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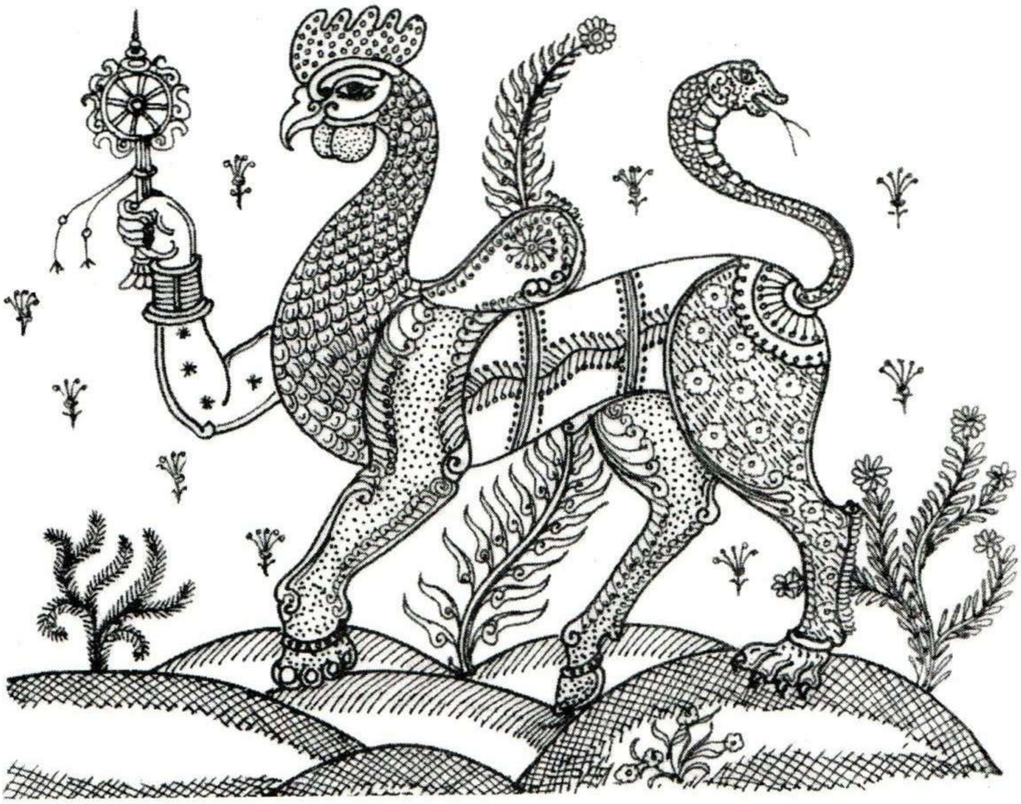

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PRANIKEE

Journal of Zoological Society of Orissa

Department of Zoology
Utkal University, Vani Vihar
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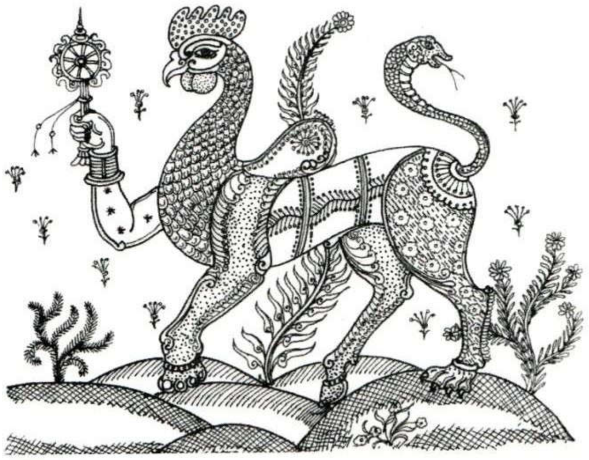
The emblem of Pranikee



The emblem "***NABAGUNJARA***" is a chimeric animal and a common motif of Odishan art and literature. It literally means "Nine form". This form has been described by poet Sarala Das in the Odia version of the epic Mahabharata. Apparently, Lord Krishna appeared in Nabagunjara form consisting of the body of an elephant, a leg each of a horse, a deer and a tiger respectively; throat of a peacock, tail in the form of a serpent, waist of a lion, hump of a bull and head of a cock, to fool his friend Arjuna. The Chimera was holding a lotus flower in a human hand. Arjuna had never seen such a creature in his life and guessed that this could not be a real animal but a form assumed by Lord Krishna and immediately bowed down at his feet. It is said that the human hand with the lotus provided the clue. In the paintings and sculptures however, the lotus is often replaced by a "Chakra" or the "stylized discus" of Lord Krishna. Chimeric forms are encountered in literature and art all over the world. However, a chimera of nine animals is uniquely Odishan. Therefore, it was considered to be an appropriate emblem for the Journal of Zoological Society of Odisha.

Padma Shri Prof. Priyambada Mohanty-Hejmadi

Former Editor

From the Editor's desk

The present edition of the Journal (Volume XXXVI) includes various aspects of Zoology. The first article describes avian diversity in urban and peri-urban areas of the Eastern ghats of southern Odisha. The natural history, distribution, brood occurrence and ethnoentomology of the only periodical cicada, *Chremistica ribhoi* in the Indian subcontinent has been covered in the second article. Decoding nature songs: exploring the influence of rainfall and atmospheric temperature on the presence and daily behaviors of annual cicadas has been discussed in the third article. The fourth article reflects the effects of light on the antioxidant defence system in brain and testis of goldfish (*Carassius auratus*). A TUNEL based study on DNA fragmentation in the regressing tail during metamorphosis in the tadpoles of the Indian tree frog *Polypedates maculatus* has been discussed in the concluding article.

I believe the present volume will augment the existing knowledge in Zoology.

PK Mahapatra

Zoological Society of Orissa

Editorial Board

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CONTENT

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AVIAN DIVERSITY IN URBAN AND PERI-URBAN AREAS OF THE EASTERN GHATS OF SOUTHERN ODISHA, INDIA

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ABSTRACT

Increasing urbanisation has been a major challenge for the long-term survival and maintenance of the genetic heterogeneity of birds. The present study comprehensively assessed avian diversity across five study sites (urban and peri-urban) in Koraput District in the Eastern Ghats of southern Odisha, India. The study documented 96 bird species representing 15 orders, 44 families, and 75 genera. The Passeriformes order was the most dominant, contributing 60 species. The species richness and diversity metrics revealed significant variations among the study sites. Based on the observation, four bird species showed higher relative abundance (RA): House crow (*Corvus splendens*) (RA=16.84%), followed by Asian Pied Starling (*Gracupica contra*) (RA=12.09%), Common Myna (*Acridotheres tristis*) (RA=10.82%) and Spotted Dove (*Spilopelia chinensis*) (RA=10.54%). A comparative analysis of habitats demonstrated that peri-urban areas supported higher avian species richness (88 species) and diversity (H′=2.94) compared to urban areas (49 species, H′=2.18). The higher dominance of avian species in urban sites (D=0.15) compared to peri-urban (D= 0.09) indicated the dominance of a few species. The observed dominance of a few human-associated bird species further indicated reduced heterogeneity and a progressive homogenization of birds in the area. Conservation measures are imperative to enhance these habitats and promote sustainable urban biodiversity management and restoration of natural peri-urban areas.

Keywords: Bird Diversity**,** Urbanisation**,** Homogenisation, Loss of Heterogeneity, Human Commensals, Urban Green Space, Korapu

INTRODUCTION

Urbanization is one of the impactful human land-use changes worldwide and is expected to expand in future. Currently, over half of the global population lives in urban areas, with projections suggesting that by 2050, at least two-thirds will reside in cities (Bairlein, 2017). The global urban land area percentage of the Earth's land surface rose from 0.22 % to 0.69 % between 1992 and 2020 (Zhao et al., 2022). The expansion of urban areas increasingly encroaches on natural ecosystems, particularly wildlife habitats (Aronson et al., 2017). The process of urbanization leads to the fragmentation and loss of habitats, resulting in a decline in biodiversity in various aspects, including taxonomic diversity, species diversity, functional diversity, and phylogenetic diversity (Tran et al., 2006; Morelli et al., 2016; Ibáñez-Álamo et al., 2017; Lepczyk et al., 2017). Further, the world's rapid urbanization profoundly affects global biodiversity, and urbanization has been counted among the processes contributing to the homogenization of the world's biota (Lepczyk et al., 2017; Morelli et al., 2017).

Geographically, cities/urban areas form complex systems that differ markedly from those systems present before the urbanizing process began (Berkowitz et al., 2003; McKinney, 2006). Such changes can present an ecological barrier for some animal species that cannot traverse or utilise an urban area (*urban avoiders*). In contrast, other species can use some urban resources (*urban utilisers*), and a few are highly successful at exploiting urban resources and conditions (*urban dwellers*) (Croci et al., 2008; MacGregor-Fors et al., 2010; Puga-Caballero et al., 2014; Fischer et al., 2015).

The urbanized areas offer a wide variety and mosaic of habitats and structures, from almost vegetation-free city centres to various kinds of gardens in the suburbs and residential areas, including city parks, cemeteries, forest remnants, and fallow land with ruderal vegetation. Birds are among the most conspicuous urbanisers and best studied (Hedblom and Margui, 2017).

Birds are widely regarded as a flagship group for conservation due to their ecological and evolutionary significance, and their prominent role in shaping people's perception of nature. Their high sensitivity and mobility make them particularly well-suited for examining the effects of human-induced disturbances on biodiversity (Gibson et al., 2011). The presence and distribution of bird species inside a city depend, among other factors, on the biogeographic species pools, the natural history of species, and the nature and distribution of habitat-related traits (Lepczyk et al., 2008; MacGregor-Fors and Scondube, 2011; McCaffrey et al., 2012). In general, omnivorous, granivorous, and cavity-nesting species have shown the strongest associations with urban areas in temperate regions (Chace and Walsh, 2006). However, insectivorous, frugivorous, and nectarivorous species are predominant in some tropical and subtropical urban areas of Brazil, Mexico, Singapore and Australia (Ortega-Alvarez and MacGregor-Fors, 2011a, b).

The structure, resource availability, and environmental pressures of urban ecosystems are significantly different from those of natural environments. Dispersed green spaces, pollutants, changed microclimates, and unusual food sources are frequently found in these places. Numerous bird species, especially those classified as generalists, have demonstrated exceptional behavioural adaptability under these circumstances (Sol et al., 2002; Marzluff, 2017). A global study conducted in 54 cities identified house sparrows (*Passer domesticus*), pigeons (*Columba livia*), starlings (*Sturnus vulgaris*), and barn swallows (*Hirundo rustica*) as some of the most common urban species worldwide (Aronson et al., 2014). These species thrive by utilizing human-provided resources, including food waste and nesting opportunities in buildings and infrastructure (Sol et al., 2002; Marzluff, 2017). In these cities, the Accipitridae family was the most prevalent, while the highest species-level diversity was observed in the Anatidae and Scolopacidae families (Lepczyk et al*.,* 2017). Cities are reported to provide habitats for threatened and endangered species (Aronson et al., 2014). However, urban areas are hotspots for species introductions, with 97% of the world's 31 most invasive bird species recorded in cities (Lepczyk et al., 2017). Urbanisation often accelerates extinction rates and the decline of native species, resulting in the homogenization of local bird communities (François et al., 2008; Maller, 2009; Shochat et al., 2011; Jadczyk and Drzeniecka-Osiadacz, 2013; Ciach and Fröhlich, 2016). This process has been described to typically reduce the prevalence of specialists while favouring generalist species, ultimately driving biotic homogenization (Kerk et al., 2007; Kowarik, 2011; Seto et al., 2012).

Koraput in southern Odisha is one of the biogeographically rich regions in the Eastern Ghats of India and is home to many avian species, some of which are globally threatened (Purohit et al*.,* 2021). During the last several decades, Koraput has undergone profound changes in its landscape due to land use and land cover (LULC) changes like large-scale deforestation, increase in the agricultural area (Reddy et al., 2012; Dash et al., 2018), increasing industrialization, and consequent rise in urbanization, leading to an increase in the built environment at the expense of natural forest cover. Although urban areas are on the rise in Koraput, there are peri-urban areas surrounded by patches of forest cover and scrublands. Though some studies have been conducted in Odisha on habitat use by urban and peri-urban birds (Panda et al., 2020; Karjee et al., 2022), studies on the impact of urbanization on birds in the southern part of Odisha is insignificant. Thus, aim of the present study was to assess the avian diversity across the selected urban and peri-urban areas of the Koraput district of southern Odisha, and evaluate the effect of urbanization on bird diversity and richness in urban and peri-urban habitats. We hypothesized that increasing urbanization reduces species heterogeneity and increases homogenization with few human-associated species in contrast to periurban habitats, which retain natural elements with higher species diversity.

MATERIALS AND METHODS

Study area

The study was carried out in Koraput district (18°N and 19°N latitude and 82°E and 83°E longitude) in the Eastern Ghats of India. It has discontinuous hill ranges of the northern Eastern Ghats, ranging in elevation from 123 to 1655 m above mean sea level (Mahata et al., 2019). The forest cover of Koraput is divided into two categories: tropical moist deciduous and tropical dry deciduous (Champion and Seth, 1968); with an annual precipitation of 1521 mm. The monsoon season (July–October) is the most dominant in the region, followed by winter (October -February) and a brief spell of mild summer (March-May). The climate is seasonal and generally humid, with temperatures ranging from 12°C to 38°C (Mahata et al., 2019) and 1452.2 mm of precipitation annually (Adhikary et al., 2019). Due to fast industrialization, shifting cultivation (known as "Podu" by tribal people), and an expansion in human settlements, Koraput has seen extensive deforestation and an increase in agricultural land and scrub areas over time (Reddy et al., 2012; Dash et al., 2018).

The present study was carried out at the selected five sites of Koraput (Figure 1), which were HAL Township (S1), Semiliguda Township (S2), NALCO Township (S3), Kanta Baunshuni Sacred Grove (S4), and Central University of Odisha Campus (S5). These five sites were classified into two categories, (i) Urban area (S1, S2, and S3) and (ii) Peri-Urban area (S4 and S5). The details of study sites under urban and peri-urban areas have been presented in Table 1.

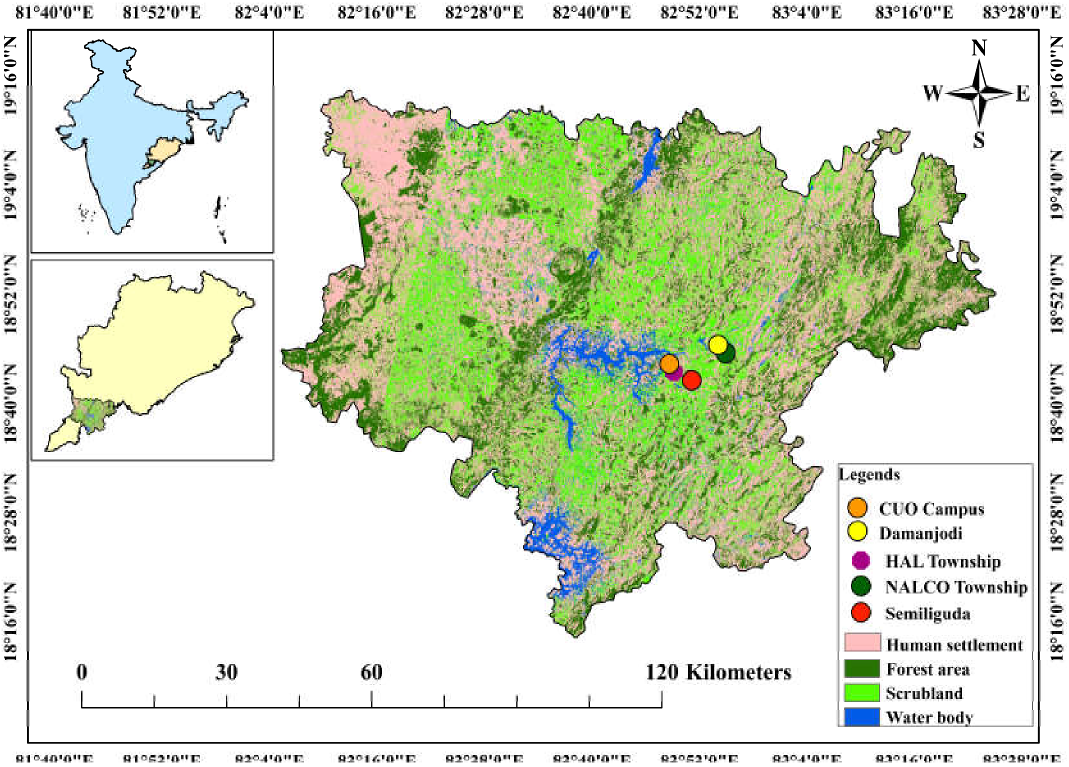

Figure 1 Map showing location of study sites of Koraput district, Odisha

Table 1 Habitat characteristics of five selected urban and peri-urban areas in Koraput District, Odisha

Study sites	Geographical Coordinate	Study site description	Habitat characteristics
HAL Township (S1)	82°81'88.62"E 18°72'42.39"N	A town surrounded by residential areas for employees of the Hindustan Aeronautics Limited (HAL)	Market area, urban settlements, parks, waterbodies with vegetation covers like *Jacaranda mimosifolia, Delonix regia, Mangifera indica, Artocarpus heterophyllus, Bougainvillea spectabilis, Psidium guajava, Peltophorum pterocarpum, Acacia auriculiformis, Araucaria columnaris*
Semiliguda Township (S2)	82°85'20.79"E 18°70'87.6"N	Market and residential areas with fewer green space and higher human activity	Human-dominated area with market complex, urban settlements, temple area, scrub patches, vegetation covers like *Bombax ceiba, Ficus benghalensis, Couroupita guianensis,* and *Psidium guajava*
NALCO Township (S3)	82°90'98.7"E 18°46’47” N	Plant area and residential areas for employees of the National Aluminium Company (NALCO)	Market area, urban settlements, temple, scrub patches, parks, water body with vegetation covers like *Psidium guajava, Callistemon citrinus, Quisqualis indica, Peltophorum pterocarpum, Acacia auriculiformis, Michelia champaca and Araucaria columnaris*
Kanta Baunshuni SG (S4)	82°53'35"E 18°45'39"N	Sacred Grove in the outskirts of NALCO Township	Sacred groves with waterbody and some natural patches with vegetation covers like *Bambusa bambos*, *Ipomoea cairica, Wedelia chinensis, Aegle marmelos, Neolamarckia cadamba, Magnolia champaca and Acacia mangium,*
CUO Campus (S5)	82°48’28” E 18°44’15” N	Rocky area with degraded scrubland, surrounded and ongoing construction activities	Human dominated with some degraded Scrub patches, vegetation covers like *Acacia mangium, Artocarpus heterophyllus, Azadirachta indica, Delonix regia, Madhuca longifolia, Lanneacoro mandelica, Magnolia champaca, Melia azedarach, Neolamarckia cadamba, Psidium guajava, Butea superba, Peltophorum pterocarpum, Saraca asoca, Acacia auriculiformis*

Data Collection

The study was conducted from December 2022 to April 2023, and the period was divided into two seasons, i.e., post-monsoon (December to February) and pre-monsoon (March to April). Birds were observed in the early morning between 0630 hrs to 0930 hrs and in the afternoon from 1530 hrs to1830 hrs using the point count method (Bibby et al., 1998). During the bird survey, 12 transect walks were undertaken randomly at each site. The length of each transect measures 200 m, and observations were made at three points on the transect, at the start, at the end, and in the middle at 100 m. All birds seen and heard were counted within 25m on both sides of the transect. For the survey, NIKON MONARCH 8×42 binoculars were used. Bird photographs were taken using Nikon Coolpix P900 cameras and Nikon DSLR 5200 with a tripod and Canon ET83D zoom lens. Foraging was recorded if the bird was found feeding on fruits/nectar or observed catching its prey by the preferred foraging strategy (such as perch and swoop, glean, and sallying) of each species (Ali et al., 2002).

Data Analysis

The birds were identified following standardised methods (Ali, 2002; Grimmet et al., 2011). Birds recorded were categorized according to their migratory status, IUCN Red List status (IUCN, 2024), feeding guild, and habitat types. The statistical analysis was done using Microsoft Excel 2007 and PAST Software Version 3.3[40]. Venn diagram and heatmap were calculated using OriginPro 2024 (Origin (Pro) v.2024).

