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Preliminary analysis indicates dietary similarities in the prey composition of *Hipposideros hypophyllus* Kock and Bhat, 1994 (Chiroptera: Hipposideridae) and sympatric congeneric species

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Abstract

Much is known about bats from other parts of the globe regarding factors such as homogeneity and heterogeneity of habitats, prey abundance influencing the predator-prey relationship, etc. Sadly, minuscule literature on bat ecology is available for India. The current study investigated diet of the Kolar leaf-nosed bat *Hipposideros hypophyllus*, a Critically Endangered (CR) insectivorous bat in IUCN Red List of Threatened Species, known only from a single cave where it shares its roosting site with congeneric species, namely Durgadas's leaf-nosed bat *H. durgadasi* Khajuria and Schneider's leaf-nosed bat *H. speoris* (Schneider), which are Vulnerable (VU) and Least Concerned (LC), respectively. Because *Hipposideros hypophyllus* is on the brink of extinction with no baseline ecological data available, the study analyzed the dietary compositions of the species. It relied on the morphological cataloguing of post-digested prey fragments in fecal pellets of the species and its congeners. Fresh fecal pellets of all three species of *Hipposideros* were analyzed to understand morphological differences and prey composition at the only known site for *H. hypophyllus*, Hanumanahalli village, Kolar district, India, between November 2022 to February 2023. A total of 29,793 prey remnants were examined from all three species belonging to 11 insect orders (Blattodea, Coleoptera, Mantodea, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Odonata, Orthoptera, and Thysanoptera). All three species of *Hipposideros* fed mainly on Coleoptera, followed by Diptera and Lepidoptera, showing a strong overlap in their prey composition. Our results warrant a long-term study across seasons and identifying prey to the species level which can provide more detailed understanding and lead to the conservation of *Hipposideros hypophyllus*, *H. durgadasi*, and *H. speoris*.

Key words: Coleoptera, diet analysis, *Hipposideros durgadasi*, *Hipposideros speoris*, Kolar

Introduction

Ecosystems like the tropics not only harbor a lot of species diversity but also patterns enabling species coexistence (Brown, 2014). Investigating species coexistence parameters like resource allocation, usage of similar microhabitats, prey-predator relationships, a wide range of diets, foraging strategies, and sensory abilities, etc., thus becomes interesting for morphologically similar and sympatric species (Fleming et al., 2020).

The prey-predator relationship particularly in the natural environment has been dubbed as one of the most challenging interactions to observe and thus scientists worldwide rely on the morphological classification of prey remains (Clare et al., 2009). Generalist predators whose excreta contain merged and disintegrated prey fragments are the ones that pose a significant challenge in the identification of the prey organisms. The documentation of prey organisms to the species level is problematical particularly in the cases such as insectivorous bats who thoroughly chew their food. Such fragments are difficult to ascertain beyond the level of order (Sheppard et al., 2004; Clare et al., 2009). Identifying prey composition merely from fecal pellets has its own limitations, and many prey organisms like insects that are fully digested in the stomach cannot be determined (Rabinowitz and Tuttle, 1982; Bohmann et al., 2011; Zeale et al., 2011).

Congeneric bat species with slight variations in call frequency, body size, and wing morphology can show differences in prey taxa (Pavey and Burwell, 2000). Concepts such as hunting styles, predator preferences, prey defenses (Acharya and Fenton, 1999), the effectiveness of cryptic coloration, sound emanations in bats (Ratcliffe et al., 2008), etc., become increasingly arduous to comprehend in the absence of specific prey identification from the fecal droppings of the predators. However, prey taxa identification through morphological analysis of prey remnants in the fecal pellets is advantageous since it is inexpensive in comparison to the molecular approach (Ware et al. 2020). Also, it is a non-invasive method hence not disturbing critically endangered species.

