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Habitat type influences the choice of sampling method in a butterfly survey

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Abstract
Urban landscapes can support the diversity of beneficial organisms like butterflies if they are well managed. Proper management may vary with different habitat types which may involve habitat specific sampling and conservation approach. This study investigated butterfly diversity in different habitat types in an urban landscape. Different sampling methods were compared across the varying habitat types. Forty-two species of butterflies were reported in the study area with an abundance of 720 butterflies. There was a significant difference in the mean abundance and species richness of butterflies across sites and sampling methods. Furthermore, sweep netting method had the highest mean abundance and species richness of butterflies sampled across study sites. Species composition of butterflies also varied significantly across study sites. While sweep netting is a preferred sampling method, there is need to give consideration to the habitat type to be sampled when making the choice of sampling method in butterfly surveys.

Keywords: Butterflies, urban landscapes, sampling, habitat management, sweep net

Introduction
In recent years, there has been rapid increase in anthropogenic activities which has led to a growing rate of habitat loss [1, 2, 3] and ultimately species loss [1, 4]. With this loss comes a need to protect, modify or manage existing landscapes in ways promoting diversity [5]. Although, management of existing urban landscapes might appear to be poor choices, previous research has reported the roles played by such habitats in conservation of biological communities [6, 7, 8, 9, 10, 11]. Examples of such habitats are: green spaces, allotments etc. [9].

Several studies have been done on butterflies because of their easy recognition, migratory behavior, habitat fragmentation sensitivity and popularity as compared to other insects [12, 13, 14] which made them useful as bioindicators [15, 16]. Butterfly assessment may involve the use of several survey/sampling methods such as Blendon trap, Pollard transects etc. [17, 18]. These varieties ensure that all butterfly species are represented on the study site due to difference in species behavior [19, 20] and the diverse nature of habitats on one site. For instance, different butterfly species have been known to be found in both fields [21, 22] and forests [23, 24]. However, in most reported cases [25, 26], only one sampling method seems to be most effective which implies that using all methods may be counterproductive. Therefore, effective sampling/survey must take into cognizance methods that will make maximum representation of butterflies possible [26, 27, 28].

In developing nations like Nigeria which is in the tropical region, the urban areas have been degrading and conservation planning is uncertain [29, 30]. Therefore, there is a need for proper understanding of the ecology, habitat requirements and appropriate sampling method of all relevant taxa to improve management of such areas [31]. In this study, we assessed the diversity of butterflies using varying sampling methods and traps on managed urban habitats in a University campus in Nigeria.

Materials and Methods
Study Area
The study was carried out at the Obafemi Awolowo University, Ile-Ife, Nigeria. The campus is located in Ile-Ife with coordinates (Latitude: 7°31′06″N, Longitude: 4°31′22″E). Four urban habitats were selected (Parks and Gardens (PG), Regrowth forest (RG), Biological Gardens (BG) and Research Farm (RF)) as sites on the campus. In each site, four sub-plots sized 25m x 25m were demarcated on which sampling was carried out.

Sampling of Butterflies

Sampling of butterflies was done fortnightly from September, 2019 to January, 2020 with the combined use of blendon traps, flight intercept traps and sweep nets. Sampling by sweep nets was done for 15 minutes under favourable weather with mild wind on each sampling day between the hours of 9:00 and 14:00 after which the traps were set in each plot. The traps were removed after 24 hrs. and the butterflies collected were handled with a pinch on the thorax before setting them on setting-boards in the laboratory. This was followed by keeping them in a dark place to dry for one week [32]. The properly set and pinned butterflies were then transferred to an insect box and identified. Butterfly identification was undertaken with the aid of appropriate identification keys such as Butterflies of West Africa [33] and Common Butterflies of IITA [34], and with the help of expert taxonomists. Identification of the collected species was done to species level, but where species level identification was not possible, it was done to family or generic levels and labelled as morphospecies. Reference specimens of the insects were deposited in the Entomology collection of the Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Statistical Analysis

The abundance and species richness of the butterflies recorded over the sampling period were pooled for the analyses. Species rarefaction was done using the *vegan* package [35] and Chao1 and Jack1 were calculated using the *fossil* package in R.3.6.1 software [36] to determine sampling adequacy. Generalized Linear Model using the MASS package in R.3.6.1 software [36] was then used to compare the mean abundance and species richness of butterflies across sites and methods.