The Margalef Species Richness Index (R), Shannon Diversity Index (H′), Dominance Index (D), Evenness Index (J), and Density (d) was estimated to analyse the community structure of birds of each study site using the following formulas:

Margalef Species Richness Index (R): This index was used as a simple measure of species richness (Margalef, 1958).

$$R = \frac{S-1}{lnN}$$

Where,

S = total number of species
N = total number of individuals in the sample
ln= natural logarithm

Shannon-Weiner Diversity Index (H′): This is an abundance-based diversity index to measure the diversity of species in a community (Shannon and Wiener, 1949).

$$H = -\sum_{i=1}^{S} pi \ln pi$$

Where,

S = number of species
pi = proportion of total sample belonging to i^{th} species (ni/N)
ni= number of individuals of i^{th} species in the sample
N = total number of individuals in the sample
ln = logarithm to base *e*

Dominance Index (D): The dominance index quantifies the dominance of one or few species in a community (Simpson, 1949). Greater values indicate higher dominance. Dominance indices are in general negatively correlated with alpha diversity indices (species richness, evenness, diversity, rarity). More dominant communities are less diverse. The value of D ranges between 0 and 1.

$$D = \sum_{i=1}^{S} \left(\frac{ni}{N}\right)^2$$

Where,

S = number of species;
ni = number of individuals of i^{th} species in the sample;
N = total number of individuals in the sample.

Pielou's Evenness Index (J): It measures the community evenness by using Shannon's diversity index. The range of Pielou's evenness is from 0 (no evenness) to 1 (complete evenness).

$$J = \frac{H}{\ln(S)}$$

Where,

H = Shannon's diversity index;
S = Total number of species;
ln = logarithm to base *e*

Bird density (d): It is defined as the number of individuals of a bird species per unit area in a given habitat.

$$d = \frac{n}{A}$$

Where,

n = Total number of birds observed;
A = Area surveyed (per km)
A one-way ANOVA test was run to check the variation of birds' richness and abundance in the study area.

RESULTS

The present study recorded 96 avian species belonging to 15 orders and 44 families and 75 genera across the selected five sites including three urban areas and two peri-urban areas of Koraput district (Table 2). Among the observed birds, Passeriformes was the most dominant order, with 60 species represented under 25 families. In terms of migration patterns, 81 (84.38%) species were identified as resident, while 15 (15.63%) species were classified as migratory (Grimmett et al., 2014) (Table 2). Among the observed species, 94 were categorized as Least Concern (LC) by the IUCN Red List, with only one species, the Green Munia (*Amandava formosa*), listed as Vulnerable (VU) and Alexandrine Parakeet (*Psittacula eupatria*), listed as Near Threatened (NT) (IUCN, 2024).

Of all birds observed in five sites, four bird species have comparatively much higher relative abundance, House crow (*Corvus splendens*) (RA=16.84%), followed by Asian Pied Starling (*Gracupica contra*) (RA=12.09%), Common Myna (*Acridotheres tristis*) (RA=10.82%) and Spotted Dove (*Spilopelia chinensis*) (RA=10.54%), respectively (Table 2).

The study revealed significant variations in avian species richness, abundance, and diversity across the selected sites. The highest number of 68 species was recorded at CUO Campus (S5), followed by 49 species in the Kanta Baunshuni SG (S4), 36 species in NALCO Township (S3), 28 species in HAL Township (S1), and the lowest richness with only 11 species was recorded in Semiliguda Township (S2) (Table 2).

Table 2 Check List of Birds of selected urban areas of Koraput District, Eastern Ghats of Odisha, India

Sl. No.	Common Name	Scientific Name	Migratory Status	IUCN Status	Feeding Guild	Relative Abundance (%)				
						S1	**S2**	**S3**	**S4**	**S5**
	Order Anseriformes									
	Family Anatidae									
1	Ruddy Shelduck	*Tadorna ferruginea*	M	LC	OV	-	-	5.67	-	-
	Order Galliformes									
	Family Phasianidae									
2	Red Junglefowl	*Gallus gallus*	R	LC	OV	-	-	-	-	0.19
	Order Columbiformes						-	-	-	-
	Family Columbidae									
3	Rock Pigeon	*Columba livia*	R	LC	GV	17.54	40.60	0.91	0.34	0.56
4	Spotted Dove	*Streptopelia chinensis*	R	LC	GV	0.96	0.97	36.21	2.46	1.55
5	Laughing Dove	*Streptopelia senegalensis*	R	LC	GV	-	-	-	-	2.14
	Order Cuculiformes									
	Family Cuculidae									
6	Greater Coucal	*Centropus sinensis*	R	LC	CV	0.96	0.25	0.11	0.51	0.16
7	Pied Cuckoo	*Clamator jacobinus*	M	LC	IV		-	-	-	0.38
8	Asian Koel	*Eudynamys scolopaceus*	R	LC	FV	0.32	-	0.08	0.42	0.54
9	Banded Bay Cuckoo	*Cacomantis sonneratii*	R	LC	IV	-	-	-	-	0.08
10	Large Hawk Cuckoo	*Hierococcyx sparverioides*	M	LC	IV	-	-	-	0.08	-
11	Common Hawk Cuckoo	*Hierococcyx varius*	R	LC	IV	-	-	0.04	0.08	0.05
	Order Caprimulgiformes									
	Family Apodidae									
12	Indian House Swift	*Apus affinis*	R	LC	IV	-	-	-	-	0.05
	Order Gruiformes									
	Family Rallidae									
13	White-breasted Waterhen	*Amaurornis phoenicurus*	R	LC	OV	-	-	-	0.17	-
	Order Charadriiformes									
	Family Charadriidae									
14	Yellow-wattled Lapwing	*Vanellus malabaricus*	R	LC	IV	-	-	-	-	0.05
15	Red-wattled Lapwing	*Vanellus indicus*	R	LC	IV	-	-	0.08	-	0.05
	Family Scolopacidae									
16	Wood Sandpiper	*Tringa glareola*	M	LC	CV	-	-	0.04	-	-
	Order Pelecaniformes									
	Family Ardeidae									
17	Great Egret	*Ardea alba*	R	LC	CV	-	-	-	0.08	0.19
18	Intermediate Egret	*Ardea intermedia*	R	LC	CV	-	-	0.19	-	0.11
19	Little Egret	*Egretta garzetta*	R	LC	CV	-	-	-	-	0.03

20	Cattle Egret	*Bubulcus ibis*	R	LC	CV	8.45	0.36	-	-	0.38
21	Indian Pond Heron	*Ardeola grayii*	R	LC	CV	-	-	0.15	0.08	0.11
	Family Threskiornithidae									
22	Red-naped Ibis	*Pseudibis papillosa*	R	LC	CV	6.22	-	-	-	0.05
	Order Accipitriformes									
	Family Accipitridae									
23	Black-winged Kite	*Elanus caeruleus*	R	LC	CV	-	-	0.04	-	0.11
24	Oriental Honey Buzzard	*Pernis ptilorhynchus*	R	LC	CV	-	-	-	-	0.05
25	Shikra	*Accipiter badius*	R	LC	CV	0.16	-	0.04	-	0.03
	Order Strigiformes									
	Family Strigidae									
25	Spotted Owlet	*Athene brama*	R	LC	CV	-	-	-	-	0.05
	Order Bucerotiformes									
	Family Upupidae									
27	Common Hoopoe	*Upupa epops*	R	LC	IV	-	-	-	0.08	-
	Order Coraciiformes									
	Family Alcedinidae									
28	Common Kingfisher	*Alcedo atthis*	R	LC	PV	-	-	-	-	0.03
29	White-throated Kingfisher	*Halcyon smyrnensis*	R	LC	CV	0.32	-	0.04	0.25	0.13
30	Pied Kingfisher	*Ceryle rudis*	R	LC	PV	-	-	-	0.17	0.08
	Family Meropidae									
31	Green Bee-eater	*Merops orientalis*	R	LC	IV	0.8	-	0.38	-	1.26
	Family Coraciidae						-	-	-	-
32	Indian Roller	*Coracias benghalensis*	R	LC	CV	-	-	-	-	0.03
	Order Piciformes									
	Family Megalaimidae									
33	Coppersmith Barbet	*Psilopogon haemacephalus*	R	LC	FV	0.64	-	-	1.27	0.03
	Family Picidae									
34	Yellow-crowned Woodpecker	*Leiopicus mahrattensis*	R	LC	OV	-	-	-	0.08	-
	Order Psittaciformes									
	Family Psittacidae									
35	Alexandrine Parakeet	*Psittacula eupatria*	R	NT	FV	-	-	-	-	0.03
36	Rose-ringed Parakeet	*Psittacula krameri*	R	LC	FV	1.59	-	-	-	0.16
	Order Passeriformes									
	Family Campephagidae									
37	Small Minivet	*Pericrocotus cinnamomeus*	R	LC	IV	-	-	0.04	-	0.21
38	Scarlet Minivet	*Pericrocotus speciosus*	R	LC	IV	-	-	-	0.08	0.13
39	Rosy Minivet	*Pericrocotus roseus*	M	LC	IV	-	-	-	0.00	0.03
40	Large Cuckooshrike	*Coracina macei*	R	LC	IV	-	-	-	0.08	-

	Family Oriolidae									
41	Indian Golden Oriole	*Oriolus kundoo*	R	LC	OV	-	-	0.04	0.08	-
	Family Dicruridae									
42	Black Drongo	*Dicrurus macrocercus*	R	LC	IV	4.47	0.86	0.34	1.10	0.99
43	White-bellied Drongo	*Dicrurus caerulescens*	R	LC	IV	0.16	-	-	0.17	-
44	Bronzed Drongo	*Dicrurus aeneus*	R	LC	IV	-	-	-	0.34	-
	Family Monarchidae									
45	Indian Paradise-flycatcher	*Terpsiphone paradisi*	R	LC	IV	-	-	-	0.17	-
	Family Laniidae									
46	Brown Shrike	*Lanius cristatus*	M	LC	CV	-	-	-	0.42	0.03
47	Long-tailed Shrike	*Lanius schach*	R	LC	CV	-	-	-	-	1.47
	Family Corvidae									
48	Rufous Treepie	*Dendrocitta vagabunda*	R	LC	OV	0.96	-	0.98	0.51	0.40
49	House Crow	*Corvus splendens*	R	LC	OV	33.49	13.72	27.63	28.61	4.31
50	Large-billed Crow	*Corvus macrorhynchos*	R	LC	OV	0.96	0.51	0.23	0.17	1.05
	Family Stenostiridae									
51	Grey-headed Canary-flycatcher	*Culicicapa ceylonensis*	R	LC	CV	-	-	-	0.08	-
	Family Paridae									
52	Cinereous Tit	*Parus cinereus*	R	LC	CV	-	-	-	0.59	-
53	Indian Black-lored Tit	*Machlolophus splionotus*	R	LC	OV	-	-	-	0.59	0.05
	Family Cisticolidae									
54	Common Tailorbird	*Orthotomus sutorius*	R	LC	IV	0.64	-	0.30	2.55	-
55	Grey-breasted Prinia	*Prinia hodgsonii*	R	LC	IV	-	-	0.04	0.17	-
56	Ashy Prinia	*Prinia socialis*	R	LC	IV	-	-	0.04	0.08	0.05
57	Plain Prinia	*Prinia inornata*	R	LC	IV	-	-	0.04	-	0.27
58	Zitting Cisticola	*Cisticola juncidis*	R	LC	IV	-	-	-	-	0.16
	Family Acrocephalidae									
59	Booted Warbler	*Iduna caligata*	M	LC	IV	-	-	-	0.08	0.03
60	Sykes's Warbler	*Iduna rama*	M	LC	IV	-	-	-	-	0.03
61	Clamorous Reed Warbler	*Acrocephalus stentoreus*	M	LC	IV	-	-	-	-	0.03
	Family Hirundinidae									
62	Barn Swallow	*Hirundo rustica*	M	LC	IV	-	-	-	-	0.32
63	Red-rumped Swallow	*Cecropis daurica*	M	LC	IV	-	-	0.04	-	-
	Family Pycnonotidae									
64	Red-vented Bulbul	*Pycnonotus cafer*	R	LC	FV	2.07	-	-	2.97	24.38
65	Red-whiskred Bulbul	*Pycnonotus jocosus*	R	LC	FV	3.99	-	5.40	15.87	8.36
	Family Phylloscopidae									
66	Greenish Warbler	*Phylloscopus trochiloides*	M	LC	IV	-	-	0.04	1.70	-

	Family Zosteropidae								
67	Indian White-eye	*Zosterops palpebrosus*	R	LC	OV	0.64	-	-	2.12	-
	Family Timaliidae									
68	Indian Scimitar Babbler	*Pomatorhinus horsfieldii*	R	LC	OV	-	-	-	0.17	-
	Family Pellorneidae									
69	Puff-throated Babbler	*Pellorneum ruficeps*	R	LC	IV	-	-	-	0.25	0.05
	Family Leiothrichidae									
70	Brown-Cheeked Fulvetta	*Alcippe poioicephala*	R	LC	IV	-	-	-	0.25	-
71	Jungle Babbler	*Argya striata*	R	LC	IV	-	-	0.08	5.09	3.70
	Family Sturnidae									
72	Asian Pied Starling	*Gracupica contra*	R	LC	OV	1.28	33.03	-	8.15	12.67
73	Brahminy Starling	*Sturnia pagodarum*	R	LC	OV	-	-	-	-	0.64
74	Chestnut-tailed Starling	*Sturnia malabarica*	R	LC	OV	-	-	-	-	0.21
75	Common Myna	*Acridotheres tristis*	R	LC	OV	4.78	3.35	19.95	13.16	8.55
76	Jungle Myna	*Acridotheres fuscus*	R	LC	OV		-	0.08	1.61	0.64
	Family Muscicapidae									
77	Asian Brown Flycatcher	*Muscicapa dauurica*	R	LC	IV	-	-	0.04	-	-
78	Indian Robin	*Copsychus fulicatus*	R	LC	IV	-	-	-	-	4.61
79	Oriental Magpie Robin	*Copsychus saularis*	R	LC	IV	0.32	-	-	4.16	0.91
80	Pale-chinned Flycatcher	*Cyornis poliogenys*	R	LC	IV	-	-	-	0.17	-
81	Blue Rock Thrush	*Monticola solitarius*	M	LC	OV	-	-	-	-	0.16
82	Pied Bushchat	*Saxicola caprata*	R	LC	IV	-	-	0.08	-	0.40
	Family Dicaeidae									
83	Pale-billed Flowerpecker	*Dicaeum erythrorhynchos*	R	LC	FV	-	-	0.08	-	-
	Family Nectariniidae									
84	Purple-rumped Sunbird	*Leptocoma zeylonica*	R	LC	NV	0.80	-	0.26	-	-
85	Purple Sunbird	*Cinnyris asiaticus*	R	LC	NV	2.55	-	-	1.87	2.52
86	Crimson Sunbird	*Aethopyga siparaja*	R	LC	NV	-	-	-	0.08	-
	Family Estrildidae									
87	Green Munia	*Amandava formosa*	R	VU	GV	-	-	0.19	-	-
88	Red Munia	*Amandava amandava*	R	LC	GV	2.07	-	-	-	2.20
89	Indian Silverbill	*Euodice malabarica*	R	LC	GV	-	-	-	-	2.33
90	White-rumped Munia	*Lonchura striata*	R	LC	GV	-	-	0.15	-	-
91	Scaly-breasted Munia	*Lonchura punctulata*	R	LC	GV	0.80	-	-	-	7.58
	Family Passeridae									
92	House Sparrow	*Passer domesticus*	R	LC	GV	2.07	6.25	-	-	0.94
	Family Motacillidae									
93	Grey Wagtail	*Motacilla cinerea*	M	LC	IV	-	-	-	0.25	-
94	White Wagtail	*Motacilla alba*	M	LC	IV	-	-	-	0.08	0.13

95	Paddyfield Pipit	*Anthus rufulus*	R	LC	IV	-	-	-	-	0.75
	Family Emberizidae									
96	Crested Bunting	*Emberiza lathami*	R	LC	GV	-	-	-	-	0.03

R-Resident, M-Migratory, VU-Vulnerable, LC-Least Concern, S1-HAL Township, S2-Semiliguda, S3- NALCO Township, S4-Damanjodi, S5-CUO Campus, CV-Carnivore, FV-Frugivore, GV-Granivore, IV-Insectivore, OV-Omnivore, NV-Nectarivore, PV-Piscivore

On comparing bird abundance among sites, CUO Campus (S5) had the highest number of individual birds (n=3732), followed by NALCO Township (S3) with 2673 individuals. Semiliguda Township (S2) and Kanta Baunshuni (S4) recorded 1968 and 1178 individuals, respectively, while HAL Township (S1) had the lowest abundance with 627 individuals. The Semiliguda Township (S2), with the highest Dominance Index (D=0.30) and lowest Shannon diversity (H'=1.45), indicated a community dominated by fewer species. CUO Campus (S5) had the lowest dominance (D=0.10), and the highest Shannon diversity (H'=2.83), indicating a well-balanced and diverse avian community. The Margalef Index, which accounts for species richness and sample size, was the highest (R=8.15) in CUO Campus (S5), highlighting its rich species composition. Semiliguda Township (S2) recorded the lowest value (R=1.32), reflecting limited species richness despite a relatively high number of individuals. Evenness was highest in HAL Township (J= 0.389) and lowest in NALCO Township (J=0.147) (Table 3).

Table 3 Diversity indices of Birds in selected urban patches

	S1	S2	S3	S4	S5
Taxa (N)	28	11	36	49	68
Individuals (n)	627	1968	2673	1178	3732
Dominance (D)	0.16	0.3	0.26	0.14	0.1
Shannon (H)	2.39	1.45	1.66	2.52	2.83
Margalef (R)	4.19	1.32	4.44	6.79	8.15
Evenness(J)	0.389	0.386	0.147	0.254	0.248

S1-HAL Township, S2-Semiliguda, S3- NALCO Township, S4-Kanta Baunshuni SG, S5-CUO Campus

Based on ANOVA, bird abundance and species richness across sites revealed contrasting patterns. There were no significant differences ($p > 0.05$) in bird abundance. In contrast, it showed a highly significant variation ($p < 0.05$) in species richness across sites (Table 4).

Table 4 One-way ANOVA test for both abundance and richness of five study sites (S1 to S5)

<table>
<tr><th colspan="7">ANOVA for Abundance (Sites: S1 to S5)</th></tr>
<tr><td></td><td><i>SS</i></td><td><i>df</i></td><td><i>MS</i></td><td><i>F</i></td><td><i>P-value</i></td><td><i>F crit</i></td></tr>
<tr><td>Site</td><td>62233.51</td><td>4</td><td>15558.38</td><td>1.69</td><td>*0.15</td><td>2.39</td></tr>
<tr><td>Residuals</td><td>4363562.99</td><td>475</td><td>9186.45</td><td></td><td></td><td></td></tr>
<tr><th colspan="7">ANOVA for Richness (Sites : S1 to S5)</th></tr>
<tr><td>Site</td><td>18.73</td><td>4</td><td>4.68</td><td>20.39</td><td>**1.61E-15</td><td>2.39</td></tr>
<tr><td>Residuals</td><td>109.05</td><td>475</td><td>0.23</td><td></td><td></td><td></td></tr>
</table>

*p-value >0.05: Not Significant, **p-value <0.05: Significant

Bird diversity and distribution in Urban Areas (UA) as compared to Peri-urban (PUA) Areas

The avian diversity and density comparison across Urban Areas (UA) consisting of S1, S2, S3, and Peri-urban Areas (PUA) consisting of S4 and S5 revealed significant variations in key biodiversity metrics. The species richness, Dominance, Shannon diversity index (H), and density showed higher values in Peri-urban areas compared to urban areas. The species richness of 88 species in Peri-urban Areas (PUA) was nearly double that of 49 species in Urban Area (UA). Further, UA exhibited higher dominance (D=0.15) of avian species in UA compared to PU (D= 0.09) which indicated the dominance of a few species, such as House Crow (*Corvus splendens*), Spotted Dove (*Spilopelia chinensis*), and Rock Pigeon (*Columba livia*) which were the most abundant species in the urban area. The Shannon diversity index (H′) was higher in PUA (2.94) as compared to UA (2.18) (Table 5). Similarly, the average bird density was also higher in PUA (10.65 ± 29.5) as compared to UA (7.58 ± 28.21) (Table 5).

