transect were maintained in the field to repeat pitfall and beating samples (30 samples of each method, 60 in total) in similar locations. This was applied in three sites of the fragment Terra Brava (TETB).

All Araneae, Opiliones, Pseudoscorpiones, Myriapoda and Insecta (excluding Diptera and Hymenoptera) were considered for this study. Several taxonomists (see Acknowledgments) checked the identifications made.

Data analyses

Turquin and Ethylene pitfall traps were considered as independent sampling methods for analyses because they differ in their attracting ability and may vary in their efficiency to capture epigean arthropod species. For most analyses, samples from each of the three dominant plant species of each transect were also analysed separately. Thus, five units of sampling methods were considered, except where noted.

Two datasets on the arthropod species composition and abundance per sample were used for analyses: 1) an archipelago database and 2) a Terceira Island database.

(1) A large database at the archipelago level was used to evaluate the efficiency of different sampling methods and effort. A total of 109 transects were considered. Of those, 11 transects had a single dominant woody plant species, 24 had two dominant species, while 74 transects had three. Overall, there were 3,270 pitfall trap samples (1,635 samples for each Turquin and Ethylene traps) and 2,810 beating samples (1,090 samples of the most dominant plant species, 980 samples of the second most dominant species and 740 samples of the third most dominant plant species).

(2) A smaller database based on additional work was used to evaluate the performance of alternative sampling methods and efforts. Transect extension was carried out in three sites, resulting in 360 samples: 90 Ethylene traps, 90 Turquin traps, and 60 beating samples of each of the three most dominant plant species. The addition of non-dominant plants was performed in four sites, with a total of 360 samples: 60 Ethylene traps, 60 Turquin traps, and 40 beating samples for each of the three dominant and the three non-dominant plant species. Re-sampling in a different year was applied in three sites, resulting in 360 samples: 90 Ethylene traps, 90 Turquin traps, and 60 beating samples for each of the three most dominant plant species. In all transects, it became evident that whenever *Juniperus brevifolia* occurred as one of the three most dominant plant species, it was always the most dominant species and thus considered as such for analyses.

The EstimateS 7.5 program (Colwell 2005) was used to calculate randomized observed species accumulation curves. Mao Tau randomized curves were preferred rather than the classical mean observed curves, since 95% confidence intervals are calculated directly with EstimateS (Colwell 2005) and curves were found to be accurate for interpolation (Colwell et al. 2004; Mao et al. 2005). This allowed the visual comparison of curves at a significance level of 0.05, by comparing the 95% intervals of one sampling strategy with the observed curve of other sampling strategy. The accumulation of observed species was compared using different units of sampling effort: individuals (species richness, Colwell et al. 2004), samples (species density, Colwell et al. 2004) and time (based on the minutes needed to complete one sample, from field work to sorting process, identification took similar time among sampling units so it was not considered).

Sample completeness was defined as the number of species observed, for a given number of samples, in relation to the estimated number of species for the overall number of samples. The Chao 1 species richness estimator was chosen as it is commonly used in the literature to calculate sample completeness (Sorensen et al. 2002; Scharff et al. 2003; Colwell 2005; Cardoso et al. 2008a, b, 2009a, b). Also, due to its formula (based on singletons and doubletons), it is inherently insensitive to the way in which species are aggregated in samples. This was also shown in a previous study with arthropods in native forests of the Azores, on the performance of different estimators across spatial grains (Hortal et al. 2006). The classic formula of Chao 1 with its corresponding 95% confidence limits was calcu-

lated using EstimateS (Colwell 2005). Correlation analyses to compare species richness between pairs of sampling units were carried out with Spearman's rank correlation using SPSS 12.0 software (SPSS Inc 2004).

The addition of sampling effort for a given transect was compared with the base transect using observed species accumulation curves (EstimateS, Colwell 2005). For the three additions of effort (transect extension, non-dominant plants and re-sampling in a different year), the analysis procedure was similar: the accumulation curve for a base transect combined with the addition of effort was compared with the accumulation curve of the combination of the base transect with another base transect. This way, it was possible to determine what captured more species, additional sampling effort to that employed in a base transect or adding another base transect in a different location within the same fragment. Since several transects were available for each addition of effort, an accumulation curve was made for each base transect plus the addition of effort for that transect, and for all possible combinations of one base transect with another. Averages of the accumulation curves were then determined by extracting the mean of the interpolated observed species richness for each sample point and then plotting the resulting average curve. Confidence intervals (95%) were determined for the means at each sample point. The use of a single curve with the total of combined transects instead of an average curve would not be accurate as a previous combination of transects would cause species overlap (also pointed by Standen 2000).