To assess butterfly species composition across sites and methods, Principal Coordinate Analysis (PCO) was done using the *vegan* package in R.3.6.1 software [36]. Then differences in species composition across sites and methods were analysed with PERMANOVA also using the *vegan* package in R.3.6.1 software [36].

Results

A total of 720 butterflies, belonging to 42 species and five families (Hespiiridae, Lycaenidae, Nymphalidae, Papillionidae, and Pieridae) were collected over the sampling period. Family Nymphalidae had the highest number of species with 35 species and Papillionidae had the least number of species with one species (Table 1). Overall, the rarefaction curve (n = 42, Chao1 = 43.5, Jack1 = 45) of the butterflies was almost at asymptote (Appendix S1).

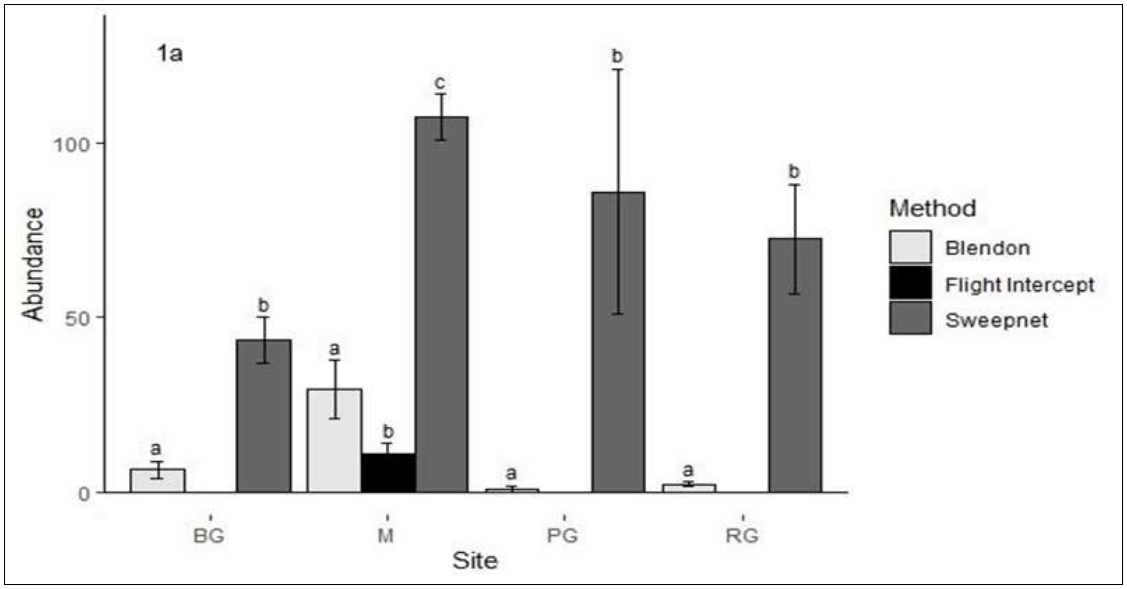
Table 1: Species list and IUCN status of butterflies during the study