Table 5 The density and diversity of species in Urban Areas (UA) and Peri-urban Areas (PUA)

Habitat	Birds' density	Species richness	Shannon Index (H)	Dominance (D)
UA	7.58 ±28.21	49	2.18	0.15
PUA	10.65±29.5	88	2.94	0.09

UA: Urban Area, PUA: Peri-urban Area

The results of the ANOVA for bird abundance between two habitats revealed no significant variation (p> 0.05) in abundance, whereas a significant difference (p<0.05) was observed in species richness across habitats. These findings suggest that while bird abundance does not vary meaningfully between habitats, habitat differences strongly influence species richness (Table 6).

Table 6 One-way ANOVA test for both abundance and richness of two habitats (Peri-Urban Area (PUA) and Urban Area (UA)

ANOVA for Abundance (Habitat: PUA and UA)						
	SS	*df*	*MS*	*F*	*P-value*	*F crit*
Habitat	570.63	1	570.63	0.02	*0.89	3.89
Residuals	5824335.11	190	30654.4			
ANOVA for Richness (Habitat: PUA and UA)						
Habitat	7.92	1	7.92	48.05	**6.29E-11	3.89
Residuals	31.32	190	0.16			

*p-value >0.05: No Significant, **p-value <0.05: Significant

Based on the value of relative abundance (RA), in urban areas (UA), most of the human-associated birds showed higher RA: House Crow had the highest (RA= 23.11%), followed by Spotted Dove (RA= 18.76%), Rock Pigeon (RA=17.8%), Asian Pied Starling (RA= 12.55%) and Common Myna (RA= 11.91%) respectively. In the peri-urban areas (PUA), RA was highest for Red-vented Bulbul (RA= 19.25%) followed by Asian Pied Starling (RA= 11.59%), Red-whiskered Bulbul (RA= 10.16%), House crow (RA= 10.14%) and Common Myna (RA= 9.65%), respectively (Table 2).

In the five sites surveyed, 41 bird species were common in both habitats. In contrast, only eight species were recorded from the urban area (UA). Which is suggested to be due to the presence of some specific opportunities like parks and waterbodies. However, 47 species were exclusively recorded in the peri-urban areas (PUA); most of them were forest birds or scrubland birds (Figure 2).

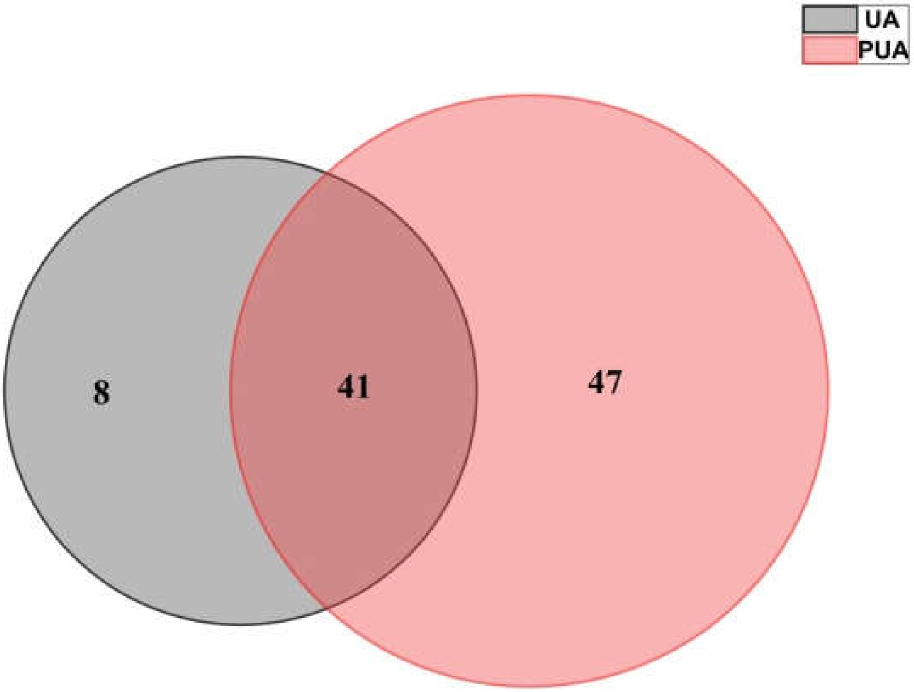

Figure 2 Venn diagram showing the number of unique and shared species among the two habitats; UA: Urban areas, PUA: Peri-urban areas

Based on feeding guilds in both habits (Table 2), among the recorded 47 exclusive species in PUA, a maximum of 23 species were insectivores (IV), followed by 09 carnivores (CV), 04 omnivores (OV), 03 granivores (GV), 02 piscivores (PV) and one each of frugivore (FV) and nectarivore (NV) species. Compared to this, among eight (08) species exclusive in UA, a maximum of 02 species each were recorded under insectivores (IV) and granivores (GV), 01 species each under carnivore (CV), omnivore (OV), frugivore (FV) and nectarivore (NV) categories.

The overall distribution of species under different feeding guilds (IV, OV, GV, FV, PV, CV, and NV) across two habitat types: UA (Urban Area) and PUA (Peri-Urban Area) has been presented in Figure 3. In the heat map, Z-values indicated on the colour bar, range from blue (low values) to red (high values), reflecting species richness in each habitat. The insectivores (IV) were the most dominant groups in both habitats, with 40 in PUA and 17 in UA, indicating a preference or higher number of species in peri-urban areas than urban areas. Carnivores (CV) species richness was highest (17) in PUA in comparison to UA (9). The number of granivores GV (7), frugivores FV (4), and omnivores (10), were recorded in UA, whereas, PUA recorded comparatively higher richness of 15 species of omnivores (OV), followed by 08 species of granivores (GV), four species of frugivores (FV), 02 species of piscivores (PV), respectively. The species richness of nectarivores (NV) (02) was the same in both habitats (Figure 3a).

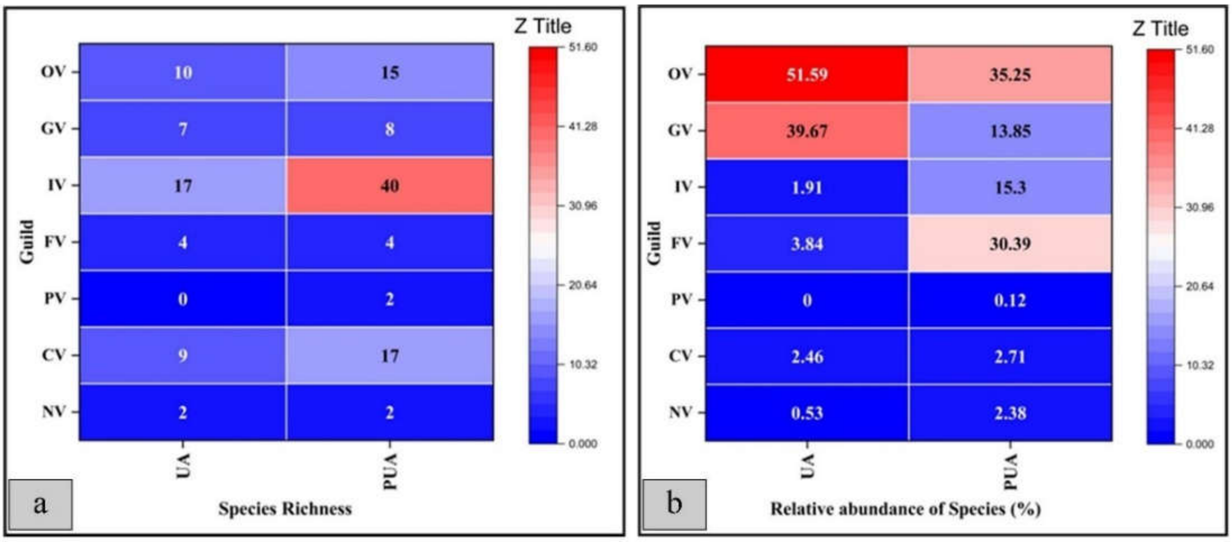

Figure 3 Heat map showing the distribution pattern of species (a) abundance of species (b) in various feeding guilds in two habitats (Blue colour represent low value and red indicate higher value). (OV- Omnivore, GV-Granivore, IV-Insectivore, FV- Frugivore, PV- Piscivore, CV-Carnivore, NV-Nectarivore)

Considering the abundance of species concerning feeding guilds in both habitats (Figure 3b), when the urban area (UA) was considered, the RA of 51.59% (n=2740) in omnivores (OM) was highest, followed by 39.67% (n=2079) of granivores (GV), and 3.84% (n=201) of frugivores (FV), 2.46% (n=129) of carnivores (CV), 1.91% (n=100) of insectivores (IV), and 0.53% (n=28) of nectarivores (NV) respectively. In the peri-urban area, most of the species were omnivores (OV) 35.3% (n=1731), followed by frugivores (GV) 30.39% (n=1492), 15.30% (n=751) insectivores (IV), and 13.85% (n=680) granivores (GV), respectively. Few birds were recorded from other feeding guilds like carnivores (CV)- 2.71% (n=133), nectarivores (NV)- 2.38% (n=117) and piscivores (PV) 0.12% (n=6) (Figure 3).

DISCUSSION

Urbanization is the most significant and noticeable human-driven factor impacting urban ecosystems and biodiversity (Deng et al., 2009; Zhou et al., 2018). It directly converts natural land into man-made areas, influencing the quantity, distribution, and quality of natural habitats (Zhu et al., 2020). A mosaic of landscape types, from densely populated metropolitan centres to natural or semi-natural areas, is the outcome of converting natural habitats into urbanized areas (McDonnell et al., 1993). Among wildlife groups, birds have long been considered bioindicators of urbanization's effects on biodiversity (Savard et al., 2000; Fors et al., 2009). As a result of replacing natural habitats with built-up areas, urbanization has led to the extinction of birds (Marzluff, 2001; Jokimäki and Suhonen, 1998). There has been evidence that urbanization causes an increase in exotic species and a reduction in native species diversity (Marzluff, 2001; Turner et al., 2004).

The dominance of a few bird species, such as Rock Pigeon (*Columba livia*), Spotted Dove (*Spilopelia chinensis*), House Crow (*Corvus splendens*), Red-vented Bulbul (*Pycnonotus cafer*), Asian Pied Starling (*Gracupica contra*), and Common Myna (*Acridotheres tristis*), was observed to be high. The Shannon Diversity Index was lower in urban areas which may be due to two possible factors: availability of enough food for birds and a higher rate of detection as compared to peri-urban areas. This indicates that homogenisation has been taking place in urban habitats. The present study documented 96 species of birds belonging to 15 Orders, 44 families, and 75 genera from the selected five urban and peri-urban areas of Koraput. Most of the birds recorded were predominantly Passeriformes, which comprised 60 species, highlighting the dominance of this order in urban and peri-urban ecosystems. The findings align with previous studies that report high species richness within Passeriformes in anthropogenically altered landscapes due to their adaptability and dietary diversity (Grimmett et al., 2014).

An investigation in Kolkata by Sengupta et al. (2014) revealed that bird diversity declined closer to the urban centres and was negatively associated with the proportion of built-up areas. A study on avian diversity in G.B. Pant Institute of Himalayan Environment and Development (GBPIHED) Campus at Almora, Uttarakhand, India by Palita et al. (2011), recorded 61 species of birds, of which suburban habitats showed higher species richness (50 species) compared to non-human habitats (44 species). Jungle Myna, Common Myna, and Red-billed Blue Magpie were more abundant in suburban areas. At the same time, House Sparrows (*Passer domesticus*) and Blue Rock Pigeons were restricted to these sub-urban habitats, likely due to better feeding and nesting opportunities (Palita et al*.,* 2011).

Pal et al. (2019), described the House crow (*Corvus splendens*), Common Myna (*Acridotheres tristis*), House sparrow (*Passer domesticus*) and Red-vented Bulbul (*Pycnonotus cafer*) as dominant species in urban habitats in Kolkata, India. The peri-urban area had lower dominance and higher diversity than the urban area. These bird species are human commensals and attain high densities in urban areas (Sodhi et al., 1999; Pauchard et al., 2006). It also indicates that the progressive homogenization of birds is taking place in urban areas very fast.

Among the peri-urban areas of the present study, the CUO Campus (S5) emerged as the most biodiverse site, with the highest species richness (N=68), abundance (n=3732), and highest diversity (H′=2.83). This aligns with studies indicating that habitats with complex vegetation structures and lower disturbance levels support higher bird diversity (Grimmett et al., 2014; Koparde and Raote, 2016). In contrast, the Semiliguda Township (S2) recorded the lowest richness (N=11) and diversity (H′=1.45), likely due to limited habitat heterogeneity and higher human disturbances. The dominance index (D) was also highest at S2 (D=0.30), suggesting a bird community dominated by a few human-associated species. Conversely, CUO Campus (S5) had the lowest dominance (D=0.10), reflecting a more even distribution of individuals across species. Such patterns are consistent with studies showing that more natural or less disturbed habitats support a balanced avian community (Clarke and Warwick, 2001).

In our study, the insectivores (IV) were the most dominant groups in both habitats based on species richness, with 40 species in PUA and 17 species in UA, indicating a preference or higher number of species in peri-urban areas than urban areas. In PUA, insectivorous are followed by carnivorous, omnivorous, granivorous, frugivorous, nectarivorous, and piscivorous, respectively. In both residential and agricultural environments, it has been discovered that insectivorous species richness and food availability are correlated (Chatterjee et al., 2013; Gatesire et al., 2014; Mukhopadhyay and Mazumdar, 2019). The predominance of insectivorous species may be due to the fact that certain groups of arthropods, such as generalist ground arthropods, plant-feeding arthropods, and generalist pollinating and jumping spiders, are more prevalent in cropland and urban areas, according to a number of studies (Faeth et al., 2005; Jokimäki et al., 2016). However, the insectivore group did not exhibit greater guild abundance, indicating a decline in their numbers, particularly in residential areas; likely as a result of anthropogenic disturbances such as poor plant cover and air pollution (Melles et al., 2003; Chamberlain et al., 2018; Xu et al., 2022).

On comparing the abundance of birds among feeding guilds in our study, omnivorous and granivorous birds in urban habitats far exceeded their peri-urban counterparts (Figure 3b), indicating that there is a progressive homogenisation due to an increase in a number of few species due to their adaptability to this changed habitat condition. Our findings corroborate many earlier studies, which show that species numbers decline with urbanization, and highly abundant species dominate the remaining species group (Melles et al., 2003**;** Chace and Walsh, 2006; Garaffa et al., 2009; Ortega-Álvarez and MacGregor-Fors, 2009)**.** In our study, frugivorous bird abundance in peri-urban areas also far exceeded that of urban areas (Figure 3b), which also corroborates earlier studies that indicated fruiting trees to attract frugivorous birds in urban areas (Gomes et al., 2008; Suhonen and Jokimäki, 2015; Curzel and Leveau, 2021; Karjee et al., 2022).

Regarding biodiversity metrics, peri-urban areas exhibited higher species richness, greater diversity, and lower dominance. This suggests that these areas offer a more varied and balanced habitat than the more homogenized urban centres. These results support the hypothesis that peri-urban zones due to their proximity to natural habitats and lower levels of disturbance, provide better conditions for sustaining bird populations.

Our finding from the study of birds in urban and peri-urban habitats at Koraput conforms to our hypothesis that peri-urban habitats support high species richness and diversity compared to urban habitats. Further, the presence of the vulnerable Green Munia (*Amandava formosa*) and near-threatened Alexandrine Parakeet (*Psittacula eupatria*) in the study sites highlights the ecological importance of urban green spaces, such as the CUO Campus. These findings align with Ives et al. (2016), identifying cities as critical refuges for threatened species. Conservation strategies should focus on enhancing habitat complexity in urban and peri-urban areas, particularly in sites like Semiliguda Township (S2) and NALCO Township (S3), to promote avian diversity and support species of conservation concern.

CONCLUSION

The study on avian diversity in urban and peri-urban areas of Koraput District highlights the importance of these areas as habitats for a variety of bird species. The dominance of few human-associated species in urban habitats indicates reduced heterogeneity and a progressive homogenisation of bird species. The study, therefore, emphasizes the role of urban green spaces in supporting a rich avian community, including vulnerable species, underlining the need for conservation efforts in these environments.

Ultimately, the findings underscore the significance of peri-urban and urban habitats in maintaining avian diversity, particularly for species adapted to human-modified environments. Conservation efforts should focus on enhancing these habitats to support both resident and migratory bird populations, ensuring the protection of key species and the overall ecological balance. This study highlights the need for continued monitoring and managing urban and peri-urban environments as crucial refuges for avian biodiversity in Koraput District.

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CHREMISTICA RIBHOI HAJONG AND YAAKOP 2013 - THE ONLY PERIODICAL CICADA IN THE INDIAN SUBCONTINENT: ITS NATURAL HISTORY, DISTRIBUTION, BROOD OCCURRENCE AND ETHNOENTOMOLOGY

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ABSTRACT

Chremistica ribhoi is the only known periodical cicada in the Indian subcontinent so far. Their nymphal development take place entirely underground over a period of four years and ultimately the final instars emerge en masse synchronously above-ground transforming into adults The paper reports for the first time the presence of two distinct populations in the form of Broods, viz., Brood- I and Brood- II. A distribution map is plotted based on records of their occurrences from various sites of Meghalaya in north-eastern India. As the Brood- I mass emergence coincides with the FIFA (Fédération Internationale de Football Association) World Cup tournament, which is generally held every four years, the indigenous people have named this cicada as Ñiangworldcup' or World cup insect. Brood- II was observed to emerge later after a phase lag of two years. The acoustic analysis in the form of waveform and frequency spectrum of the recorded male call or tymbalisation is also provided. Besides, a note on the ethno-entomological aspect among the indigenous Bhoi people (Khasi subtribe) is presented.

Keywords: *Chremistica ribhoi***,** periodical cicadas, brood, mass emergence, acoustic call analysis, entomo-tourism.

INTRODUCTION

Chremistica ribhoi or locally known as the Ñiangtaser is a periodical cicada species that remains underground during development for a period of four years and emerges over-ground in the form of a phenomenon known as mass emergence after it completes its nymphal development. This mass emergence of cicada from Ri-Bhoi district of Meghalaya in north-eastern India (N25°51'37.1'' E091°51'16.3'') was first reported by Hajong (2013). Subsequently, Hajong and Yaakop (2013) described this cicada as a new species under the genus *Chremistica*.

Only a few periodic cicadas are known that exhibit mass emergence after completing several years of development underground to complete their life cycle. This period may range from as low as two to up to an extended seventeen years (Hiromu et al., 2014). This phenomenon of mass emergence is unique to the periodical cicadas and is quite unlike the annual cicadas, which have a yearly emergence. Around 3000 species of cicadas are known worldwide, and the majority of them are annual, while only a few are known to be periodical (Hiromu et al., 2014).

Several periodical cicadas have been studied from various parts of the globe. The most famous of which is that of the seventeen-year cicada of the United States, belonging to the genus *Magicicada* (Snodgrass, 1919; Marshall and Cooley, 2000; Karban et al., 2000 and Karban, 2021). There are altogether seven known species of *Magicicada,* of which three species, *viz., Magicicada septemdecim, Magicicada cassini,* and *Magicicada septendecula,* emerge after a period of seventeen years. They are distributed in eastern, upper Midwestern, and the Great Plains states. The other four species are *Magicicada tredecim, Magicicada neotredecim, Magicicada tredecassini* and *Magicicada tredecula* which emerge around the Southern and Mississippi valley states within the United State. Each species has more than one Brood and are distributed in specific areas (Williams and Simons, 1995). One of the striking events about periodic cicadas is that they have synchronized emergence, strong site tenacity, and unusually long-life cycle of more than a year (Yoshimura, 1997). Periodical cicada is quite different from the annual cicadas, for they emerge after a given specific period of time in terms of 4 to 17 years. Periodical cicadas are the longest-lived insects as a juvenile and develop into an adult, which has a short life span of about one month (Williams and Simons, 1995). One of the most well-known and studied cases of periodic mass emergence is that of the *Magicicada septendecim*, which emerged after every 13 and 17-year period in the United States of America (Snodgrass, 1919).

