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Combining sampling techniques aids monitoring of tropical butterflies

MARIA F. CHECA,[1] DAVID A. DONOSO,[2] JACQUELINE RODRIGUEZ,[1] ELISA LEVY,[1] ANDREW WARREN[3] and KEITH WILLMOTT[3] [1]Museo QCAZ de Invertebrados, Pontificia Universidad Católica del Ecuador, Quito, Ecuador, [2]Departamento de Biología, Escuela Politécnica Nacional, Quito, Ecuador and [3]McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA

Abstract. 1. We compared the performance of three common techniques for sampling butterflies in order to better understand any bias associated with each method. This information is still scarce for the Neotropics where butterfly diversity reaches a peak.

2. These techniques included use of hand nets, carrion traps with fermented shrimp, and fruit traps with fermented bananas. We examined which taxonomic groups were sampled by each technique and determined the intra-annual and inter-annual (two continuous years) differences in the collection of butterflies from each approach.

3. Surveys of butterflies were taken every 2 months, in dry and wet seasons, over a 2-year period, and were carried out in two forests (one wet and one dry) in western Ecuador.

4. A total of 2289 butterflies of 231 species were collected. Hand-netting collected the most species (57% and 60% of total species in the dry and the wet forest, respectively), followed by carrion traps (24% and 23%), and then fruit traps (19% and 16%). Methods differed with respect to the butterfly species they collected most frequently. Moreover, each sampling technique resulted in significant differences in species composition across seasons and years.

5. Because our sampling techniques differed in their performance, our study suggests that implementing all the methods together can improve estimates of species diversity and result in more accurate characterisation of butterfly communities.

6. While budget and logistics might constraint the utilisation of multiple techniques, minimally we recommend using both carrion and fruit baits to alleviate the bias of each bait.

Key words. Bait traps, biological monitoring, butterflies, carrion baits, dry forest, fruit baits, hand nets, sampling techniques, tropical forests, western Ecuador.

Introduction

The conservation of insect biodiversity is a challenge at both local and global scales, with the primary factors being habitat loss (i.e. deforestation, Laurance, 2010), and climate change (Vitousek, 1994; Basset *et al.*, 2015; Baez *et al.*, 2016). Butterflies have often been used as biological indicators (Kremen, 1992, 1994; Bonebrake *et al.*, 2010; Wallis *et al.*, 2017), owing to their abundance and species richness, relatively resolved taxonomy, ease of sampling, and sensitivity to environmental change (Kremen, 1992, 1994; Brown & Freitas, 2000; Valtonen *et al.*, 2013). This is particularly true for bait-attracted butterflies, which have been used as a model system to study theoretical aspects of butterfly ecology (reviewed by Freitas *et al.*,

Correspondence: Maria F. Checa, Museo QCAZ de Invertebrados, Pontificia Universidad Católica del Ecuador, Apartado postal 17-01-21-84, Quito, Ecuador. E-mail: mfcheca@puce.edu.ec

2014). For example, results from previous studies suggest that bait-attracted butterfly diversity is correlated with total butterfly diversity at local scales (Ribeiro & Freitas, 2012), and it is also correlated with the diversity of trees and birds (Schulze *et al*., 2004). Thus, monitoring of butterfly communities over time is a high priority and can aid in understanding the factors that affect insect population dynamics (Basset *et al*., 2017; Donoso, 2017).

Two methodologies are commonly used to sample tropical butterflies: collecting using entomological hand nets, which tend to sample most butterfly guilds (Lamas *et al*., 1991; Daily & Ehrlich, 1995; Robbins *et al*., 1996); and bait traps, typically baited with fermented banana, used to sample fruit-feeding species (Freitas *et al*., 2014). The fruit-feeding butterfly guild is usually dominated by Nymphalidae (mainly Satyrinae, Charaxinae and some Nymphalinae; DeVries, 1988). Most other butterfly groups, including Papilionidae, Pieridae, Lycaenidae, Riodinidae, Hesperiidae and some subfamilies of Nymphalidae, likely obtain carbohydrates through nectar feeding. These groups are therefore not sampled by fruit-baited traps as frequently as they are sampled with nets. The utilisation of fruit-baited traps has increased sharply in recent years for ecological research (see Freitas *et al*., 2014; Grøtan *et al*., 2014; DeVries *et al*., 2016; Graca *et al*., 2016), while the use of hand nets has remained restricted largely to non-quantitative field inventories. To permit comparisons among datasets across space and time, and to develop standardised, affordable, and efficient sampling methods, we need to understand the biases associated with different sampling techniques.