Most studies on the prey composition of insectivorous bats are limited with more studies being conducted in Europe and the Americas than in Asia (Heim et al., 2021). Studies from India are limited to just a few species like *Hipposideros ater* Templeton, 1848, *Hipposideros speoris* (Schneider, 1800), *Lyroderma lyra* E. Geoffroy, 1810 (*Megaderma lyra* Geoffroy, 1810), *Rhinolophus rouxii* Temminck, 1835, *Pipistrellus mimus* Wroughton, 1899 (*Pipistrellus tenuis* Temminck, 1840), *Taphozous melanopogon* Temmnick, 1841, *Rhinopoma microphyllum* Brunnich, 1782 and *Scotophilus kuhlii* Leach, 1821 (Advani, 1981; Whitaker et al., 1999; Ramanujam and Verzhutskii, 2004a, b; Srinivasulu and Srinivasulu, 2005; Rekhasalvi et al., 2010; Sophia, 2010; Bharti and Elangovan, 2021). Also, numerous studies have identified prey organisms from the prey remnants recovered from fecal pellets only to the order level and not to the species level (Ramanujam and Verzhutskii, 2004a, b; Srinivasulu and Srinivasulu, 2005; Rekhasalvi et al., 2010; Bharti and Elangovan, 2021). From the available data, some of the *Hipposideros* bats are known to feed on agricultural pests (Sophia, 2010; Kasso and Balakrishnan, 2013), thereby regulating their populations. *Hipposideros* bats are known to feed more on Coleoptera and Lepidoptera (Eckrich and Neuweiler, 1988; Ramanujam and Verzhutskii, 2004b; Sophia, 2010; Weterings et al., 2015; Aguiar et al., 2021) which have been determined as major insect pests.

Kolar leaf-nosed bat, *Hipposideros hypophyllus* Kock and Bhat, 1994, is a Critically Endangered (CR) bat in the IUCN Red List of Threatened Species that is currently known from only single cave located in Hanumanahalli village, Kolar district of Karnataka state, India and left with only around 150−200 individuals (Srinivasulu et al., 2014). The subterranean cave roost of *Hipposideros hypophyllus* also hosts other *Hipposideros* species, including *Hipposideros durgadasi* Khajuria, *Hipposideros speoris* (Schneider), *Hipposideros ater* Templeton, and *Hipposideros fulvus* Gray (Srinivasulu et al., 2014). Our knowledge about *H. hypophyllus* and *H. durgadasi* are restricted only to their taxonomic characteristics like morphology, anatomy, and characteristic echolocation call details, apart from their geographical distribution (Kock and Bhat, 1994; Srinivasulu et al., 2014; Srinivasulu et al., 2016). However, limited information on the diet of *H. speoris* tells us it feeds on mosquitoes, flies, beetles, and other low-flying insects, especially termites (Bates and Harrison, 1997) in twilight (Pavey et al., 2001).

As a part of our broader study, we now understand that these *Hipposideros* have preferred foraging areas that are shared by all species. Hunting habitat contains Tamarind (*Tamarindus indica*) and Millettia trees (*Millettia pinnata*), and small streams lined with trees and bushes as flyways (Under Publication). However, almost no useful ecological information like prey-predator relationship with respect to diet is available on *H. durgadasi*, *H. speoris* and *H. hypophyllus.* To this end, we aimed to investigate fecal samples to identify prey organisms in *H. hypophyllus* and two congeneric sympatric species *H. durgadasi* and *H. speoris* giving us information on bat ecology. Because these three species share the same roosting cave (Hanumanahalli) and foraging habitat, we hypothesized that there could be an overlap between the taxa of focus and congeneric species given the fact they all roosted in the same cave. We also looked at the differences in the morphological characters of fecal pellets of these three *Hipposideros* species studied.

