DOI: https://doi.org/10.3329/jbcbm.v10i2.82235 *J. biodivers. conserv. bioresour. manag.* **10**(2), 2024

INDIGENOUS TECHNIQUES FOR SAMPLING BUTTERFLIES IN THE BHAWAL AND MADHUPUR SAL FORESTS OF BANGLADESH

Chowdhury, M. A. K.* and M. A. Bashar[1]

Department of Zoology, National University, Gazipur-1704, Bangladesh; [1]Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh

*Corresponding author: chowdhurym.ashek@gmail.com

Abstract

A comprehensive assessment of butterfly diversity requires effective sampling techniques tailored to species behavior and habitat conditions. This study evaluates butterfly populations and their distribution in two deciduous moist Sal forests of Bangladesh—Bhawal and Madhupur—dominated by the tree *Shorea robusta*. As part of a broader conservation initiative, we conducted year-round surveys using four sampling techniques: sweep netting, visual observation, fruit-feeding, and sap-feeding. Sampling was performed twice monthly at ten sites (five per forest) between 7:30 a.m. and 6:00 p.m. Visual observation recorded the highest mean butterfly abundance (158.38±29.51), while sap-feeding yielded the lowest (0.23±0.23). Variability was highest for sap-feeding (coefficient of variance: 781.02) and lowest for sweep netting (136.53). Sweep netting effectively captured fast-flying species, visual observation facilitated non-invasive identification, and fruit- and sap-feeding targeted specific butterfly guilds, enhancing species inventory completeness. Our findings highlight the importance of integrating multiple techniques to reduce species-specific biases and ensure robust biodiversity assessments in tropical forests.

Key words: Sweep netting; Distance sampling; Fruit-feeding; Sap-feeding; Madhupur and Bhawal forests.

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 *et al*. 2010), climate change (Medina-Baez *et al*. 2016), fragmentation of habitats (Cousins *et al.* 2015), increased use of pesticide (Ehrlich and Harte 2015), and rapid increase in anthropogenic activities (Harrison and Winfree 2015). Thus, insect diversity is declining worldwide (Kral *et al.* 2018), and ultimately, species are losing (Böhm *et al*. 2013). With this loss comes a need to protect, modify, or manage existing landscapes in the ways that promote diversity (Chong *et al*. 2014). Therefore, halting the process of biodiversity decline, or at least significantly reducing its rate, is one of humankind's global challenges (Nowicki *et al*. 2008).

Butterflies are consistently becoming the focus of conservation research (Kral *et al*. 2018) and are among the most studied non-pest insects globally (Thomas *et al*. 2009). Because of their contribution to ecosystem service particularly pollination (Rader *et al.* 2016), ease of sampling, relatively resolves taxonomy, abundance, species richness, migratory behavior, sensitivity to habitat fragmentation and environmental change, short life cycles (Thomas *et al*.

2004); breeding even in small habitat patches (van Swaay *et al*. 2006) and popularity as compared to other insects butterflies constitute themselves the predominant fraction of biodiversity (Thomas 2005). Butterfly diversity is linked to total butterfly diversity at local scales (Ribeiro and Freitas 2012) and is also associated with tree diversity (Schulze *et al.* 2004). Thus, monitoring butterfly communities over time is a high priority as a potential tool for assessing large-scale biodiversity trends (van Swaay *et al.* 2006) and can aid in understanding the factors that affect their population dynamics (Basset *et al.* 2017).

Butterfly monitoring schemes are recording programs initiated to monitor butterfly abundance and distribution patterns (van Swaay *et al.* 2015). This requires proper survey designs as well as reliable methods of data collection and statistical analysis (Roy *et al.* 2001). Both sampling (collecting individuals physically) and surveying (observing individuals visually) are used interchangeably to monitor and research butterflies (Zaman *et al.* 2015). Four main methods are used in butterfly research and monitoring: (1) trapping (i.e. chemical, visual, or form lure used to attract butterflies) and netting (i.e. nothing used to attract butterflies), (2) mark-recapture, (3) transects (Pollard walks), and (4) distance sampling (Kral *et al.* 2018). However, two common methodologies are used to sample tropical butterflies: collecting them with entomological hand nets, which effectively capture most butterfly guilds (Robbins *et al.* 1996), and employing bait traps to target fruit-feeding species (Freitas *et al*. 2014). No comparative study on the performance of different sampling techniques has been done yet in the tropics (Robbins *et al*. 1996). Whereas, quantitative comparisons between net-sampling and baited trap techniques are critically needed, as both methods are likely biased toward different taxonomic groups (Iserhard *et al.* 2013). The analysis of temporal variation in the performance of each sampling technique is also key to understanding the biases associated with each method. For example, nectar-feeding butterflies have, on average, shorter life spans than fruit-feeding butterflies (Beck and Fiedler 2009); thus, the abundance of nectar-feeders may fluctuate more than that of fruit-feeding butterflies.

Bangladesh, a developing nation in tropical Asia, is facing rapid degradation of its forest areas (Bashar 2015), while conservation planning remains uncertain. Furthermore, the butterfly fauna in the country is underrepresented, primarily due to limited detection by existing monitoring schemes, presenting an opportunity to improve the butterfly monitoring efforts that could significantly enhance forest management strategies. To address this issue, there is a critical need for a comprehensive understanding of appropriate sampling techniques for butterfly taxa to identify and mitigate biases associated with specific techniques and habitats in butterfly monitoring. In this study, we evaluated various sampling techniques and survey methods applied along transects to quantify butterfly abundance in the forests of Bangladesh. Our objective was to assess the overall performance of these techniques in estimating butterfly community diversity, determine the feasibility of using a single technique as a benchmark for overall butterfly diversity in ecological and conservation studies, and explore unique sampling approaches to guide the design and standardization of butterfly-monitoring networks. This research aims to improve the management and conservation of butterflies in their natural habitats, ensuring the continuation of gene flow in nature. We analyzed butterfly diversity across taxonomic groups over different seasons and years, employing a range of sampling methods and designs across the Bhawal and Madhupur Sal Forests of Bangladesh.

MATERIAL AND METHODS

The present survey methodology was designed using various sampling tools to evaluate the relative population size of butterflies over the years and their distribution in two deciduous moist sal forests: Bhawal and Madhupur, both dominated by the plant *Shorea robusta*. This effort is part of an ongoing broader conservation program aimed at enhancing butterfly and wildlife diversity in the natural habitats of Bangladesh. The butterfly sampling programs are designed to ensure unbiased and precise data collection, allowing for robust survey designs that enable statistical analysis and scientific reliability. These initiatives aim to use butterflies as environmental change indicators by monitoring their occurrence trends, abundance, and variations in spatiotemporal distribution within the study areas.