Aside from nectar and fermenting fruit, butterflies feed commonly on a rich variety of other substrates, including sweat, animal excretions, mud puddles, carrion and excrement (Norris, 1936; Gilbert & Singer, 1975; Boggs & Jackson, 1991; Beck *et al*., 1999), a behaviour that is often called ‘puddling’. Researchers have taken advantage of these behaviours to develop a wide variety of alternative baits, including faeces, urine, and ‘imitation’ bird droppings (Lamas *et al*., 1991; Austin *et al*., 1993; Robbins *et al*., 1996; Montero *et al*., 2009). Carrion (usually decaying shrimp or fish) has been used as a bait in previous ecological studies, sometimes in combination with fermented banana (Checa *et al*., 2009, 2014). Using different baits may benefit studies on butterfly community structure since fruit and decaying fish/shrimp likely attract different taxonomic groups of butterflies (Hall & Willmott, 2000; Hamer *et al*., 2006; Checa *et al*., 2014). Furthermore, in some regions and habitats, carrion baits may attract a more diverse butterfly community, including fruit-feeding (e.g. nymphalids, Molleman *et al*., 2005a), and nectar-feeding taxa (Hall & Willmott, 2000; Checa *et al*., 2009; Holloway *et al*., 2013).

Despite the general awareness of the particularities of each sampling method, information about bait attractiveness is scarce (Freitas *et al*., 2014). Comparative studies that have quantitatively tested for differences in performance of baits have been carried out in Asia (a comparison of carrion vs. fruits, Hamer *et al*., 2006; Holloway *et al*., 2013) and Africa (a comparison among different fruits, Molleman *et al*., 2005b). To date no comparative study on the performance of different sampling techniques has been done in the Neotropics, where global butterfly diversity reaches its peak (Emmel & Austin, 1990; Lamas *et al*., 1991; Robbins *et al*., 1996). The relative performance and biases of carrion and fruit baits thus remains poorly understood in the Americas. Furthermore, quantitative comparisons between net-sampling and baited traps techniques are critically needed, as both sampling methods are likely biased towards different taxonomic groups (Sparrow *et al*., 1994; DeVries & Walla, 2001; Caldas & Robbins, 2003; Pozo *et al*., 2005, 2008; Iserhard *et al*., 2013).

Analysis on the temporal variation in the performance of each sampling technique is also key for understanding biases associated with each method. This is mostly true because measures of butterfly abundance in the field may be biased by changes in the effectiveness of the traps through time, which may vary among sampling techniques. Furthermore, some ecological aspects of butterflies may mask true temporal dynamics. For example, nectar-feeding butterflies have, on average, shorter life spans than fruit-feeding butterflies (Beck & Fiedler, 2009); thus, the abundances of nectar-feeders may fluctuate more than those of fruit-feeding butterflies. Similarly, the non-feeding moths more often display outbreak dynamics than nectar-feeding moths (Tammaru & Haukioja, 1996). Finally, most long-term studies of tropical communities have only considered fruit-bait trapping (Grøtan *et al*., 2012, 2014; Valtonen *et al*., 2013). As only this technique has been studied, there is a need to understand whether it accurately estimates the presence of other butterfly feeding guilds.

Our study examined (i) which taxonomic groups are sampled by these different techniques and (ii) what are the intra-annual (seasonal) and inter-annual (two continuous years) differences in the collection of butterflies from each of these techniques. Understanding these aspects would allow us to assess whether differences in performance between methods have a significant effect on estimates of community diversity, sampled guilds, and temporal dynamics. In other words, can the suite of butterfly taxa sampled by one technique be used as an indicator of overall butterfly diversity in ecological and conservation studies? Or instead, are multiple techniques needed to better represent overall patterns? An improved understanding of how different sampling techniques perform should help design and standardise butterfly-monitoring networks, and set up guidelines for regional efforts that can help build globally comparable datasets.

Methods

Study area

This study was carried out in a wet forest of northern Ecuador (Canandé River Reserve, Esmeraldas Province), and in a dry forest of southern Ecuador (Jorupe Reserve, Loja Province). Canandé (00°28′N, 079°12′W) consists of 2000 ha of continuous evergreen forest (Sierra, 1999) and is part of the Chocó-Darién biogeographic region, one of 21 global hotspots of biodiversity and endemism (Myers *et al*., 2000). Canandé is hilly (200–500 m) and wet (mean annual precipitation at 2787 mm; WorldClim Version 1, Hijmans *et al*., 2005) with a wet season from January–June (2210 mm) and a drier season from July–December (674 mm). In Canandé, we sampled butterflies at altitudes between 300 and 400 m. Jorupe (4°18′S, 079°37′W) consist of 1400 ha of deciduous forest, within a river valley and up the adjacent slopes, within the Tumbesian biogeographic region. Jorupe is even hillier with elevations ranging from 480–2440 m, with a mean annual precipitation of 937 mm (WorldClim Version 1, Hijmans *et al*., 2005); a wet season from January–April (685 mm) and a pronounced dry season from May–December (252 mm). In Jorupe, we sampled butterflies at altitudes between 600 to 700 m.

Census techniques

Butterflies were sampled with bait-traps and hand nets at each study site from mid-2011 to mid-2013. We sampled communities in the dry and wet seasons. Wet seasons in both reserves were sampled in January, March and May. Dry seasons were sampled in July, September and November. In each sampling month the sampling period lasted 7 days.

