

# Coexistence of two sympatric and morphologically similar bat species *Rhinolophus affinis* and *Rhinolophus pearsoni*

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## Abstract

Ecologists have long focused on the coexistence of sympatric species. Here, we investigated two horseshoe bat species, *Rhinolophus affinis* and *Rhinolophus pearsoni* inhabited in the same cave, for their foraging strategies, niche differentiation, prey selection, and their coexistence status. These two species of horseshoe bats were different in the dominant frequency of their echolocation calls, but similar in their morphology. We found evidence for prey selectivity although there was a high degree of overlap in prey categories and sizes. *R. affinis* and *R. pearsoni* foraged on 16 and 7 categories insects, respectively, with Pyralidae, Geometridae, Melolonthidae dominating their diets. The degree of trophic niche overlap was 0.69. Pairwise comparisons suggested that there was no obvious differentiation in prey categories and size. However, high prey availability in the environment (Simpson diversity index = 0.79 and Margalef richness index = 4.12) contributed to their coexistence by dampening the interspecific competition. Since there are one or more mechanisms facilitating species coexistence in a community, our results suggest that the spatial niche differentiation in foraging microhabitats and in foraging habitats at landscape scale may promote the coexistence of the two bat species. However, additional field data are needed to confirm this speculation.

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**Keywords:** Bats; Species coexistence; Foraging strategies; Niche differentiation; Prey richness

## 1. Introduction

Niche differentiation has long been invoked as a mechanism of species coexistence [1]. Coexisting animal species frequently differ in resource use in at least one niche dimension and thus avoid competition [1–5]. For instance, competition by expanding pipistrelle bat populations (*Pipistrellus pipistrellus*) leads to the decline of *Rhinolophus hipposideros* for no food niche differentiation [6]. Such niche differentiation involves resources, predator, temporal and spatial dimensions, as well as different responses to conspecifics via either direct or indirect mechanisms [7].

A number of studies on closely related species have confirmed that sensory differences lead to trophic niche differentiation [8,9]. In a community of savannah-dwelling bats, correlation was recorded between the maximum bite force of the bats and the hardness of their prey's exoskeletons [10]. Differences in the prey detection systems of *Nycteris thebaica* and *Hipposideros caffer* might also lead to trophic niche differentiation [11]. One study demonstrated that difference in the sensory cues used to detect prey led to differentiation in diet composition during the summer between the two sympatric bat species *Myotis nattereri* and *Plecotus auritus* [12]. Moreover, differences in call frequency and pulse bandwidth also contributed to resource partitioning [9,13,14]. However, these studies only considered differences in sensory cues that promote trophic niche differentiation of morphologically similar bat species and the

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differentiation of prey categories, the differentiation of prey size was ignored which also be an important fact leading to species coexistence.

Trophic niche differentiation is only one mechanism of species coexistence. Many investigations have demonstrated that spatial niche partitioning also contributed to the coexistence of morphologically similar species. One study demonstrated that white-footed mice (*Peromyscus leucopus*) and golden mice (*Ochrotomys nuttalli*) partitioned in three-dimensional foraging space, which promoted long-time coexistence [15]. Other studies indicated that different foraging microhabitats between the two sympatric bats species *Myotis myotis* and *Myotis blythii* facilitated spatial niche partitioning [16], and two morphologically similar bat species *Pipistrellus pygmaeus* and *P. pipistrellus* had different food preference which led to resource partitioning and contributed to their coexistence [17].

However, niche theory only emphasizes quantitatively different effects on species coexistence based on different ecological dimensions, but does not addresses how prey richness affects the degree of trophic niche overlap. When food resources become less abundant, an intensive interspecific competition could cause a decrease in trophic niche overlap [18–24], like González-Solís et al. [25] found that the abundance of resources led to high trophic niche overlap between two sympatric gulls without affecting their coexistence, and Christopher and Barrett [15] reported that abundant prey resources promoted the coexistence of white-footed mice (*P. leucopus*) and golden mice (*O. nuttalli*) by dampening the interspecific competition. Consequently, species coexistence is not only determined by the degree of niche differentiation but also by how prey richness influences niche overlap.

In this study, we investigated two bat species *R. affinis* and *R. pearsoni* distributed in the southeastern and southwestern areas of China for elucidating how niche differentiation resulted in the existence of the two horseshoe bats based on their echolocation calls, wing morphology and prey abundance and diversity. The results provided the evidence for that the differences in their sensory cues promoted trophic niche differentiation and their coexistence.