Material and Methods

Study site

Hipposideros hypophyllus (*Hh*), roosts in a subterranean cave of a granite hillock in Hanumanahalli village (13º09'32.89" N; 78º17'30.20" E), Kolar district of Karnataka, India (Fig. 1). *H. hypophyllus* shares roost with four congeneric species – *H. speoris, H. durgadasi*, *H. fulvus*, and *H. ater* (Srinivasulu et al., 2014). However, only *H. durgadasi* (*Hd*) and *H. speoris*

(*Hs*) were found in larger numbers (*Hd* around 400 and *Hs* around 500 individuals) inside the cave while *H. fulvus* (*Hf*), and *H. ater* (*Ha*) were very few (~ 25). Hence only *H. hypophyllus*, *H. durgadasi* and *H. speoris* were considered in this study. The landscape comprises tropical dry shrubland interspersed with agricultural and horticultural land, mango, eucalyptus, and cashew plantations, rocky outcrops, water bodies, and built-up areas like roads and houses. The vegetation other than horticulture includes *Tamarindus indica* (Tamarind), *Ficus benghalensis* (Banyan), *Ficus religiosa* (Peepal), *Ficus racemosa* (Cluster Fig), *Azadirachta indica* (Neem), *Acacia* spp. (Acacia), *Millettia pinnata* (Millettia), *Lantana camara* (Common Lantana), *Eupatorium odoratum* (*Chromolaena* odorata) and *Bambusa arundinacea* (Bamboo).

Study site mapping

Study site was mapped using Google Earth Pro and ArcMap-10.3 tools with WGS 84 maps for Country, State and District boundaries.

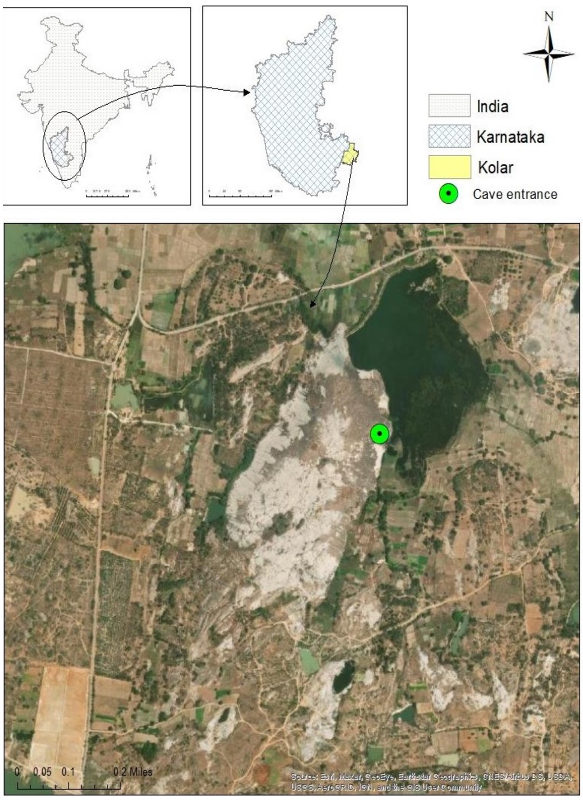

Figure 1: Study site map: Study site is in Kolar District of Karnataka State, India as shown in the figure. The study cave is in the granite hillock of the conservation reserve where all three *Hipposideros* species of the current study (*H. hypophyllus*, *H. durgadasi* and *H. speoris*) are roosting.

Sample collection

We made about 30–40 visits and spent around 20 hours inside the cave across seasons to understand the niche partitioning of species inside the cave. We observed each *Hipposideros* species using a night vision monocular (Night Owl Optics) to identify the species and noticed that they have their own niche areas within the cave. When disturbed some individuals roosted along with other *Hipposideros* species, however, the bats would fly back to their primary roost after some time. This long-term and frequent observation helped us to identify a few spots where each species of *Hipposideros* roosted exclusively. This allowed us to distinguish pellets of a particular species from others. Fecal pellets were collected by a non-invasive method every week from November 2022 to February 2023. A clean polythene sheet was spread on the cave floor right below the roosting site of each species in the evening and the freshly fallen pellets on the sheet were collected the following morning. Fecal pellets were stored in 70% ethanol in the laboratory (Sophia, 2010; Shetty, 2013) except for the photographed pellets which were brought dry in airtight containers and photographed within an hour of collection. An average of 20 pellets were collected for each species during every sampling night across the study period.