In each reserve, Van Someren-Rydon bait traps were set in two transects at least 500 m apart, with eight sampling positions located within each transect (16 sampling positions per reserve). Each transect comprised of alternating four banana traps and four shrimp traps. Prior to going to the field, bananas fermented for 2 days and shrimp fermented for 13–18 days. The entrance of bait traps were suspended 1.5 m above the ground. In each transect, the distance between neighbouring positions was at least 40 m. Traps were opened and baited on the first trapping day (we did not close them at night), and butterflies found within the traps were identified and counted during the next 6 days. Total sampling effort for each bait type was approximately 12 h of daylight × 8 traps × 6 days × 12 sampling trips = 6912 trap h per reserve. We acknowledge that 12 h is an average, with total sampling effort depending on day-to-day light variability. Besides, 12 h may under represent twilight species of the community. Baits were renewed each day. Most trapped butterflies were collected and killed by a pinch to the thorax and placed in glassine envelopes, except for some very common species that were marked with a unique number on the wing underside, using a marker pen, and released.

Net sampling was also performed along the transects. To reduce potential changes in the sampled fauna that might result from sampling at different times of the day or on different days with different weather conditions, net sampling was conducted immediately after checking traps from 9 am to 3 pm. One collector remained in the sampling position for 15 min collecting all butterflies flying inside a 30 m diameter circle (with the trap located at the centre) and at a maximum height of 5 m above the ground. Total sampling effort by hand netting was therefore 15 min × 8 sampling positions × 7 days × 12 sampling trips = 168 h per reserve. We sampled butterflies with hand nets, using this point-count approach, rather than the Pollard transect walk commonly used in temperate regions (see Pollard, 1977; Pollard & Yates, 1993). All collected material was examined and identified to species, with the higher classification following Wahlberg *et al.* (2009). Identifications were made using reference collections at the Museo QCAZ de Invertebrados (QCAZ, Pontifical Catholic University of Ecuador) and the McGuire Center for Lepidoptera and Biodiversity (FLMNH, Florida Museum of Natural History), and our own research on the systematics of the butterflies of Ecuador conducted over the last few decades. AW identified Hesperiidae, and MFC and KRW identified remaining groups. Specimens collected were deposited and vouchered in the QCAZ, and duplicates of some species were also deposited in the FLMNH.

Statistical analyses

To investigate whether different sampling techniques recovered different butterfly communities we used Generalised Linear Models (GLMs). GLMs tested if butterfly abundance and observed species richness differed with respect to sampling technique (hand net, carrion traps, and fruit traps) and seasons (see temporal analyses below). GLMs are ideal for count-based data (Hoffmann, 2004) where the assumptions of the standard linear model (LM) (e.g. normality) do not hold (Zuur *et al*., 2009). We selected among possible models using Akaike's information criterion values and residual variance. The negative binomial distribution fitted our field data better than a Poisson or Normal distribution. Scatterplots of the residuals and fitted values were checked to examine whether the model met the assumption of homogeneity (see Dobson, 2002). Butterfly diversity (Simpson diversity index) was evaluated with LMs. The Simpson index is among the most robust for diversity estimation (Magurran, 2004), being independent of sample size (Lande *et al*., 2000). We analysed each forest type, wet and dry forests, independently. Each replication in the analyses thus consisted of butterfly abundance, species richness or estimated Simpson diversity index recorded at a given trap, which in total summed 16 points (8 sampling positions × 2 transects) per sampling technique within each forest type. GLMs and LMs were used from the MASS packages in R 3.3.0 (R Core Team, 2016).

Then, at each forest type, one PERMANOVA (non-parametric MANOVA) was performed on Euclidean distances to test for significant differences in the structure of butterfly communities with respect to sampling technique. PERMANOVAS were done with the R package Vegan (Oksanen *et al.*, 2018). To visualise community composition with respect to sampling technique in each forest type, we used a non-Metric Multidimensional Scaling (NMDS), with Euclidean distances and log-transformed abundance data. Log-transformation allowed common and rare species to contribute more equally in the analyses.

To determine if the effects of sampling techniques (hand nets, shrimp traps and banana traps) varied across seasons we used GLMs (see above). Here, we examined the estimate and associated *P*-values of the interaction effect technique × season. An additional PERMANOVA test was performed to test for significant differences in the structure and composition of butterfly communities sampled by each technique across seasons. An NMDS was performed to visualise differences in community structure sampled by each technique between dry and wet seasons.

Finally, to test for inter-annual patterns of species richness over time, according to sampling technique, we used repeated-measures ANOVA. An independent analysis was performed for each forest type. A repeated measures design is useful where multiple observations are made on the same replicate at different times (Gotelli & Ellison, 2004); replicates in this study corresponded to each of the sampling positions within transects. Species records from each sampling day were pooled together for each sampling position and used as the dependent variable. The repeated factor corresponded to the species richness registered for each of the 12 sampling months. The *F* value and associated *P* used to interpret results corresponded to the interaction method × month. Species richness data were log-transformed to more readily meet the assumption of normality in the residual distribution. Moreover, the assumption of homogeneity of variances was tested using Mauchly's test of sphericity, and when this assumption was violated, we used the Lower-bound test in order to correct values. Mauchly's test tests whether the variances between observations in time are equal, which is a key assumption for a repeated measures analysis to control for Type I errors (Gotelli & Ellison, 2004). This test calculated an appropriate adjustment to the degrees of freedom of the *F*-test. Analyses were carried out with SPSS 15.0 (SPSS Inc., Chicago, IL, USA)