The sampling of butterflies was conducted weekly throughout the year at 10 selected study sites (five in each forest) twice a month, following a yearly survey schedule. This phase of the survey took place from July 2007 to June 2022. The Bhawal Sal Forest (24°01' N, 90°20' E) in Gazipur District is 40 km north of Dhaka city along the Dhaka-Mymensingh highway. The Madhupur Sal Forest, situated (23°50'-24°50' N, 89°54'-90°50' E) at Madhupur Upazila in Tangail District, lies 120 km north of Dhaka City. The soil type in both forest ranges from sandy loam to silty loam, with organic matter contents varying from low (<1.5%) in grassland to moderate (2–5%) under forest cover. The soil is acidic, with a pH of 5.2–5.5 (Dhar and Mridha 2006). The climate in the deciduous forest region is relatively uniform, with annual rainfall between 1,830 mm and 2,300 mm and average maximum and minimum temperatures of 34°C and 11°C, respectively, depending on the season, making January the coldest month and from April to September the warmest period. The relative humidity averages (78.88%) throughout the year, ranging from 60% to 90%, with September being the most humid month and March the least.

Fig. 1. Categories of insect sweeping nets of different sizes used for butterfly samplings: **a**. Small size insect net for capturing small butterflies available on soil surface; **b**. Net for capturing butterflies on herbs, shrubs and hedges at man-height level; and **c**. Net used for capturing butterflies on trees above man-height level.

Sampling was conducted following the "Pollard walks" method (Pollard 1977), combined with handheld sweep nets of different sizes (Fig. 1) to capture flying, foraging, and resting butterflies, alongside visual observations of butterflies engaged in displaying behaviors, such as foraging, resting, puddling, feeding on fallen ripe fruits, sap-feeding and fruit traps. Each sampling session lasted an hour at each site along a 0.5 km line transect, with a 5-meter radius on either side, conducted under favorable weather conditions (no heavy rain or strong wind). Alarape's transect walking techniques (Alarape *et al*. 2015) guided efforts to catch or count every butterfly observed, while random sweep netting facilitated rapid biodiversity assessments. The sampling occurred between 7:30 a.m. and 6:00 p.m. Bangladesh Standard Time (BST), occasionally extending to 7:00 p.m., as detailed in Table 1.

Table 1. Varieties of equipment/techniques used for sampling butterflies.

Recording features	Equipments/techniques used for sampling			
	Sweep netting	Distant sampling (Visual recording)	Fruit-feeding	Sap-feeding
Flying butterfly	√	√	X	X
Foraging butterfly	√	√	X	X
Resting butterfly	√	√	X	X
Puddling butterfly	X	√	X	X
Fruit-feeding butterfly	X	√	√	X
Sap-feeding butterfly	X	√	X	√

After capturing the butterflies, they were placed into killing jars containing wads of cotton soaked in chloroform (Trichloromethane) for anesthetization and transported to the laboratory. The specimens were classified into various families based on their primary morphological features in the laboratory. The butterflies were handled carefully, piercing the thorax with insect needles before being placed on a setting board to dry in a dark area for one week. Once dried, the properly set and pinned specimens were stored in well-sealed wooden insect boxes with naphthalene balls for preservation. The samples were then labeled and stored in the Environmental Biology and Biodiversity Laboratory (EBBL) of the Department of Zoology at the University of Dhaka. The identification of the butterflies was carried out using identification keys from various sources, including Bingham (1905 and 1907), Evans (1932), Talbot (1947), Wynter-Blyth (1957), Borror *et al.* (1989), Pinratana (1992), Ek-Amnuay (2012), Kehimkar (2008) and Bashar (2014). The specimens were identified at the species level, and the butterflies were photographed both in the field and the laboratory using Sony Cyber-Shot DSC-H50 and Olympus E-1 Zoom Digital Cameras.

Statistical analyses

To investigate whether different sampling techniques yield distinct butterfly communities, we used Least Squared Difference (LSD), One-way ANOVA, and other relevant statistical tests to analyze differences in butterfly abundance and observed species richness. The analysis was made on the data obtained following four sampling methods- sweep netting, distant sampling through visual recording, fruit-feeding and sap-feeding over different seasons. The data were analyzed using SPSS 27 and PAST 5.01 statistical softwares. The Simpson's D, Shannon H, and Margalef Index were employed to estimate diversity for each forest, season, and species richness. Butterflies recorded on each sampling day were pooled for each sampling location throughout the entire study period, and these pooled data were used as the dependent variable in the analysis.

RESULTS AND DISCUSSION

Butterfly diversity across the Bhawal and Madhupur Sal Forests (BSF and MSF) was vibrant, encompassing 8 families, 17 subfamilies, 23 tribes, and 61 genera (Table 2). The family Nymphalidae exhibited the highest diversity followed by Lycaenidae and Pieridae with BSF hosting 85 species and MSF 73 species, resulting in a combined total of 109 species.

Table 2. A list of different taxa of butterflies captured using various sampling methods from the Bhawal and Madhupur Sal Forests during the study period.

Butterfly Taxa			Sampling methods	Biotopes		Total no. of species	Simson's Diversity Index (D)
Subfamily	Tribe	Genus		BSF	MSF		
			PAPILIONIDAE				
Papilioninae	Papilionini	*Chilasa*	SN, VR	1	2	2	0.0065
		Papilio	√	4	4	5	0.0001
	Leptocercini	*Graphium*	√	2	2	2	0.0340
			PIERIDAE				
Pierinae	Pierini	*Appias*	√	2	2	2	0.0105
		Cepora	√	2	2	1	0.0041
		Delias	√	4	4	4	0.0019
		Leptosia	√	1	1	1	0.0266
		Pareronia	√	1	2	2	0.00345
		Pieris	√	0	3	3	0.0024
		Pontia	√	0	1	1	0.0054
Coliadinae	Coliadini	*Catopsillia*	√	2	2	2	0.0054
		Eurema	√	4	4	4	0.0060
			DANAIDAE				
Danainae	Danaini	*Danaus*	√	3	2	3	0.0039
		Euploea	√	2	2	3	0.00851
		Tirumala	√	0	1	1	0.0072
			SATYRIDAE				
Satyrinae	Elymniini	*Elymnias*	√	1	0	1	0.0071
		Lethe	√	2	2	3	0.0170
	Melanitini	*Melanitis*	FF, VR	2	0	2	0.0040
	Satyrini	*Mycaleis*	SN, VR	1	3	4	0.0029
		Orinoma	√	1	0	1	0.0013
		Orsotrioena	√	1	0	1	0.0021
		Ypthima	√	1	2	2	0.0004
			NYMPHALIDAE				
Charaxinae	Charaxini	*Charaxes*	SN, VR, SF	0	2	2	0.0169
Apaturinae	Apaturini	*Stibochiona*	SN, VR	1	0	1	0.0156
Biblidinae	Biblidini	*Ariadne*	√	2	0	2	0.0053
Limenitidinae	Limenitidini	*Athyma*	√	0	2	2	0.0089
	Adoliadini	*Euthalia*	√	2	0	2	0.0111
	Limenitidini	*Moduza*	√	1	0	1	0.0033
	Neptini	*Neptis*	√	1	2	2	0.01493
Nymphalinae	Junoniini	*Hypolimnas*	SN, VR, FF, VR	2	0	2	0.0169
		Junonia	SN, VR	4	5	6	0.0037
Heliconiinae	Vagrantini	*Phalantha*	√	1	0	1	0.0659
			ACRAEIDAE				
Acraeinae	Acraeini	*Acraea*	√	1	0	1	0.0004
			LYCAENIDAE				
Theclinae	Theclini	*Arhopala*	√	3	2	4	0.0021