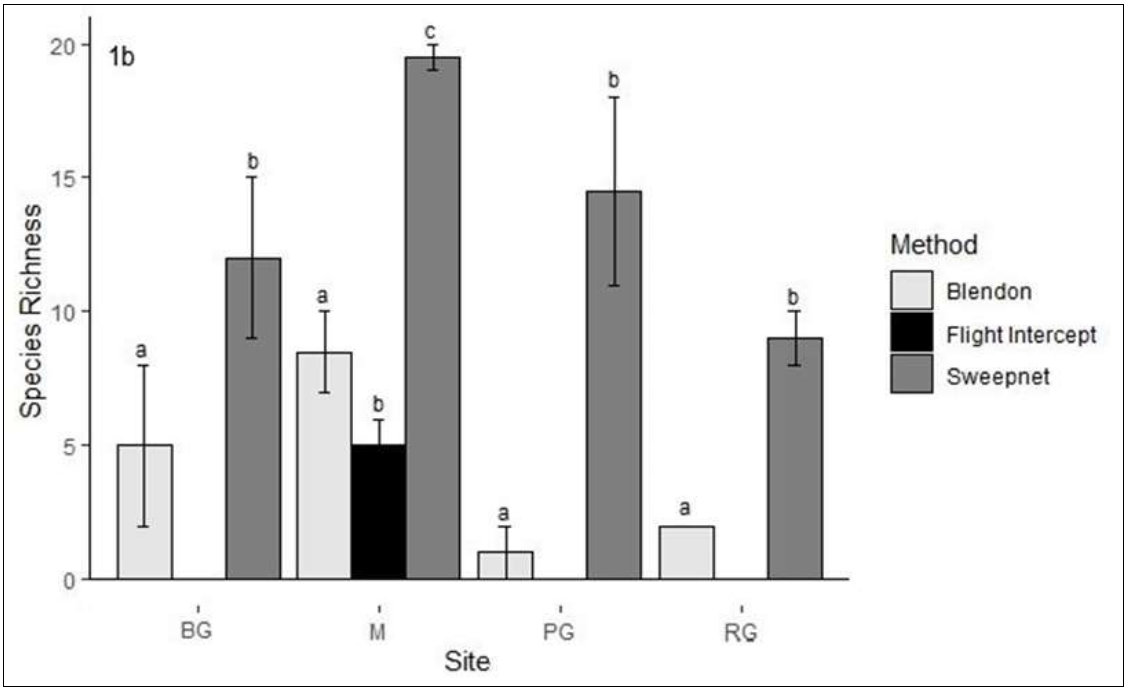
Family	Species	Sites	Method	Common Name	IUCN Status
Hesperiidae	*Celaenorrhinus galenus* (Fabricius, 1793)	BG	Blendon	common orange Sprite	NA
	Pardaleodes oedipus	RF, PG	Blendon, Sweep net	skipper	NA
	sp. 1	PG	Sweep net	Nil	NA
Lycaenidae	*Anthene larydas* (Cramer, 1780)	RG	Sweep net	spotted hairtail	NA
	Mimeresia libentina (Hewitson, 1866)	RG	Sweep net	common harlequin	NA
Nymphalidae	*Acraea alciope* (Hewitson, 1852)	BG, RF, PG	Sweep net	Hewitson Acraea	NA
	Acraea lycoa lycoa (Godart,1819)	BG, RF	Flight Intercept, Sweep net	Nil	NA
	Acraea pseudegina (Westwood, 1852)	RF, RG	Blendon, Flight Intercept, Sweep net	Nil	NA
	Acraea serena (Fabricius, 1775)	RF	Sweep net	dancing acraea	NA
	Acraea sp.	BG, PG	Sweep net	Nil	NA
	Aterica gelane (Brown, 1776)	BG, RF, PG	Sweep net	forest glade nymph	NA
	*Bicyclus*sp. 1	BG, RF, PG	Blendon, Sweep net	Nil	NA
	*Bicyclus*sp. 2	RF	Sweep net	Nil	NA
	Byblia anvatara (Boisduval, 1833)	RF	Blendon, Flight Intercept, Sweep net	common joker	NA
	Catuna angustatum (Felder & Felder, 1867)	BG	Sweep net	large pathfinder	NA
	Catuna crithea (Drury, 1773)	BG	Sweep net	common pathfinder	NA
	Danaus chrysippus (Linnaeus, 1758)	RG	Blendon, Sweep net	African Monarch	LC
	Euphaedra sp. 1	BG, PG	Blendon, Sweep net	Nil	NA
	Euphaedra sp. 2	BG, RF, PG	Blendon, Sweep net	Nil	NA
	Euphaedra sp. 3	BG, RF, PG	Blendon, Sweep net	Nil	NA
	Euphaedra sp. 4	PG	Sweep net	Nil	NA
	Euphaedra themis (Hubner, 1807)	PG, RG	Sweep net	common Themis forester	LC
	Junonia chorimene (Guerin-Meneville, 1844)	RF	Blendon, Flight Intercept, Sweep net	golden pansy	NA
	Junonia oenone (Linnaeus, 1758)	BG, RF, PG, RG	Blendon, Flight Intercept, Sweep net	dark blue pansy	LC
	Junonia orithya (Linnaeus, 1758)	RF	Sweep net	Nil	NA
	Junonia sophia (Fabricius, 1793)	BG, RF, PG	Sweep net	little commodore	NA
	Junonia terea terea (Drury, 1773)	BG, PG	Sweep net	soldier commodore	NA
	Precis octavia octavia (Cramer, 1777)	RF	Blendon, Sweep net	gaudy commodore	LC
	Precis pelargia (Fabricius, 1775)	BG, RF, PG, RG	Sweep net	fashion commodore	NA
	Pseudaacrae sp.	RF, PG	Sweep net	Nil	NA
	sp. 1	BG, RF, PG	Blendon, Sweep net	Nil	NA
	sp. 2	BG, PG	Sweep net	Nil	NA
	sp. 3	BG, PG	Blendon, Sweep net	Nil	NA
	sp. 4	BG	Blendon	Nil	NA
	sp. 5	BG, PG	Blendon	Nil	NA
Papillionidae	*Papilio menestheus* (Drury, 1773)	RG	Sweep net	western emperor	NA