Results

A total of 2289 butterflies and 231 species were collected using all three sampling methods. The hand net technique recorded 794 individuals and 179 species, while carrion traps registered 681 individuals and 68 species, compared to 814 individuals and 52 species collected with fruit traps across study sites. A few species dominated the samples within the dry forest. *Fountainea ryphea* (Cramer, 1775) (Nymphalidae), *Hamadryas amphichloe* (Boisduval, 1870) (Nymphalidae) and *Cissia* sp. (Nymphalidae) comprised 54% of butterflies collected in the dry forest with 854 individuals. The most abundant species in the wet forest, namely *Haetera piera* (Linnaeus, 1758), *Cithaerias pireta* (Stoll, 1780) and *Nessaea aglaura* (Doubleday, [1848]) (Nymphalidae), accounted for only 21% of individuals (Appendix S1). Raw data showed that the hand net technique sampled a larger number of butterfly species across ecosystems. In dry forest, hand-netting recorded more than twice the number of species (73 species, or 57% of total species across all techniques) compared to carrion trapping (30 species, 24%) and fruit trapping (24 species, 19%). In wet forest the differences were even more conspicuous, where hand-netting recorded 112 species (60% of total species), whereas carrion trapping recorded 43 species (23%), and fruit trapping recorded 30 species (16%) (Table 1).

Traps were more taxonomically selective in the species they recorded, sampling mainly Nymphalidae and to some extent Hesperiidae. Hand-netting sampled families not recorded with traps such as Papilionidae, Lycaenidae, Pieridae and Riodinidae. These results were consistent across sites (Appendices S1 and S2). A few species of Riodinidae (5 out of 35) were also collected with traps in wet forest. The taxonomic groups sampled by traps differed between the bait types. Hesperiidae, and to a lesser extent Riodinidae, were exclusively collected with carrion in wet

Table 1. Abundance, observed species richness and diversity (Simpson diversity index) recorded, using hand net and traps baited with carrion or fruit during dry and wet season within wet and dry forests.

	Abundance			Species richness			Simpson diversity index		
Season/bait	Shrimp	Banana	Hand net	Shrimp	Banana	Hand net	Shrimp	Banana	Hand net
Wet forest									
Dry	44	51	153	22	23	59	0.711	0.736	0.767
Wet	105	112	239	33	23	87	0.778	0.822	0.930
Total	149	163	392	43	30	112	0.744	0.779	0.849
Dry forest									
Dry	259	374	295	28	21	63	0.798	0.684	0.899
Wet	273	277	107	17	17	40	0.731	0.620	0.630
Total	532	651	402	30	24	73	0.764	0.652	0.765

forest, whereas in dry forest Riodinidae was not collected in traps and Hesperiidae showed no preference for one bait (meaning both baits captured similar number of species of this family). In the case of Nymphalidae, both baits collected similar numbers of butterfly species in dry forest and wet forest, but in the wet forest, a higher species richness was recorded with fruit, due to the higher number of satyrines collected with this bait compared to carrion (20 and 7, respectively). Finally, some Danainae were also collected with traps, but only in dry forest.

Abundance, richness and diversity: comparison among sampling techniques and seasons

In wet forest, we found significant differences among sampling techniques for abundance (GLM Estimate = 0.722, $P = 0.020$), but not for species richness or Simpson diversity index (Table 2). Hand-netting collected 392 individuals, more than twice the number collected with carrion traps (149) and fruit traps (163). In wet forest, seasonality only had a minor effect on butterfly communities; pooling data across sampling techniques, there was a tendency for higher species richness, abundance and diversity to occur during the wet season compared to the dry season (Table 1), but the statistical models only detected significant differences in butterfly abundance (Estimate = 0.993, $P = 0.021$) (Table 2).

In dry forest, by contrast, sampling techniques significantly differed in terms of species richness (GLM Estimate = 0.693, $P = 0.003$) and Simpson diversity index (LM Est. = 0.208, $P = 0.048$), but not abundance (Table 2). Hand-netting collected 73 species, more than twice the number of species collected with carrion traps (30) and fruit traps (24) (Table 1). Seasonality patterns in dry forest showed an opposite pattern to that found in wet forest; pooling data across sampling techniques, we found a non-significant tendency for higher species richness and diversity during the dry season compared to the wet season (Tables 1 and 2).

The interaction between sampling techniques and season (intra-annual variation) was not significant, and all sampling techniques resulted in a similar inferred seasonal pattern of abundance, observed species richness, and Simpson diversity index for wet and dry forests (Table 2).

Table 2. Estimates of Linear Models for the independent variables: method, season and the interaction term (method × season). Several models were run for each dependent variable per ecosystem (wet and dry forests): butterfly species richness, abundance and diversity (Simpson diversity index).

	Sampling method	Season	Method × Season
Wet forest			
Species richness	0.43546	0.36251	0.06569
Abundance	0.72161*	0.99375*	-0.13574
Simpson diversity	−0.02093	0.03097	0.03639
Dry forest			
Species richness	0.6929**	0.0922	−0.2419
Abundance	0.1515	0.1846	−0.2709
Simpson diversity	0.20769*	0.06894	−0.1001

Asterisks represent significant effect of independent variables (*$P < 0.05$; **$P < 0.001$).