Finally, we assessed the efficiency of the base transect of the BALA protocol compared with a theoretical protocol optimized for capturing maximum species richness with minimum effort, based on the guidelines and procedures provided by Cardoso (2009). For each of the six sites where either a transect extension or re-sampling in a different year was done (with a total of 120 samples at each site) we resampled the datasets to 60 samples, the number used in the base transect of the BALA protocol. Beating, Ethylene and Turquin traps were considered as different methods, so that we had 60 + 30 + 30 samples per site to resample. The efficiency of the protocols was tested by running 1000 sampling simulations per site. For each simulation we calculated the average and confidence intervals of the species richness observed for each protocol. Specific software (www.ennor.org/pro_software.html) was used for the purpose (Cardoso, 2009).

RESULTS

A total of 128,101 identifiable specimens, distributed amongst 21 taxonomic orders, 104 families, 348 genera, and representing 440 species were collected in 18 native forest fragments from seven islands of the Azores archipelago.

At least 41.5 hours were needed to complete a base transect, composed of 30 pitfall traps and 30 beating samples. Four hours were required in the field (Table 1), 22.5 hours sorting samples (Table 1), and 15 hours identifying specimens (15 minutes per sample). Pitfall samples required less time in the field and laboratory and fewer human resources than beating samples (pitfall traps: 16.5 hours, one person required, beating samples: 25 hours, two persons required in the field, Table 1).
The species richness captured per transect in Ethylene traps was significantly correlated with that of the remaining sampling methods, with a greater correlation coefficient and significance for Turquin traps and Plant 3 (Table 2). Conversely, Turquin traps did not show a significant correlation with any of the other sampling methods except Ethylene (Table 2). Species richness per transect from Plant 1, Plant 2 and Plant 3 were significantly correlated among each other, with similar coefficients and significance values (Table 2).

The comparison of the average number of species found per transect using different combinations of sampling methods showed that sampling one of the most dominant plant species captured more species (23.6 ± 1.2 spp) than Ethylene (17.7 ± 1.0 spp) or Turquin traps (19.9 ± 1.1 spp, Fig. 1). However, when Ethylene and Turquin traps were combined, the number of species caught (25.6 ± 1.4 spp) was not different from the species richness found for one plant species (Fig. 1).

Table 1. Minimum time (in minutes), human and financial (consumable material) resources needed to accomplish each of the sampling methods applied in the field and in the laboratory (sorting and identification) for a base transect (i.e. 15 samples of Ethylene, 15 samples of Turquin and three plants each with 10 samples).

		Time (min.)			N. Persons		Material	
	N. samples	**Field**	**Sort.**	**Ident.**	**Field**	**Lab**	**Field**	**Lab**
Ethylene	15	45	225	225	1	1	Anti-freezing	Alcohol
Turquin	15	45	225	225	1	1	Beer, preservatives	Alcohol
Plant 1	10	50	300	150	2	1	-	Alcohol
Plant 2	10	50	300	150	2	1	-	Alcohol
Plant 3	10	50	300	150	2	1	-	Alcohol

Table 2. Spearman correlation coefficients and their significance values for species richness among sampling methods (n=74; Note: * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001).

	Ethylene	**Turquin**		**Plant 1**		**Plant 2**		**Plant 3**	
Ethylene	—	0.663	***	0.352	**	0.281	*	0.424	***
Turquin		—		0.196		0.197		0.178	
Plant 1				—		0.505	***	0.675	***
Plant 2						—		0.546	***
Plant 3								—	