		Dacalana	√	0	2	2	0.0076
		Deudorix	√	1	1	1	0.0142
		Hypolycaena	√	1	1	1	0.0029
		Loxura	√	1	1	1	0.0026
		Rapala	√	3	2	3	0.0018
		Rathinda	√	1	0	1	0.0033
		Remelana	√	1	0	1	0.0016
		Spindasis	√	3	2	3	0.0040
		Tajuria	√	1	0	1	0.0039
Polyommatinae	Polyommatini	*Castalius*	√	1	0	1	0.0002
		Catochrysops	√	1	1	1	0.0040
		Chilades	√	2	2	2	0.0004
		Discolampa	√	1	0	1	0.0083
		Euchrysops	√	1	1	1	0.0073
		Everes	√	1	1	1	0.0025
		Jamides	√	1	0	1	0.0067
		Lampides	√	1	0	1	0.0036
		Neopithecops	√	1	1	1	0.0006
		Pseudozizeeria	√	1	1	1	0.0032
		Zizina	√	1	1	1	0.0024
			HESPERIIDAE				
Coeliadinae	Coelidini	*Badamia*	√	0	1	1	0.0053
Pyrginae	Pyrgini	*Sarangesa*	√	1	0	1	0.0071
		Tagiades	√	1	0	1	0.0099
Hesperiinae	Hesperiini	*Borbo*	√	1	0	1	0.0148
		Cephrenes	SN, VR	1	0	1	0.0031
		Matapa	√	1	0	1	0.0056
		Udaspes	√	1	1	1	0.0002
17	23	61		85	73	109	0.45289

BSF = Bhawal Sal Forest; MSF = Madhupur Sal Forest; SN = Sweep netting; VR = Visual recording; FF = Fruit-feeding; and SF = Sap-feeding.

Statistical analyses revealed significant differences in diversity between the two forests ($F = 19.22$, $P < 0.01$) with notable variations in species richness ($F = 264.66$, $P < 0.01$), family-level species count ($F = 266.11$, $P < 0.01$), and butterfly abundance ($F = 784.19$, $P < 0.01$). Certain genera were forest-specific, such as *Ariadne* and *Elymnias* in BSF and *Pieris* and *Tirumala* in MSF, while others, including *Junonia*, *Papilio* and *Graphium*, were widespread across both. The genera like *Chilasa* and *Papilio* showed higher diversity in MSF, whereas dominant genera, such as *Delias* and *Eurema* under Pieridae, were evenly distributed. The genus *Junonia* of Nymphalidae showed the highest species richness. These findings highlighted the unique ecological compositions of BSF and MSF as biodiversity hotspots. The Diversity Index is D = 0.4562, reflecting moderate diversity in deciduous sal forests. The genera like *Lethe* (D = 0.0170), *Graphium* (D = 0.0340) and *Phalantha* (D = 0.0659) showed relatively high individual contributions to diversity. The taxa with low Simpson diversity values, such as *Udaspes* and *Castalius*, highlight potential vulnerability or specific habitat needs.

A detailed account of the number of butterfly species recorded using various sampling methods across different families and subfamilies is presented in Table 3. The Sweep netting and Visual recording methods consistently captured the highest number of species across families and subfamilies (108 species each). The Fruit-feeding method recorded four species, indicating limited applicability to certain families/subfamilies. The Sap-feeding method recorded two species, showing minimal efficiency or relevance for capturing species compared to other methods. No species of the Families Papilionidae, Pieridae, Danaidae and Lycaenidae showed fruit or sap-feeding habits; the sweep netting and the visual recording were effective for them. Some species of Satyridae and Nymphalidae were observed engaging in fruit-feeding or sap-feeding, especially in the subfamilies Satyrinae, Charaxinae, and Nymphalinae. Two species of the subfamily Satyrinae were fruit-feeding, demonstrating its relevance for this group. Two species of the subfamily Charaxinae under the family Nymphalidae showed sap-feeding habits, highlighting its dependence on specific resources. Two subfamilies, Limenitidinae and Nymphalinae of Nymphalidae, recorded one species each through fruit-feeding, showing occasional dependence on alternative methods. The Lycaenidae family, especially the Theclinae subfamily, recorded the highest number of species (19) both in the Sweep netting and Visual recording methods, followed by Pierinae (14) of Pieridae, Satyrinae (12) of Satyridae, Polyommatinae (12) of Lycaenidae and Nymphalinae (8) of Nymphalidae (Table 3).

Table 3. Species count by four different sampling methods.

Family	Subfamily	No. of species recorded using various sampling methods			
		Sweep netting	Visual recording	Fruit-feeding	Sap-feeding
Papilionidae	Papilioninae	09	09	-	-
Pieridae	Pierinae	14	14	-	-
	Coliadinae	06	06	-	-
Danaidae	Danainae	07	07	-	-
Satyridae	Satyrinae	12	12	02	-
Nymphalidae	Charaxinae	02	02	-	02
	Apaturinae	01	01	-	-
	Biblidinae	02	02	-	-
	Limenitidinae	07	07	01	-
	Nymphalinae	08	08	01	-
	Heliconiinae	01	01	-	-
Acraeidae	Acraeinae	01	01	-	-
Lycaenidae	Theclinae	19	19	-	-
	Polyommatinae	12	12	-	-
Hesperiidae	Coeliadinae	01	01	-	-
	Pyrginae	02	02	-	-
	Hesperiinae	04	04	-	-
08	17	108	108	04	02

A significant difference was observed in the mean abundance (mean ± standard error) of butterfly species captured and recorded using different sampling methods across the BSF and the MSF (Table 4). The mean abundance values for each method were as follows: sweep netting (58.56 ± 10.24), fruit-feeding (1.34 ± 0.80), sap-feeding (0.23 ± 0.23) and visual observation (158.38 ± 29.51). Among these, visual observation recorded the highest mean abundance of butterflies (158.38), whereas sap-feeding yielded the lowest mean abundance (0.23). The coefficient of variance varied significantly across sampling methods, with sap-feeding exhibiting the highest variability (781.02) and sweep netting showing comparatively low variability (136.53) (Table 4).

Statistical analysis confirmed significant differences among the means, as indicated by Omega2 = 0.2201 and Welch's F-test ($F = 24.46$, $P < 0.0001$). Furthermore, the Bayes factor

(7.113E15) provides decisive evidence supporting the presence of significant variations in species abundance across the sampling methods followed.

Table 4. Mean abundance (± SE) of butterflies captured and recorded using different sampling methods.