Community composition: comparison among sampling techniques and seasons

Our NMDS analysis showed differences among sampling techniques, particularly for wet forest (Fig. 1, stress = 0.097), where all sampling techniques formed distinct, separate clusters. This pattern was not recovered in dry forest (Fig. 1, stress = 0.091), which mainly showed two clusters, one for hand-netting, and one for trapping. Samples from hand-netting resulted much more variable among each other (more spread cluster) than samples of the traps (either fruit or carrion) are among each other (less spread clusters). PERMANOVA analyses indicated that these differences were significant for both ecosystems (wet forest $F = 5.008$, d.f. = 1, $P = 0.001$; dry forest $F = 12.938$, d.f. = 1, $P = 0.001$), meaning communities sampled by each technique significantly differed in terms of structure.

The NMDS revealed that community structure differed among sampling techniques and between seasons for wet (stress = 0.121) and dry forests (stress = 0.121), particularly for hand-netting (Fig. 2). These differences in community structure according to sampling techniques and season were significant for both ecosystems (wet forest PERMANOVA, $F = 5.017$, d.f. = 1, $P = 0.001$; dry forest PERMANOVA, $F = 6.229$, d.f. = 1, $P = 0.001$). In other words, different species were sampled with hand nets, fruit traps and carrion traps during dry and wet seasons.

Models comparing sampling techniques across time

The repeated-measures ANOVA showed that recorded species richness was significantly different among techniques × month in the wet forest ($F = 2.221$, d.f. = 22, $P < 0.001$, Fig. 3) and in the dry forest ($F = 5.356$, d.f. = 2, $P = 0.013$, Fig. 3). Although temporal patterns of species richness showed some broad similarities across sampling techniques in our study, visual inspection of patterns showed that hand net butterfly communities exhibited more conspicuous and variable peaks of species richness (Fig. 3). Both baits, and hand nets (to a lesser extent) resulted in a general pattern of the highest numbers of species being recorded during the wet season of the wet forest, but the opposite pattern was observed for the dry forest.

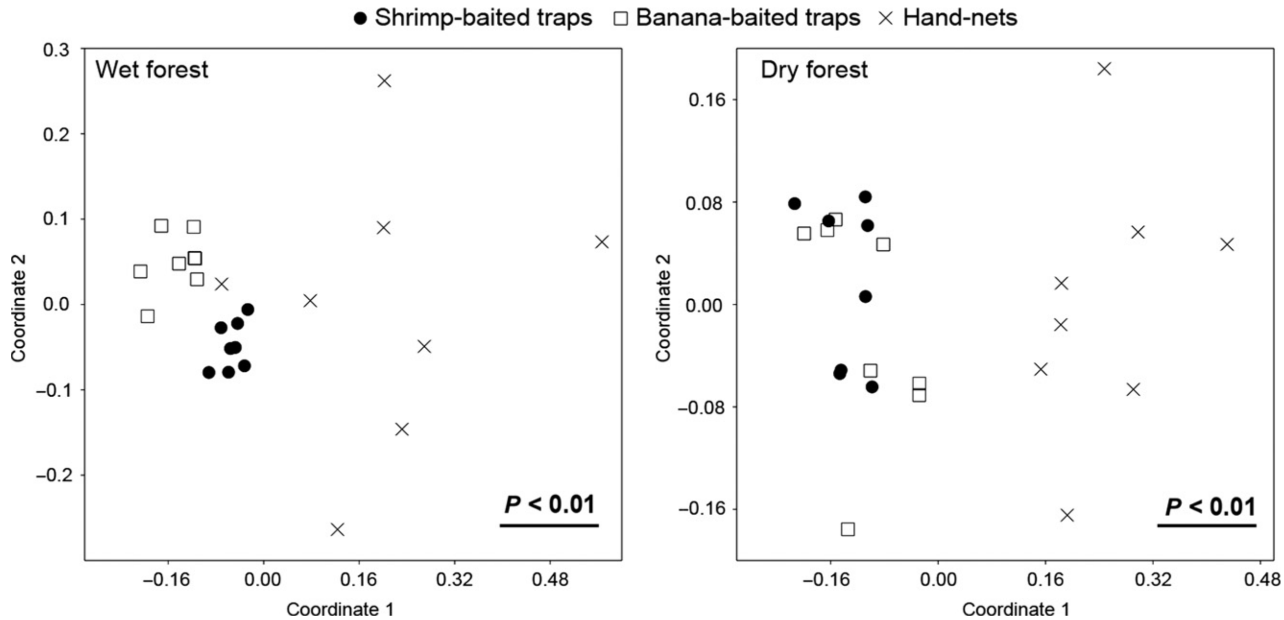

Fig. 1. Non-metric Multidimensional Scaling (NMDS) analysis comparing the structure and composition of butterfly communities collected with hand nets, fruit traps and carrion traps in Canandé (wet) and Jorupe (dry) forest. Points represent the sampling positions within transects. Here, *P*-values corresponded to those estimated by a PERMANOVA test.