The other known case of periodic cicada exhibiting mass emergence is that of the eight year periodic cicada of Fiji known as the '*Nanai*' or *Raiateana knowlesi* (Watling, 1986; Tikoca, 2018). Salmah and Zaidi (2002); Gogala and Trilar (2004) from Malaysia have described mass emergence of *Chremistica guamusangensis.* Leong et al. (2011) reported a synchronous emergence of *Chremistica umbrosa* at the Labrador Nature Reserve in Singapore.

In the Indian subcontinent; which includes India, Bangladesh, Bhutan, Maldives, Nepal, Pakistan and Sri Lanka, only three species of cicadas from the genus *Chremistica* are known viz., *C. seminiger* from Nilgiri Hills, South India; *C. mixta* from Sri Lanka and *C. ribhoi,* from Meghalaya in north-eastern India. However, mass emergence has been described only in *Chremistica ribhoi* (Hajong, 2013). This species is found in the Ri-Bhoi district of Meghalaya, and therefore, is the only known periodical cicada within India as well as the Indian subcontinent. It has a four-year periodicity and exists as two known broods. This cicada is known by the local ethnic Bhoi community, and is commonly referred to as *Ñiangtaser.* There also exists a folklore, which is popular among the Bhoi community explaining as to how this cicada appeared.

MATERIALS AND METHODS

Observations and survey were done across the Ri-Bhoi district of Meghalaya. Day and night observations on various aspects of this cicada were carried out mainly surrounding the Nongkhyllem Wildlife Sanctuary. Observations were made mostly from Iewsier (N25˚52.060` & E091˚51.112`; 431m), and Warmawsaw (N25˚48.018` & E091˚31.486`; 314m) located in Ri Bhoi district which are closer to the southern border of Assam (Figure 1). Specimens of both the nymphs and adults were collected from the emergence sites. They were either preserved in 70% ethanol or air-dried, stretched, and mounted in insect display boxes for observation and identification. Photos were taken with a DSLR camera.

The habitat of *C. ribhoi* were verified by the presence of nymphs around bamboo grooves and also by gadding around bamboo grooves and retrieving the developing nymphs from excavated soil. Information on mass emergence sites were obtained from various places by interviewing villagers about past emergence sites. The GPS co-ordinates for these locations were recorded with the help of a Garmin (eTrex) receiver. GPS co-ordinate data were used to plot a distributional map using QGIS 3.20.3 Software.

Male calls which is known by the term tymbalisation were recorded in WAV format at a 44 KHz sampling rate using a handheld digital sound recorder (Zoom H1 and Zoom H2). Recorded sound was analysed using the Raven Pro software Ver. 1.6. A sound spectrum was prepared as per Boulard (2007, 2013).

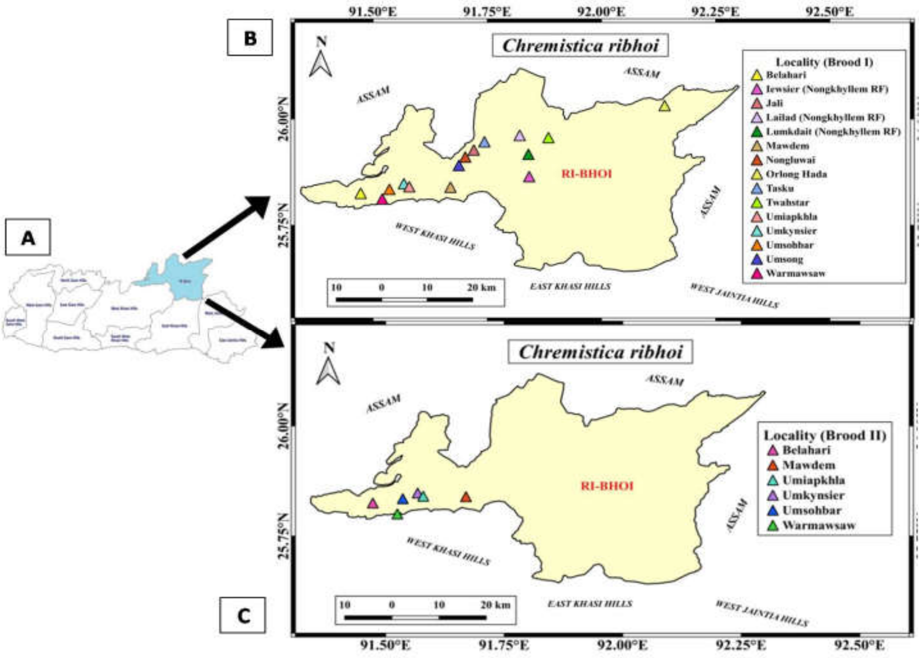

Figure 1 (**A**) Map showing mass emergence sites of *Chremistica ribhoi* in Meghalaya, (**B**) Brood- I mass emergence sites, (**C**) Brood- II mass emergence sites. Brood- I emergence extended to a much larger geographical area, including the East and Western parts of Ri-Bhoi; whereas Brood- II appeared only in the western part of the Ri-Bhoi district.

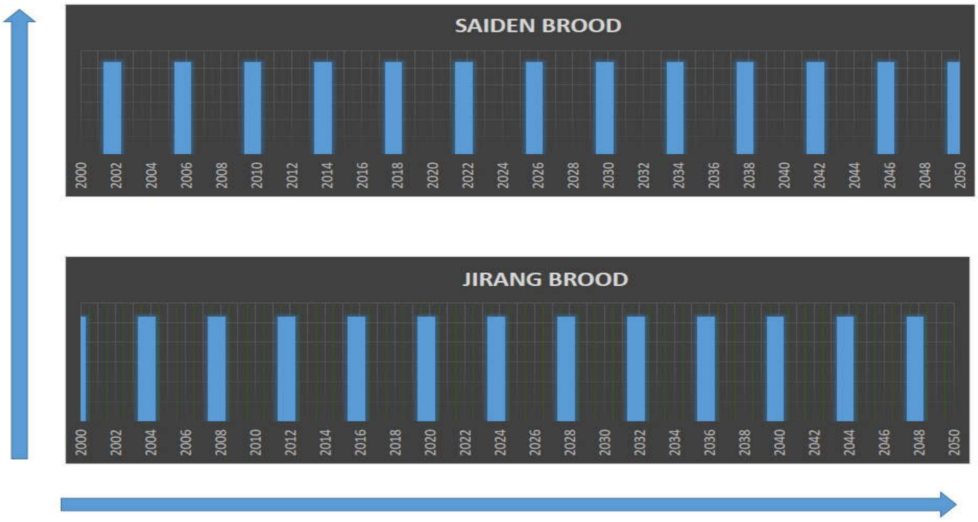

Figure 2 Past, present and future Mass emergence years of *Chremistica ribhoi*. There is a 2 years phase lag in Mass emergence of the two known broods. Saiden Brood emerges 2 years ahead of the Jirang Brood

RESULTS AND DISCUSSION

Distribution

The distribution of this species is not widespread like other cicadas, but is restricted to only few specific localities within the Ri-Bhoi district. The GPS coordinates of several mass emergence sites and localities were recorded. Some of these localities are near forest habitats close to villages, including Saiden, Belahari, Iewsier (Nongkhyllem RF), Jali, Lailad (Nongkhyllem RF), Lumkdait (Nongkhyllem RF), Mawdem, Nongluwai, Orlong Hada, Tasku, Twastar, Umiapkhla, Umkynsier,Umsohbar, Umsong and Warmawsaw. From all of these sites, mass emergence was observed during the year 2006, 2010, 2014, 2018 and 2022 (Figure 1, Table 1). This particular population was thus designated as Brood- I or ‘Saiden Brood’.

The other emergence sites of *C. ribhoi* were found to be located mainly northwest, bordering the state of Assam, These places include Belahari, Mawdem, Umiapkhla,Umkynsier, Umsohbar and Warmawsaw. In these localities, we observed mass emergence in the year 2016, 2020 and 2024. Based on reports obtained from local villagers, mass emergence had also been said to have occurred in these places every four years before the year 2016, ie., 2008 and 2012. This particular population of *C. ribhoi*, which has mass emergence timing two years after the Saiden brood, was therefore considered as Brood- II or ‘Jirang Brood’

Table 1 Table showing the mass emergence years of Brood- I and Brood- II (along with the future years when mass emergence is predicted to occur)

Name of Brood	**Year of emergence**						
Brood I (Saiden Brood)	2006	2010	2014	2018	2022	2026	2030
Brood II (Jirang Brood)	2008	2012	2016	2020	2024	2028	2032

Interestingly, we observed an overlap of these two *C. ribhoi* brood populations. Brood- I population were also observed to emerge in those areas where Brood- II had emerged in previous mass emergence years. Brood -I thus appears to have a distributional area that extends and overlaps with that of the range of Brood- II (Table 2).

These habitats are characterised by the presence of Bamboo grooves along with other subtropical tree species. All these localities have an elevation ranging from 150 to 700m above sea level., with temperatures ranging from 25° to 35°C and humidity ranging from 70 to 80 % during the period of mass emergence in the month April- June. Rainfall during this period was form moderate to heavy torrential.

Mass emergence and Brood

According to Dybas and Lloyd (1974) broods are those populations of periodical cicadas that emerge during the same year and have a contiguous geographical range. In case of *C. ribhoi* two distinct broods thus appear to exist as per observations on the mass emergences during different years and from various geographic sites within the Ri-Bhoi district of Meghalaya. Brood, I emerged in the year 2006, 2010, 2014, 2018, and in 2022. For this brood the subsequent mass emergence is therefore predicted to occur during the year 2026 and after every four years hence. However, Brood- II emergence occurred in the year 2008, 2012, 2016, 2020 and 2024 and the subsequent mass emergence will happen in 2028 (Figure 2). Brood- I occupied a larger geographical area than Brood- II, which had a smaller geographical distribution; and its appearance was mainly confined to the western part of Ri-Bhoi District. Brood- I, however, has an overlapping distribution and was also found to occur in areas of Brood- II emergence, but the emergence was synchronous and two years ahead of Brood- II (Figure 2).

Table 2 Localities with GPS co-ordinates of Brood- I and Brood- II mass emergence.

Brood- I (SAIDEN BROOD)			**Brood- II (JIRANG BROOD)**	
Sl no	**Locality**	**GPS Coordinates**	**Locality**	**GPS Coordinates**
1	Belahari	N25˚49.514` E091˚28.377	Belahari	N25˚49.514` E091˚28.377`
2	Iewsier (Nongkhyllem RF)	N25˚51.884` E091˚50.512`	Mawdem	N25˚50.380` E091˚40.168`
3	Jali	N25˚55.625` E091˚43.231`	Umiapkhla	N25˚50.440` E091˚34.760`
4	Lailad (Nongkhyllem RF)	N25˚57.698` E091˚49.265`	Umkynsier	N25˚50.870` E091˚34.055`
5	Lumkdait (Nongkhyllem RF)	N25˚55.026` E091˚50.406`	Umsohbar	N25˚50.101` E091˚32.175`
6	Mawdem	N25˚50.380` E091˚40.168`	Warmawsaw	N25°48.018` E091°31.487`
7	Nongluwai	N25˚54.633` E091˚42.115`		
8	Orlong Hada	N26˚1.899` E92.8.296`		
9	Tasku	N25˚56.793` E091˚44.622`		
10	Twastar	n/a		
11	Umiapkhla	N25˚50.440` E091˚34.760`		
12	Umkynsier	N25˚50.870` E091˚34.055`		
13	Umsohbar	N25˚50.101` E091˚32.175`		
14	Umsong	N25˚53.457` E091˚41.268`		
15	Warmawsaw	N25°48.018` E091°31.487`		

n/a: GPS data not recorded

After completing their nymphal stage, the penultimate nymph emerges in considerable numbers in the form of 'Mass emergence', which starts from the last week of April and continues till about the end of May. The adults, however, can still be found till June. During the period after emergence, hundreds of adults are seen flying and resting on various trees and bamboo branches. One can also observe several mating pairs on branches; there constant whistling chorus can be heard as one approaches the forest.

Incidentally, the Brood- I mass emergence coincides with the Fédération Internationale de Football Association (FIFA) international football World cup event which is generally held every four years. The nymphs usually start emerging a month ahead of this event and for this reason locals popularly call this as Ñiangworldcup'. The word *Ñiang* - in Khasi language mean insects, and hence the use of the appropriately the catchy term 'World cup insect' or World Cup cicada.

Elsewhere, other than Ri-Bhoi, the distribution of *C. ribhoi* has not been recorded. There are unconfirmed reports of adults sighting in the nearby state of Assam and Duars of North- Bengal. However, no information on the actual mass emergence other than what has been observed and reported from the Ri-Bhoi district of Meghalaya exists.

Based on our observation, *C. ribhoi* appears to be an endemic species with distribution range only limited to the Ri-Bhoi district of Meghalaya. No reports of their occurrence in other parts of North-east India exists It would be interesting to know whether the other *Chremistica* species viz., *C. seminiger and C. mixta,* found in the Indian subcontinent are also periodic and exhibit mass emergence. A future bio-geographical and phylogenetic study among these *Chremistica* species is therefore required to shed light on the present-day distribution of this species.

Over-ground emergence of nymphs, moulting into adults, mating and life cycle

Immediately after mass emergence, thousands of adult cicadas are seen. Adult males and females are sexually matured and are ready for mating. The males emit a mating call in the form of a constant shrill whistling. The females are devoid of producing any sound. They are seen to fly and settle down on branches or twigs where the males sing and approach the calling males. Mating then follows, and the female becomes ready to lay eggs. The females can be seen making tiny slits or cuts using their pointed and serrated ovipositor on the surface of twigs or bark of trees. Some weeks later (around 2-5 weeks), the eggs hatch, and the hatching nymph, or the first instars, drops down to the ground. The nymphs are positively geotropic and once on the surface of the ground, burrow under the soil by using their sharp forelimb claws. The young nymphs developing underground are pale white without any pigmentation; however, later instars are more brownish in appearances. The tiny young nymphs are seen adhering to the rootlets (Figure 3A).

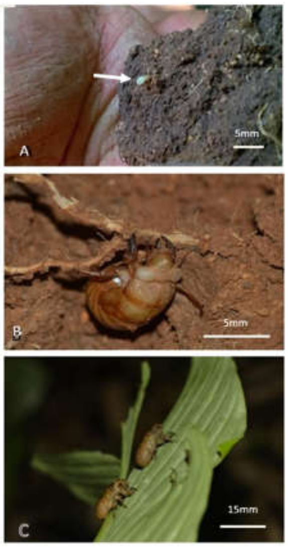

Figure 3 (A) The tiny 2nd instar nymphs dug out from the ground are seen associated with rootlets within the soil **(B)** The fully-grown penultimate nymphs during the beginning of fourth year seen feeding on root sap. They are brownish at this stage **(C)** The final instar nymphs known locally as '*Ñiangpyrkok'* after mass emergence on vegetation.

The prolonged development process then occurs underground. The instars solely feed on the sap from tiny rootlets of bamboo. They remain underground from a few inches to as deep as 3-4 feet below the earth. The nymphs stay underground for four years, during which time the nymphs grow and moult into four instars. In the fourth year, they reach the penultimate 5th instar stage (Figure 3B) and start to tunnel through towards the surface of the ground with the help of their sharp digging foreleg claws. At this stage they become negatively geotropic and are finally ready to come out of the ground and moult into the teneral adult. The fifth instar nymphs that emerge overground are known by the local Bhoi Khasis as Ñiangpyrkok (Figure 3C).

The emergence of Ñiangpyrkok only occurs during the evening hours, well after darkness. The intensity of mass emergence is seen to be maximum during a brief window, from around 6 pm to around 7.30 pm. Once these Ñiangpyrkok emerge on the surface of the ground, they start crawling sluggishly seeking for any vertical object usually any vegetation nearby, then climb upward to few centimetres to as high as 2 to 3 meters upward on to trunks and branches of trees. Once they find a secure spot, they stop moving and fix themselves on the surface by clinging with the help of their foreleg claws. The nymphs then remain motionless for about half an hour. The slow process of moulting then begins, which can be seen with the longitudinal splitting of the integument along the thoracic midline (Figure 4). The split line broadens gradually, and the thoracic portion pushes out of the exuvium, exposing the head and the middle thoracic region. Once the upper part of the teneral adult pushes out of the exuvium, the wings then gradually uncurl. At this moment, simultaneously it begins to make a slow turn upward with the abdominal tip still anchored to the exuvium.

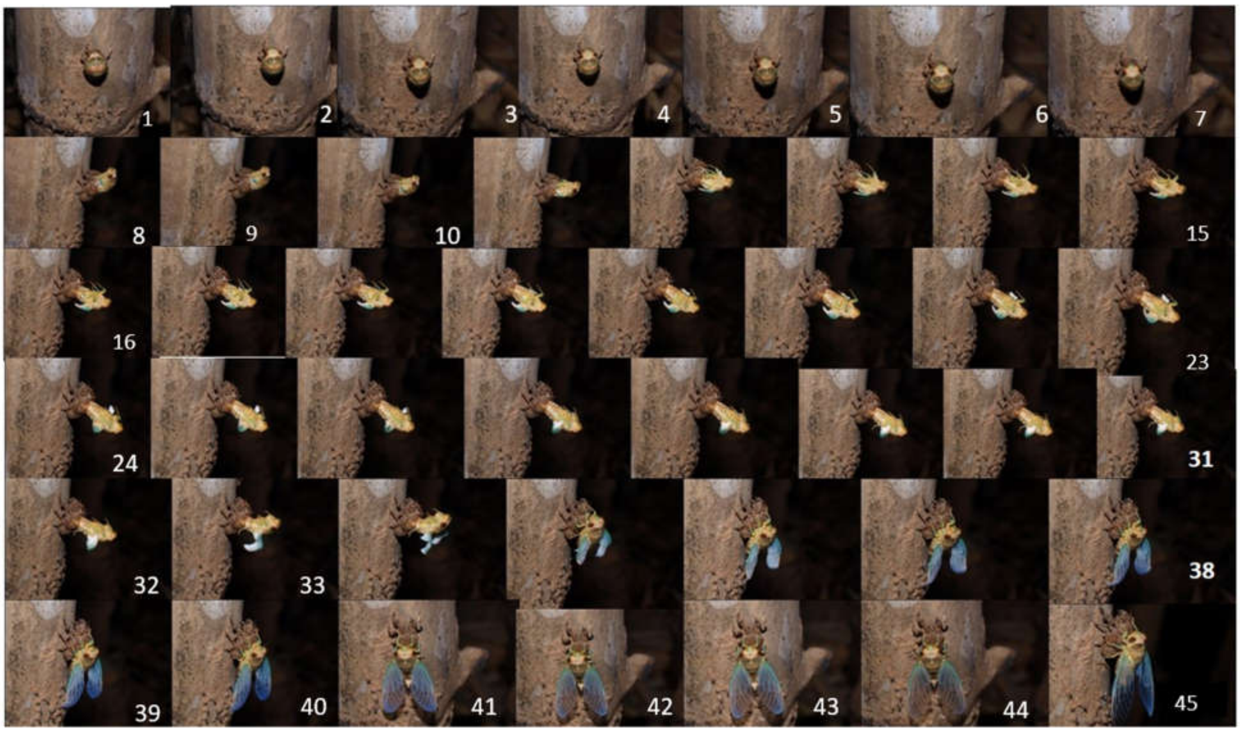

Figure 4 Showing time-lapse photo sequence of events leading to emergence of imago from the penultimate Instar stage: Photo 1:7:45 pm, Photo 7:7:51 pm, Photo 8-7:52 pm, Photo 9 -7:57 pm Photo 16 -7:59pm, Photo 23:8:00 pm, Photo 24-8:07pm, Photo 32: 8:16pm, Photo 33-8:18pm, Photo 38; 8:23, Photo 39-8:29, Photo 45- 8:40pm

Once the head approaches the exuvial skin, it attaches itself to it by the forelegs. Subsequently it frees itself from the exuvium. The abdomen then becomes free, and now the teneral stage adult is totally outside of its exuvium. The sequence of these process lasts from around 45 minutes to more than one hour and usually appear to occur between 6.30 pm to 8.30 pm. This timing may be important, as it would enable the still soft, teneral adults to become fully hardened and turn into an active adult well before the approaching morning hours. The process of moulting into a teneral adult requires the nymphs to crawl upwards on any vegetation.