				swallowtail	
Pieridae	*Colotis euippe* (Linnaeus, 1758)	BG, PG	Sweep net	Nil	NA
	Colotis sp.	RF	Blendon, Sweep net	Nil	NA
	Eurema hecabe (Linnaeus, 1758)	BG, RF, PG, RG	Blendon, Sweep net	common grass yellow	NA
	Eurema sp.	RF	Blendon, Flight Intercept, Sweep net	Nil	NA
	Leptosia alcesta alcesta (Stoll, 1782)	BG, RF, PG, RG	Blendon, Flight Intercept, Sweep net	African wood white	NA
	Mylothris chloris (Fabricius, 1775)	BG, RG	Sweep net	common dotted border	NA

Sites: BG = Biological Garden, M = Teaching and Research Farm, PG = Parks and Gardens, RG = Regrowth forest.

There was a significant difference in the mean abundance across sites (Z = 4.051, p < 0.05; BG = 16.67 ± 8.75, RF = 49.33 ± 18.92, PG = 29 ± 20.17, RG = 25 ± 15.55) and methods (Z = 5.194, p < 0.05; Blendon = 9.88 ± 4.67, Flight Intercept = 2.75 ± 1.89, Sweep net = 77.38 ± 11.50). Similarly, there was a significant difference in the species richness across sites (Z = -2.078, p < 0.05; BG = 5.67 ± 2.46, RF = 11 ± 2.80, PG = 5.17 ± 3.10, RG = 3.67 ± 1.74) and methods (Z = 2.326, p < 0.05; Blendon = 4.13 ± 1.29, Flight Intercept = 1.25 ± 0.84, Sweep net = 13.75 ± 1.71). Highest abundance and species richness of butterflies sampled was recorded with sweep netting across all sites (Figure 1a and 1b).