Sampling methods	Mean Abundance ± SE	Coefficient of Variance
Sweep netting	58.56 ± 10.24	136.53
Visual observation	158.38 ± 29.51	145.54
Fruit-feeding	1.34 ± 0.80	465.73
Sap-feeding	0.23 ± 0.23	781.02

Statistical summary:

- Levene's Test for homogeneity of variance (Omega2): 0.22 ($P < 0.0001$)
- Welch's F-Test (for unequal variances): 24.46 ($P < 0.0001$)
- Bayes Factor: 7.113E15 (decisive evidence for unequal means)

Various sampling methods, including sweep netting (SN), visual recording (VR), fruit-feeding (FF) and sap-feeding (SP) were employed to document butterfly species within the Bhawal and Madhupur Sal Forests (BSF and MSF). Statistical analyses revealed significant differences among sampling methods in capturing butterflies across different taxonomic groups ($F = 21.66$, $P < 0.000$), indicating substantial variation in method efficacy. Specifically, sweep netting ($F = 10.87$, $P < 0.000$) and visual observation ($F = 9.73$, $P < 0.000$) demonstrated statistically significant differences across groups, confirming their effectiveness in capturing a diverse array of butterfly species (Table 5).

Table 5. One-way ANOVA test results.

Sampling methods	F-Statistic	Significance (P-Value)
Overall Sampling Methods	21.66	<0.000
Sweep netting	10.87	<0.000
Visual observation	9.73	<0.000
Fruit-feeding	0.54	0.746
Sap-feeding	0.579	0.716

Additional statistical metrics:

- Levene's Test for homogeneity of variance (from means): 1.86E-19
- Levene's Test (from medians): 1.82E-11
- Welch's F-Test (for unequal variances): 24.52 ($P = 4.123E-18$)

Bayes Factor: 5.819E15 (decisive evidence for unequal means)

Conversely, fruit-feeding ($F = 0.54$, $P = 0.746$) and sap-feeding ($F = 0.579$, $P = 0.716$) did not exhibit significant differences across groups, suggesting that these methods are less effective and exhibit lower variability in butterfly counts compared to sweep netting and visual observation. Furthermore, variance across sampling methods was found to be non-homogeneous ($P \ll 0.05$), as indicated by Levene's test. The intraclass correlation coefficient

(ICC) suggests that group-level differences account for a moderate proportion (25.3%) of total variance. The Bayesian analysis strongly supports the presence of unequal means, reinforcing the observed statistical disparities.

The results from the One-sample t-test presented in Table 6 reveal significant differences in the effectiveness of various butterfly sampling methods. The sweep netting shows a highly significant result ($t = 5.720$, $p < 0.001$), with a mean difference of 58.56 and a 95% confidence interval ranging from 38.08 to 79.03, indicating its high effectiveness in capturing butterflies. In contrast, the fruit-feeding ($t = 1.677$, $p = 0.099$) and the sap-feeding ($t = 1.000$, $p = 0.321$) methods are not statistically significant, with p-values above 0.05. The mean differences of 1.34 (fruit-feeding) and 0.23 (sap-feeding) suggest that there was a minimal impact of feeding on sampling and the confidence intervals for both methods span values close to zero. The visual observation is, however, highly significant ($t = 5.366$, $p < 0.001$), showing a mean difference of 158.38 and a 95% confidence interval ranging from 99.34 to 217.41, highlighting its exceptional effectiveness in butterfly recording. These findings suggest that the sweep netting and visual observation should be prioritized in future butterfly surveys, as these two methods provide the most reliable results.

Table 6. One-sample t-test results.

Sampling methods	t	Sig. (2-tailed)	Mean difference	95% Confidence interval of the difference	
				Lower	Upper
Sweep netting	5.720	.000	58.56	38.08	79.03
Fruit-feeding	1.677	.099	1.34	-.2592	2.9477
Sap-feeding	1.000	.321	.23	-.2296	.6886
Visual observation	5.366	.000	158.28	99.3417	217.4124

A detailed visualization of the distribution of data across the groups is presented in Fig. 2, which combines the features of a violin plot with a box plot. The blue violin represents data

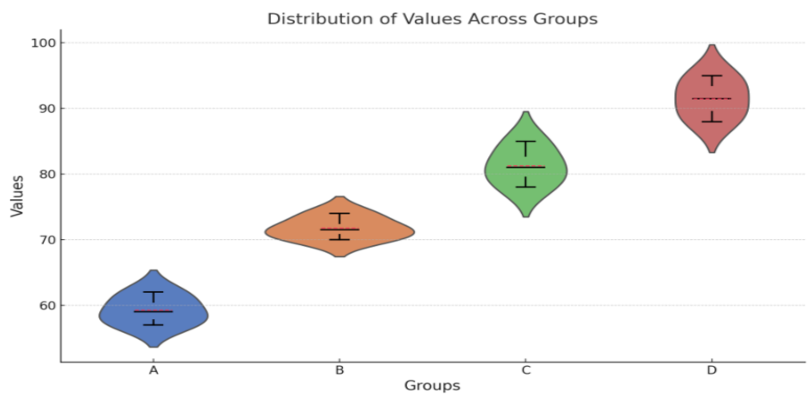

Fig. 2. Illustrating the distribution of values across different groups using a violin plot overlaid with a boxplot.

from the sweep netting sampling method, while the green violin corresponds to data from the visual observation sampling method. The orange violin is used for the fruit-feeding sampling method and the purple violin represents data from the sap-feeding method. The height of the violins indicates the variability within the groups, with taller violins suggesting higher variability and an uneven distribution of data. Differences in the overall width, shape, or position of the violins provide valuable insights into the comparative differences across the groups. These characteristics allow for a clear understanding of the distribution of values and how each method performs in terms of variability and central tendency.

The results presented in Fig. 3 highlight the correlation between various sampling methods and the number of butterflies and species recorded. The sweep netting and the visual observation methods exhibit a strong and significant correlation with both the number of butterflies and the species recorded, suggesting that these methods are highly effective in capturing butterfly diversity. However, while these two methods are effective individually, they show only a weak correlation with each other, indicating that they may capture different aspects of butterfly populations. In contrast, the fruit-feeding and the sap-feeding methods show an insignificant correlation either with the number of butterflies or with the species recorded. Despite this, these two methods do demonstrate a weak correlation with each other, suggesting a slight overlap in their effectiveness.

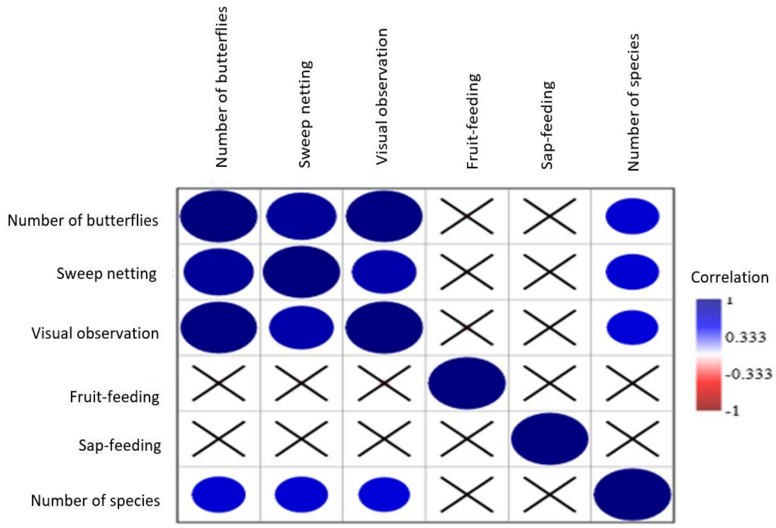

Fig. 3. Illustrating correlations among different sampling methods by which the number of butterflies and their number of species were recorded from the Bhawal and Madhupur Sal forests.