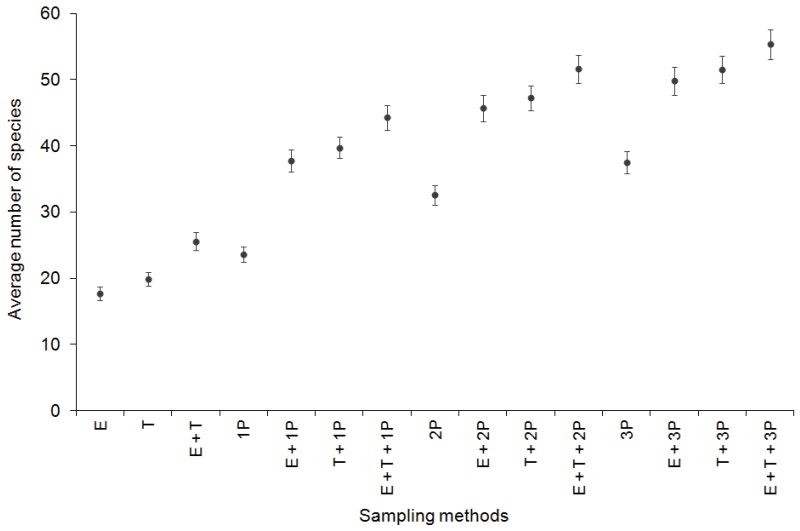
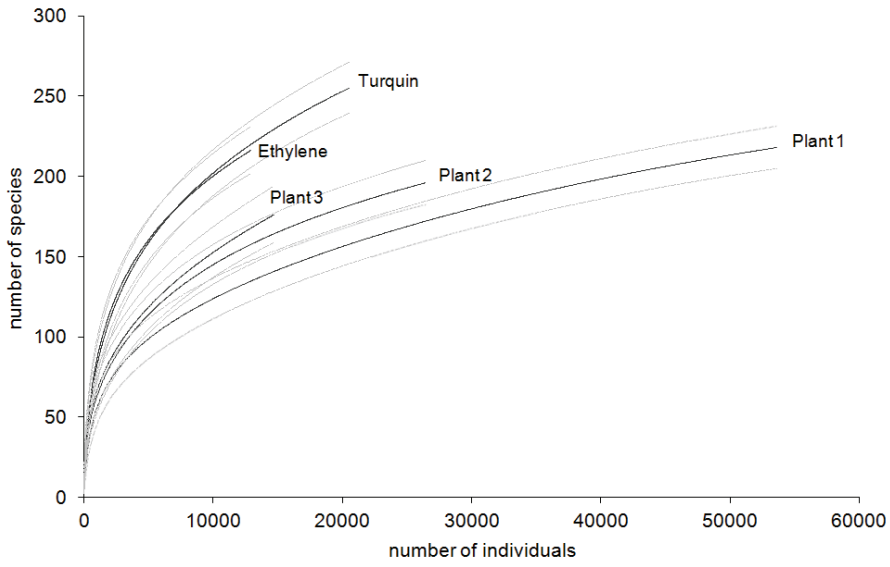


Fig.1. Average number of species per transect (with 95% confidence limits) using different combinations of sampling methods (n=109 for combinations including 1P, n=98 for those including 2P, n=74 for 3P). E-Ethylene traps, T-Turquin traps, P-Beating samples (average of all combinations of one, two or three dominant plant species).

The combination of Ethylene (37.8 ± 1.7 spp) or Turquin traps (39.7 ± 1.6 spp) with one plant species substantially increased the number of species (Fig. 1). When combined, Ethylene, Turquin and one plant (44.3 ± 1.9 spp) were not different in number of species from the combination of Ethylene (45.6 ± 2.0 spp) or Turquin (47.2 ± 1.9 spp) with two plants (Fig. 1). The average number of species captured with all methods combined (55.3 ± 2.3 spp) was not different from the combination of Ethylene, Turquin and two plants (51.6 ± 2.2 spp), or of Turquin and three plants (51.5 ± 2.0 spp, Fig. 1).

Fig. 2. (below and following page) Observed species accumulation curves (dark lines) and their corresponding 95% confidence limits (dashed lines) for the sampling methods studied, using different units of sampling effort: a) number of individuals (species richness), b) number of samples (species density), and c) number of minutes (time). Figures b) and c) do not show the 95% confidence intervals for the accumulation curves of Plant 1 and Plant 3, due to their redundancy and for clarity.

a)



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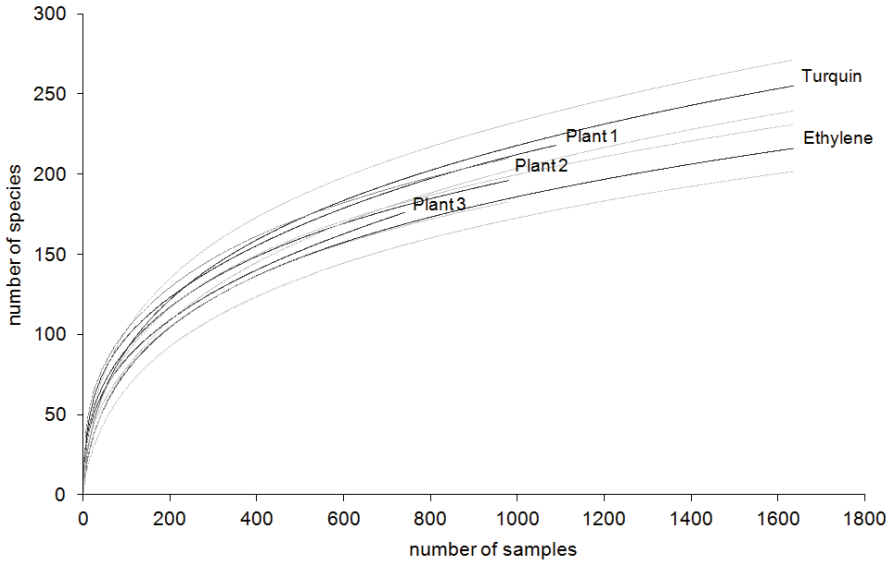