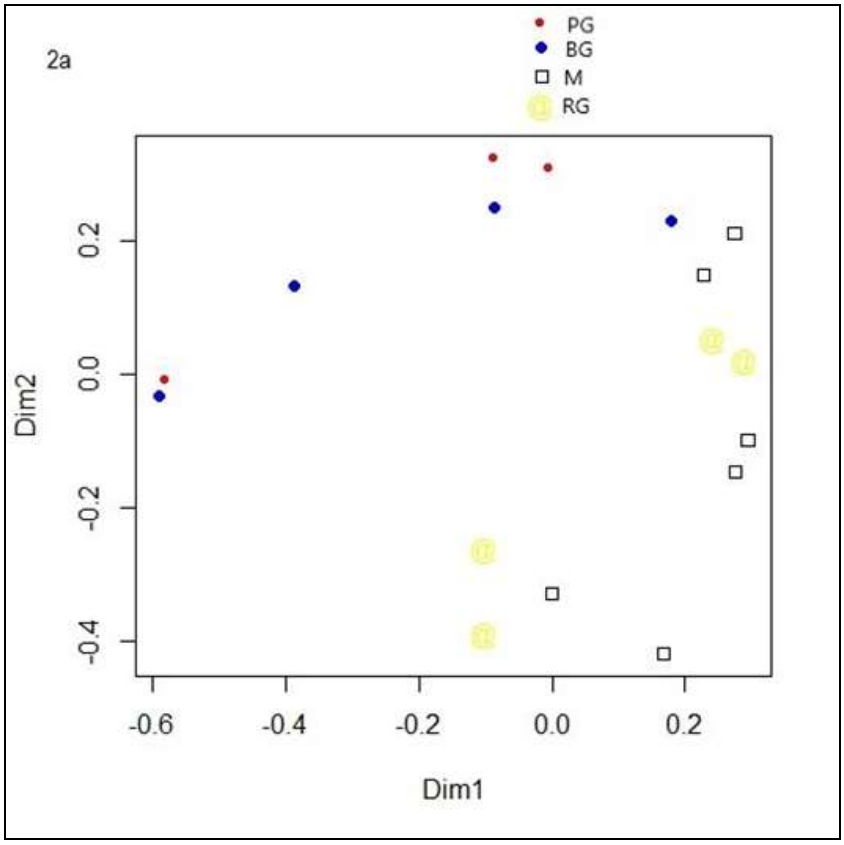
BG = Biological Garden, M = Teaching and Research Farm, PG = Parks and Gardens, RG = Regrowth forest.

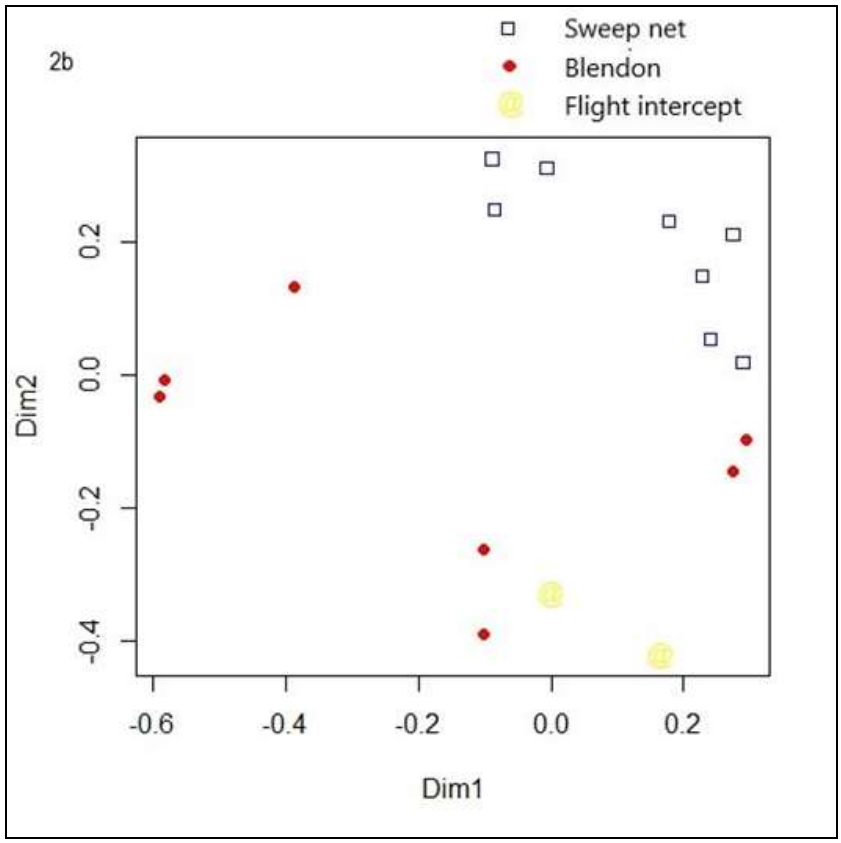
Fig 1: a Mean (±SE) abundance of butterflies sampled with different methods in different habitat types. b) Mean (±SE) species richness of butterflies sampled with different methods in different habitat types. Methods with different alphabets are significantly different (P < 0.05).