Table 7. Results of different α-diversity and β-diversity tests for various sampling methods.

Sampling methods	Sweep netting	Visual observation	Fruit-feeding	Sap-feeding
Dominance D	0.04619	0.05045	0.3583	1
Shannon H	3.511	3.478	1.061	0
Margalef	7.334	6.539	0.4539	0
P-value	< 0.0000	< 0.0000	< 0.0000	< 0.0000

The butterfly species richness observed in the Bhawal and Madhupur Sal forests demonstrates a high level of diversity, as shown by Simpson's diversity (D) and Shannon α-diversity indices (Table 7). The biodiversity of species was assessed across four different sampling groups: sweep netting, visual observation, fruit-feeding and sap-feeding. Among these groups, sap-feeding showed the highest dominance value (D=1), indicating a highly uneven distribution of species, with one or a few dominant species. In contrast, the other sampling groups showed much lower dominance values (sweep netting: D = 0.04619, visual observation: D = 0.05045, fruit-feeding: D = 0.3583), suggesting a more even distribution of species. Furthermore, sweep netting and visual observation exhibited the highest Shannon H values (3.511 and 3.478, respectively), indicating a more diverse species composition. In contrast, sap-feeding had a significantly lower value (1.061), reflecting reduced diversity within this group. Regarding species richness, the Margalef Index values were highest for sweep netting (7.334) and visual observation (6.539), suggesting that these methods host a greater variety of species. On the other hand, sap-feeding had a very low Margalef Index value (0.4539), reflecting fewer species overall. All differences observed across the groups were highly statistically significant ($p < 0.0000$), confirming that the variations in species diversity, evenness and richness are not due to random chance, but are likely driven by ecological or behavioral factors inherent to the sampling strategies.

Butterflies from different families exhibited significant differences in their feeding habits, leading to the categorization of the species into two distinct groups. Nectar-feeding butterflies were observed visiting a variety of flower species across both the Bhawal and Madhupur Sal forests throughout different seasons. These butterflies were primarily collected using a sweep net or recorded visually. However, comparisons between different types of sweep nets were not feasible due to the pooling of data. On the other hand, fruit-feeding butterflies were predominantly observed through visual observation.

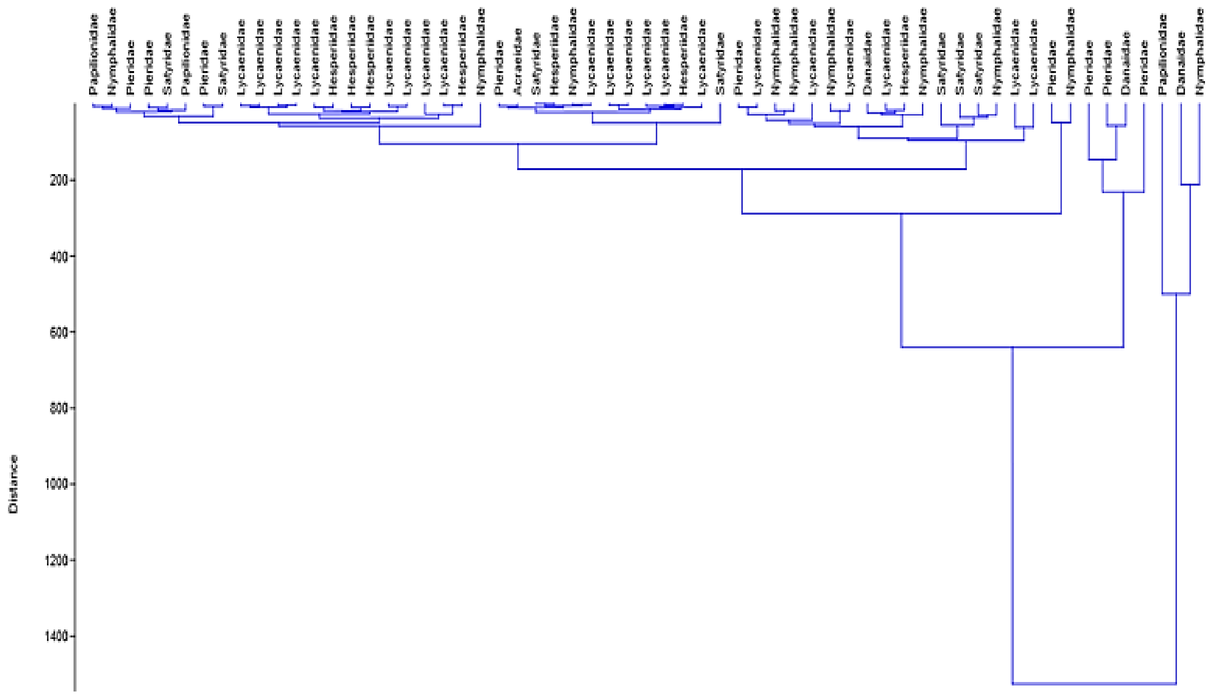

Fig. 4. Feeding relationships among different taxa of butterflies recorded from the Bhawal and Madhupur sal forests.

Interestingly, butterflies within the same family often displayed different feeding preferences, highlighting the diversity of feeding strategies even within closely related species. Additionally, butterflies from different families sometimes exhibited similar feeding habits at a higher taxonomic level, particularly at the Tribe level. For instance, butterflies from the Genus *Melanitis* (Tribe Melanitini, Subfamily Satyrinae, Family Satyridae) and those from the

Genus *Euthalia* (Tribe Adoliadini, Subfamily Limenitidinae, Family Nymphalidae) and *Hypolimnas* (Tribe Junoniini, Subfamily Nymphalinae, Family Nymphalidae) displayed striking similarities in their feeding habits, as revealed by Cluster Analysis (Fig. 4). These results underline the complexity of feeding behavior among butterfly species and emphasize the importance of taxonomic relationships in understanding ecological interactions.

The Principal Component Analysis (PCA, Fig. 5) provides valuable insights into the effectiveness and significance of different butterfly sampling methods. The clustering along PC1 indicates that active strategies**,** such as sweep netting and visual recording, are significantly more effective in capturing butterfly abundance and diversity than passive methods, such as sap-feeding and fruit-feeding, represented along PC2**.** Notably, PC1 accounts for 98.50% of the variance, while PC2 explains only 1.45%, underscoring the dominant role of active methods in driving overall variation in butterfly sampling.