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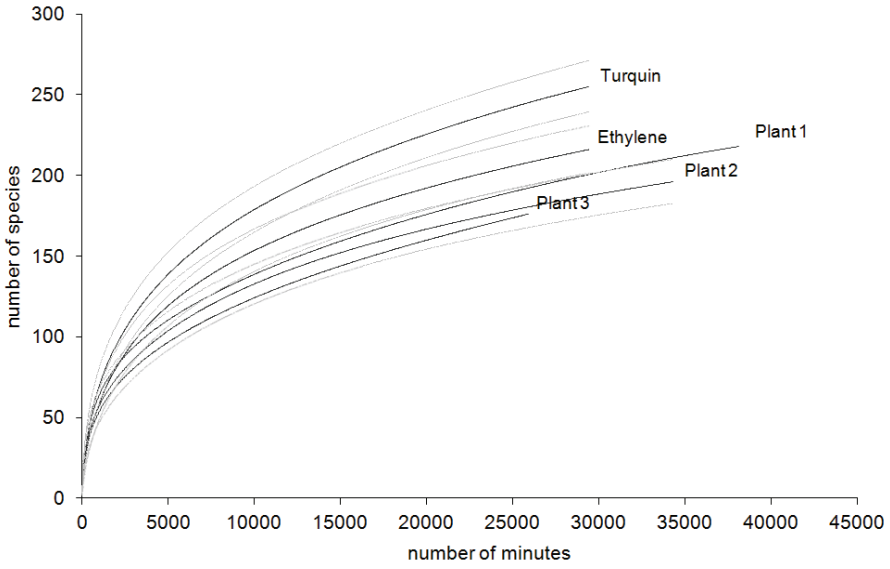

A significantly smaller number of individuals from Turquin or Ethylene traps were needed to accomplish the same species richness as for plant samples (Fig. 2a). For example, to attain 202 arthropod species on average, 10,000 individuals from Turquin traps were needed, while 41,044 individuals from Plant 1 were necessary (Fig. 2a). Turquin and Ethylene traps did not show significant differences in the number of species recorded per number of individuals (Fig. 2a). Sampling of Plant 3 captured significantly more species with a smaller number of individuals than Plant 1 (Fig. 2a).

Comparing the species density among sampling methods, Turquin caught significantly more species with a smaller number of samples than Ethylene traps (Fig. 2b). No significant differences were found among plants nor between plants and Ethylene or Turquin traps (Fig. 2b).

Regarding the time needed in order to complete a sample as a measure of sampling effort, Turquin traps caught significantly more species in less time than any other method (Fig. 2c). Ethylene and Plant 1 captured a similar number of species in the same time (Fig. 2c), while Plant 2 and Plant 3 required more time to capture the same number of species than Ethylene traps (Fig. 2c).

Plant 1 and Plant 2 achieved the highest sample completeness using the lowest number of samples (Fig. 3). Ethylene traps, Turquin traps and Plant 3 required a similar number of samples for the same proportions of sample completeness of each method (Fig. 3).

The most dominant plant species, Plant 1, was more efficient than Ethylene or Turquin methods to represent the overall estimated species richness of the largest native forest fragment of Terceira Island (Fig. 4). In fact, Plant 1 needed a smaller number of samples to achieve the same sample completeness as the other two methods (Fig. 4). Turquin together with Plant 1 were the most efficient combination of two methods (Fig. 4). Joined with the second and the third dominant plant species, they formed the most efficient combination groups of three and four sampling methods (Fig. 4).