## 2. Materials and methods

### 2.1. Study area and design of flight tent

We conducted a field survey at Shiyan cave (114°12'E, 26°36'N) in Liping village of Jinggangshan Natural Reserve, Jiangxi Province, China, from June to August in 2006. There the average temperature and the mean rainfall were 14 °C and 1865 mm per year, respectively, with a subtropical wet climate, a high vegetation coverage of 70% and a high biodiversity [26]. The standard sunset time was 19:20. The study site was dominated by a forest consisting of *Phyllostachys heterocycla*, *Cunninghamia lanceolata*, *Schima Superba* and *P. heterocycla*, and the Shiyan cave

located at the study site consisted of a main cave and a side branch of only 20 m long. *R. affinis* was found roosted in the side branch, whereas *R. pearsoni* was in the main cave. Their morphology is very similar except for the length of ear and morphology of noseleaf [27]. The numbers of individuals for *R. affinis* and *R. pearsoni* were about 1500 and 800, respectively. Both species dominated over the remaining bat species, such as *Rhinolophus macrotis* ( $n = 100$ ), *Hipposideros armiger* ( $n = 200$ ) and *Myotis davidii* ( $n = 150$ ) in the cave.

A flight tent in size of 9 m × 9 m × 4 m was erected approximately 500 m from the Shiyan cave entrance on a grassy area, where *R. affinis* and *R. pearsoni* actively foraged. The flight tent was made of a wire netting with a mesh width of 25 mm × 25 mm that allowed insects to fly in and out of the tent. A 200 W light bulb was attached to the top of the tent to attract insects but turned off for the behavioral observations and sound recordings (see below).

### 2.2. Morphometrics

Bats were captured with mist nets between 19:00 and 21:00 at the entrance of the Shiyan cave and were recognized as adults or juveniles by inspecting epiphyseal fusions of the bats' forearms [28]. Since morphology and echolocation calls between adults and juveniles are significantly different, only adults were measured. All bats were grouped by sex and weighed on an Ohaus LS 200 balance (precision 0.1 g), and their forearm and ear length were measured with a ruler at a precision of 1 mm. Using the criteria layed out by Bowie and Jacobs [11] and Norberg and Rayner [29], wingspan, wing area, wing loading, tip length ratio, tip area ratio and tip shape ratio of the bats were measured and calculated. The data were obtained from 14 adults of *R. affinis* (11 female, 3 male) and 15 adults of *R. pearsoni* (14 female, 1 male).

### 2.3. Echolocation call recordings and analysis

Echolocation calls of all bats were recorded in the flight tent under a semi-free flight condition with an ultrasonic detector (U30, ultra sound advice, UK). After processing with an ultra sound device PUSP (UK), the data were input into a notebook PC and subsequently analyzed using "Batsound professional" software (Batsound 3.10). Spectrogram and power spectra were produced using a 1024-sample Fast Fourier Transform (FFT) at sampling frequency of 44.1 kHz with a Hanning window. The number of harmonics, frequencies of the fundamental constant frequency component (CF1) and of the second harmonic (CF2), pulse duration, inter-pulse interval, bandwidth of downward frequency-modulated terminal component of the second harmonic (FM) and duty cycle were calculated [30]. Additionally, following Lawrence and Simmons [31] and Kingston and Rossiter [32], the maximal detection distance for the two horseshoe bats was calculated.

#### 2.4. Observation of foraging behavior of bats and the collection of culled parts of prey

Foraging behavior of *R. affinis* and *R. pearsoni* was observed in the flight tent. The light was turned on in order to trap insects at approximately 19:30 every night. When a large number of insects were trapped in the flight tent, the light was turned off for not to disturb the foraging performance of the bats. We observed foraging behavior of the bat with a night vision binoculars (YU-NV-25022, Yukon) and documented them with an infrared camera (Sony PC-101E). All bats (14 *R. affinis* and 15 *R. pearsoni*) observed were the individuals whose external morphology had been measured. Five bats were observed every night and each bats was observed for five nights. Only one bat at a time was allowed to forage in the tent and was observed for 1 h. During the observation, insect fragments dominated by Lepidoptera and Coleoptera that were rejected after bat took food were collected, such as forewing, underwing, antennae, crura, and head. After the end of the behavioral observations every night the bats were left to continue foraging in the flight tent and the insect fragments were collected at the next morning. Since insectivorous bats eat only relatively soft insect parts, the remaining harder chitin fragments reflect major food habits of bats [33]. The foraging behavior of *R. affinis* and *R. pearsoni* was observed over a total period of 10 and 13 nights, respectively. To minimize differences in the diversity and the abundance of the insects, behavioral observation of the two horseshoe bat species was alternated from day to day. In addition, the following two experiments were designed. In the first one, a foam pad (50 cm × 30 cm × 10 cm) was placed on the ground in the center of the flight tent, then a live moth was tethered to a nylon thread (0.1 mm in diameter), which was then attached to the foam pads with pins. When the moth was fluttering, we observed whether the bats would exhibit gleaning behavior close to the ground. In the second experiment, when the bats were actively foraging in the flight tent, live fluttering moths were held against the grass on the flight tent floor or against the wall of the flight tent with a pair of blunt forceps and the reactions of the bats to the wing-beating moths were observed.