Some nymphs can be seen several feet above on tree trunks and branches. The process of moulting cannot occur on the plain ground surface as they require their wings to develop and spread out in a proper shape, which requires them to manoeuvre. During this process the emerging stages come out with their head and thorax first. The head, which is towards the ground, then has to be bent in an upward position; to achieve this, it performs a loop and clings on to the exuvium with its foreleg claws. In this position, the wings are then free to spread out (Figure 4, sequence 32-38). During this process of moulting, the old exuvial skin of the fifth instar nymphs, are fully shed. After this the teneral adult, which appears greenish, takes several hours to finally transform into a fully active adult (Figures 5A and B). The ‘exuviate’ or the shed exoskeleton can be seen in hundreds on tree trunks and branches still clinging for weeks after the Mass emergence is over.

Figure 5 (**A**) Newly moulted (**B**) and teneral adults of *Chremistica ribhoi*

Bioacoustics and Tymbalization of male *C. ribhoi*

The individual male call or tymbalization of *C. ribhoi* appear as sharp whistling. Several male individuals tymbalise in chorus. For instance, as one walks towards Iwesier from Saiden village, their tymbalising chorus can be heard suddenly as one approaches the area of their mass emergence. The tymbalization pattern consists of a series of continuously repeating undulating waveforms or phrases, each with a duration of 10 second. Each of this phrase consists of a series of spaced out echemes that are of around 5-6 seconds in duration. This is followed by a close packed and continuous echemes of 5-6 seconds. The initial echemes in the spaced-out section consists of individual spike of 0.03 second. Spectrum analysis shows a main frequency band ranging from a lower 2400 Hz to a higher 4500 Hz, the upper frequency going up to a frequency of 48000 Hz (Figure 6).

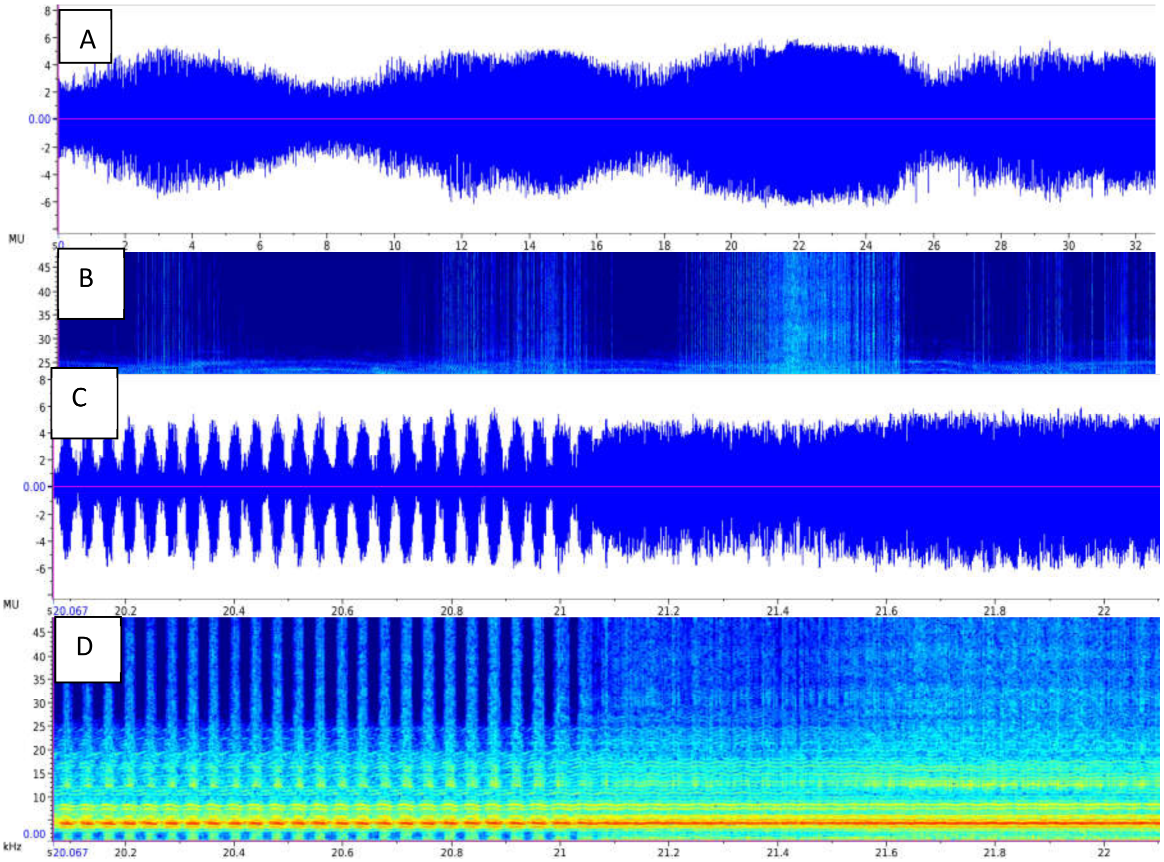

Figure 6 (A) Waveform of *Chremistica ribhoi* tymbalization recorded for around 33 second. Note the series of a repeating undulating phrases 10 second duration. **(B)** Frequency spectrum of above. **(C)** A single phrase expanded to show an initial Echemes which are drawn out, followed by a closely packed echemes. **(D)** Spectrum analysis shows a main frequency band ranging from a lower 2400 hz to a higher 4500 hz, the upper frequency going up to a frequency of 48000 hz.

Ethnoentomological aspects of *C. ribhoi* among the Bhoi Khasis

The Bhoi Khasis is one of the main communities that inhabit these areas where *C. ribhoi* are distributed. The mass emergence and the practice of gathering this cicada as a rare delicacy is a community practice that have been going on among the Bhoi people since the time of unwritten records. *C. ribhoi* is considered a rare and unique item of delicacy and forms one of the main edible insects. The recent focus and widespread social media coverage have led to a huge rush of people flocking during the emergence period eager to gather these natural delicacies. They gather them not only for their personal consumption but also for selling it in the market which fetch an attractive price. This rush of people has raised serious concern for their over-exploitation and conservation. People typically collect large quantities, either for keeping them longer in the form of dry preservation for later consumption or to prepare various form of pickles (Figures 7 and 8). It provides natural nutritional supplements rich in many dietary components such as protein, carbohydrate, fatty acid, vitamins and minerals like iron, calcium, etc. (Hajong and Syiemlieh, unpublished report). It may also be mentioned that the teneral forms collected are sold for as much as Rs. 400 to Rs. 800 a kilogram.

Figure 7 Pickles prepared and preserved for latter consumption from the collected teneral

Figure 8 (A) The collected teneral adults, **(B)** Roasted and ready for consumption dried under the sun for preserving about 2- 3 months.

Ecological significance of the mass emergence phenomena of *C. ribhoi*

During the mass emergence, huge numbers of emerging nymphs are transformed into teneral adults, which are soft and vulnerable to predation by several forest-dwelling organisms. The vast number of individuals form an essential and abundant food source for several creatures who directly benefit from this sudden food availability. Several organisms, including vertebrate predators like snakes, chameleons, frogs, birds, mice, rodents, and several predatory insects like praying mantis, ants, crickets, wasps and spiders, etc., have been observed to feed the teneral adults (Figure 9).

Figure 9 (A) Insects like crickets and **(B)** ants forage on the emerging tenerel adult

Habitat and its destruction

Mass emergence of *C. ribhoi* have been observed to occur mainly in forested areas near bamboo groves and other subtropical tree species like *Castanopsis* sp., *Artocarpus* sp., *Dillenia pentagyna* etc. Even when large numbers of them are eaten by their predators, there are still plenty more ready to mate and lay eggs for the next generation as long as their habitat are not destroyed. But unfortunately, due to economic and population pressure there has been a continuous reduction in the natural habitats of *C.ribhoi*. Natural habitat of this cicada is cleared for agricultural cultivation which is one of the leading factors of habitat reduction of *C.ribhoi* (Figures 10A, B, C, D). For instance, villagers from the village Iewsier (N25˚57.380` and E091˚53.037`), have narrated how there were huge emergence only a few decades back, but have presently ceased altogether in several places, attributed due mainly to habitat clearing for agriculture. The undisturbed Nongkhyllem wildlife sanctuary, however, can be one of the vital conservation habitats for this unique cicada. In this respect, we propose that the government of Meghalaya should consider this cicada as the state insect of Meghalaya and declare Nongkyllem Wildlife sanctuary as the conservation site for *C. ribhoi* or Ñiangtaser.

Entomotourism and Cicada festival

Recent popularisation of this cicada in media and social media has led to people flocking in large numbers to witness the event and enjoy the delicacy of this cicada during the mass emergence. The Village council of Saiden along with the local NGO in the form of Ri-Bhoi Tourism and Environmental Development Forum (RBTEDF) and the Saiden Socio-cultural and Sport Club have started organisation of the 'Ñiangtaser' festival. The first such 'Ñiangtaser' festival was held in the year 2010, and then held every four years hence during the month of May. The festival is organised at a place called Iewsier under village of Saiden (N25˚51.797` and E091˚50.782`) in the Ri-Bhoi district. It is about 6 km from the Nongpoh town which is the district headquarter of Ri-Bhoi viz., -Nongpoh town. The event is attended by hundreds of people including the government official, researchers and locals. This festival features indigenous food stalls, jungle camping, folksongs along with many other traditional cultural programs.

Figure 10 (A-D) Destruction of original forest habitats of Chremistica

Various other activities like fishing, traditional fashion show, talk by resource persons along with stalls selling local wine and Ñiangtaser delicacies are the main highlights of this one-of-a-kind Cicada festival. This festival thus unofficially has the distinction of being the only festival in celebration of the periodical cicada *C. ribhoi*. It is thus a promising initiative by the community stakeholders and has a future potential for boasting Entomotourism, associated with insects. Further, festival like this can help boost and promote sustainable nature tourism and bring about conservation awareness, as well as highlight this unique and exciting insect as a valuable bioresource. According to local stakeholder and former headman of Saiden village, the main motto behind the cicada festival is to make people aware of the importance of conserving forests, their inhabitants and the environment.

Mass aggregation and suicidal behaviour of adults

Large aggregates of the adults are seen on trees and bamboo branches during and towards the end of their mating around end of May and beginning of June. They are seen squirting fluid or urine from their rear, and often when there are hundreds of them in tree tops, the squirting fluid falls like a continuous rain. Towards the end of their mating, they become sluggish and appear to spend-out and the constant whistling calls once loud and pervading the forest, ceases suddenly. Many die out and some could be seemed remaining fixed to the twigs with their rostrum still embedded in the tree twigs. Some fall down lifeless on the ground.

Another unique phenomenon of *C. ribhoi* that the locals often narrate is the suicidal tendencies of the adults which plunge into turbulent or fast-flowing river Umtrew. This event is seen towards the end of their mating process. The phenomena of this unusual behaviour of *C. ribhoi* was observed by one of the authors (SRH) and recorded during the 2006 mass emergence at Lailad in the river Umtrew. Several adult individuals were seen emerging from the nearby forest and made short circles around the roaring and fast-flowing river before plunging into the water which caused their death.

Conservation effort

It was observed that most mass emergence occurred in undisturbed areas, consisting of pristine forests. Undisturbed forest habitat is required for the young nymphs to develop underground. However, most of their natural habitats are shrinking due to various anthropogenic activities such as deforestation, shifting cultivation, urbanization and forest fire. Thus, efforts should be initiated to conserve the habitat.

CONCLUSION

C ribhoi or Ñiangtaser is a unique and valuable bioresource that needs to be preserved, including its habitat. In Meghalaya, the habitat is located only in the Ri -Bhoi district. Various anthropogenic activities are continuously destroying its habitat, which may lead to extinction of this species. Moreover, this insect has ethnological importance because it is associated with the myth and legend of the indigenous Bhoi (Khasi subtribe) people. They have, from time immemorial, been utilizing this insect as a delicious food item. The collection and consumption of this periodical cicada as food has become a social ritual where almost everyone participates enthusiastically.

The unique mass emergence phenomena of *C. ribhoi* or Ñiangtaser or Ñiangworldcup cicada as they are called fondly by the locals, have brought publicity and limelight to the Ri-Bhoi district, the state of Meghalaya, and as well as to the whole country. Unfortunately, their habitats are shrinking and getting destroyed, which might threaten their existence and survival. Therefore, an initiative to create awareness among the stakeholders is the need of the hour to conserve this unique and valuable natural bioresource.

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DECODING NATURE SONGS: EXPLORING THE INFLUENCE OF RAINFALL AND ATMOSPHERIC TEMPERATURE ON THE PRESENCE AND DAILY BEHAVIORS OF ANNUAL CICADAS

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ABSTRACT

Certain cicadas display unique emergence and activity patterns, categorized as either periodical cicadas that appear infrequently or annual cicadas. This study investigates the ecological dynamics of cicada diversity in the Ri Bhoi District of Meghalaya, North East India, over three consecutive years (2021-2023), employing a thorough analysis of ecological factors, including rainfall and atmospheric temperature. The research explores the temporal and environmental influences on cicada behavior, highlighting the relationship between atmospheric temperature, rainfall, and cicada diversity. Notable differences in abundance between the Sub-Family Cicadinae and Cicadettinae prompt further exploration into their ecological implications. The findings indicate a correlation between increased atmospheric temperature and rainfall during July to September and heightened cicada diversity. This underscores the interconnectedness of these climatic factors. Additionally, cicadas demonstrate a preference for tree axis, potentially associated with the well-defined xylem system in trees. Further, the study documented five cicada species with varying abundances and impacts on diversity. *Pomponia linearis* was the most abundant, significantly enhancing diversity, while *Cryptotympana corvus* and *Pomponia ramifera* also contributed positively. In contrast, *Scieroptera splendidula* and *Eopycna repanda* had lower abundances and minimal or negative impacts on diversity. These findings emphasize how various species contribute differently to the overall diversity of the community. In conclusion, this research provides valuable insights into the intricate ecological dynamics and adaptive behaviors of cicadas.

Keywords: Activity, Cicada, Diversity, Habitat, Meghalaya

INTRODUCTION

Cicadas are hemipteran insects distinguished by the complex acoustic signals or calling songs produced by males, which play a vital role in the ambient sounds of the forest. It has been reported that these insect-generated sounds which harmonize with those of other creatures like crickets, frogs, and birds (Myers, 1929; Boulard, 2013). Some cicadas display unique emergence and activity patterns, with certain species appearing infrequently, while others are designated as annual cicadas (Yoshimura et al., 2009; Koenig and Liebhold, 2013; Hajong and Yaakop, 2013; Hajong and Thangkiew, 2018; Hajong and Limatemjen, 2021; Marshall et al., 2011, 2018).

Periodical cicadas, characterized by their extended life cycles, commonly emerge in synchronized broods after spending multiple years underground as nymphs. In contrast, annual cicadas have shorter life cycles and emerge on a yearly basis. Cicadas exclusively feed on tree sap, forming a close association with plants. They are the insect group capable of extracting and unlocking the essential xylem fluids, making this resource available to the subsequent trophic level (Williams and Simon, 1995).

Recent research indicates that a significant number of cicadas show distinct preferences for particular habitats (Goswami, 2024). Despite the fascinating behavior of cicadas and their vital role in ecological food webs, a considerable amount of fundamental ecological information remains undisclosed. Existing literature lacks sufficient information regarding the impact of ecological factors on the diversity and behavior of cicadas in Meghalaya, North East India. Therefore, this study delves into the ecological dynamics of cicada diversity in the Ri Bhoi District over three consecutive years (2021-2023). The investigation involves a comprehensive analysis of ecological factors such as rainfall and atmospheric temperature, exploring their temporal and environmental influences on cicada diversity and behavior. The study yields valuable insights into the intricate ecological dynamics and adaptive behaviors of cicadas, potentially informing the development of more realistic conservation strategies for habitats and ensuring long-term persistence of the entire cicada community.

METHODOLOGY

The research was conducted from July to December over three consecutive years (2021-2023) at the experimental garden of the Botanical Survey of India and its surrounding areas situated in Umiam (25.677182ºN, 91.904213ºE elevation 1000 m; Figure 1) in the Ri Bhoi District of Meghalaya, North East India (Figure 2). During field investigation, acoustic and visual tracking techniques were used to detect and identify cicadas. Detailed observations of behavioral and physical characteristics were facilitated through the use of Nikon binoculars (ACULON A211 model) and a One Plus Nord camera. The activity behavior of cicadas was meticulously observed, taking into account spatial adaptation and other activities. This observational approach aimed to unveil the ecological interactions and behavioral dynamics occurring within the natural habitat of cicadas.

Atmospheric temperature was recorded using a Borosilicate Glass Mercury Thermometer, while rainfall data was collected with the RGR126N Oregon Rain Gauge (Figure 3A), providing insights into precipitation patterns. After observation, a delicate insect telescope-catching net (Figure 3B), was employed for capturing some of these insects to minimize harm to specimens. Subsequently, the captured cicadas were carefully transported in sealed insect jars. Cicadas collected in the field were euthanized initially by sealing them in a jar containing ethyl acetate. Subsequently, their wings and legs were carefully stretched on an insect stretching board. The specimens were then placed in an oven set at 40°C to 45°C for 3 to 6 days. Alternatively, some cicadas were euthanized by immersion in 70% to 95% ethanol. Upon reaching the laboratory, these specimens were properly labeled with the appropriate information (Arensburger et al., 2004; Kumar and Saxena, 2021).

The study also involved analyzing the species abundance and diversity of some selected cicada species within a specific ecological community. For each species, the number of individuals and calculated several diversity metrics has been recorded. To determine the proportion (p) of each species, the number of individuals of each species is divided by the total number of individuals observed. The natural logarithm of this proportion [ln(p)] was then calculated, followed by the product of the proportion and its natural logarithm [p*ln(p)]. These values were utilized in calculating the Shannon-Wiener index (H'), which measures species diversity by accounting for both abundance and evenness. The formula for H' is:

$H' = -\sum [p * \ln(p)]$, Where $\sum$ denotes the sum across all species.

Additionally, we calculated the maximum possible diversity (H_{max}) using the natural logarithm of the number of species [ln(S)]: $H_{max} = \ln(S)$

Pielou's evenness index, which indicates how evenly individuals are distributed among the different species, was determined as the ratio of H' to H_{max}: $J'=H'/H_{max}$

RESULTS

The investigation indicates the presence of five cicada species, classified into two sub-families, over the duration of the study spanning the years 2021, 2022, and 2023. Four species, namely *Pomponia linearis*, *Pomponia ramifera, Cryptotympana corvus* and *Eopycna repanda*, were identified under the sub-family Cicadinae Latreille, 1802, while *Scieroptera splendidula* belonged to the sub-family Cicadettinae Buckton, 1889 (Table 1).

Table 1 Showing the list of cicada species and their habitat (vegetation) preferences (for 3 consecutive years i.e. 2021-2023) recorded from Ri Bhoi District, Meghalaya

Sub-Family	Cicadas	Habitat preference	Vegetation Preference	Part of the plants	Specimen observed in the field	Associated trees species / flora
Cicadinae Latreille, 1802	*Pomponia linearis*	Extends across a spectrum of habitats, ranging from dense forests to arboreal settings within human settlements.	Tall Trees	Stem	110	*Pterospermum sp., Myrica sp., Actinodaphne sp., Dysoxylum sp, Pinus khasiana*
	Pomponia ramifera	Broad distribution encompassing various ecological niches, extending from sylvan habitats to suburban areas	Stunted Trees	Axis	70	*Artocarpus sp., Anthocephalus sp., Castanopsis sp.*
	Cryptotympana corva	Resides within the woodland areas characterized by towering trees.	Tall Trees	Axis	120	Engelhardtia *sp.,Dysoxylum sp.Chukrasia sp.*
	Eopycna repanda	Trees alongside the road and within the open forest.	Moderate size Trees	Axis	90	*Prunus sp,Cordia sp.Tectona sp.*
Cicadettinae Buckton, 1889.	*Scieroptera splendidula*	The shrub to grass population within the thin forest area	Shrub	Axis	15	*Hedychium sp., Boehmeria sp., Gmelina sp., Triumfella sp.*

Pomponia linearis (Fig. 4) was observed in July throughout the three years, exhibiting adaptability to various habitats, including dense forests and arboreal settings within human settlements, with a preference for tall tree stems. Notably, *Pomponia linearis* displayed crepuscular behavior, engaging in vocal communication over considerable distances (approximately 8 meters). *Pomponia ramifera* (Figure 5) was recorded in July, displaying a broad distribution across wooded habitats and suburban areas, particularly in the axis of stunted trees. Calling activities of *Pomponia ramifera* were predominantly matutinal, forming choruses during early morning and noon. *Scieroptera splendidula* (Figure 6) was recorded in July, inhabiting shrub axes within thin forest patches. The species exhibited diurnal activity, emitting solitary calls during the day and noon. *Cryptotympana corvus* (Figure 7) displayed an extended occurrence from August to October. This species exhibited diurnal behavior, with males engaging in chorus calls. *Eopycna repanda* (Figure 8) occurred from October to December, confined to the axes of moderate-sized trees along roads and within open forests. The species displayed diurnal behavior, engaging in chorus calls (Table 1). *Cryptotympana corvus* were observed to be residing in tall tree axes within woodland areas (Figure 9).