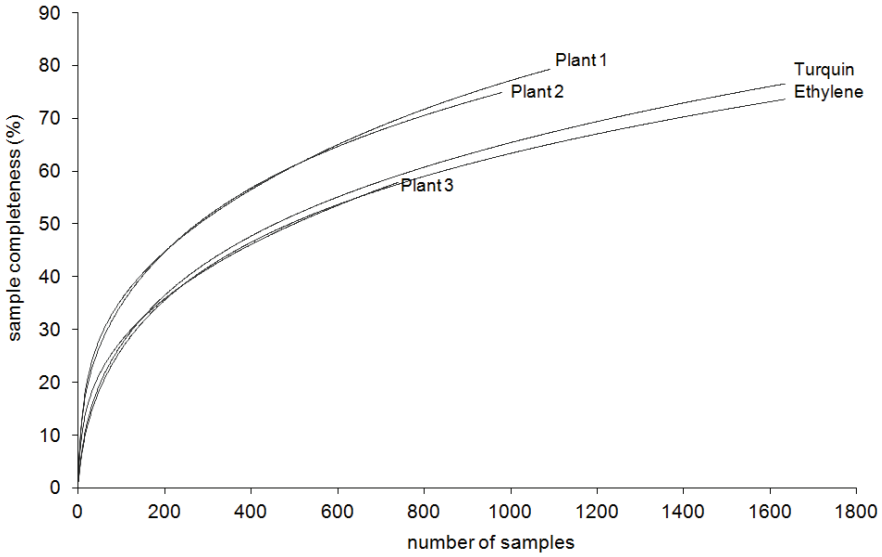

Fig. 3. Number of samples needed to achieve a given proportion of sample completeness (number of species observed in relation to the estimated species richness for each method) for each sampling method (data from 109 transects of seven islands).

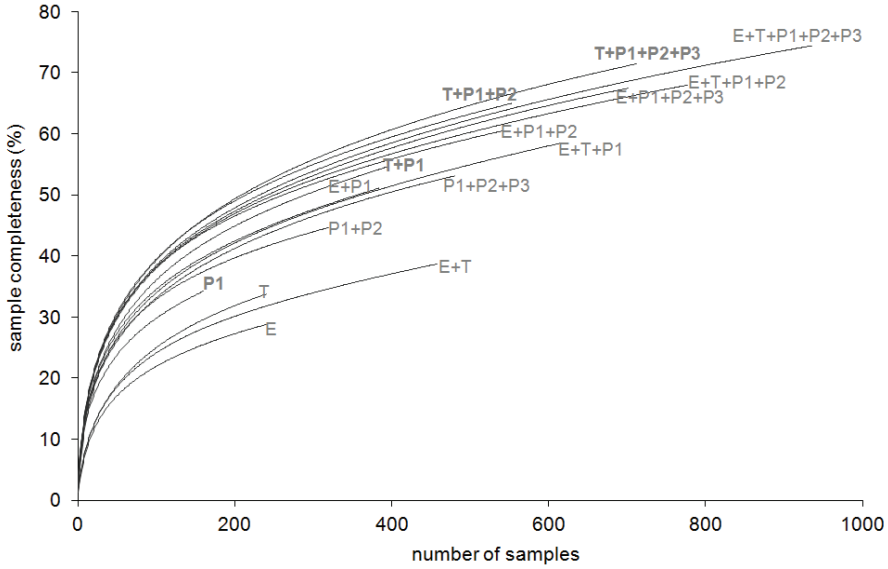

Fig. 4. Number of samples needed to achieve a given proportion of sample completeness (number of species observed for each combination of methods in relation to estimated species richness for all methods combined) using different combinations of the sampling methods studied: E-Ethylene traps, T-Turquin traps, P1, P2, P3-first, second and third most dominant plant species (data from 16 transects from the largest fragment of Terceira Island, TESB).

The number of species gained from doubling the extension of a given transect was not significantly different to that gained by adding another transect at another location in the same fragment (Fig. 5). Similarly, the addition of non-dominant plant species to a given transect or adding dominant plants from another transect at a different location within the fragment showed similar species richness (Fig. 6). The same pattern was found when adding the same transect in a different year or adding another distinct transect (Fig. 7). Overall, none of the combinations of sampling methods and effort tested, including the average of a base transect and the accumulation of all transects with their additions of effort showed significant differences in the species richness observed (Figs. 5-7).