After the end of all these experiments, all bats were released at the capture site.

#### 2.5. Diet analysis

The insects were trapped during 10 nights from 20:00 to 23:00 using incandescent security lights set up in different locations around the flight tent (probably 0–2 km away from the flight tent) in order to investigate the abundance and the diversity of insect species and to calculate the insects diversity and richness index of the study area. Prey fragments collected and insects trapped were identified in the laboratory to a special order or family level. Further, the total number and percentage of each insect categories eaten by the two horseshoe bats were calculated as the rep-

resentation of insects in the flight tent in order to be compared with light-trap samples in the environment. In addition, the forewings length of insects were measured, and based on that the insects were divided into eight groups with a 5 mm interval.

The food-niche Breadth (FNB) [34] for the two horseshoe bats was calculated by

$$\text{FNB} = 1 / \sum_{i=1}^n P_i j^2 \quad (1)$$

where  $P_i$  is the proportion of the  $i$ th prey category of species  $j$ .

The degree of trophic niche overlap between the two horseshoe bats was calculated using Schoener's niche overlap index [35]:

$$\text{NOI} = 1 - (1/2) \left( \sum_{i=1}^n |P_{ij} - P_{ik}| \right) \quad (2)$$

where  $P_i$  is the proportion of the  $i$ th prey category of species  $j$  and  $k$ .

Prey resource diversity index in the environment was calculated using the Simpson index ( $D$ ) [36]:

$$D = 1 / \sum_{i=1}^n (P_i)^2 \quad (3)$$

where  $P_i$  is the proportion of the  $i$ th prey category in the environment.

Richness index of respective diet, total diet of *R. affinis* and *R. pearsoni* and available insects in the environment was calculated using the Margalef richness index ( $d$ ) [37]:

$$d = (S - 1) / \ln N \quad (4)$$

where  $S$  is the number of insect categories and  $N$  is the total number of individual of all insect categories.

#### 2.6. Statistical analysis

Because some data sets could deviate significantly from normal distribution (Kolmogorov–Smirnov test using Lilliefors adaptation;  $P < 0.05$ ), a Mann–Whitney  $U$ -test was used to test for the differences between the two horseshoe bats in morphology and echolocation call characteristics. Forewing length difference in the dietary composition of the two bat species was tested using a contingency  $\chi^2$ -test and a  $G$ -test [38]. In addition, the difference of insect categories between the flight tent and the environment was tested using  $G$ -test.

### 3. Results

#### 3.1. Morphometrics

Among 10 morphological parameters measured (see Table 1), significant differences between *R. affinis* and *R. pearsoni* were detected in ear length, wing area and aspect

Table 1  
Comparison of the morphological characteristics (means  $\pm$  SD) of *Rhinolophus affinis* and *Rhinolophus pearsoni*

	<i>Rhinolophus affinis</i>	<i>Rhinolophus pearsoni</i>	Mann–Whitney <i>U</i> -test ( <i>P</i> )
<i>n</i>	14	15	–
Mass (kg)	0.013 $\pm$ 0.008	0.014 $\pm$ 0.001	64.0 (0.073)
Forearm length (m)	0.052 $\pm$ 0.002	0.053 $\pm$ 0.015	78.0 (0.209)
Ear length (m)	0.019 $\pm$ 0.014	0.023 $\pm$ 0.001	6.5 <sup>a</sup>
Wingspan (m)	0.349 $\pm$ 0.008	0.354 $\pm$ 0.007	60.5 (0.052)
Wing area (m <sup>2</sup> )	0.018 $\pm$ 0.001	0.019 $\pm$ 0.001	28.5 <sup>b</sup>
Aspect ratio	6.871 $\pm$ 0.148	6.589 $\pm$ 0.368	45.0 <sup>c</sup>
Wing loading (N/m <sup>2</sup> )	7.284 $\pm$ 0.565	7.129 $\pm$ 0.593	92.0 (0.570)
Tip length ratio	1.222 $\pm$ 0.039	1.223 $\pm$ 0.044	79.5 (0.266)
Tip area ratio	0.092 $\pm$ 0.084	0.921 $\pm$ 0.042	83.0 (0.335)
Tip shape ratio	3.189 $\pm$ 0.819	3.164 $\pm$ 0.589	93.0 (0.600)

<sup>a,b</sup> There are significant differences between *Rhinolophus affinis* and *Rhinolophus pearsoni* ( $P < 0.001$ ).

<sup>c</sup> There is significant difference between *Rhinolophus affinis* and *Rhinolophus pearsoni* ( $P < 0.01$ ).

ratio. *R. pearsoni* had longer ears than *R. affinis*, a larger wing area, and a smaller aspect ratio (Table 1).