Sample analysis

I. Properties of fecal pellets

For each species, 30 pellets were selected from the collection and examined for their morphological features through direct observation and under stereo microscope (Leica MZ75). Pellets were measured using Vernier calipers (Mitutoyo).

II. Dietary prey composition

Each fecal pellet was separated under the stereo microscope and observed under a binocular microscope (Leica DM1000 and Walter Products 50 Series Binocular Microscope), searching for undigested prey remnants. In each sample, 20 pellets were randomly selected and analyzed for remnants. Prey remnants observed under the microscope were identified to Order level with the help of available literature (Borror and DeLong, 2005; Pokhrel and Budha, 2014; Shetty, 2013; Misra and Elangovan, 2016; Malar, 2020).

Statistical analysis and data interpretation

Identified prey remnants were counted for each category (Order) and the percentage of occurrences was calculated using the following formula: Percentage frequency (%F) = number of occurrences of the category/ number of samples analyzed × 100. Results were classified into one of the four categories: Primary (>20%), Secondary (5–20%), Supplementary (1–5%), and Opportunistic (< 1%) (cf. Ramanujan and Verzhutskii, 2004a, b). Data visualization (graphs) was achieved using RStudio 4.3.0.

Results

I. Properties of fecal pellets

Fecal pellets of each species had unique characteristics though they roughly appeared similar. Pellets of *H. hypophyllus* were smaller and more slender compared to the other two species (*H. durgadasi* and *H. speoris*). The color varied from dark green and brown to black; *H. durgadasi* pellets were black and usually shiny, whereas *H. speoris* pellets were like those of *H. durgadasi* but were usually not shiny. Also, typically there were well-marked constrictions in pellets of *H. durgadasi* (0–2), and *H. speoris* (1–2), whereas there was no such constriction in *H. hypophyllus* pellets (Table 1; Fig. 2).

II. Dietary prey composition

A total of 29,793 prey remnants were recorded from fecal samples of *H. hypophyllus*, *H. durgadasi*, and *H. speoris* (Table 2). The majority of the insect parts observed in the pellets were exoskeletons, legs, and antenna. In addition, we also observed fragments of wings and heads. From the current study, it was found that all three species, *H. hypophyllus*, *H. durgadasi*, and *H. speoris*, predominantly consumed Coleoptera, followed by Diptera, Lepidoptera, and Hymenoptera (Fig. 3; Table 2).

Dietary prey

In the diet of *H. hypophyllus*, prey organisms belonged to eight orders of insects: Coleoptera dominated with 57.30%, followed by Diptera-27.38%, Lepidoptera-12.13%, and Mantodea-0.02%. In the diet of *H. durgadasi* nine orders of insects were found: Coleoptera were dominant with 60.14%, followed by Diptera-19.71%, Lepidoptera-17.56%, and Orthoptera-0.01%. And finally, in the diet of *H. speoris*, a total of 10 orders of insects were found: Coleoptera dominated at 71.00%, being followed by Diptera-17.87%, Lepidoptera-7.89%, and Neuroptera-0.03% (Table 2).

Table1: Length and width of fecal pellets and the morphology of fecal pellets of all the *Hipposideros* species under study: *Hh- H. hypophyllus*; *Hd- H. durgadasi*; *Hs- H. speoris*. For each species 30 pellets were measured (sample size, n= 30).

Species	**Properties of fecal pellet (n= 30)**		
	Length; Largest diameter (Average ± Standard Deviation) (cm)	**Color**	**Morphology**
Hh	0.72±0.06; 0.11±0.03	Dark green, pale to dark brown, to black	Small in size; fresh pellets were soft to slightly hard; mostly smooth outline; small divots; no major or well-marked constriction.
Hd	1.02±0.10; 0.18±0.04	Black and usually shiny	Medium in size; very hard; small to medium divots usually with 0–2 major constrictions.
Hs	1.27±0.12; 0.21±0.07	Brown to Black; sometimes shiny	Medium in size; medium hard; small to medium divots usually with 2 major constrictions.