The recorded monthly mean temperatures for the consecutive years 2021, 2022, and 2023 exhibited ranges from 22.6 to 29.7°C, 22.1 to 29.5°C, and 22.0 to 29.1°C, respectively. Simultaneously, the monthly rainfall measurements span from 0 to 115.1 mm, 14 to 302 mm, and 33 to 687 mm for the corresponding years (Table 2).

Table 2 The recorded monthly rainfall (mm) and atmospheric temperature (˚C) and the number of species recorded in each month during the study duration from the study site (2021-2023)

Year	Months	Monthly Rainfall (mm)	Atmospheric temperature (˚C)	Number of species recorded
2021	July	115.1	29	3
	August	72.15	27.8	1
	September	20.05	29.7	1
	October	13.2	28.3	2
	November	0	25.4	1
	December	1.4	22.6	1
2022	July	302	29.5	3
	August	232	27.2	1
	September	132	26.1	1
	October	79	25.3	2
	November	54	24.9	1
	December	14	22.1	1
2023	July	567	28.8	3
	August	477	29.1	1
	September	687	27.5	1
	October	342	26.9	2
	November	121	25.5	1
	December	33	22	1

Figure 1 Illustrates a forest patch along with a pond in the BSI Experimental Garden, Umiam, Meghalaya (25.677182ºN, 91.904213ºE)

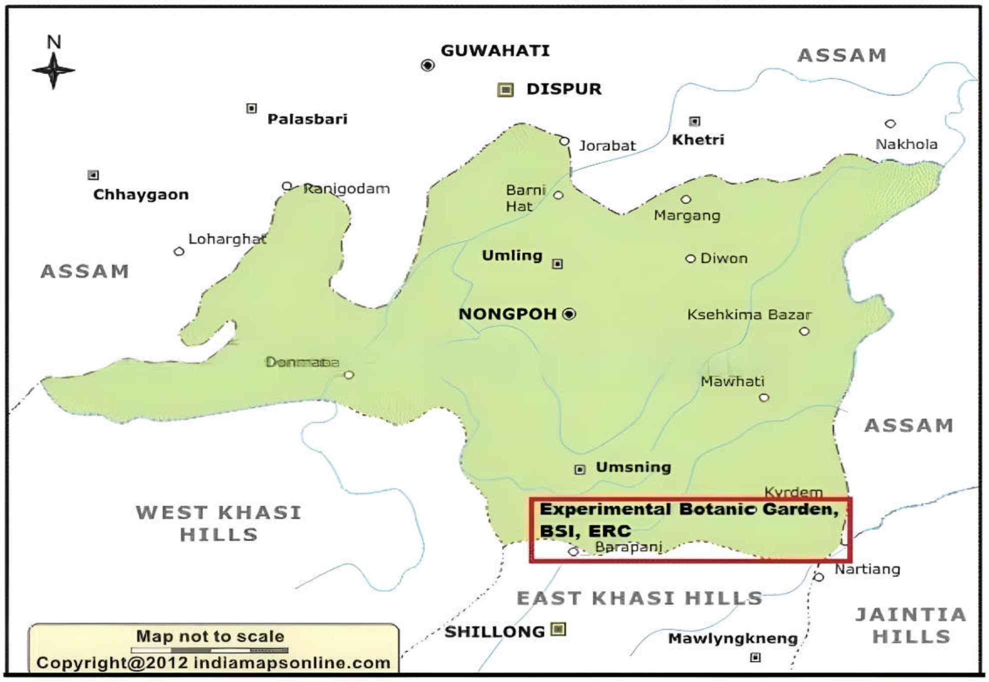

Figure 2 Showing map of BSI Experimental Garden, Umiam, Meghalaya. (Bora and Meitei, 2014)

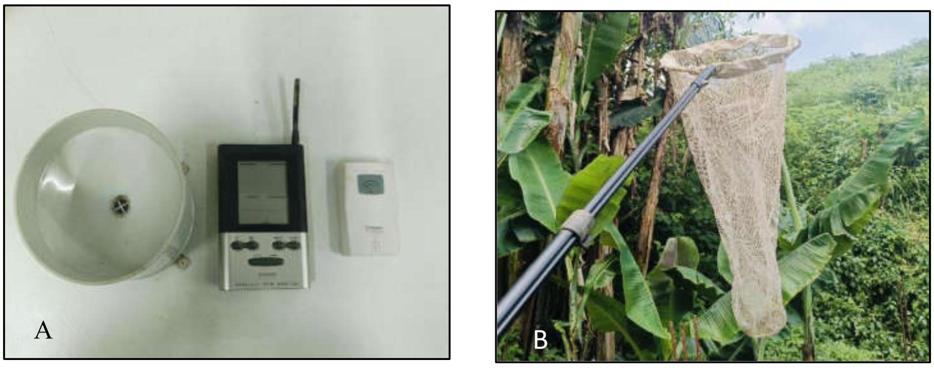

Figure 3 (A) RGR126N Oregon Rain Gauge, (B) Insect telescope-catching net

Figure 4 *Pomponia linearis*

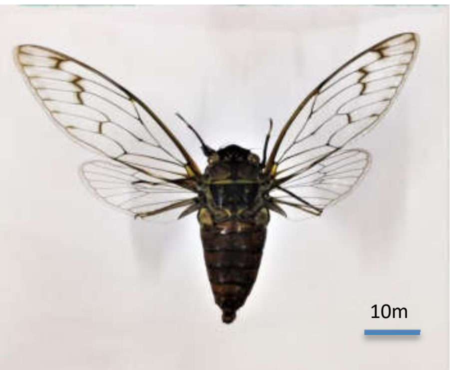

Figure 5 *Pomponia ramifera*

Figure 6 *Scieroptera splendidula*

Figure 7 *Cryptotympana corvus*

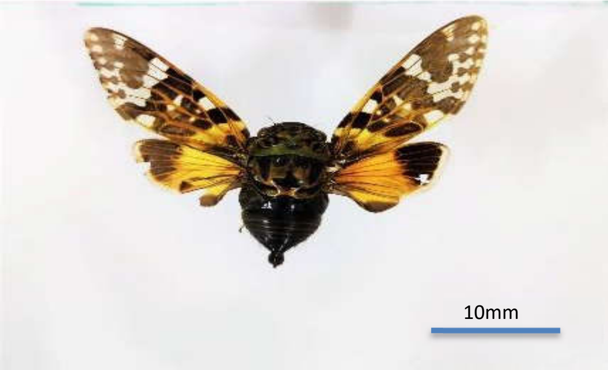

Figure 8 *Eopycna repanda*

Figure 9 Cicada on the Stem of the Tree

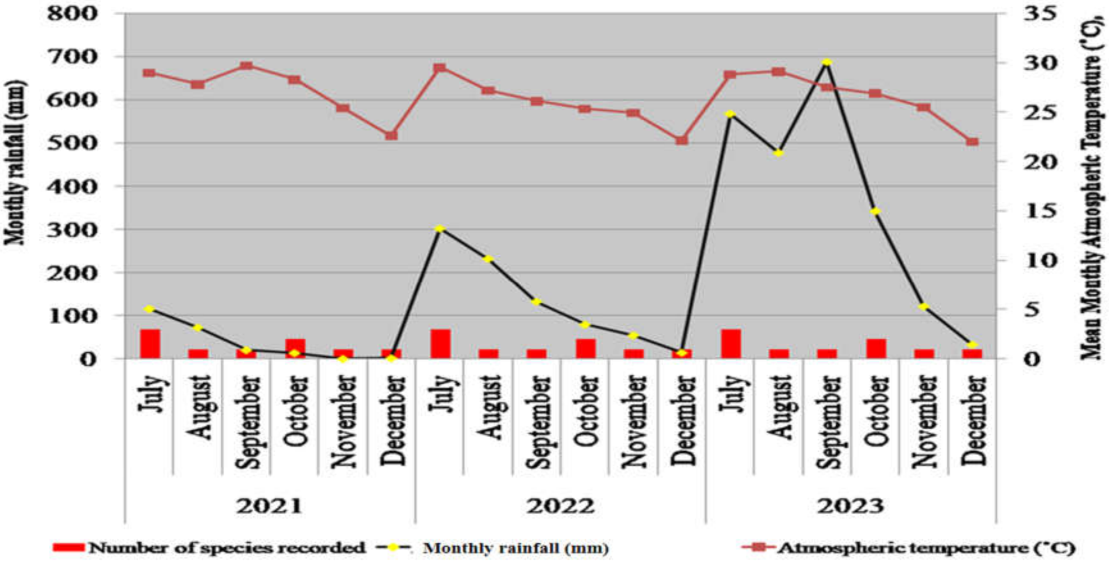

Figure 10 Graphical representation of the recorded monthly rainfall (mm) and atmospheric temperature (˚C) and the number of species recorded in each month during the study duration from the study site.

Notably, the month of July consistently demonstrated the highest species diversity, featuring three distinct species throughout the entire study duration (Figure 10). Upon comparing monthly rainfall, it was observed that July exhibited peak precipitation in both 2021 and 2022 (Table 2 and Figure 10). Conversely, in 2023, there was a substantial increase, reaching a recorded rainfall of 567 mm. The mean atmospheric temperature recorded in July is notably high, falling within the optimal range of 28.8°C to 29.5°C, suggesting favorable conditions for the survival of cicada species.

Table 3 Diversity Indices for Sample Cicada Species of the studied area

Cicada species	**Number of Individual**	**p**	**lnp**	**p*lnp**	**H (shannon -Weiner)**	**Hmax**	**Pielou's eveness**
Scieroptera splendidula	14	0.016	-4.15	-0.065	1.274	1.609	0.791
Cryptotympana corvus	229	0.258	-1.355	-0.349			
Eopycna repanda	45	0.051	-2.982	-0.151			
Pomponia linearis	382	0.43	-0.844	-0.363			
Pomponia ramifera	218	0.245	-1.404	-0.345			
Total	888		-10.736	-1.274			

Further, the study recorded five cicada species, with varying levels of abundance and contributions to diversity. *Scieroptera splendidula* had 14 individuals, comprising a small proportion (p = 0.016) of the total and contributing minimally to diversity (p*lnp = -0.065). *Cryptotympana corvus* was represented by 229 individuals, making up a significant proportion (p = 0.258) and positively influencing diversity (p*lnp = -0.349). *Eopycna repanda* had 45 individuals, with a proportion of 0.051 and a negative diversity contribution (p*lnp = -0.151). *Pomponia linearis* was the most abundant species, with 382 individuals and a notable positive impact on diversity (p*lnp = -0.363). *Pomponia ramifera* had 218 individuals, also contributing positively to diversity (p*lnp = -0.345) (Table 3).

DISCUSSION

This study represents a comprehensive effort to examine the distribution and diversity of cicadas in parts of Ri Bhoi District of Meghalaya, North East India. The research highlights a significant concentration of cicada species in July, with three main species—*Pomponia linearis*, *Pomponia ramifera*, and *Scieroptera splendidula*—dominating this period. The findings suggest that higher temperatures are correlated with increased cicada diversity. Furthermore, the study indicates a noticeable impact of rainfall on cicada diversity, with instances of rainfall exceeding 150 mm being associated with a decline in cicada diversity. This indicates the potential role of rainfall in influencing the ecological dynamics of cicada populations. The data reveals a greater abundance of the subfamily Cicadinae compared to the subfamily Cicadettinae, prompting a need for further investigation into the ecological implications and potential factors influencing these observed abundance patterns. The disparity in diversity between Cicadinae and Cicadettinae may be linked to factors such as habitat preferences, reproductive strategies, and ecological niches, each of which warrants thorough exploration.

The research revealed that cicadas display a preference for the axis, specifically the stem and branches of trees, as opposed to leafy shrubs (Figure 9). This inclination can be attributed to the distinct xylem system that is notably prominent in trees. Trees typically possess a robust and varied vascular system, particularly in their stems and branches, facilitating efficient water transport and providing mechanical support. The behavior of xylem-feeding, unique to specific cicada species, has consequences for both the cicadas and the targeted plants, as outlined by Williams and Simon (1995). The observed habitat choice of cicadas appears to be influenced by the structural characteristics of trees, where the pronounced xylem is conducive to the insects' ecological needs. In contrast, the study indicates that many shrubs often exhibit a more compact and bushy growth form. The vascular systems of shrubs are adapted to suit their smaller stature, and this adaptation may impact the attractiveness of the shrub axis to cicadas.

Additionally, this study revealed that cicadas predominantly exhibit a diurnal activity pattern, showing a clear preference for the morning to noon time frame as opposed to crepuscular and matutinal periods. This discovery strongly suggests that cicadas rely on solar energy for their physiological and behavioral activities, including habitat site selection (Bosnak, 2020). This dependency on sunlight operates not only at broader between-host scales but also potentially influences microsite preferences within hosts. The role of light availability as a critical determinant of habitat is not exclusive to cicadas; it extends to various other insect herbivores, although it's crucial to acknowledge that the specific direction of these preferences can vary significantly across different ecological systems (Yang, 2006; Sheppard et al., 2020). Furthermore, the quality of habitat for cicadas could be influenced by the illumination conditions in the vicinity of host trees (Cole and Lorimer, 1994). The present study also suggests that cicadas may optimize their energy acquisition and utilization by aligning their peak activity with the period of maximum sunlight exposure during summer. This strategic temporal adaptation may confer advantages related to foraging efficiency, communication, and reproductive activities, all of which could be influenced by the availability of solar energy.

The prevalence of diurnal activity in cicadas indicates a reliance on solar energy for their ecological and physiological functions. Researchers, including Dybas and Lloyd (1974); White et al., (1979); White (1980); Karban (1982, 1997); Karban et al. (2000); Williams et al., (1993); Rodenhouse et al., (1997); and, Oberdorster and Grant (2006) have suggested that temperature variations associated with daytime conditions play a crucial role in shaping the activity patterns and metabolic processes of cicadas. Delving into the specific mechanisms underlying this diurnal behavior and exploring the potential advantages conferred by temperature-related factors could significantly enhance our comprehensive understanding of cicadas' adaptive strategies and ecological roles.

Furthermore, the research indicated that cicada species prefer to engage in group calling rather than emitting distant calls far from each other. This behavior appears to contribute to the overall amplification of the cicada song, a crucial factor in outdoor environments like forests or wooded areas where sound transmission can be hindered by vegetation. Additionally, the collective noise produced by a chorus of cicadas may serve the practical purpose of reaching greater distances. This extended reach becomes essential for attracting distant females dispersed within the habitat (Hou et al., 2022).

The findings of this study reveal significant differences in the abundance and contributions to diversity among the five cicada species recorded. The high abundance of *Pomponia linearis* translates into a substantial positive impact on overall diversity, suggesting a crucial role of *Pomponia linearis* within the community. The notable presence of *Cryptotympana corvus* and *Pomponia ramifera*, evidenced by their relatively high numbers, enhances species richness and evenness, thereby supporting the stability and resilience of the ecosystem. Conversely, *Scieroptera splendidula* and *Eopycna repanda* had much lower abundances, indicating that these species have a lesser influence on the community's overall diversity, possibly due to their smaller populations and the ecological niches they occupy.

The varying contributions of these cicada species to diversity highlight the complexity of ecological interactions within the community (Moriyama and Numata, 2015). The dominance of *Pomponia linearis* and its significant positive impact on diversity emphasizes its predominant role within the community. In contrast, the lower abundances and minimal impacts of *Scieroptera splendidula* and *Eopycna repanda* suggest they may occupy more specialized or less competitive niches. Understanding these dynamics is crucial for conservation efforts, as it emphasizes the need to preserve not just species richness but also the ecological roles and interactions that sustain biodiversity.

In summary, this study highlights the significance of both abundant and less common cicada species in maintaining ecological diversity. Species such as *Pomponia linearis*, *Cryptotympana corvus*, and *Pomponia ramifera* contribute significantly to community diversity and ecosystem stability. Meanwhile, less abundant species like *Scieroptera* *splendidula* and *Eopycna repanda* also play crucial roles, potentially within more specialized niches. Effective conservation strategies should aim to preserve the intricate balance of species contributions to sustain biodiversity and ecosystem health. Future research could delve deeper into the specific ecological roles and interactions of these cicada species to enhance our understanding of their contributions to biodiversity. This study provides essential baseline information to enrich the cicada community database in the region and offers valuable insights into the complex ecological dynamics and adaptive behaviors of cicadas. These insights could inform the development of more effective conservation strategies for habitats, ensuring the long-term persistence of the entire cicada community.

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VARIATION IN LIGHT/DARK EXPOSURE IMPAIRS ANTIOXIDANT DEFENCE SYSTEM IN BRAIN AND TESTIS OF GOLDFISH (*CARASSIUS AURATUS*)

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ABSTRACT

This study aimed to assess the overall health of goldfish (*Carassius auratus*) by analysing the effects of light and dark conditions on the antioxidant defense system in the brain and testis. Thirty acclimated fish were equally divided into three aquaria where in control group the fish exposed to normal light and dark phase as designated 12hD/12hL. Second group fish were exposed to complete darkness (24hD) and the third group was exposed to continuous light (24hL) for 30 days. The results showed an enhanced level of lipid peroxidation (by 1.4-fold) in brain tissues of fishes of 24hD, whereas its level was decreased in both 24hD and 24hL in testis (by 2.1 and 1.4 fold, respectively) indicating the differential production of reactive oxygen species (ROS). The activity of superoxide dismutase (SOD) was declined (by 2.1 fold) in brain of the fish exposed to continuous light, whereas glutathione peroxidase (GPx) activity was increased under continuous exposure of light or darkness (by 1.2 and 1.3 fold respectively) as compared to control. On the other hand, the activity of SOD in testis was increased under consistent darkness (by 1.3 fold). However, the activity of catalase (CAT) and GPx in testis was declined under continuous light exposure (by 1.5 and 1.2 fold) indicating imbalance in the redox system under experimental condition. Based on the present findings, it is reasonable to conclude that deviation from normal photoperiod disrupt the cellular antioxidant defence system in goldfish.

Keywords: Light/dark, brain, testis, oxidative stress, antioxidant enzymes.

INTRODUCTION

Living organisms are exposed to several environmental elements, such as light, temperature, and food, which are necessary for maintaining their physiological functions (Weia et al., 2019). Light continues to play a crucial role in their existence. Nevertheless, light exhibits significant variability, especially in aquatic environments. Lighting circumstances have a significant impact on fishes, affecting their hormonal and reproductive state, color pigmentation, circadian cycles, and ambient temperature (Mäthger and Hanlon, 2007; Kolmos and Davis, 2007; Villamizar et al., 2014). Also, light play a crucial role in fishes for enhancing the feeding efficiency and avoiding the predators (Pitcher and Parrish, 1993). In addition, the schooling behavior and movement towards the light source are also affected by artificial light cues (Marchesan et al., 2005).