### 3.2. Characteristics of echolocation calls

Both *R. affinis* and *R. pearsoni* produced long constant-frequency echolocation calls, which were preceded and followed by brief frequency-modulated components (FM-CF-FM). The bat emitted calls with a high duty cycle while foraging in the tent (Fig. 1). Each call of *R. affinis* included two harmonics with the second harmonic being the strongest, whereas calls of *R. pearsoni* only contained one, presumably the second, harmonic component (Fig. 1).

The calls of *R. affinis* had higher dominant frequencies than those of *R. pearsoni* (Table 2), and there were significant differences in the bandwidth of the terminal FM of the second harmonic ( $13.867 \pm 1.548$  kHz for *R. affinis* vs  $17.321 \pm 1.806$  kHz for *R. pearsoni*;  $U_{30,28} = 67.500$ ,  $P < 0.001$ ) (Table 2). The other call parameters, however, did not show any significant differences. In addition, the maximal detection distances of *R. affinis* and *R. pearsoni* were 3.0 and 4.3 m, respectively.

### 3.3. Foraging behavior of the bats

Both horseshoe bat species foraged using aerial hawking and flycatcher style, and responded preferably to echoes from fluttering insects. When the bats encountered fluttering Coleoptera, both the species immediately approached the insects and captured them. *R. affinis* (50%) had a higher foraging success rate (foraging attempt of bats divided by the number of foraging success) than *R. pearsoni* (20%). Even though there were a great number of insects in the flight tent, *R. pearsoni* was less actively flying around. It also appeared that *R. pearsoni* preferably scanned for prey closer to the ground, but did not prey upon insects.

We virtually never observed ground gleaning for insects in this study, only in one instance: *R. affinis* detected a Geometridae that was fallen to the ground and remained there beating its wings. The bat immediately approached the moth but ultimately failed capturing it. In addition, in all instances when we held live fluttering moths against the grass covering the floor or against the sidewalls of the flight tent, both the horseshoe bat species showed no obvious reaction.

### 3.4. Diet analysis

Culled parts of prey consumed by *R. affinis* and *R. pearsoni* and of all insects trapped in the immediately surrounding area are summarized in Table 3. A total of 29 insect categories and 894 individuals (25 families of 4 orders) were trapped in the area. The dominant categories were Pyralidae (242, 27.07%), Geometridae (236, 26.39%), Noctuidae (214, 23.93%) and Melolonthidae (58, 6.48%). The Simpson diversity index and the Margalef richness index for all insects in the area were 0.79 and 4.12, respectively. Moreover, the latter was much higher than that of respective diet, total diet of *R. affinis* and *R. pearsoni*, which indicated a very high degree of diversity and availability of insects (Table 3). The prey fragments of *R. affinis* (272) and *R. pearsoni* (228) were collected,

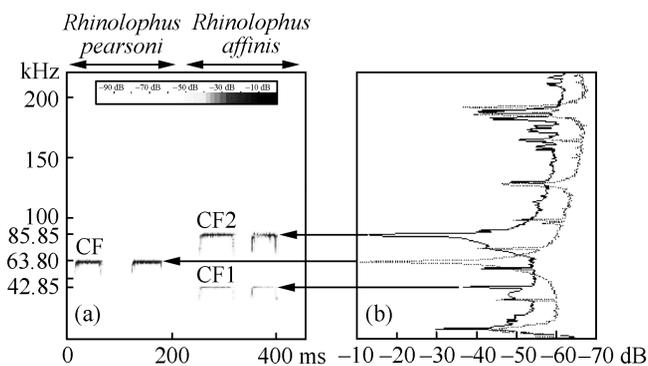


Fig. 1. Echolocation calls emitted by *Rhinolophus affinis* and *Rhinolophus pearsoni*. (a) Spectrogram; (b) power spectrum (the solid line and dash line represent *Rhinolophus affinis* and *Rhinolophus pearsoni*, respectively).

Table 2

Comparison of echolocation calls characteristics (means  $\pm$  SD) of *Rhinolophus affinis* and *Rhinolophus pearsoni* using Mann–Whitney *U*-test

Species	<i>n</i>	Spectrogram	1st harmonic (kHz)	2nd harmonic (kHz) <sup>a</sup>	Duration (ms)	Inter-pulse interval (ms)	Bandwidth of down-FM (kHz) <sup>b</sup>	Duty cycle (%)
<i>Rhinolophus affinis</i>	28	FM-CF-FM	42.86 $\pm$ 0.30	85.86 $\pm$ 0.59	46.48 $\pm$ 3.78	45.19 $\pm$ 4.42	17.32 $\pm$ 1.81	50.70 $\pm$ 2.72
<i>Rhinolophus pearsoni</i>	30	FM-CF-FM	–	63.80 $\pm$ 0.55	45.78 $\pm$ 4.80	46.09 $\pm$ 3.77	13.87 $\pm$ 1.55	49.82 $\pm$ 3.53

<sup>a,b</sup> There are significant differences between *Rhinolophus affinis* and *Rhinolophus pearsoni* ( $P < 0.001$ ).