Taxon Species Composition

There was a significant difference in species composition of butterflies across sites ($F_{(3,13)}$ = 1.7485, P = 0.008, Fig. 2a) and methods ($F_{(2,14)}$ = 2.0277, P = 0.003, Fig. 2b). There were four butterfly species unique to Biological Gardens (BG), eight butterfly species unique to Teaching and Research Farm (RF), two butterfly species unique to Parks and Garden (PG) and four butterfly species unique to the Regrowth Forest (RG). Also, out of the 42 species recorded on all study sites, 21 species were sampled with the sweep net (Table 1).

The PCO diagram showed the similarity of species composition across sites and methods (Fig. 2a and 2b). It also showed the species composition of butterflies across sites using different methods (Fig. 2c).





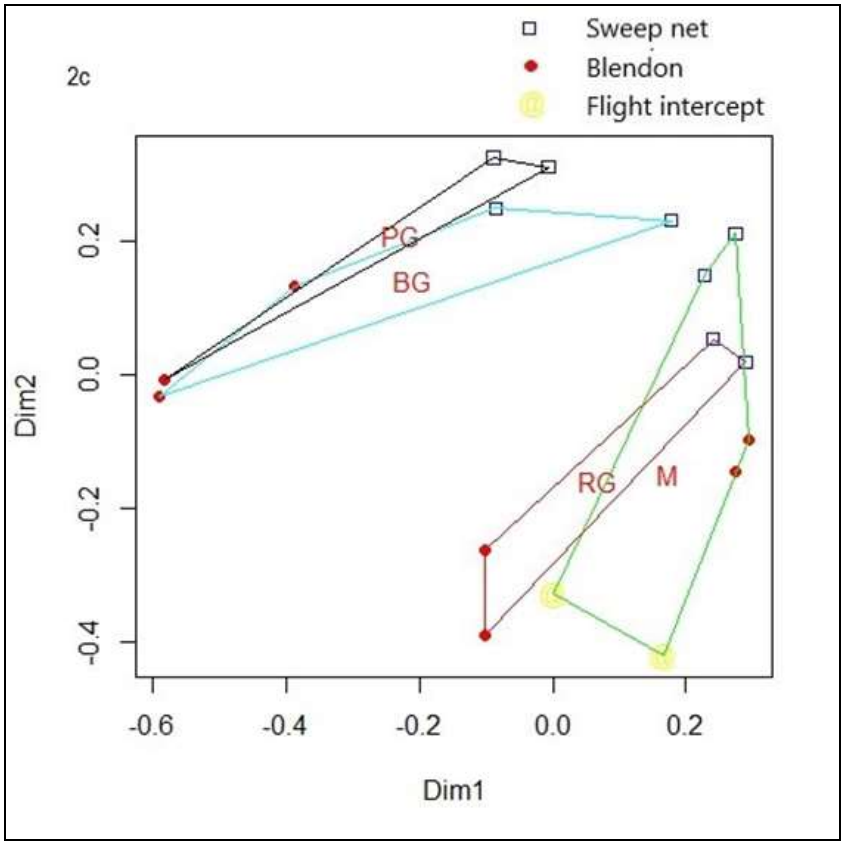
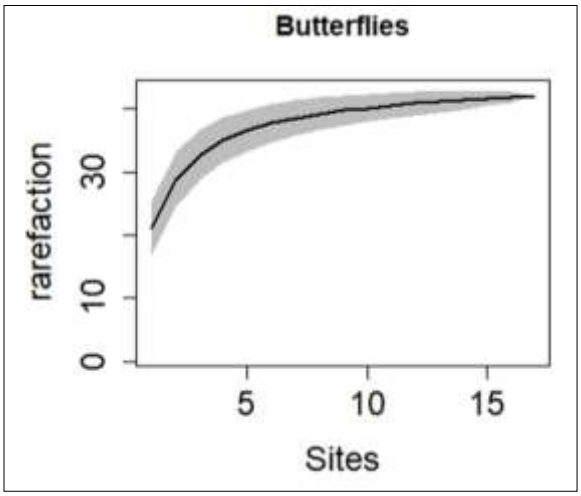