Goldfish holds a unique position among ornamental fishes due to its appealing color, shape, and social significance (Ota and Abe, 2016). These fish exhibit high sensitivity to factors such as temperature, light intensity, pH levels, and water salinity. Variations in light intensity impact the circadian rhythm of goldfish, thus leading to the development of stressful circumstances. The stress conditions include sleep deprivation, changes in behavior, and other physiological states (Kim et al., 2014). The presence of light impacts the circadian cycle of goldfish, leading to elevated levels of thermal stress in the surrounding water. Therefore, the fluctuation in this state causes oxidative stress in goldfish by producing reactive oxygen species (ROS) in the electron transport chain of mitochondria (Choi et al., 2014). These ROS such as superoxide radicals, hydroxyl radicals, hydrogen peroxides, etc. are responsible for oxidative stress by imparting its effect on cellular biomolecules like lipid, protein and nucleic acid. In aerobes, the cellular system is also endowed with antioxidant defence system which comprises both non enzymatic antioxidants like reduced glutathione, ascorbic acid, etc. and enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidise (GPx). Thus, the antioxidant defense system employs many mechanisms to mitigate oxidative stress by counteracting the surplus of free radicals (Bagnyukova et al., 2006; Halliwell and Gutteridge, 2007; Sulfath et al., 2013; Deepa et al., 2016).

Light is an environmental cue linked to seasonal developmental processes in fish, regulating their circadian rhythms and various other physiological and behavioural changes (Biswas et al., 2005, Boeuf and Le Bail, 1999, El-Sayed and Kawanna, 2004). Although several literatures are available on effect of light on fish physiology (Boeuf and Le Bail, 1999; Bruning et al., 2018; Khan et al., 2020; Czarnecka et al., 2019; Ruchin, 2021; Barbarossa et al., 2021; Bassi et al., 2022; Hsieh et al., 2023), but sufficient information on status of oxidative stress and antioxidant defences under such conditions is not available in fish model. Therefore, the present investigation was aimed to evaluate the long term lighting effect (30 days of light or dark) on the oxidative stress biomarker, lipid peroxidation (LPx) and the activities of antioxidant enzymes (SOD, CAT and GPx) in brain and testis of goldfish.

MATERIALS AND METHODS

Experimental design

Healthy Adult goldfishes (*Carassius auratus*) of approximately equal length (7-9 cm) and weight (5-7 g) were taken from State Fisheries Museum, Bhubaneswar, Odisha. The fishes were acclimatized for 15 days prior to the experiment in laboratory conditions. Fifty litre capacity aquarium having size 60x30x40cm were selected for rearing and experimentation in normal tap water. The fishes were conditioned to 12h dark/12h light cycle during acclimatization and the standard feed pellet was added two times in a day. All aquaria were maintained in ideal conditions and the quality of water like dissolved oxygen (6.0-6.8 mg/L), hardness (130-160mg/L), alkalinity (94.8- 103.5 mg/L), pH (6.9-7.1) and temperature (28±2ºC) were measured to ensure the optimum rearing state. Thirty percent water of the aquaria was changed in every alternative day. Thirty goldfishes were divided randomly into three groups each having ten in number and kept in three aquaria with constant aeration. The fishes in the aquarium were designated as group I: Control with normal exposure of 12h light and 12h dark (12h L/12h D); group II: the animals exposed with 24h dark (24h D); group III: the fishes exposed with 24 h light (24h L). The lighting condition was maintained with usual fluorescent light and the dark condition was maintained in a dark room for an experimental period of 30days.

Tissue processing and sample preparation

The fish were captured carefully and anesthetized with immersion anaesthesia alfaxalone (6 mg/L^{-1}) following Leonardi et al. (2019). Following measurement (body length and weight), the animals were sacrificed to collect the brain and testis. These organs were immediately washed in potassium phosphate buffer (PB, 50mM, pH 7.4) and pat dry in blotting paper. The organs of different groups were kept in labelled microcentrifuge tubes and stored at -20 ºC till further use. The tissues were homogenized (10%) in 50mM Phosphate buffer (pH 7.4) with the help of hand homogenizer in ice. The crude homogenate was used for biochemical assay of LPx. A part of the crude homogenate was subjected for Triton X-100 treatment (final concentration 0.1%) and centrifuge at 10,000 × g for 15 min. The obtained supernatant was used for the measurement of antioxidant enzymes. The supernatant samples were passed through a 1 ml of Sephadex G-25 column, and elutes were used for the assays of SOD and GPx. The activity of CAT was estimated using the original supernatant (without column-pass).

Biochemical analysis of the samples

Protein estimation

Protein concentrations in different samples of the crude homogenate and supernatant samples were estimated following Bradford (1976) taking BSA (Bovine Serum Albumin) as standard.

Estimation of Lipid Peroxidation (LPx)

Determination of Lipid peroxide level was done by monitoring the formation of thiobarbituric acid reactive substances (TBARS) following Okhawa et al. (1979). LPx level was calculated from its extinction co-efficient 1.56 × 105 $M^{-1}cm^{-1}$ and expressed as nmoles TBARS formed per mg protein.

Measurement of the activities of antioxidant enzymes (SOD, CAT and GPx)

The activity of SOD was measured following the method developed by Das et al. (2000). One unit of enzyme activity was defined as amount of SOD capable of inhibiting 50% of nitrite formation under assay condition and the activity was expressed as unit/ mg protein. Catalase was assayed following the methods of Aebi (1974) and Cohen et al. (1970). The enzyme activity was calculated by taking 43.6 $M^{-1}cm^{-1}$ as molar extinction coefficient of H_2O_2 and expressed as nkatal/mg protein. 1 nkatal is defined as the amount of enzyme able to catalyse the degradation of 1 nmole of H_2O_2 per second. The activity of GPx was measured following the method of Paglia and Valentine (1967). Total GPx activity was calculated using molar extinction coefficient for NADPH (6.22×10^3 $M^{-1}cm^{-1}$) and expressed as nmoles NADPH oxidized per minute per mg protein.

Statistical analyses

The data were presented as mean ± S.D. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range tests to find out the level of significance among the mean values (Duncan, 1955). The minimal statistical significance was considered at $p \leq 0.05$ levels.

RESULTS

Level of oxidative stress marker, LPx (nMoles TBARS formed /mg protein)

An increased level of LPx (by1.4 fold; 2.24 ± 0.33) was observed in brain of 24-hour dark exposed (24h-D) fishes in comparison to control (1.63 ± 0.13). Whereas, an unchanged level of LPx was found in 24-hour light exposed (24h-D) fishes as compared to control. LPx level of testis was declined in both groups of 24h-D (3.05 ± 0.74) by 2.1 fold and 24h-L (4.67 ± 0.74) by 1.4 fold with respect to control (6.33 ± 0.72) (Figure 1).

Activities of antioxidant enzymes (SOD, CAT and GPx)

Activity of SOD (Unit/mg protein)

The activity of SOD was significantly declined (by 2.1 fold; 0.52 ± 0.13) in brain of 24h-L group fold as compared to Control (1.12 ± 0.16). However, an unchanged activity of SOD was observed in 24h-D group in comparison to control. SOD activity in testis was significantly increased (by 1.3 fold; 2.17 ± 0.17) in 24 h-D with respect to control (1.67 ± 0.13), whereas the activity of the enzyme in testis of 24h-L was remained same with that of control group (Figure 2).

Activity of CAT (nKatal/min/mg protein)

Catalase activity in testis was decreased (by 1.5 fold; 168.22 ± 23.5) in 24h-L group with respect to control (252.34 ± 21.4), whereas an unaltered CAT activity was observed in testis of 24h-D group with regard to control fish. Catalase activity in brain tissues (150.25 ± 18.9) of different groups was not affected in response to dark/light conditions (Fig.3).

Activity of GPx (nMoles NADPH oxidized)

The activity of GP$_x$ in brain tissues was increased in both groups of 24h-D (146.1 ± 11.2) and 24h-L (156.91 ± 13.2) by 1.2 and 1.3 fold respectively as compared to control (121.04 ± 6.41). On the other hand, GP$_x$ activity in testis was significantly decreased (by 1.2 fold; 105.53 ± 2.56) in 24h-L group with respect to control (123.19 ± 8.84). An unchanged activity of GPx in testis was recorded in 24h-D group as compared to control (Figure 4).

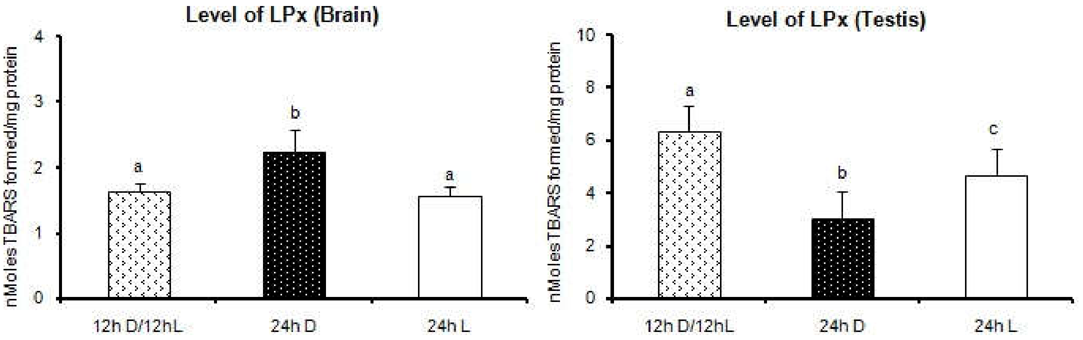

Figure 1 Effect light/dark exposure on oxidative stress parameter, LPx in brain and testis of goldfish. Group I: Control with exposure of normal light and dark phase, Group II: fishes exposed with 24h dark, Group III: fishes exposed with 24h light every day for a period of 30days. Data are expressed as mean ± S.D. of ten animals. Data having different superscripts differ significantly ($p \leq 0.05$) from each other.

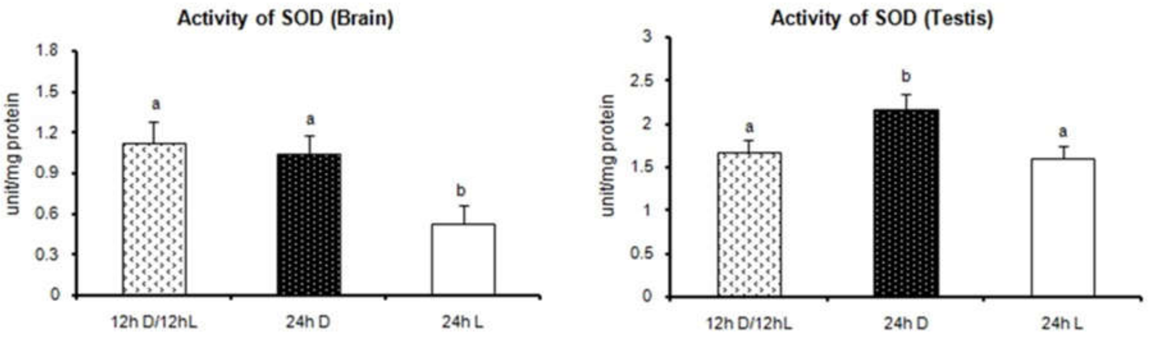

Figure 2 Effect light/dark exposure on the activity of antioxidant enzyme, SOD in brain and testis of goldfish. Group I: Control with exposure of normal light and dark phase, Group II: fishes exposed with 24h dark, Group III: fishes exposed with 24h light every day for a period of 30days. Data are expressed as mean ± S.D. of ten animals. Data having different superscripts differ significantly ($p \leq 0.05$) from each other.

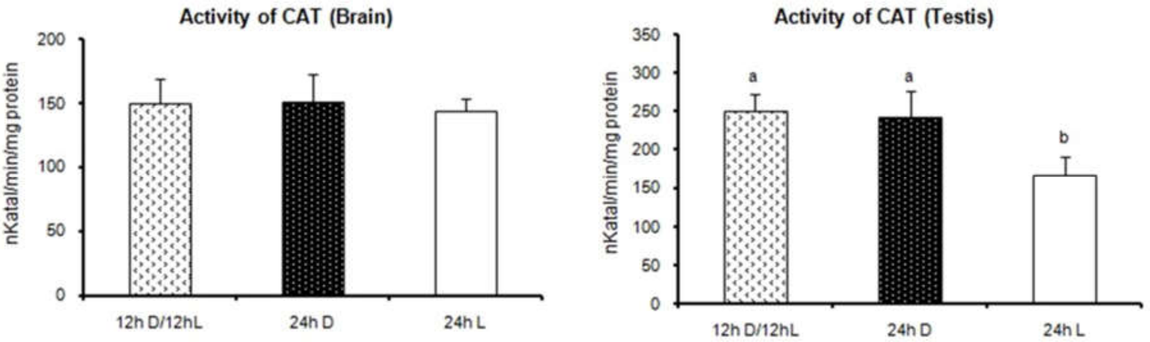

Figure 3 Effect light/dark exposure on the activity of antioxidant enzyme, CAT in brain and testis of goldfish. Group I: Control with exposure of normal light and dark phase, Group II: fishes exposed with 24h dark, Group III: fishes exposed with 24h light every day for a period of 30days. Data are expressed as mean ± S.D. of ten animals. Data having different superscripts differ significantly ($p \leq 0.05$) from each other.

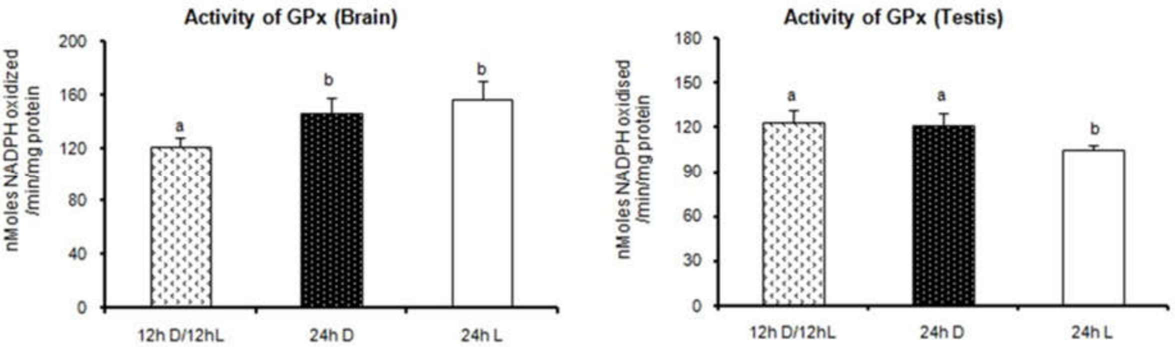

Figure 4 Effect light/dark exposure on the activity of antioxidant enzyme, GPx in brain and testis of goldfish. Group I: Control with exposure of normal light and dark phase, Group II: fishes exposed with 24h dark, Group III: fishes exposed with 24h light every day for a period of 30days. Data are expressed as mean ± S.D. of ten animals. Data having different superscripts differ significantly ($p \leq 0.05$) from each other.

DISCUSSION

Light is one of the important factors that hugely influence the living beings including aquatics organisms (Bassi et al., 2022). Light induces signalling of pineal gland which is responsible for activation of hypothalamus, the master gland which in turn regulates the hormonal activity of body. Melatonin, a hormone that is sensitive to light, is secreted by the pineal gland and enters the bloodstream. It is then absorbed by the testis, where it directly influences testicular function (Reiter, 1981, 1991). Light-induced changes in hormone levels can trigger many signalling pathways, leading to the generation of free radicals in the brain and its target organs. Light has a greater impact on the brain and testis compared to other organs in the body, making them more susceptible to its effects (Fonken and Nelson, 2014; Bedrosian and Nelson, 2017). Stress is a physiological state of body and it reflects the overall health of the organism. Among the oxidative stress biomarkers, LPx is one of the major indices. However, in this investigation, LPx level was enhanced in brain and declined in testis of 24 hour dark exposed (24h-D) fishes in comparison to control. Moreover, LPx level in testis of 24h-L group was also declined with respect to Control (12h-D/12h-L), but the magnitude of decrease was higher in 24h-D group by 0.7 fold than 24h-L group. Lipid peroxides could be the result of ROS generation and among them hydroxyl radical is the most prominent initiator of lipid peroxidation (Halliwell and Gutteridge, 2007). Enhanced LPx level in brain under dark condition may due to over production of free radicals. In contrast, LPx level in testis was decrease in both light and dark exposure showing the differential generation of free radicals in these tissues.

To counteract these harmful free radicals, cellular system developed a well organised defence systems termed as antioxidant defence system which comprises enzymatic and non-enzymatic defences. The important enzymatic scavengers responsible for the impediment of ROS formation and oxidation are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx). SOD catalyses the dismutation of superoxide radical to hydrogen peroxide and is known as first line of antioxidant enzyme. The formation of hydrogen peroxide is further catalysed by two enzymes such as CAT and GPx in to water and oxygen. CAT coverts its substrate H_2O_2 directly into O_2 and H_2O, but GPx catalyses H_2O_2 into H_2O by oxidizing another substrate reduced glutathione (GSH) into oxidized glutathione (GSSG) (Bagnyukova et al., 2006; Halliwell and Guttridge, 2007). In the present study, the activity of SOD was declined in brain of 24h-L group as compared to Control and 24h-D group. Whereas, SOD activity in testis was significantly increased in 24 h-D with respect to control and 24h-L group. The SOD activity found to be decreased in brain of 24h-L groups, whereas its activity was enhanced in testis of 24h-D groups. It shows the lighting-effect on the activity of SOD was not uniformly influenced, particularly in these tissues. Catalase activity in testis was decreased in 24h-L group with respect to control and 24h-D groups. However, CAT activity in brain tissues of different groups was not affected in response to dark/light conditions. The activity of GPx in brain tissues was increased in both 24h-D and 24h-L groups as compared to testis of control fishes. On the other hand, GPx activity in testis was significantly decreased in 24h-L group by 1.2 fold in comparison to control. Although CAT and GPx have the same role to neutralise the H_2O_2, activity of CAT was not altered by the lighting conditions. In contrast, GPx activity was enhanced in brain under both the conditions (24h-D and 24h-L). This may be due to the availability of GSH which is an additional substrate for GPx under such lighting conditions. However, the level of GSH and GSSG not measured in this study needs further investigation. In testis, the activity of CAT decreased which is concomitant with the activity of GPx in response to 24h lighting condition. These results revealed difference in the activity of antioxidant enzymes in response to long-term exposure of light/dark variation in two different organs. However, based on the findings it is reasonable to conclude that variation in light and dark cycle causes oxidative stress by modulating the activities of antioxidant enzymes in goldfish.

CONCLUSION

Light is one the important abiotic factors that play crucial roles in controlling the fish physiology and metabolism. In this study, it was concluded that prolonged and consistent exposure to light or darkness impacts on photoperiodism of the fishes that affects metabolic status which ultimately causes oxidative stress and modulates the antioxidant system, particularly in brain and testis.

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DNA FRAGMENTATION IN THE REGRESSING TAIL OF *POLYPEDATES MACULATUS* DURING METAMORPHOSIS: A TUNEL BASED STUDY

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ABSTRACT

Amphibian metamorphosis is intricately associated with apoptosis to carry out the tissue lysis and remodelling best suited for a habitat change. Based on this factual backdrop, the aim of the present study was to investigated cell death manifested by DNA fragmentation in tail of anuran tadpoles of Indian tree frog, *Polypedates maculatus*. Apoptotic cells were detected *in situ* in the regressing tadpole tail using terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL). We identified, TUNEL positive cells in the epidermis, muscle cells and notochord. However, such cells were absent in the caudal spinal cord during the climax stage in the regressing caudal cord of *P. maculatus* tadpoles which deviates from earlier report in *X. laevis* suggesting DNA fragmentation is not manifested during cell death in the regressing spinal cord in *P. maculatus*. Thus, indicative of differences in the cell death process during tail regression among anuran species.

Keywords: Anurans, Apoptosis, DNA fragmentation, Metamorphosis, Regression, Tail, TUNEL.