Table 3

Dietary composition of *Rhinolophus affinis* and *Rhinolophus pearsoni* and the total number and percentage of insects eaten by the two horseshoe bats and trapped in the environment

	<i>Rhinolophus affinis</i> <i>N</i> (%)	<i>Rhinolophus pearsoni</i> <i>N</i> (%)	<i>Rhinolophus affinis</i> and <i>Rhinolophus pearsoni</i> <i>N</i> (%)	Light-trap samples in the environment <i>N</i> (%)	<i>G</i> -test ( <i>P</i> ) <sup>a</sup>
Geometridae	44(16.17)	32(14.04)	76(15.20)	236(26.39)	3.05(0.080)
Sphingidae	4(1.47)	–	4(0.80)	2(0.22)	–
Lithosiidae	–	–	–	6(0.64)	–
Lymantriidae	8(2.94)	–	8(1.60)	28(3.13)	0.50(0.477)
Arctiidae	–	4(1.76)	4(0.80)	10(1.11)	–
Thyatiridae	–	–	–	4(0.47)	–
Limacodidae	4(1.47)	–	4(0.80)	2(0.22)	–
Lasiocampidae	4(1.47)	4(1.75)	8(1.60)	8(0.89)	–
Noctuidae	32(11.76)	20(8.77)	52(10.40)	214(23.93)	5.48 <sup>b</sup>
Pylalidae	88(32.35)	128(56.14)	216(43.20)	242(27.07)	3.73(0.53)
Nymphalidae	4(1.47)	–	4(0.80)	4(0.47)	–
Tortricidae	4(1.47)	–	4(0.80)	6(0.67)	–
Saturniidae	–	–	–	2(0.22)	–
Rutelidae	16(5.88)	–	16(3.20)	16(1.78)	0.41(0.521)
Melolonthidae	36(13.23)	32(14.04)	68(13.60)	58(6.48)	2.58(0.108)
Cetoniidae	8(2.96)	–	8(1.60)	2(0.22)	1.18(0.277)
Scarabaeidae	–	–	–	4(0.43)	–
Cerambycidae	–	–	–	6(0.67)	–
Carabidae	–	–	–	2(0.22)	–
Chrysomelidae	–	–	–	4(0.47)	–
Corydalidae	4(1.47)	–	4(0.80)	6(0.67)	–
Tipulidae	–	–	–	4(0.47)	–
Tettigoniidae	–	–	–	2(0.22)	–
Ichneumonidae	4(1.47)	–	4(0.80)	4(0.47)	–
Chrysopidae	4(1.47)	–	4(0.80)	6(0.67)	–
Hemiptera	8(2.95)	–	8(1.60)	4(0.44)	0.70(0.402)
Trichoptera	–	–	–	2(0.22)	–
Plecoptera	–	8(3.50)	8(1.60)	4(0.47)	0.65(0.419)
Blattaria	–	–	–	6(0.67)	–
Trophic niche breadth	5.88	2.76	–	–	–
Trophic niche overlap	0.69	–	–	–	–
Simpson diversity index	–	–	–	0.79	–
Richness index	2.68	1.11	2.74	4.12	–

<sup>a</sup> Difference of insect categories between the flight tent (as the representation of total insects eaten by the two horseshoe bats) and in the environment was tested using *G*-test.<sup>b</sup> There is significant difference in Noctuidae between the flight tent and in the environment ( $P < 0.05$ ).

respectively. *R. affinis* foraged on 18 prey categories (17 families and 1 order), with Pylalidae (88, 32.35%), Geometridae (44, 16.17%), Melolonthidae (36, 13.23%) and Noctuidae (32, 11.76%) representing the dominant categories (Table 3). Diet fragments of *R. pearsoni* belonged to 7 prey categories (6 families and 1 orders), most of them from Pylalidae (128, 56.14%), Geometridae (32, 14.04%)

and Melolonthidae (32, 14.04%). Interestingly, the diet of *R. pearsoni* contained Arctiidae (4, 1.76%) and Plecoptera (8, 3.50%), the insects that were never found in the diet of *R. affinis*. In addition, the insects in the flight tent (representing as the sum of total prey eaten by the two horseshoe bats) also demonstrated that the dominant categories were Pylalidae (216, 43.20%), Geometridae (76,