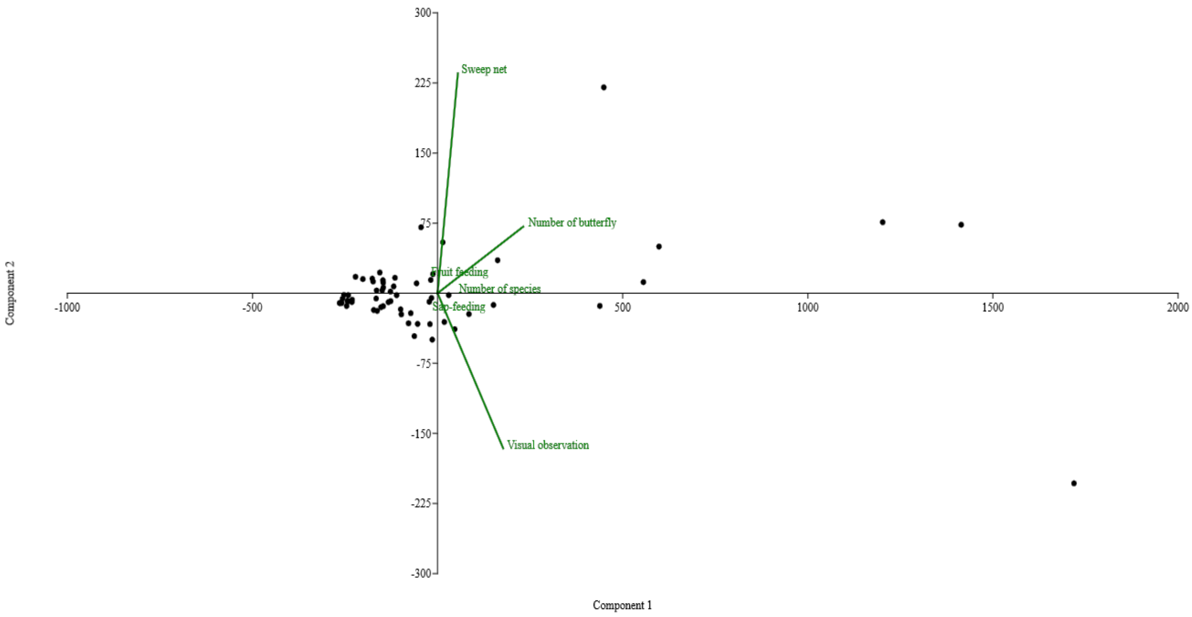

Fig. 5. PCA plotting to illustrate the effectiveness and importance of various butterfly sampling methods.

The positioning of these sampling methods along the PCA axes reflects clear differences in their ecological niches and sampling efficiency. Sweep netting and visual recording are recognized as the primary sampling methods due to their substantial contribution to variation (high PC1 contribution), making them the most effective strategies for butterfly assessments. In contrast, the sap-feeding and fruit-feeding methods play a secondary role, contributing less to overall variation but still capturing unique aspects of butterfly behavior and habitat preferences. These findings reinforce the need for a strategic combination of methods to maximize biodiversity assessments and account for the ecological diversity of butterfly species.

The findings of this study underscore significant variations in butterfly diversity between the Bhawal and Madhupur Sal Forests (BSF and MSF), highlighting the effectiveness of different sampling methods in capturing species richness and abundance. A multi-method approach, including sweep netting, distant sampling, fruit-feeding and sap-feeding, proved essential for a comprehensive assessment. The reliance on multiple methods aligns with previous studies (Haddad *et al.* 2008, Kral *et al*. 2018) emphasizing the importance of diverse sampling techniques to ensure representative biodiversity data.

Among the techniques employed, sweep netting and distant sampling through visual recording emerged as the most effective, capturing 108 species each, particularly from the families Papilionidae, Pieridae, Danaidae and Lycaenidae. These methods successfully targeted butterflies with active flight and nectar-feeding behaviors, as demonstrated by statistically significant F-values ($F = 10.87$, $P < 0.000$ for sweep netting; $F = 9.73$, $P < 0.000$ for visual observation). The effectiveness of these active methods is consistent with earlier research, where netting and visual surveys were found to be reliable in detecting species in both open and forested habitats (Amusan *et al.* 2014, Ojianwuna and Amusan 2019).

Despite their effectiveness, the netting and visual recording methods are often criticized for their inherent biases, such as the potential for non-randomized sampling and site-specific constraints (Hamm 2013). Although alternative methods, such as distance sampling, offer greater inferential strength by incorporating detection functions and randomization (Henry and Anderson 2016), they remain underutilized in butterfly research, even as studies increasingly advocate for their integration (Kitahara and Fujii 1994, Nowicki *et al*. 2008). One of the key limitations of distance sampling is its potential inefficacy in areas with exceptionally high species abundance, where individual counting becomes impractical (Isaac *et al.* 2011).

In contrast, passive methods, such as fruit-feeding and sap-feeding, proved significantly less effective, recording only four and two species, respectively. These methods primarily captured species from the Nymphalidae and Satyridae families, confirming earlier findings that certain butterfly families, such as Lycaenidae and Pieridae, are not easily attracted to baited traps (Ojianwuna and Amusan 2019). The limited success of passive techniques highlights the need to tailor sampling methods to species-specific behaviors and habitat characteristics (Gracia *et al.* 2017). This suggests that while sweep netting is generally preferred, habitat-specific considerations should inform the choice of survey methods to maximize species representation.

Statistical analyses reinforced these observations. Welch's F-test ($F = 24.46$, $P < 0.0001$) and Omega2 (0.2201) confirmed significant differences in species abundance across sampling methods, while the high Bayes factor (7.113E15) indicated that these differences were not due to random variation. Levene's test ($P \ll 0.05$) further supported the heterogeneity in sampling effectiveness, with sweep netting and visual observation displaying higher variability due to their broader species coverage. The intraclass correlation coefficient (ICC = 25.3%) underscored the influence of taxonomic group characteristics on sampling outcomes. The findings align with previous studies suggest that, in most cases, a single sampling method may be the most effective, reinforcing the importance of selecting the most suitable method for each study (Caldas and Robbins 2003, Lang *et al*. 2019).

Principal Component Analysis (PCA) revealed a distinct separation between active and passive sampling techniques, with sweep netting and visual recording accounting for 98.5% of the variance. These results further emphasize the superiority of active sampling methods in assessing butterfly diversity, while passive techniques serve as supplementary tools for specific taxa with specialized feeding behaviors. Additionally, biodiversity indices (Simpson's D, Shannon's H, and the Margalef Index) showed significant differences in diversity, evenness, and richness across sampling methods ($p < 0.0000$). The higher values associated with sweep netting and visual observation highlight their applicability across diverse habitats and environmental conditions.

Overall, the study underscores the necessity of integrating multiple sampling techniques to enhance the accuracy of butterfly diversity assessments. Given the ongoing global decline in butterfly populations, optimizing sampling approaches become increasingly important for conservation planning. Future research should focus on refining sampling methodologies, incorporating seasonal variations, and addressing habitat-specific influences to improve biodiversity assessments in tropical forest ecosystems. By adopting a balanced approach that combines active and passive methods, researchers can obtain a more comprehensive and representative understanding of butterfly diversity, ultimately contributing to more effective conservation strategies.