INTRODUCTION

The tadpole tail is a transient structure in anurans that regresses during metamorphosis. Thus, regression of the tail provides insights into programmed cell death (PCD) which is an essential part of embryonic development (Yoshizato, 1989; Mukai et al., 1995; Yaoita, 2019; Domínguez-Bautista et al., 2021). Previous studies on tail regression in *P. maculatus* tadpoles have been indicative of cells with apoptotic morphology (Mahapatra and Mahapatra, 2011, 2015). DNA fragmentation is a hallmark event of apoptosis (Collins et al.,1997; Kitazumi and Tsukahara, 2011). Apoptotic cells characterized by small cytoplasmic fragments containing condensed nuclei (Kerr et al., 1972) can be identified by TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling) in histological sections. TUNEL detects DNA fragmentation representing the end-point of apoptotic DNA degradation (Kalyuzhny, 2011). The extent of apoptosis revealed by TUNEL technique is considerably greater than apoptosis detected by nuclear morphology (Rojo and Gonzalez, 1998). Furthermore, Negoescu et al., (1998) have reported TUNEL as a more sensitive and specific technique to study apoptosis in comparison to in situ nick translation (ISNT). Since, metamorphosis includes a massive structural reorganization in the tadpoles by the loss of tail, hence apoptosis is hypothesised to be an integral event in course of the tail loss. Thus, localization of apoptotic bodies is important in this context. In the present investigation we have localized apoptotic bodies by in situ DNA fragmentation during tail regression in metamorphosing tadpoles of *P. maculatus*.

MATERIALS AND METHODS

Collection of egg mass, staging of the embryos and tail amputation

Egg nests of the Indian tree frog, *Polypedates maculatus* were collected during monsoon period in the month of July and Augusts. Tadpoles were reared in the laboratory following the standardized procedure (Mohanty-Hejmadi, 1977). Staging of the tadpoles was done in accordance with Taylor and Kollros, (1946). Tail amputation was carried out by first anesthetizing the tadpoles with 0.003% MS222 (Tricaine methane sulphonate). Tadpoles were positioned laterally on a pre-sterilized porcelain plate while the tails were amputated from the base. Further, blood loss was prevented by transferring the tadpoles immediately to the amphibian ringer solution. All procedures were approved by the Animal Care Review Committee at Utkal University, Odisha, India.

DNA Extraction and Agarose Gel Electrophoresis

The tail tissues of *P. maculatus* tadpoles in different stages of development such as TK stage V, X, XVII, XX, XXI, XXII and XXIV of less than 25 mg weight were cut into small pieces and collected in sterilized micro-centrifuge tubes for investigation. DNA extraction was done as per the instructor’s manual (DNeasy Blood and Tissue Kit, Qiagen). The isolated DNA samples were quantified using a spectrophotometer (Eppendorf Biospectrometer ® kinetic). For the visualization of the ladder, isolated DNA samples 6 µl each were electrophoretically separated on 1.2% agarose gel containing ethidium bromide (0.5µg/ml). Eight wells were selected for the analysis. DNA ladder of 1 kb (Gene Ruler TM, Fermentas, Life Sciences) was loaded in the first one while the subsequent wells were loaded with samples of TK stages V, X, XVII, XX, XXI, XXII and XXIV respectively. The power pack set at 80V was connected to the horizontal gel electrophoresis apparatus (GeneiTM, Sanmar Speciality Ltd., Bangalore, India) and was allowed to run for 1 hour continuously. Gel was carefully removed and laddering was visualized under an UV gel documentation system (BIO-RAD, USA).

Histology and TUNEL staining

Tail tissues were fixed in formalin for 24 hours and embedded in paraffin wax. Pre-coated (gelatinized) glass slides were used for the retention of tissue sections on to the slides. Tissue sections of 5µm thickness were obtained and Mallory’s triple staining was carried out for the histological analysis. Serial sections were selected for histology and TUNEL staining. We used the TACS® 2 TdT-Fluor In Situ Apoptosis Detection Kit (Trevigen, Inc., Gaithersburg) in the paraffin embedded tissue sections specific for apoptotic fragments. The TUNEL based labelling procedure was carried out as per the instructor’s manual. Sections were incubated first in proteinase K for 30 minutes at room temperature followed by the labelling reaction mix for an hour inside a humidity chamber. Later, the tissue sections were covered with Strep-Fluor solution in the dark for 20 minutes at room temperature. The slides were then mounted with a fluorescence mounting medium, containing DAPI (4', 6-diamidino-2-phenylindole), a fluorescent dye that labels DNA (Collins et al., 1997) obtained from Santa Cruz biotechnology, Santa Cruz. Apoptotic cells and fragmented DNA were observed and photographed using Leica DFC450 C camera fitted to a fluorescence microscope (Leica DM3000 LED). Adobe Photoshop software was used for annotations and assemblage of images into plates.

RESULTS

Chromatin cleavage during tail regression

DNA fragmentation was detected by agarose gel electrophoresis. A distinct ladder pattern with four bands having 1000, 750, 500 and less than 500 base pairs were marked in the TK stage XXIV sample indicating chromatin cleavage (Figure 1). However, ladder was not evident in the samples of the rest of the TK stages V, X, XVII, XX, XXI, XXII (Figure 1). Based on this finding TK stage XXIV was selected for further investigations (Figure 1 G).

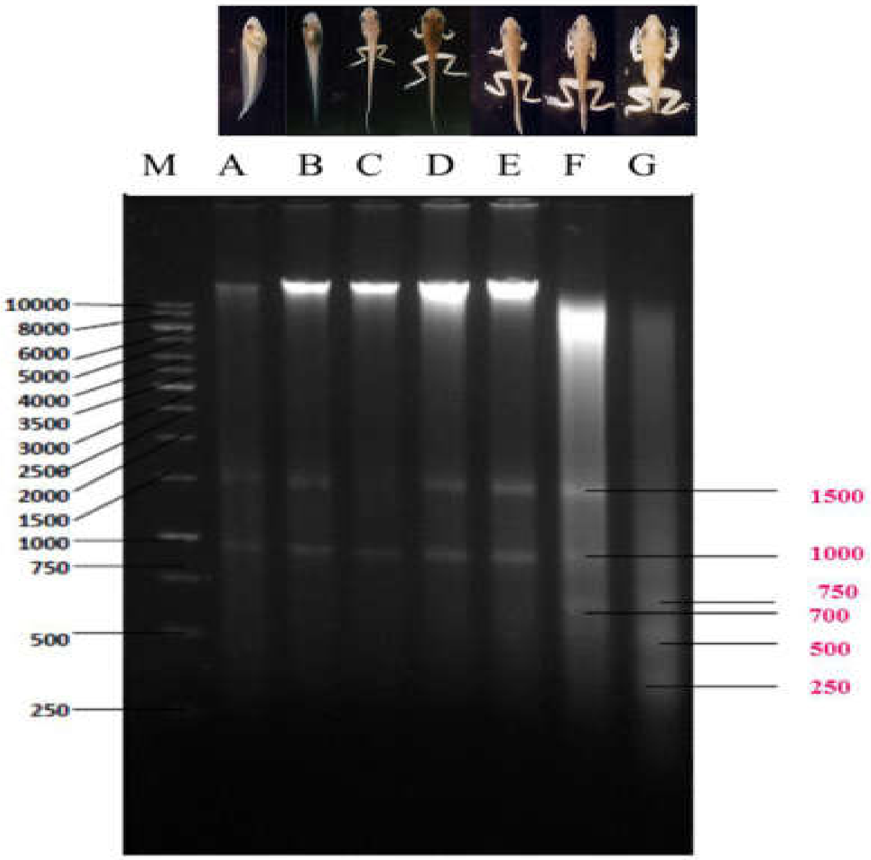

Figure 1 Agarose gel electrophoresis of genomic DNA from the tail tissues of *Polypedates maculatus* tadpoles of different developmental stages. (M) stands for molecular marker. (A-G) represents DNA extracts from tail tissues of the TK developmental stages V, X, XVII, XX, XXI, XXII, XXIV, respectively.

Histological analyses

The histology of the regressing tail (TK stage XXIV) revealed tissue degradation (Figures 2 A-F). The tail tip was blunt and cup shaped packed with degraded cell masses (Figure 2A). Melanocytes (m) (Figure 2 B, C) were found in large numbers beneath the thick basement membrane (bm) (Figure 2B). A stratified epidermis (e) (Figures 2B, C) lined by loosely arranged cuticle (c) (Figure 2C) contained cells that exhibited apoptotic morphology (ap) (Figure 2

C). Such cells failed their connections from their neighboring cells. The regressing notochord (n) appeared in patches under higher magnification (Figure 2D). The spinal cord (sc) was no more continuous and appeared as patches (Figure 2E). Sarcolytes (sl) or the degraded muscles and compact muscle patches (cmp) were more prominent at the distal portion of the tail (Figure 2F).

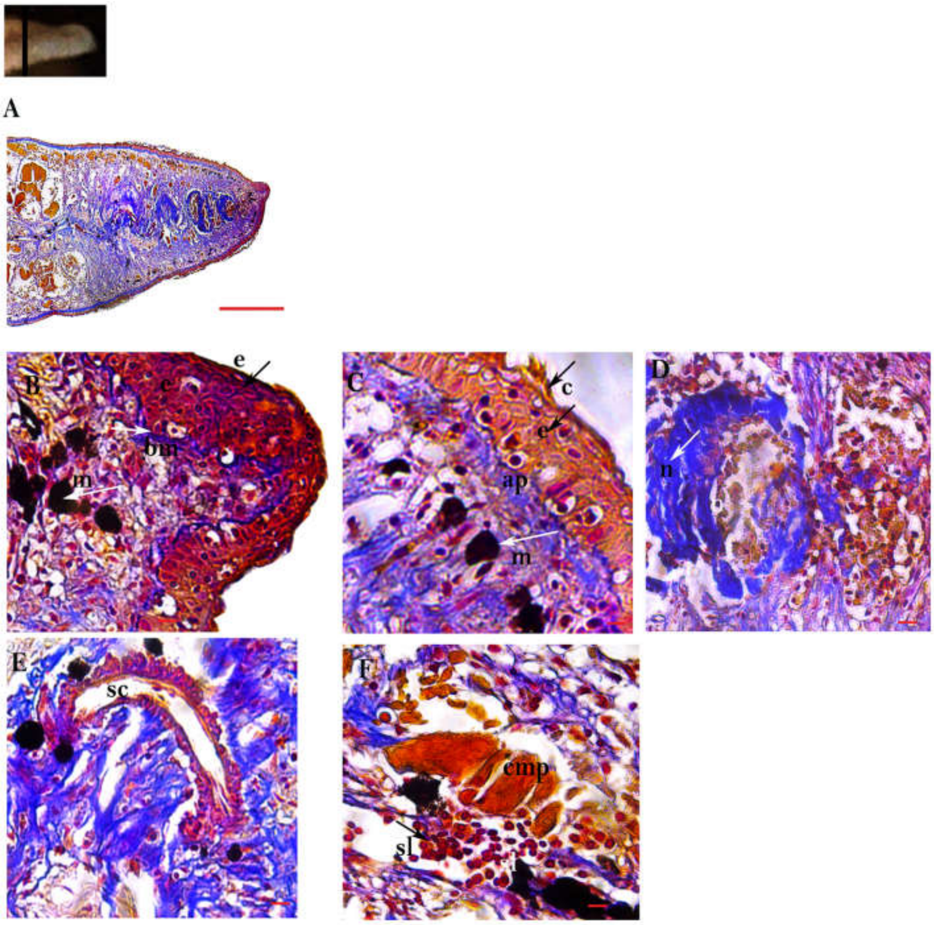

Figure 2 Histology (Longitudinal section) of the regressing tadpole tail of *Polypedates maculatus* TK stage XXIV. (**A**) Mallory staining of TK stage XXIV regressing tail (lower magnification); (**B**) Tail tip region showing epidermis (e) and melanocytes (m) and basement membrane (bm); (**C**) Tail tip showing cuticle (c) and cell showing apoptotic morphology (ap); (**D**) Notochord (n) of the regressing tail; (**E**) Spinal cord (sc); (**F**): Compact muscle patches (cmp) also showing sarcolytes (sl). Scale bars: (A) = 500μ; (B-I) = 25μ.

Localization of cell death by TUNEL

TUNEL positive cells were seen in the epidermis of the tail tip region of TK stage XXIV tadpole (marked by arrows in Figure 3A). Apoptotic bodies in clusters were also evident in the notochord. (Figure 3B). However, the regressing spinal cord in the TK stage XXIV tadpoles lacked TUNEL positive cells (Figure 3C) while apoptotic cells that stained positive for TUNEL were localized in the degenerating, regressing muscles (Figure 3D).

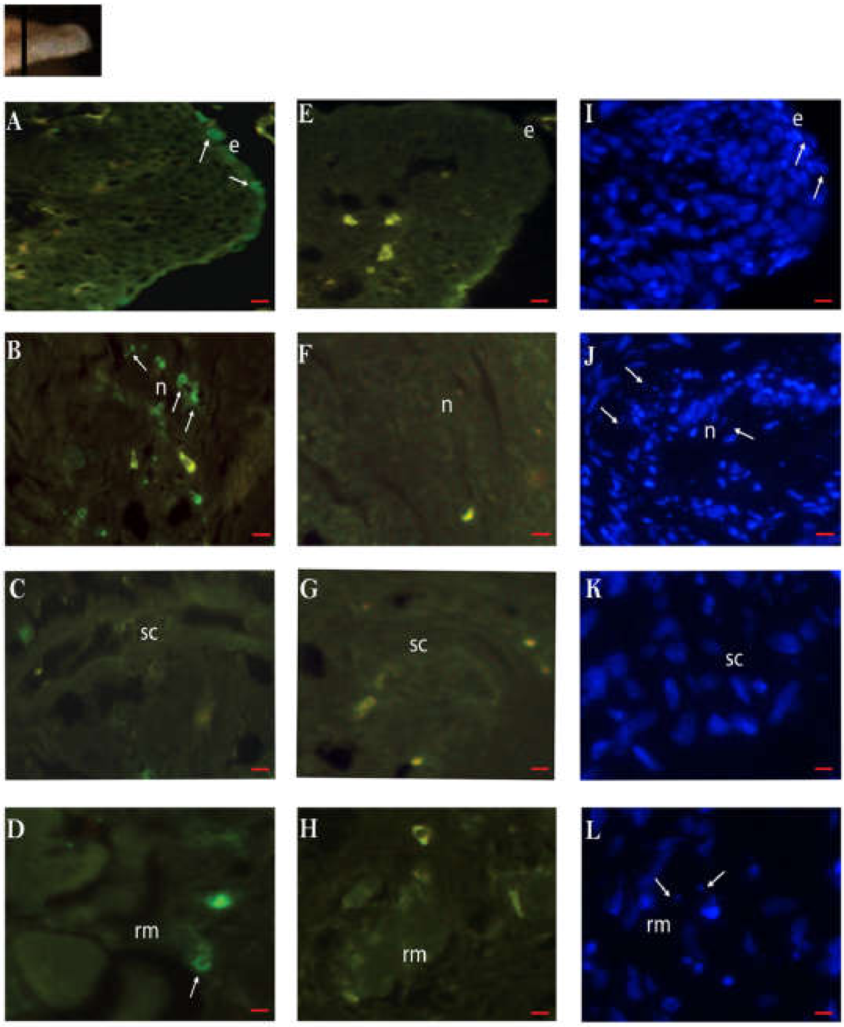

Figure 3 TUNEL staining of the regressing tadpole tail of *Polypedates maculatus* of TK stage XXIV. (**A**) TUNEL positive cells at the tail tip region in the epidermis (e). (**B**) TUNEL stained nuclei of notochord (n). (**C**) Negative TUNEL staing of the nerve cord (sc) in the TK stage XXIV tadpole tail. (**D**) TUNEL labelling in the regressing muscle (rm). (**E**) Tail tip region of Unlabelled control showing epidermis (e). (**F**) Unlabelled control showing notochord (n). (**G**) Unlabelled control showing nerve cord (sc). (**H**) Unlabelled control TK stage XXIV showing regressing muscle (rm). (**I**) DAPI stained condensed and fragmented nuclei at the tail tip in the epidermis (e). (**J**) DAPI stained fragmented nuclei at notochord (n) region. (**K**) DAPI stained nuclei at the nerve cord (sc) region. (**L**) DAPI stained fragmented nuclei of the regressing muscles (rm) tadpole tail. (TUNEL stained nuclei and DAPI stained fragmented nuclei are represented by arrows). Scale bars: (A-L = 25µ).

The unlabelled experimental control where the TdT (Terminal deoxynucleotidyl transferase) enzyme was omitted during the staining procedure (Figures 3E - H) lacked labelling and apoptotic cells were not visualized. The nuclei labelled for DNA breaks were localized at the distal part of the tail, mostly at the site of extensive tissue damage (Figures 3I-L). The fragmented nuclei were evident in the epidermis (Figure 3I) while such fragmented nuclei were found as clusters in the degenerating notochord (Figure 3J). DAPI stained fragmented nuclei were absent near the spinal cord (Figure 3K). In the disintegrating muscles distinct fragmented nuclear staining (Figure 3L) was observed.

DISCUSSION

During the metamorphosis of anuran amphibians, the tail resorption process is a necessary and crucial change (Zhang et al., 2024). Cell death during amphibian metamorphosis is reportedly mediated through apoptosis (Ishizuya-Oka et al. 2010). Shi et al., (2001) and Tamura et al., (2015) have reported the involvement of apoptotic cell death during elimination tail in the African clawed frog *Xenopus laevis*. Likewise, Estabel et al. (2003) have suggested severe cell death to be crucial for the complete removal of tail during the metamorphic climax in *Xenopus laevis*. Cell death has been described in the epidermis of the Japanese brown frog *Rana japonica*, during TK stages XIX-XX (Kinoshita et al., 1985 and Ishizuya-Oka et al., 2010). TUNEL labelling have also been detected in the epidermis of *the frog Microhyla fissipes* during metamorphosis (Wang et al., 2019). Likewise, cytoplasmic condensation of epithelial cells, the fragmentation of the nuclei and also formation of compact apoptotic bodies in the epidermis have also been reported in regressing tadpole tail of dwarf tree frog, *Litoria glauerti* (Kerr et al., 1974; Nakajima et al., 2005). In *X. laevis* apoptotic cells were visualized towards the end of the climax at NF stage 64 (Estabel et al., 2003). Apoptotic cells have been observed in the tail epidermis in *Pelophylax ridibundus* (Başımoğlu and Koca, 2014). The epidermal cells in the present investigation exhibited apoptotic morphology and TUNEL stained apoptotic nuclei were evident in TK stage XXIV. Apoptotic cells have also been reported in the notochord of tails of different lengths in *Pelophylax ridibundus* larvae during the expression and localization studies of Bax/Bcl-2 proteins during metamorphosis (Başımoğlu and Koca, 2014). Likewise, TUNEL labelling have also been detected in the notochord of *M. fissipes* in the regressing tail (Wang et al., 2019). TUNEL positive cells in the notochord observed in the present study suggests a possible role of DNA fragmentation during lysis of notochord during tail regression in tadpoles of *P. maculatus*.

Striated muscle fibre loss in tadpole tail as a result of fragmentation of the cytoplasm into apoptotic bodies have been previously documented (Kerr et al., 1974; Nakajima et al., 2005; Başımoğlu and Koca, 2014). Such apoptosis in the muscles have reportedly known to begin at NF stage 57 in *X. laevis* before the shortening of tail at stage 62 (Nakajima and Yaoita, 2003) while the number of apoptotic cells in the muscles peaks towards the climax at NF stages 63/64 (Nishikawa and Hayashi, 1995). Estabel et al. (2003) also reported the detection of TUNEL positive muscle cells during NF stage 62 in *X. laevis* tadpoles. In the present investigation, TUNEL labelling in the regressing muscles during the metamorphic climax further confirms DNA fragmentation mediated cell death in *P. maculatus*. Estabel et al. (2003) have reported appearance of TUNEL positive apoptotic cells in the caudal spinal cord of *X. laevis* in NF stage 58 with the onset of metamorphic climax However, no TUNEL positive cells were detected in the regressing caudal spinal cord of *P. maculatus* tadpoles.

Thus, the present investigation further validates DNA fragmentation manifested cell death during tail regression in the tadpoles of *P. maculatus* and provides a comparative account with other anurans in terms of differences in the occurrence and localization of apoptotic cells. Further, their localization in the regressing epidermis, muscle and notochord but not in the regressing spinal cord suggests DNA fragmentation is not manifested during cell death in the regressing spinal cord in *P. maculatus* tadpoles in contrast to *X. laevis* tadpoles. Based on the present finding, further futuristic studies on detection of apoptosis-associated gene expression will be necessary to understand the differences during tail regression.

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Chapter in book:

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