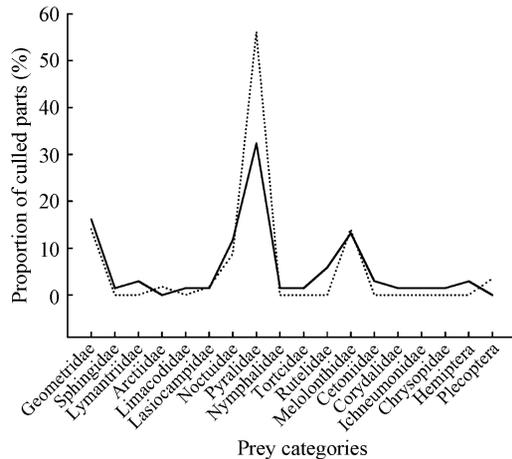


Fig. 2. The degree of trophic niche overlap between *Rhinolophus affinis* (solid line) and *Rhinolophus pearsoni* (dashed line).

15.2%), Melolonthidae (68, 13.6%) and Noctuidae (52, 10.40%) (Table 3).

The food-niche breadth of *R. affinis* was found to be considerably wider than that of *R. pearsoni* (5.88 vs 2.76; Table 3 and Fig. 2). The diet of *R. affinis* was more general whereas that of *R. pearsoni* was more limited, most notably on Pyralidae (56.14%) (Fig. 2). The trophic niche overlap index between *R. affinis* and *R. pearsoni* was 0.69.

Comparing the forewing length of 9 insect categories (>1%) (Table 1) in the flight tent with those of light-trap samples in the environment, there were no significant differences in 8 insect categories (Geometridae,  $G = 3.05$ ,  $df = 0.080$ ; Lymantriidae,  $G = 0.50$ ,  $df = 0.477$ ; Pyralidae,  $G = 3.73$ ,  $df = 0.053$ ; Rutelidae,  $G = 0.41$ ,  $df = 0.521$ ; Melolonthidae,  $G = 2.58$ ,  $df = 0.108$ ; Cetoniidae,  $G = 1.18$ ,  $df = 0.277$ ; Hemiptera,  $G = 0.70$ ,  $df = 0.402$ ; Plecoptera,  $G = 0.65$ ,  $df = 0.419$ ) except Noctuidae ( $G = 5.48$ ,  $df = 0.019$ ). Neither a contingency  $\chi^2$ -test nor a  $G$ -test of the forewing length distribution of insects hunted by *R. affinis* and *R. pearsoni* yielded a significant difference ( $\chi^2 = 11.06$ ,  $df = 7$ ,  $P = 0.14 > 0.05$  and  $G = 13.3$ ,  $df = 7$ ,  $P = 0.06 > 0.05$ ) (Fig. 3) despite the fact that there were

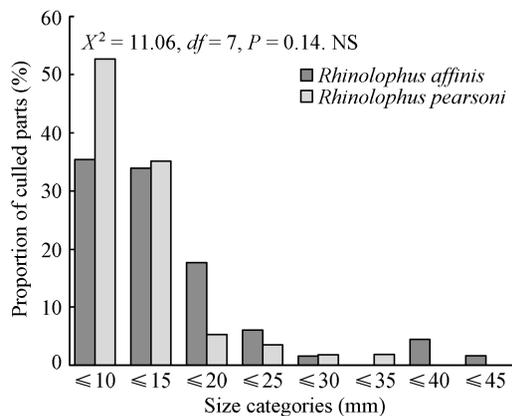


Fig. 3. The forewing length distribution of insects hunted by *Rhinolophus affinis* and *Rhinolophus pearsoni*. NS, non-significant ( $P > 0.05$ ).

significant differences between the two groups ( $\leq 20$  and  $\leq 40$  mm) ( $G = 5.78$ ,  $P = 0.016 < 0.05$  and  $G = 4.15$ ,  $P = 0.04 < 0.05$ ).