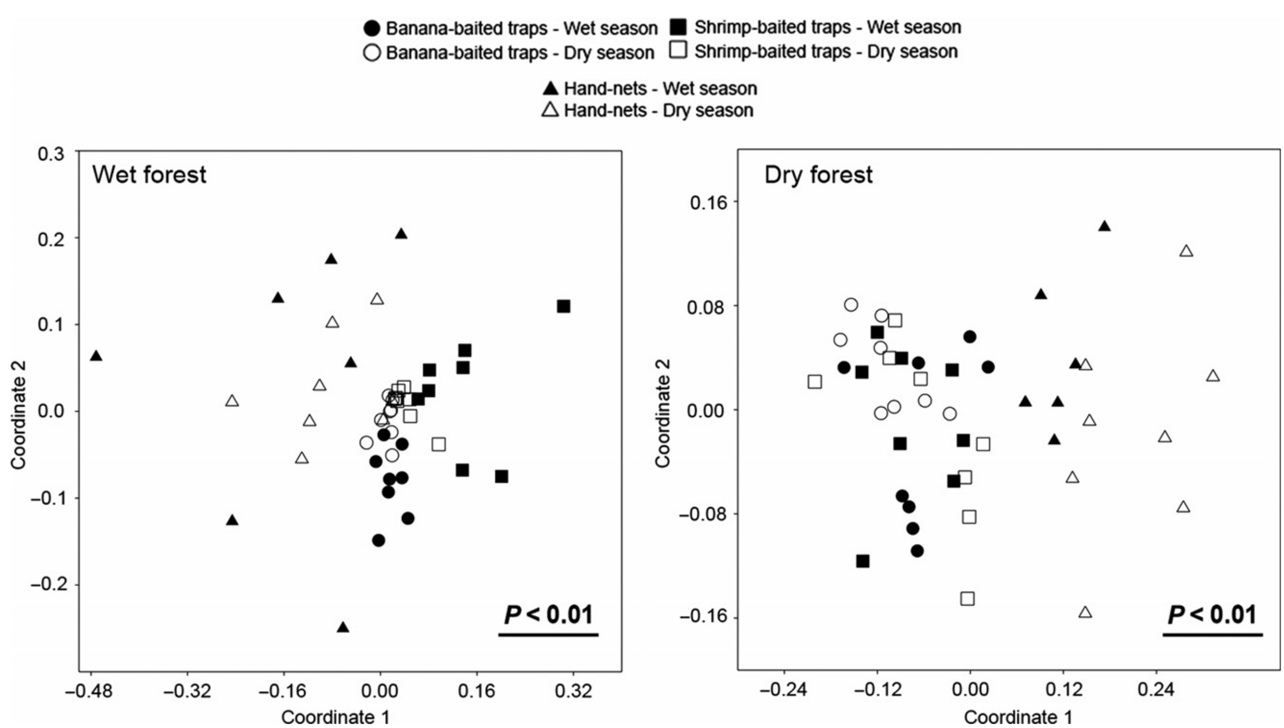

Fig. 2. Non-metric Multidimensional Scaling (NMDS) analysis comparing the structure and composition of butterfly communities collected with hand nets, fruit traps, and carrion traps during dry and wet seasons across study sites: Canandé wet forest and Jorupe dry forest. Presentation follows as Fig. 1.

Discussion

This study represents the first attempt to quantitatively compare the performance of three sampling techniques (hand-netting, carrion traps, fruit traps) for Neotropical butterflies. We showed that these techniques capture different taxonomic groups confirming the results of earlier studies (e.g. Brown & Freitas, 2000; Iserhard *et al*., 2013; DeVries *et al*., 2016). Moreover, we found significant differences with respect to the abundance, richness, and the temporal pattern of butterfly occurrence. Hand-netting collected more species compared to both carrion traps and fruit traps. Carrion traps recorded a higher number of species from a wider range of taxonomic groups compared to fruit traps. Concordant results were found for wet and dry forests. Communities sampled by each technique during wet and dry seasons significantly differed in terms of community structure for both ecosystems. Moreover, the temporal pattern of species richness significantly varied for each technique over the 2-years of sampling. These results suggest that researchers should be aware of potential biases among techniques and how they might influence results.

In our study, the use of traps with different baits (carrion and fruit) and hand nets complemented each other in terms of maximising sampled species diversity, therefore these techniques should be combined in butterfly research in order to increase local diversity sampled. Hand nets were useful to sample butterflies that are believed to obtain carbohydrates mainly from nectar, such as Pieridae, Papilionidae and Lycaenidae, and most groups of Riodinidae and Hesperiidae. These findings were consistent for wet and dry forests. Trapping was more suited for sampling most nymphalids (Biblidinae, Charaxinae, Heliconiinae, Limenitidinae, Nymphalinae and Satyrinae). More specifically, fruit bait was particularly attractive for the tribe Satyrini (subfamily Satyrinae), whereas carrion bait was more effective for attracting Hesperiidae and Riodinidae in the wet forest. Carrion traps in the dry forest did not attract Riodinidae, and were equally efficient attracting Hesperiidae.