Table 2: Dietary composition of three hipposiderid species from the study. #, Number of prey remnants identified; %, percentage of each category of prey. *Hh* = *H. hypophyllus*, *Hd* = *H. durgadasi*; *Hs* = *H. speoris.*

Bat species and prey remnants		**Coleoptera**	**Diptera**	**Lepidoptera**	**Hymenoptera**	**Thysanoptera**	**Blattodea**	**Hemiptera**	**Mantodea**	**Odonata**	**Orthoptera**	**Neuroptera**	**Total**
Hh	#	5560	2637	1177	188	108	4	7	2	0	0	0	9703
	%	57.30	27.38	12.13	1.94	1.11	0.04	0.07	0.02	0.0	0.0	0.0	
Hd	#	7311	2396	2135	241	52	12	0	0	4	1	4	12,156
	%	60.14	19.71	17.56	1.98	0.43	0.10	0.0	0.0	0.03	0.01	0.03	
Hs	#	5633	1418	626	206	23	12	5	0	3	6	2	7934
	%	71.00	17.87	7.89	2.60	0.29	0.15	0.06	0.00	0.04	0.08	0.03	
Total number of prey remnants analysed													29,793

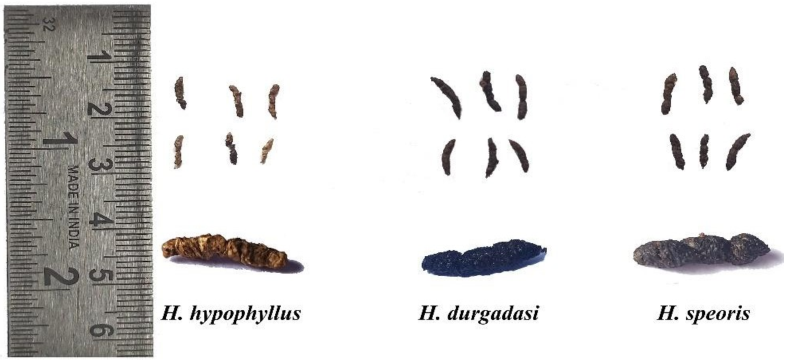

Figure 2: Comparison of fecal pellets of *Hipposideros* species under study: Fecal pellets of *H. hypophyllus* were slender and small compared to pellets of *H. durgadasi* and *H. speoris*.

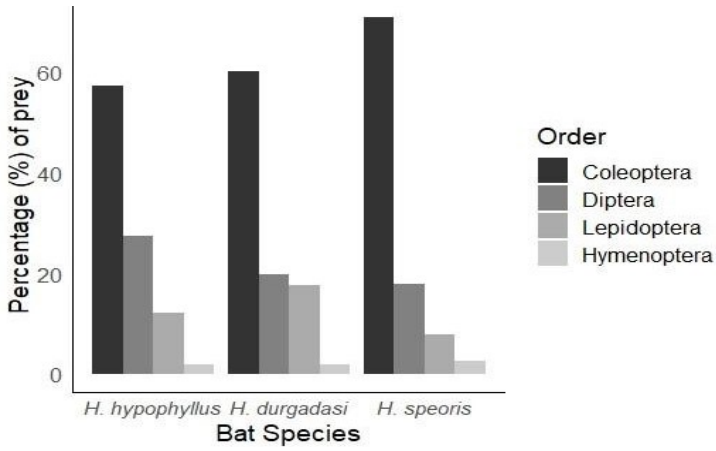

Figure 3: Dietary of three hipposiderid species: X-axis contains species names: *Hh*, *H. hypophyllus*; *Hd*, *H. durgadasi*; Hs, *H. speoris.* Y-axis contains the percentage composition of prey remnants. Diets of all three bat species are dominated by orders of Coleoptera, Diptera, Lepidoptera and Hymenoptera. Note that in the graph only the four most abundant orders are shown; the remaining data are provided in Table 2.