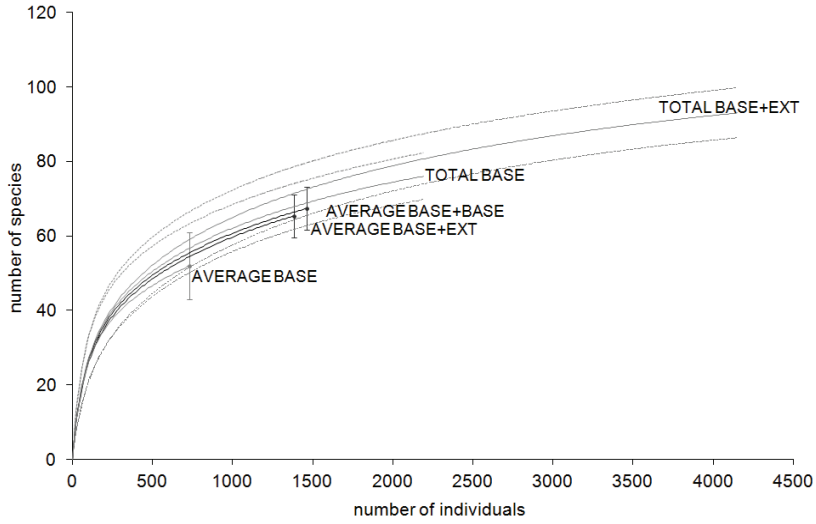

Fig. 5. Comparison of observed and average species accumulation curves (and corresponding 95% confidence intervals, dashed lines and error bars) for different additions of sampling effort and methods. AVERAGE BASE-average of base transects, TOTAL BASE-accumulation of base transects, TOTAL BASE+EXT-accumulation of base transects with their correspondent extensions, AVERAGE BASE+EXT-average of base transects with their extensions, AVERAGE BASE+BASE-average of two base transects combined.

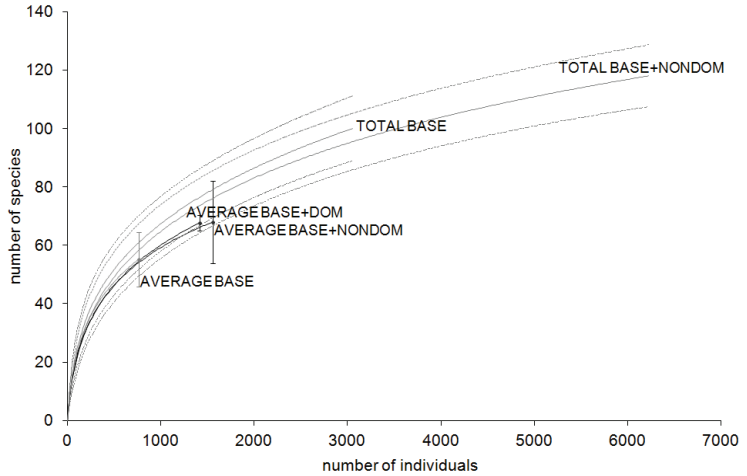

Fig.6 (previous page). Comparison of observed and average species accumulation curves (and correspondent 95% confidence intervals, dashed lines and error bars) for different additions of sampling effort and methods. AVERAGE BASE-average of base transects, TOTAL BASE-accumulation of base transects, TOTAL BASE+NONDOM-accumulation of base transects with their correspondent non-dominant plant species, AVERAGE BASE+NONDOM-average of base transects with their non-dominant plant species, AVERAGE BASE+DOM-average of base transects combined with dominant plant species of other base transects.

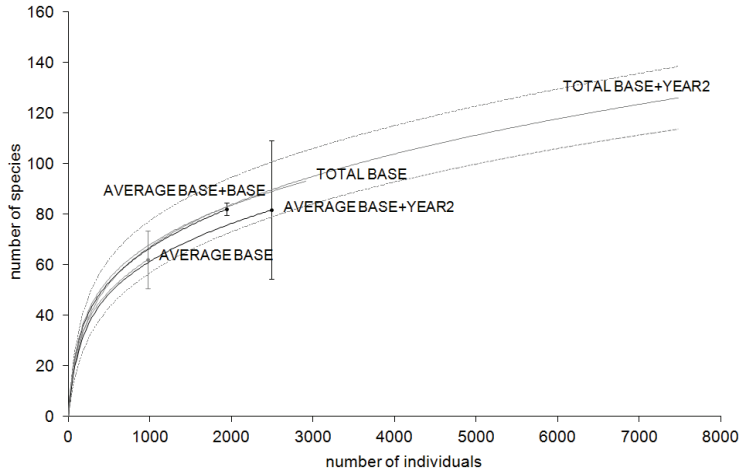

Fig.7. Comparison of observed and average species accumulation curves (and correspondent 95% confidence intervals, dashed lines and error bars) for different additions of sampling effort and methods. AVERAGE BASE-average of base transects, TOTAL BASE-accumulation of base transects, TOTAL BASE+YEAR2-accumulation of base transects with their correspondent re-sampling in a different year, AVERAGE BASE+YEAR2-average of base transects with re-sampling in a different year, AVERAGE BASE+BASE-average of two base transects combined.