Many studies have shown tropical insect populations to fluctuate over time in terms of abundance and/or species richness, with a tendency for species richness to peak through the wet season (see Wolda, 1978; Novotny & Basset, 1998; Grimbacher & Stork, 2009; for a review Kishimoto-Yamada & Itioka, 2015). We found similar results here for butterfly communities from the wet forest, but our study is the first to notice that the pattern depends on the sampling technique and ecosystem type. Our inter-annual analyses found that bait traps recover fewer peaks in species richness. These results suggest that non-nectar feeding taxa (species mainly collected with traps such as Charaxinae and Limenitidinae) are less responsive to season, and that nectar-feeding butterflies (mainly captured by hand nets) may be responding to environmental cues such as water and carbohydrate source availability (Castro & Espinosa, 2015). We conclude that community temporal analyses based solely on fruit-bait trapping are not representative for other butterfly feeding guilds or sampling techniques.

There are advantages associated with each technique that might influence the chosen technique for a given butterfly research project. A major advantage of trapping is the ability to sample inaccessible habitats, in particular the forest canopy, which is poorly sampled (DeVries *et al*., 1999; DeVries & Walla, 2001; Checa *et al*., 2009, 2014). While the use of canopy towers, walkways, and single-rope ascending techniques (e.g. Hall & Willmott, 2010) can enable hand-netting surveys to be done in the canopy, opportunities for such surveys are limited. Another advantage of trapping for ecological studies is the ability to simultaneously sample multiple locations in a standardised way, permitting accurate comparison among samples in space and time (DeVries *et al*.,1999, 2016). This is true not only for comparisons across geographically distant sites and broad time-scales, but also within a single site over short periods of time. Thus, a single person is able to check a large number of traps (e.g. 50 day^{-1}) throughout a day period, distributed over different microhabitats and strata (e.g. Checa *et al*., 2009), to document fine-scale changes in faunas. To comparably sample those same locations simultaneously with hand-netting would involve dozens of field assistants.

In comparison, differing abilities among hand net researchers in noticing and collecting butterflies can introduce substantial artifactual variation among sampling events. Although such artifacts may be reduced by using collectors with similar levels of experience, this makes hand-netting less accessible to novel researchers in the field, and in long-term projects where similarly experienced collectors would need to be maintained over a long time period. In our experience, these issues also make it more feasible to train local people to carry out trapping studies than hand-netting studies (Checa, 2015). Involving local people living close to sample sites and training them as field assistants should make logistical costs lower and conservation efforts more effective (Sekercioglu, 2012). In our case, this practice helped reduce monthly sampling costs by 49%, since hiring local people to carry out monitoring excluded both lodging and transportation costs. Another potential disadvantage of hand-netting is demonstrated by our NMDS analyses. Here, hand net communities were depicted by more spread clusters, suggesting that either this fauna presents greater sensitivity to micro-habitat, or that hand net communities were more susceptible to the weather at the moment of sampling. This is a disadvantage because the large variance among samples obtained by hand-netting (as compared to other techniques) means that a higher sampling effort is needed to obtain a representative sample of the community. Finally, our results come with a caveat. Hand-netting was performed using a point-count protocol instead of Pollard transect walks. The point-count protocol is less efficient to survey local butterfly fauna compared to Pollard transect walks, but we used point-counts because it provided