Comprehensive butterfly diversity assessments require the integration of multiple sampling techniques to address species-specific behaviors and habitat variability. This study in the Bhawal and Madhupur Sal Forests demonstrates that sweep netting, visual recording, fruit-feeding and sap-feeding methods contribute uniquely to species documentation. Sweep netting was effective for capturing small and fast-flying species, while visual recording provided a non-invasive means of identifying butterflies in different microhabitats. Fruit-feeding and sap-feeding techniques supplemented the dataset by attracting the species that rely on alternative food sources, particularly those from the Nymphalidae and Satyridae families.

The findings emphasize that no single method can comprehensively document butterfly diversity in complex ecosystems. Therefore, employing a multi-method approach is essential for minimizing observational biases and maximizing species detection. Future research could explore additional sampling techniques, such as light trapping for nocturnal butterflies or pheromone traps for specific taxa, to further enhance biodiversity assessments in these ecologically significant forests.

ACKNOWLEDGMENTS

This article is a part of the broader research program, *Butterflies of Bangladesh and Their Conservation*. The authors acknowledge the Ministry of Education, Government of the People's Republic of Bangladesh, for financial support. Gratitude is extended to the Department of Zoology, University of Dhaka, for providing access to the Environmental Biology and Biodiversity Laboratory (EBBL). The authors also thank the Forestry Division of the Ministry of Environment, Forest and Climate Change, Government of the People's Republic of Bangladesh, for their assistance in conducting field research in the forests of Bangladesh.

REFERENCES

Alarape, A. A., K. Omifolaji and G. Mwansat. 2015. Butterfly species diversity and abundance in University of Ibadan Botanical Garden, Nigeria. *Open Journal of Eco*logy. **5**(8): 352-360.

Amusan, B., C. Ojianwuna, T. Kehinde and A. Akanbi. 2014. Butterfly diversity in Obafemi Awolowo University, Ile Ife, South-West Nigeria. *The Zoologist*. **12**: 1-7.

Bashar, M. A. 2015. *Butterflies of Bangladesh: A broad approach to nature lovers (EBBL red list and butterfly activities)*. Vol. 2. 1st ed. BCTF Publication, Dhaka-1000. 177 pp.

Bashar, M. A. 2014. *Butterflies of Bangladesh: A broad approach for nature lovers*. Vol. 1. 1st ed. BCTF publications, Dhaka-1000. 514 pp.

Basset, Y., H. Barrios, J. A. Ramirez, Y. Lopez, J. Coronado, F. Perez, S. Arizala, R. Bobadilla and M. Leponce. 2017. Contrasting the distribution of butterflies and termites in plantations and tropical forests. *Biodivers. Conserv*. **26**: 151-176.

Beck, J. and K. Fiedler. 2009. Adult life spans of butterflies (Lepidoptera: Papilionoidea and Hesperioidea): broadscale contingencies with adult and larval traits in multi-species comparisons. *Biol. J. Linnean Soc*. **96**: 166-184.

Bingham, C. T. 1907. *The Fauna of British India, including Ceylon and Burma.* Butterflies-Vol. 2. Taylor and Francis Ltd., London. 480 pp.

Bingham, C. T. 1905. *The Fauna of British India, including Ceylon and Burma.* Butterflies-Vol. 1. Taylor and Francis Ltd., London. 511 pp.

Böhm, M., B. Collen, J. E. Baillie, P. Bowles, J. Chanson and N. Cox. 2013. The conservation status of the world's reptiles. *Biol. Conserv*. **157**: 372-385.

Borror, D. J., C. A. Triplehorn and N. F. Johnson. 1989. *An Introduction to the Study of Insects*. 6th ed. Saunders College Publishing, Philadelphia, USA. 875 pp.

Caldas, A. and R. Robbins. 2003. Modified Pollard transects for assessing tropical butterfly abundance and diversity. *Biol. Conserv*. **110**: 211-219.

Chong, K. Y., S. Teo, B. Kurukulasuriya, Y. F. Chung, S. Rajathurai and H. T. W. Tan. 2014. Not all green is as good: different effects of the natural and cultivated components of urban vegetation on bird and butterfly diversity. *Biol. Conserv*. **171**: 299-309.

Cousins, S. A. O., A. G. Auffret, J. Lindgren and L. Trank. 2015. Regional-scale land-cover change during the 20th century and its consequences for biodiversity. *Ambio.* **44**(1): 17-27.

Dhar, P. P. and M. A. U. Mridha. 2006. Biodiversity of arbuscular mycorrhizal fungi in different trees of Madhupur forest, Bangladesh. *J. Forestry.* **17**: 201-205.

Ehrlich, P. R. and J. Harte. 2015. Opinion: to feed the world in 2050 will require a global revolution. *PNAS.* **112**(48): 14743-14744.

Ek-Amnuay, P. 2012. *Butterflies of Thailand*. 2nd ed. Baan Lae Suan Amarin Printing, Bangkok, Thailand. 943 pp.

Evans, W. H. 1932. *The Identification of Indian butterflies*. Bombay Natural History Society, Bombay, India. X + 454 pp.

Freitas, A. V. L., C. A. Iserhard, J. P. Santos, J. Y. O. Carreira, D. B. Ribeiro, D. H. A. Melo, A. H. B. Rosa, O. J. Marini-Filho, G. M. Accacio and M. Uehara-Prado. 2014. Studies with butterfly bait traps: an overview. *Rev. Colomb. Entomol*. **40**: 203–212.

Gracia, M. B., J. L. P. Souza, E. Franklin, J. W. Morais and P. A. Pequeno. 2017. Sampling effort and common species: optimizing surveys of understory fruit-feeding butterflies in the Central Amazon. *Ecological Indicators*. **73**: 181-188.

Haddad, N. M., B. Hudgens, C. Damiani, K. Gross, D. Kuefler and A. Pollock. 2008. Determining optimal population monitoring for rare butterflies. *Conserv. Biol*. 22(**4**): 929-940.

Hamm, C. A. 2013. Estimating abundance of the federally endangered Mitchell's satyr butterfly using hierarchical distance sampling. *Insect Conserv. Divers.* **6**: 619-626.

Harrison, T. and R. Winfree. 2015. Urban drivers of plant-pollinator interactions. *Functional Ecol*. 2**9**: 879-888.

Henry, E. H. and C. T. Anderson. 2016. Abundance estimates to inform butterfly management: double-observer versus distance sampling. *J. Insect Conserv.* **20**: 505–514.

Isaac, N. J. B., K. L. Cruickshanks, A. M. Weddle, R. J. Marcus, T. M. Brereton and R. L. H. Dennis. 2011. Distance sampling and the challenge of monitoring butterfly populations. *Methods of Ecology Evolution*. **2**: 585-594.

Iserhard, C. A., K. S. Brown Jr. and A. V. L. Freitas. 2013. Maximized sampling of butterflies to detect temporal changes in tropical communities. *J. Insect Conserv*. **17**: 615-622.