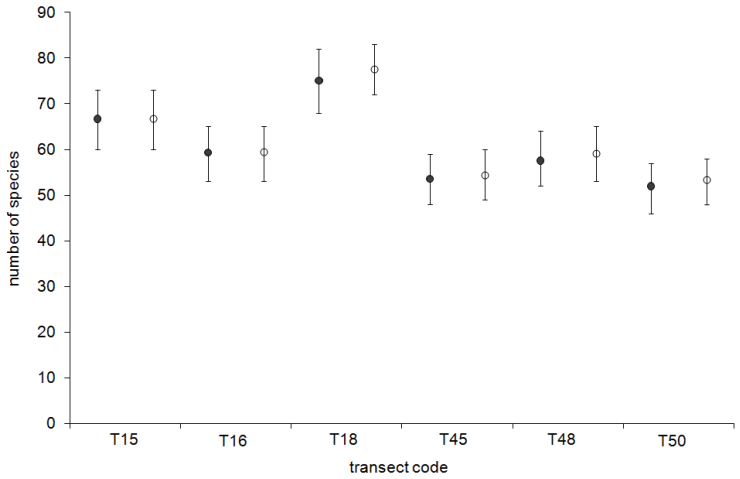

Fig.8. Comparison of the number of species collected on six transects in Terceira Island (filled dots) with the number of species attained using a theoretical optimized protocol (open dots) after 1000 randomizations of the sampling scheme to 60 samples (with 95% confidence intervals). No significant differences were found.

The optimization exercise showed that the optimal protocol consisting of 60 samples would contain 31 plant, 17 Turquin and 12 Ethylene samples. Nevertheless, the current protocol, consisting of 30 plant samples (10 from each of the three dominant plants), 15 Turquin and 15 Ethylene traps is very close to optimal, and statistically indistinguishable from the best possible solution at each site (Fig. 8). It should be noted, however, that each plant sample requires approximately 50% more effort than each pitfall sample (Table 1), and these results should thus be viewed with some caution as the method is built for optimization of equally time-consuming samples (Cardoso 2009).

DISCUSSION

Several logistic concerns were taken into consideration when choosing the sampling methods applied in the field during the BALA project, namely: a) all the material needed is affordable and most of it is reusable (e.g., cups, tubes, plastic bags, cloth), b) the material is easily transported across islands, fragments and through the forests, c) no more than two people are required to implement the methods, d) the time needed to complete a base transect in the field (4 hours) is appropriate to the rapid weather changes in these islands, e) the protocol is easily reproducible in many other sites, and f) the closed vegetation and rough volcanic ground limit movements making other common methods, such as sweeping, unfeasible. Other sampling methods were also tested in preliminary surveys (small number of replicates, Gaspar et al. unpublished data), such as Malaise traps, hand collection, light trapping and Berlese funnels. However, they required considerably more material (except hand collection) and more time to accomplish in the field and/or laboratory. Moreover, these sampling methods did not seem to provide sufficient gains for the effort applied, as only a low number of target individuals and species were observed. In particular, the exclusive arthropod species recorded using these methods for a given transect were mostly captured by beating and pitfall methods in other transects of the same fragment (Gaspar et al. unpublished data). Thus, at a fragment scale, the addition of such sampling efforts seems to be redundant. Despite the preliminary findings, these and other alternative sampling methods should be thoroughly tested in the Azorean native forests. The dense understorey cover and great abundance of bryophytes in the forests offer many microhabitats (Gabriel & Bates 2005) for arthropods that are unlikely to be collected using pitfall and beating methods.

The number of arthropod species collected in Ethylene traps per transect was significantly correlated with that of the remaining methods. Ethylene is a non-attractive method, as is beating, capturing the species that would normally occur in that location, in contrast to Turquin traps which may attract species from other microhabitats. However, Ethylene is also a passive method, as is Turquin, collecting species with different activity through time, in contrast to beating that actively captures the species that are present at a single point in time (Standen 2000). This may explain why the species richness from Ethylene was related to some extent with the number of arthropod species from plants and also with the arthropod species richness from Turquin, while plant samples (non-attractive, active method) and Turquin traps (attractive, passive method) did not show a significant correlation in the number of species between them. Plant 1, Plant 2 and Plant 3 showed a strong significant correlation across transects, regardless of the plant species considered as dominants, showing a pattern of homogeneity in the arthropod species diversity and composition of the canopy from each transect that has also been observed in previous studies (Ribeiro et al. 2005; Gaspar et al. 2008).

Ethylene or Turquin traps (15 samples each) captured fewer arthropod species than one dominant plant species (10 samples). Still, Ethylene or Turquin trapping required less time in the field and laboratory (270 min.) than sampling one dominant plant species (350 min.). Actually, when relating the number of species collected to the time needed, Turquin was more time-effective than Ethylene and dominant plants, because it captured significantly more species over the same period of time. However, when Ethylene, Turquin and one dominant plant species were combined,

this substantially increased the number of arthropod species collected. This is related to the high complementarity in species composition among methods, particularly between pitfall and beating that were found to share only one third of the overall diversity (Gaspar et al. 2008). This means that in Azores there is the need to sample arboreal and epigean (ground dwelling) arthropods to have a reliable understanding of the local diversity. This is in accordance with a number of previous studies in different regions and with different taxa, which consistently found a combination of complementary methods to be not only ideal but fundamental for sampling overall diversity (Longino & Colwell 1997; Cardoso 2009; Muelelwa et al. 2010; Tista & Fiedler 2011). The addition of a third dominant plant did not seem to add more species richness to the combination of Ethylene, Turquin and two dominant plants. The exclusion of a third plant would mean a reduction of eight hours in the total time (from 2490 min to 1990 min) to complete a transect, without sacrificing the total number of arthropod species caught. Similarly, there seems to be redundancy in adding Ethylene traps to the combination of Turquin and three dominant plants, and with a similar reduction in total time (from 2490 min to 1995 min).