Fig 2: PCO diagram showing comparison of species composition across a) Study sites b) Methods c) Study sites and Methods. Study Sites: BG = Biological Garden, M = Teaching and Research Farm, PG = Parks and Gardens, RG = Regrowth forest.



Appendix S1: Rarefaction curve of butterflies

Discussion

Butterflies belong to the Order Lepidoptera [37, 38] and about 14,500 of the total number of lepidopteran species are butterflies [38]. From this total number, about 1500 butterfly species have been recorded in West Africa and about 1000 species in Nigeria [33]; of which 42 species were reported in this study. The abundance and species richness of butterflies recorded in this study shows that urban areas may be able to support diversity of this taxon if well managed, which will help mitigate the otherwise large effect of urbanization on biodiversity [9, 39, 40]. However, *Eurema hecabe* which is an indicator of a threatened ecosystem was recorded on all study sites. Also, *Mylothris chloris* another indicator of a threatened ecosystem was recorded on Biological gardens (BG) and Regrowth Forest (RG) [24, 33]. Therefore, the management of these areas should be closely monitored to limit anthropogenic threats and promote their suitability as habitat for butterflies and other beneficial organisms.

Also, a high number of Nymphalids were recorded as opposed to other tropical butterfly families. This implies that the study areas attracted more of Nymphalids which could be due to them being the most diverse and abundant family in the Southern part of Nigeria [22, 41] or due to their nature of being strong fliers which can travel over a long distance to locate food [42] or could also be due to the trapping methods used. According to [26], Nymphalidae are more likely to be observed regardless of survey method because of their colour and size. Furthermore, other butterfly families like Lycaenidae and Pieridae are not easily attracted to traps [24]. This study also confirmed that members of families Lycaenidae and Pieridae recorded were sampled mainly with sweep netting method. Further, species composition of butterflies sampled with the different sampling methods varied across the different study sites. This suggests that while sweep netting is the preferred sampling method, there is need to give consideration to the habitat type to be sampled when making the choice of sampling method in butterfly surveys [28].

Conclusion

There is a need for more intensive study across various habitat types in urban landscapes to document in details butterfly diversity, their habitat preferences and match the various habitats with the most appropriate sampling method. This will motivate more guided butterfly surveys by both experts and citizen scientists. Consequently, resulting in holistic documentation of native, endemic and other categories of butterfly species in Nigeria and other less studied ecosystems, particularly in the tropics. That being so, threatened and endangered species of butterflies would be accounted for before they are lost in the ecosystem.

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