Dietary prey compositions

The dietary compositions of the three species were slightly different. Primary food of *H. hypophyllus* were Coleoptera and Diptera, whereas both *H. durgadasi* and *H. speoris* preyed primarily on Coleoptera. The secondary foods of *H. hypophyllus* were Lepidoptera, whereas in *H. durgadasi* and *H. speoris* they were Lepidoptera and Diptera. Supplementary food of *H. hypophyllus* were Hymenoptera and Thysanoptera whereas both *H. durgadasi* and *H. speoris* had Hymenoptera. Opportunistic food of *H. hypophyllus* included Blattodea, Hemiptera, and Mantodea, while opportunistic food of *H. durgadasi* included Thysanoptera, Blattodea, Mantodea, Odonata, Orthoptera, and Neuroptera. Opportunistic food of *H. speoris* included Thysanoptera, Blattodea, Hemiptera, Odonata, Orthoptera, and Neuroptera (Table 3).

Out of the total 11 insect orders that were identified, six Orders (Coleoptera, Lepidoptera, Hymenoptera, Diptera, Blattodea, Thysanoptera) were common in all three *Hipposideros* species; 3 orders (Odonata, Orthoptera, Neuroptera) were found only in *H. durgadasi* and *H. speoris*; 1 order (Hemiptera) was found only in *H. hypophyllus* and *H. speoris*; 1 order (Mantodea) was found only in *H. hypophyllus*; the other two hipposiderid species had no unique prey order in their diet (Fig. 4).

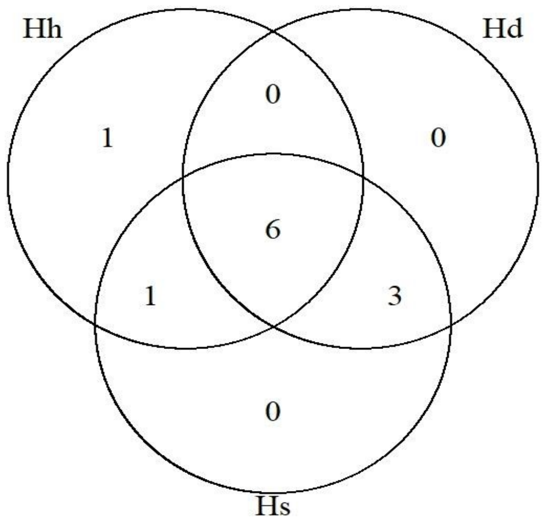

Figure 4: Overlap of prey taxa of three hipposiderid species under study: *Hh*= *H. hypophyllus*; *Hd*= *H. durgadasi*; *Hs*= *H. speoris*. Out of 11 orders of prey insect taxa identified, 6 orders were common in all three *Hipposideros* species; 3 were found only in *Hd* and *Hs*; 1 order was found only in *Hh* and *Hs*; 1 order was found only in *Hh* and other two hipposiderid species had no (0) unique prey order in their diet.

Table 3: Four categories of food preferred by three species of bats studied: *Hh* = *H. hypophyllus*, *Hd* = *H. durgadasi*; *Hs* = *H. speoris.* Primary (> 20%), Secondary (5–20%), Supplementary (1–5%) and Opportunistic (< 1%).

Bat species	Primary Food (> 20%):	Secondary Food (5–20%)	Supplementary Food (1–5%)	Opportunistic Food (< 1%)
Hh	Coleoptera, Diptera	Lepidoptera	Hymenoptera, Thysanoptera	Blattodea, Hemiptera, Mantodea
Hd	Coleoptera	Lepidoptera, Diptera	Hymenoptera	Thysanoptera, Blattodea, Mantodea, Odonata, Orthoptera, Neuroptera
Hs	Coleoptera	Diptera, Lepidoptera	Hymenoptera	Thysanoptera, Blattodea, Hemiptera, Odonata, Orthoptera, Neuroptera

Discussion

This study provides first insight into the diet of three hipposiderid bats: *H. hypophyllus*, a Critically Endangered species, *H. durgadasi*, a Vulnerable species and *H. speoris*, a Least Concerned species. This outcome was a part of an ongoing larger project focusing on the entire foraging ecology, habitat requirement, anthropogenic impact assessment and conservation approach for these rare species, with special focus on *H. hypophyllus*.