#### 4. Discussion

Many published data on prey taken by insectivorous bats are from stomach contents or fecal analysis [39], but prey fragments in the feces of bats may be identified only to order level but hardly to family level, this method may not reflect finely trophic niche of bats. Culled parts below the roosts can be usually useful in diet analysis for its reliable results and easy identification [40–47]. It is indicated that CF or FM-CF-FM bats specialize on lepidopteran or coleopteran prey based on stomach content [48–50], culled parts [46,51] and feces [51] analysis, and many culled parts are discarded. Therefore, collecting culled parts in flight tent was selected by us to study finely the trophic differentiation degree between *R. affinis* and *R. pearsoni* which both produced long constant frequency echolocation calls (FM-CF-FM) (Fig. 1). Moreover, although the total insect categories eaten by the two horseshoe bats were much less than those collected by light-trap in the environment (18 vs 29), the epactal 11 insect categories only contributed 4.9% of light-trap samples (Table 3). Simultaneously, comparing the 9 insect categories in the flight tent (representing as the total insect eaten by the two horseshoe bats) with those of light-trap sample in the environment, there were no significant differences in the 8 insect categories except Noctuidae ( $G = 5.48$ ,  $df = 0.019$ ) (Table 3). This may be the result of underestimating the insect percentage of the flight tent because not all of the insects in it were hunted by bats. So we considered that the categories and the number of insects in the flight tent could predominantly represent the natural environment. In addition, culled parts (forewing length <4 mm) were not found in the food composition of the two horseshoe bats, which may not be the reason that small preys (<4 mm) were eaten entirely. But their call frequencies confine that they seem unlikely to hunt these insects (forewing length <4 mm) because reflectivity decreases sharply when the wavelength (3.96 mm wavelength for *R. affinis* and 5.33 mm wavelength for *R. pearsoni*) exceeds the wing length of the ensouled insects [52]. So this showed that small preys have not been overlooked in diet analysis. Finally, insects (especially Lepidoptera or Coleoptera) do react to incandescent security lights, so, insofar as the area around the light trap was ecologically similar to that where the bats hunted, and the samples from the light trap may have accurately represented the Lepidoptera and Coleoptera available in the general area [46]. Though *R. affinis* and *R. pearsoni* foraged only in the flight tent, but it was erected in the high forage activity range (approximately 500 m from Shiyang cave) and had intensive illumination. And, the space of flight tent (9 m × 9 m × 4 m) was big enough for bats' normal forage and flight height of insects [33]. So, insofar as the area around the flight tent was ecologically similar to that where

the two bats hunted. Therefore, our method about diet analysis of *R. affinis* and *R. pearsoni* is reasonable and credible.

The trophic niche overlap between *R. affinis* and *R. pearsoni* was relatively high (0.69), suggesting that there was little difference in their respective trophic niche. This trophic niche overlap (Fig. 2) was large because both the species fed mainly on Pyralidae, Geometridae and Melolonthidae, although the food-niche breadth of *R. affinis* was considerably wider than that of *R. pearsoni*. Our result is consistent with the data from another study which showed that 26 sympatric and morphologically similar insectivorous bat species had similar diets [53]. However, this may be resulted from high prey diversity and availability in the environment in summer, because abundance of food resources often leads to a high trophic niche overlap by dampening the interspecific competition [18,20].

The prey detection hypothesis suggests that difference in call frequency may lead to differentiation in prey size [54,55]. The calls of higher frequency are predicted to reflect more strongly from small prey items than the calls of lower frequency [52]. However, although the dominant frequency of *R. affinis* ( $85.86 \pm 0.59$  kHz,  $n = 28$ ) was higher than that of *R. pearsoni* ( $63.80 \pm 0.55$  kHz,  $n = 30$ ), the diets of them had no distinct difference in prey size (Fig. 3) in spite of the two group insects ( $\leq 20$  and  $\leq 40$  mm, respectively) having a significant difference in forewing length ( $G = 5.78$ ,  $P = 0.016 < 0.05$  and  $G = 4.15$ ,  $P = 0.04 < 0.05$ , respectively) for they were not preyed on mainly by the two horseshoe bats (Fig. 3). Therefore, our results indicated that the difference in target strength is even less likely to distinguish *R. affinis* from *R. pearsoni* as their wavelengths differ by only 1.37 mm (3.96 mm wavelength for *R. affinis* and 5.33 mm wavelength for *R. pearsoni*). Jones and Barlow [56] suggested that a 10 kHz (1.4 mm wavelength) difference in call frequency between the two cryptic species of pipistrelles is not sufficient to influence target strengths from the major prey types.

Several studies suggest that long ears are associated with acute low-frequency hearing enabling these bats to hunt by passively listening to prey-produced sound [9,57,58]. Although *R. pearsoni* had longer ears than *R. affinis*, our diet analysis for either bat species did not find any fragments of insects producing notable noise that the bats could use to locate them passively, such as Scarabaeidae and Carabidae [9]. This was supported by the results of behavioral observation, for we found that *R. pearsoni* did not hunt Scarabaeidae and Carabidae, even though there were a large number of Scarabaeidae and Carabidae in the flight tent. So our results indicated that *R. pearsoni* may not forage by listening to prey-produced sound. Therefore, sensory ecology in *R. affinis* and *R. pearsoni* did not show difference, and both horseshoe bat species foraged using aerial hawking and flycatcher style, which led to no obvious difference in their trophic

niche. Consequently, coexistence of them cannot be explained by trophic niche, which negated our hypotheses.