Ethylene and Turquin methods captured significantly more species with a lower number of individuals than the remaining methods. Thus, if standardisation of the sampling protocol was to be made based on the number of individuals caught, those methods should be preferred. Plant 3 captured significantly more species with lower number of individuals than Plant 1. This may occur because the most dominant plant species, on many transects this was *Juniperus brevifolia*, shows a high abundance of common arthropod species, reducing the chance that other less abundant arthropod species occur on those plants. In contrast, the third most dominant plant species usually captures some individuals of more abundant species that are dispersing but also individuals of species that find an available niche on those plants, resulting in a higher number of species sampled for a lower number of individuals, a pattern also observed by Ribeiro & Borges (2010).

The Turquin method captured more species for a smaller number of samples than Ethylene. This is likely to be related to the attractiveness of Turquin traps. However, any of the dominant plants performed as well as Turquin traps. This suggests that a similar gain in number of species will occur by adding samples, regardless of which method (Turquin, Plant 1, Plant 2 or Plant 3) is used.

There were no significant differences in the number of species captured whether an alternative sampling method and effort was applied at a base transect, or whether another base transect was established in another location within the same forest fragment. The same results were observed for different additions of effort and methods: extension of base transects, sampling of non-dominant plant species, and re-sampling in different years. This is an important outcome, since it suggests that at the fragment scale, the addition of an effort equivalent to a transect will increase the number of species found but it is not relevant where that addition of effort is applied and, to some extent, which methods are used (i.e., which habitat is surveyed). This may occur because observed species accumulation curves are not saturating and are increasing at similar rates for different additions of effort. It has been suggested that an extension of a transect in these forests beyond 150m captures a different sub-habitat (Hortal et al. unpublished data) and thus, would be similar to adding a transect in a different location. The non-significant difference between different additions of effort in the same fragment may also be related with the spatial scale to which local species richness is related (in the Azores, at the fragment scale, Borges et al. in prep.).

The most dominant plant species from each transect, Plant 1 and Plant 2, showed the highest sample completeness of all methods. Beating methods seem to be more efficient at capturing their estimated arthropod diversity. This could be related to a lower number of singletons on those plants, leading to low estimated diversity. Again, the high abundance of common arthropod species on the most dominant plant species may explain the higher completeness for Plant 1 and Plant 2.

The results obtained here may offer new insights into the most effective combination of methods and effort to inventory and to monitor diversity. Inventorying of diversity should favour a combination of sampling methods that maximizes overall sample completeness. This way, it

could be said that the Turquin traps and the three most dominant plants are a good alternative to the combination of all methods. Although the inventory protocol could then disregard the Ethylene traps, the optimization results suggest that the base transect, composed of 15 Turquin traps, 30 beating samples and 15 Ethylene traps is very close to, and not statistically different from the optimal solution. In addition, using the base transect from BALA in future inventory protocols would allow full comparability with the large standardised database already available for the region, so we would recommend the use of the BALA protocol in future inventories of arthropod diversity in the Azores. In contrast, monitoring of diversity should minimize sampling effort. Minimization of sampling effort should imply a simplification of the sampling methods applied. In this case, Turquin and the most dominant plant seem to be an adequate option, as they are the combination of two methods that needs the lowest number of samples to accomplish the same proportion of sample completeness. The use of a single sampling method, for which Plant 1 should be preferred, would greatly facilitate the sampling of these forests, but the proportion of total sample completeness is very low (34% for 160 samples and 8,000 minutes needed). A compromise should be made between sample completeness and the number of samples and time needed to assess diversity. For the most effective combination of sampling methods analysed here (Plant 1, Turquin plus Plant 1, Turquin plus three plants), an increase of 20% of sample completeness would require twice the number of samples and time.

Further studies are needed regarding techniques in the field and to analyse data to evaluate the sampling efficiency of arthropods in the Azores and elsewhere. However, with the results of this study, it is believed that the optimzation of sampling effort and methods is a promising approach to shortcut assessment of diversity.

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