Firstly, morphology of the fecal pellets (length, diameter, and color) (Fig. 2 and Table 1) of *H. durgadasi* and *H. speoris* species appeared more similar to each other than to *H. hypophyllus*. This could indicate a different diet in *H. hypophyllus*. However, the data from the preliminary dietary analysis revealed a significant overlap in the prey composition of *H. hypophyllus* and sympatric congeners, contradicting this presumption. We henceforth suggest that there should be an increase in sample size with an aim to clarify whether bat fecal pellet morphology data could be used to predict bat diet (Ware et al., 2020).

Secondly, all the three *Hipposideros* species showed mostly overlapping prey taxa and shared foraging areas. As we anticipated, the diet compositions of all the three species overlapped (Fig. 4) extensively and consisted of four orders – Coleoptera, Diptera, Lepidoptera, and Hymenoptera respectively. This diet pattern might be influenced by a few factors such as:

(a) the methods used for the study limited the prey taxa identification only to the level of order;

(b) adaptation in foraging when the prey source is limited (Salinas-Ramos et al., 2015);

(c) nearly similar body size (average Forearm of *H. hypophyllus* – 38.9 mm; *H. durgadasi* – 37.0 mm; and *H. speoris* – 50.7 mm (*H. speoris* is significantly bigger than other two species)), because body size and wing morphology influence foraging of hipposiderid bats (Pavey and Burwell, 2000).

There is a need to explore whether bat diets are influenced by the availability of prey and to what extent they show preferences in prey consumption (Vesterinen et al., 2016; Brack and LaVal, 1985; Brigham and Saunders, 1990). Bats that feed mostly on Coleoptera and Diptera tend to have a longer, and a more robust fecal pellets (Stebbings, 1986), and we also observed this: *H. durgadasi* had very hard pellets, *H. speoris* had medium hard, and *H. hypophyllus* had relatively softer pellets (Table 1 and Fig. 3).

Our study data recorded that Coleoptera constituted basic food component in all the fecal samples (Table 3) with Diptera forming a primary food component only in *H. hypophyllus*. However, dipterans constituted secondary food items in the diets of *H. durgadasi* and *H. speoris* along with lepidopterans. Hymenopterans were only found to be supplementary food sources for all the three species. From the data (Figs. 3 and 4, Tables 1 and 3) it appears that *H. durgadasi* and *H. speoris* have greater overlap in diet composition in comparison to *H. hypophyllus*, which differed slightly from its congenerics. Similar analyses carried out throughout the year will aid in understanding the dietary resources and foraging ground which are fundamental in developing conservation action plans for such rare bats. Also, from the prey composition analysis, it was seen that all these bats play a significant role in controlling pest insects such as moths (Lepidoptera), beetles (Coleoptera) and disease-causing vectors such as mosquitoes (Diptera), by feeding on them as a major part of diet (Fig. 3).

The preliminary insights from the current study also do not undermine trophic niche differentiation between these three congeneric sympatric bats as an effective way to lessen competition for food resources and thus encouraging coexistence amongst them. The current study provides a basis for future studies on these three species from a trophic niche differentiation perspective. Parameters such as body size, wing morphology, echolocation calls, and bite force (Dai et al., 2023) can possibly throw light to analyze the degree of trophic niche overlap between *Hipposideros* congeners from Kolar, Karnataka aiding in devising appropriate conservation actions.

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Author contributions

Project Planning was done by Shraddha Kumari K, Rajesh Puttaswamaiah, and Chetan Nag K S. Fecal samples were collected and analyzed by Shraddha Kumari K. Data analysis, data interpretation and manuscript preparation were achieved by all the authors' viz., Shraddha Kumari K, Rajesh Puttaswamaiah, and Chetan Nag K S.

Conflict of interest

The authors declare that there are no conflicting issues related to this research article.

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