Abundance of food resources often leads to a high trophic niche overlap between two species by dampening the interspecific competition [18,20,59]. A particularly interesting case occurred in southern England and Wales where the decline of insects richness led to the decrease of the number of bats [60]. Similarly, abundant food resources contribute to the coexistence of two gull species, Audouin's gulls *Larus audouinii* and yellow-legged gulls *Larus cachinnans*, despite the fact that the trophic niche overlap index between the two species was 0.97 and 0.85 in different phase, respectively [25], also dampen interspecific competition between white-footed mice *P. leucopus* and golden mice *O. nuttalli*, thus promotes their coexistence [15]. A model simulation also revealed that two species restricted by similar power resources could coexist [61]. Different populations can coexist while maintaining growth indefinitely as long as resources are not limiting [62]. Darlington [63] indicated that when, and only when, limitation of the resource is the direct and only factor limiting the population of both species, competitive repulsion may occur. And Chesson [7] showed that coexistence requires that intraspecific competition exceeds interspecific competition. We found that the prey diversity and richness index of the insects trapped in the area surrounding the study site was 0.79 and 4.12, respectively. Simultaneously, there were much higher number richness of insects than that eaten by *R. affinis* and *R. pearsoni* in the environment, which therefore should have led to more intense intraspecific than interspecific competition between the two bat species [18,22,25], and thereby increase the overlap degree of trophic niche. Therefore, even though the overlapping degree of trophic niche was 0.69, competitive exclusion would not occur because abundant food resources availability ensures coexistence between the two horseshoe bats.

However, the richness degree of food resources often varies with the environment fluctuation and season change, so we cannot exclude that additional mechanisms, especially when concerning food shortage, may be involved.

It is commonly thought that different call frequencies represent adaptations to different forage microhabitats (the foraging habitat hypothesis) [64,65]. *R. affinis* and *R. pearsoni* both produced pure and long constant frequency (FM-CF-FM) echolocation calls with a high duty cycle, which allow them to exploit prey resources around and within dense vegetation [64]. A relatively low wing loads, aspect ratio and a relative high tip shape ratio of horseshoe bats (Table 1) also support this notion [29]. Feng [66] showed that *R. affinis* forages on insects in cluttered habitats such as branches, leaves and boskage. *R. pearsoni* often hunt insects within a 2–6 m distance in canopy and above hardwood of evergreen broadleaf and hardwood forest [67]. These observations were corroborated by our

estimation of the maximal detection distances of *R. affinis* and *R. pearsoni*, which were 3.0 and 4.3 m, respectively. This indicates that *R. pearsoni* ( $63.80 \pm 0.55$  kHz,  $n = 30$ ) is less adapted than *R. affinis* ( $85.86 \pm 0.59$  kHz,  $n = 28$ ) for foraging in cluttered spaces and is likely to prefer microhabitats that are somewhat more open. Consequently, we suggest that differences in foraging microhabitats may contribute to the coexistence of the two horseshoe bat species. This aspect, however, needs to be specifically addressed in further field studies.

Partitioning of foraging habitats not at the microhabitat level but also at landscape scale may be another mechanism for coexistence of *R. affinis* and *R. pearsoni*. This has been demonstrated in the two sibling bat species *M. myotis* and *M. blythii*, where resource partitioning is based on their preference for different foraging habitats [16]. Bats are social mammals and often form large groups, which presumably is beneficial [68–70]. Based on Lotka-Volterra's competition model, Hanski [71] proposed that group distribution contributes to species coexistence. In addition, Norberg and Rayner [29] indicated that species of Rhinolophidae having a higher aspect ratio and smaller wing area may relate to lower energy loss and much quicker flight speed. Therefore, *R. affinis* might have larger foraging range than *R. pearsoni* because the former has a higher aspect ratio and smaller wing area than the latter (Table 1). Consequently, it is conceivable that *R. affinis* and *R. pearsoni* may forage in different landscape patches and habitats, and spatial niche differentiation at the landscape scale contributes to their coexistence. But future field studies may be required to examine this prediction.

Just as “plankton paradox” of Hutchinson [72], the neutral theory proposes that species are all the same ecologically and their abundance simply fluctuates randomly over time [1,58,73,74]. Scheffer and Van Nes [75] indicated that sufficiently different or similar enough species can coexist. In this study, the morphology and the diets of *R. affinis* and *R. pearsoni* were highly similar, so they may locate the same clump in a niche axis [75] and contribute to together community dynamics [1]. Consequently, being sufficiently similar may promote coexistence of the two horseshoe bats, despite the fact that the clump may be transients [76,77].

In conclusion, our results suggest that there was no clear differentiation in the trophic niche of *R. affinis* and *R. pearsoni*, but that a relatively high abundance of prey promoted their coexistence. Moreover, they may also coexist because of their similarity in morphology and diet between them. In addition, we propose that the spatial niche differentiation in foraging microhabitats and foraging habitats at landscape scale may contribute to their coexistence, but this needs to be examined in future field work. Since species coexistence may be facilitated by one or several mechanisms [7,73], it is essential to consider each possible mechanism in an integrative way, especially in the relative long time span.

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