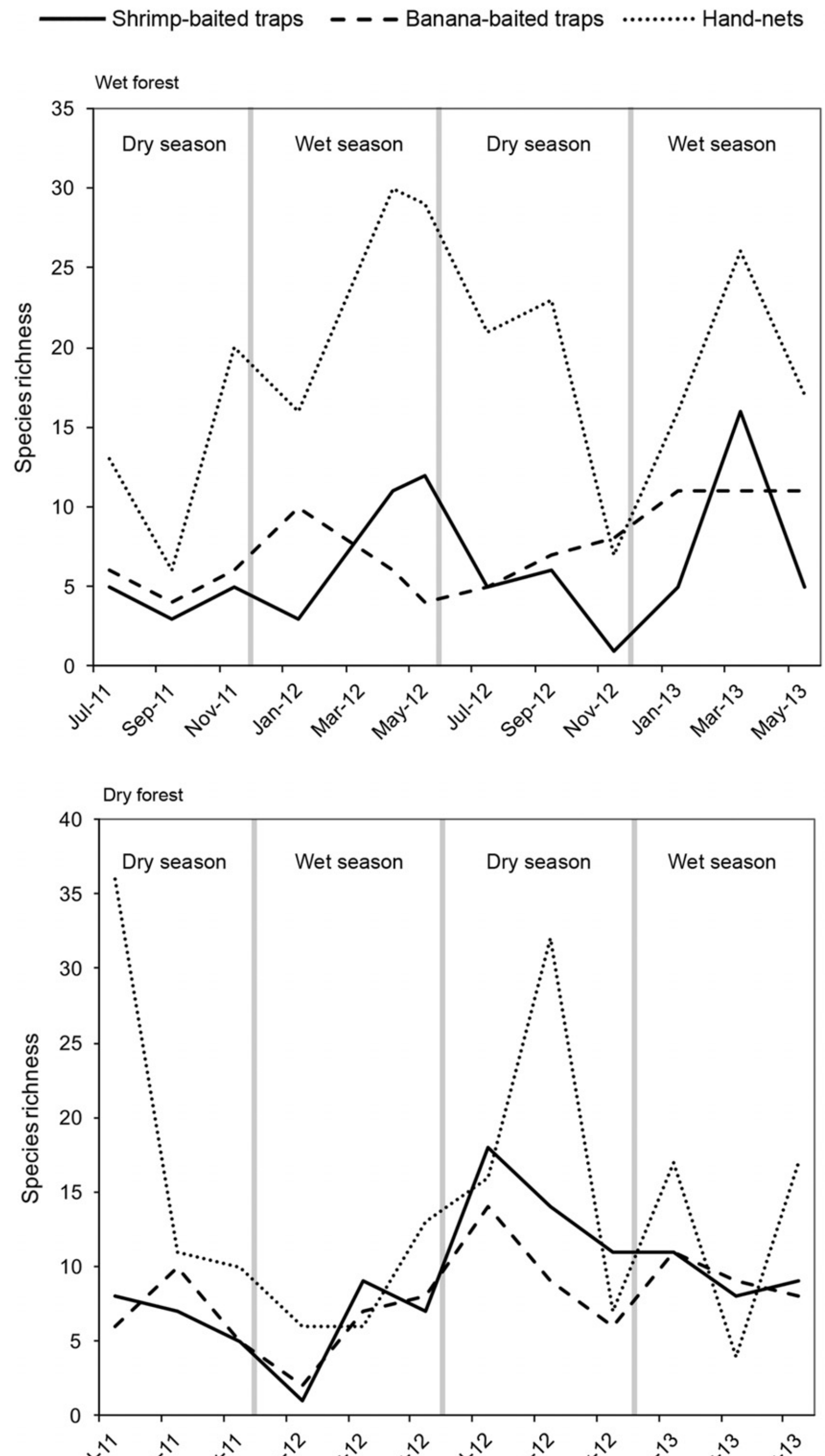

Fig. 3. Seasonal patterns of observed species richness of butterfly communities collected with carrion traps, fruit traps and hand nets across study sites: Canandé wet forest and Jorupe dry forest.

the most direct comparison between material collected by netting vs. trapping since both methods sampled the same area of forest.

Notwithstanding these points, there are some disadvantages of trapping. Trapping methods sample only a fraction of the taxonomic diversity, which may not represent the entire community. Also important is the extent to which the abundance of individuals recorded in traps reflects their overall abundance in nature. There are differences in preference for baits among butterfly species and higher taxa (as noted by numerous authors and demonstrated here), as well as within the same species across time (Torres *et al.*, 2009). Greater abundance of one species in a trap in comparison to another could therefore be a result of its stronger attraction to the bait or its greater mobility, rather than any true difference in abundance. Moreover, the fraction of the fauna sampled by traps may vary significantly among world regions. The efficiency of trapping is very limited in temperate zones compared to tropical regions (Lapkratok & Suwanwaree, 2014; Jakubikova & Kadlec, 2015), and within tropical areas, variation also occurs with, for example, low capture rates in comparison with hand-netting reported in tropical rainforest in Papua New Guinea and Panama (Basset *et al.*, 2012). Finally, the causes for geographic and habitat-scale variation in trap efficiency, such as between even geographically close sites such as eastern and western Ecuador (M. F. Checa & K. Willmott, unpubl. data) are still poorly understood and further research on this topic is urgently needed.

Further logistical disadvantages to trapping include the cost of buying bait, which can be significant for a long-term monitoring programme plus potential difficulties in obtaining bait in some areas. Buying traps represents a significant initial investment, and there are ongoing maintenance costs. A further reported disadvantage for carrion bait is its strong odour after several days of putrefaction (14–19 days in this study). We recommend that shrimp bait be prepared by liquefying fresh shrimp with water at a 1:1 proportion, with the resulting solution stored in plastic bottles. When checking traps and renewing baits, it has proven much easier to pour bait from bottles rather than serving from containers.

Given our results, and these advantages and disadvantages, we suggest that combining sampling techniques can result in significant improvements in butterfly monitoring programmes and ecological research. Combining hand net and bait trap techniques not only maximises species sampled but also likely results in more robust ecological insights, since the biases associated with each of these methods is different (Caldas & Robbins, 2003) and the data they gather are complementary (Sparrow *et al.*, 1994). While budget and other factors might pose constraints on the utilisation of multiple sampling techniques for standardised butterfly monitoring, minimally we recommend the use of both carrion and fruit baits to mitigate the differential attractiveness of these baits to different taxa and over time. The results presented here will hopefully contribute towards developing efficient and effective standard methods for long-term butterfly monitoring schemes in the tropics. These schemes have provided influential data about the effects of environmental change and highlighted priorities for conservation in Europe (Isaac *et al.*, 2011), and could do so in the Neotropics, which contains a disproportionately large share of global biodiversity, but is limited by resources and numerous conservation challenges (Sodhi & Ehrlich, 2010).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Abundance of species collected with hand nets, fruit and carrion traps within wet and dry forests.

Appendix S2. Number of individuals (N) and species (S) collected with hand nets, carrion traps and fruit traps within wet and dry forests.

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