Kehimkar, I. 2008. *The Book of Indian Butterflies*. Bombay Natural History Society and Oxford University Press, Mumbai, India. 497 pp.

Kitahara, M. and K. Fujii. 1994. Biodiversity and community structure of temperate butterfly species within a gradient of human disturbance: an analysis based on the concept of generalist vs specialist strategies. *Researches on Population Ecology*. **36**: 187-199.

Kral, K., J. Harmon, R. Limb and T. Hovick. 2018. Improving our science: the evolution of butterfly sampling and surveying methods over time. *J. Insect Conserv.* **22**(1): 1-14.

Lang, B. J., P. M. Dixon, R. W. Klaver, J. R. Thompson and M. P. Widrlechner. 2019. Characterizing urban butterfly populations: the case for purposive point-count surveys. *Urban Ecosystems*. **22**(6): 1083-96.

Laurance, W. F., J. L. C. Camargoa, R. C. C. Luizãoa, S. G. Laurancea, S. L. Pimmd, E. M. Brunae, P. C. Stoufferf, B. Williamsong, J. Benítez-Malvidoh, H. L. Vasconcelosi, K. S. V. Houtand, C. E. Zartmank, S. A. Boylel, R. K. Didhamm, A. Andradea and T. E. Lovejoyo. 2010. The fate of Amazonian forest fragments: A 32-year investigation. *Biol. Conserv*. **144**: 56–67.

Medina-Báez, O. A., A. Lenard, R. A. Muzychuk, C. R. B. da Silva and S. E. Diamond. 2016. Life cycle complexity and body mass drive erratic changes in climate vulnerability across ontogeny in a seasonally migrating butterfly. *Conserv. Physiol*. **11**(1): 1-16.

Nowicki, P., J. Settele, H. Pierre-Yves and M. Woyciechowski. 2008. Butterfly monitoring methods: the ideal and the real world. *Israel J. Ecol. Evol*. **54**: 69-88.

Ojianwuna, C. C. and B. O. Amusan. 2019. A survey of butterfly fauna in the sacred grove of Umuaja forest, Southern Nigeria. *Ethiopian J. Environment. Stud. Manage.* **12**(2):158-166.

Pinratana, Br. A. and J. N. Eliot. 1992. *Butterflies in Thailand. Papilionidae and Danaidae.* Vol. 1. 3rd rev. ed. Brothers of St. Gabriel, Thailand. 174 pp.

Pollard, E. 1977. A method for assessing changes in the abundance of butterflies. *Biol. Conserv*. **12**(2): 115-134.

Rader, R., I. Bartomeus, L. A. Garibaldi, M. P. D. Garratt, B. G. Howlett, R. Winfree, S. A. Cunningham, M. M. Mayfield, A. D. Arthur, G. K. S. Andersson, R. Bommarco, C. Brittain, L. G. Carvalheiro, N. P. Chacoff, M. H. Entling, B. Foully, B. M. Freitas, B. G. Herren, J. Ghazoul, S. R. Griffin, C. L. Gross, L. Herbertsson, F. Herzog, J. Hipólito, S. Jaggar, F. Jauker, A. M. Klein, D. Kleijn, S. Krishnan, C. Q. Lemos, S. A. M. Lindström, Y. Mandelik, V. M. Monteiro, W. Nelson, L. Nilsson, D. E. Pattemore, N. de O. Pereira, G. Pisanty, S. G. Potts, M. Reemer, M. Rundlöf, C. S. Sheffield, J. Scheper, C. Schüepp, H. G. Smith, D. A. Stanley, J. C. Stout, H. Szentgyörgyi, H. Taki, C. H. Vergara, B. F. Viana and M. Woyciechowski. 2016. Non-bee insects are important contributors to global crop pollination. *PNAS*. **113**(1): 146-151.

Ribeiro, D. B. and A. V. L. Freitas. 2012. The effect of reduced-impact logging on fruit-feeding butterflies in Central Amazon, Brazil. *J. Insect Conserv.* **16**: 733-744.

Robbins, R. K., G. Lamas, O. H. H. Mielke, D. J. Harvey and M. M. Casagrande. 1996. Taxonomic composition and ecological structure of the species-rich butterfly community at Pakitza, Parque Nacional del Manu, Peru. In: D. E. Wilson and A. Sandoval (eds.). *Manu: The Biodiversity of Southeastern Peru.* Smithsonian Institution, Washington DC, USA., pp. 217-252.

Roy, D. B., P. Rothery, D. Moss, E. Pollard and J. A. Thomas. 2001. Butterfly numbers and weather: predicting historical trends in abundance and the future effects of climate change. *J. Animal Ecol.* **70**: 201-217.

Schulze, C. H., M. Waltert, P. J. Kessler, R. Pitopang, D. Veddeler, M. Muhlenberg, S. R. Gradstein, C. Leuschner, I. S. Dewenter and T. Tscharntke. 2004. Biodiversity indicator groups of tropical land-use systems: comparing plants, birds and insects. *Ecological Applications*. **14**: 1321-1333.

Talbot, G. 1947. *The Fauna of British India, including Ceylon and Burma. Butterflies*. Vol. 2. Taylor and Francis, Ltd., London. 506 pp.

Thomas, J. A. 2005. Monitoring change in the abundance and distribution of insects using butterflies and other indicator groups. *Phil. Trans. R. Soc. B*. **360**: 339-57.

Thomas, J. A., D. J. Simcox and R. T. Clarke. 2009. Successful conservation of a threatened *Maculinea* butterfly. *Science*. **325**: 80-83.

Thomas, J. A., M. G. Telfer, D. B. Roy, C. D. Preston, J. J. D. Greenwood, J. Asher, R. Fox, R. T. Clarke and J. H. Lawton. 2004. Comparative losses of British butterflies, birds, and plants and the global extinction crisis. *Science*. **303**: 1879-1881.

van Swaay, C., E. Regan, M. Ling, E. Bozhinovska, M. Fernandez, B. Huertas, C. K. Phon, Á. Kőrösi, O. J. M. Filho, J. Meerman, G. Pe'er, S. Sáfián, L. Sam, J. Shuey, D. Taron, R. Terblanche, M. U. Prado, L. G. Underhill. 2015. *Guidelines for Standardized Global Butterfly Monitoring*. Group on Earth Observations Biodiversity Observation Network (GEO BON), Leipzig, Germany. 32 pp.

van Swaay, C., M. Warren and G. L. Thomas. 2006. Biotope use and trends of European butterflies. *J. Insect Conserv.* **10**: 189-209.

Wynter-Blyth, M. A. 1957. *Butterflies of the Indian Region*. Bombay Nat. Hist. Soc. and Oxford University Press, Mumbai, India. xx + 523 pp.

Zaman, K., C. Tenney, C. Rush and R. I. Hill. 2015. Population ecology of a California endemic: *Speyeria adiaste clemencei*. *J. Insect Conserv*. **19**: 753-763.

(Manuscript received on 23 February, 2025)