Analysis of Acoustic Behavior of Horseshoe bats of Kalakad Mundanthurai Tiger Reserve (KMTR)

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Abstract – Cramming echolocation calls is a crucial factor in identifying bat species and monitoring their habitats. Acoustic methods are used increasingly to survey and monitor bat populations. It is a new venture in Kalakad Mundanthurai Tiger Reserve (KMTR). The Reserve situated in the South Western Ghats of India is one among the 18 world biodiversity hotspots. It is bound by forests in west, north and south and by villages in the east. The aim of this study was to investigate the use of echolocation call structure to identify Horseshoe bats in the family Rhinolophidae from KMTR. Acoustic detectors have been used for monitoring flight activity of bats. This study mainly focused on analyzing pulse duration, pulse interval, peak frequency, start frequency, ending frequency, number of harmonies, and maximum power of horseshoe bats' calls. According to the frequency-time spectra, these calls were categorized into four types: broadband FM, narrowband FM, long multiharmonic and short multiharmonic. These echolocation data provide significant background information for bat conservation efforts such as surveying and future research on analyzing other bat species' representative search phase calls. Effective monitoring of echolocation calls is vital in many studies of the ecology and conservation of bats in this region.

***Key words*: Echolocation, Horseshoe bats, KMTR, Acoustic detectors, conservation efforts**

I INTRODUCTION

Electronic acoustic devices have been developed that allow investigators to hear or visualize ultrasonic echolocation calls of bats (Fenton 1995). Variation in the acoustic structure of bat echolocation calls often provides sufficient information for reliable and efficient species identification. Call identification methods are important in developing efficient cost-effective methods for large-scale bioacoustics surveys for global biodiversity monitoring and conservation planning. Bats (order Chiroptera) with over 1200 species are the second-largest order of mammals (Simmons 2005). They are considered to be important indicators of wider environmental health as they play key roles in ecosystems. Most Microchiroptera bats mainly rely on echolocation for detecting and preying on flying insects, identifying nearby obstacles, and mapping flight paths in the dark (Zhang *et al* 2005, Schnitzler *et al* 2001).

1.1 Biosonar

Echolocation, also called biosonar, is the biological sonar used by several kinds of animals. They use these echoes to locate and identify the objects. A single emission of sound is referred to as a call and a series of calls is a call sequence (Fenton 1999). Echolocation calls of bats consist of three phases: search, approach, and terminal (Griffin *et al* 1960). Search phase calls are produced to locate prey, approach phase calls are produced to identify exact locations of prey, and terminal phase calls are produced just prior to capture. Bats emit varied echolocation patterns and altered frequency of echolocation according to different environments, situations and types of prey.

1.2 Signal Types

1.2.1 FM bats:

According to Altringham (1996), bats emit echolocation sounds in pulses. The pulses vary in properties depending on the species, and can be correlated with different hunting strategies and mechanisms of information processing (Grinnell 1995). Most families use short, downward frequency- modulated (FM) sounds that sweep through about an octave. The signal bandwidth is further increased by one or more harmonics. The acoustics signal types are represented in plate 1

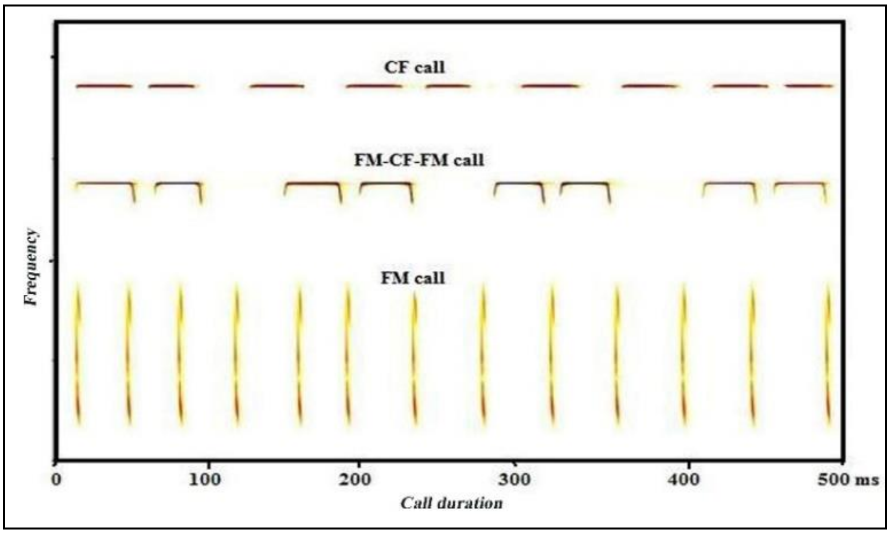
1.2.2 Short CF/FM bats

There also exists numerous species that employ an intermediate pulse design, with pulses containing a short CF component of up to 8–10 ms and terminating in a FM sweep (Grinnell 1995). It is also common for bats to modify the pulse structure according to the environment. Some species emit pure FM signals when close to vegetation, but in uncluttered environments prolong the pulse and reduce the amount of sweep to be able to detect faint echoes from remote targets.

1.2.3 Long CF/FM bats

Another common echolocation signal pattern is constant-frequency (CF) signals. Long CF/FM pulses are used by a much smaller number of species. These signals have a long (10–100 ms) constant-frequency component preceding an FM sweep (Grinnell 1995). The CF components are approximately 60–62 kHz and 83 kHz. Although individual bats exhibit slight frequency differences, the signals are very consistent in any given bat (Schuller and Pollak 1979, Grinnell 1995, Fenton 1995). Long CF/FM bats usually hunt in cluttered environments where prey detection is harder for bats that use only FM signals.

Plate 1 - Acoustics signal pattern types



1.3 Impact

As insectivorous, horseshoe bats use echolocation to adapt to the environment and prey on flying insects (Kingston 2010). However, very few studies and research have been conducted on the *Rhinolophus* species of KMTR. The present research has been conducted to analyze their basic and characteristic echolocation patterns in order to contribute to and lay fundamental data for further research on echolocation for species identification and ecological surveys on *Rhinolophus* species of KMTR.We believe that our study will help reveal the potential for using acoustic identification in rapid and efficient surveys of bats in forest ecosystem, where deforestation rates are the highest in the tropics (Achard *et al* 2002; Sodhi *et al* 2004, 2006) and biodiversity is also high (Myers *et al* 2000; Cardillo *et al* 2006; Kingston *et al* 2006). Indeed, if current deforestation rates persist, 40% of bat species within the region may become extinct by the end of the century (Kingston 2010). Species inventories need to be developed urgently to allow the prioritization of areas for research and conservation, and so monitoring protocols must be established to identify areas where conservation efforts should be prioritized.

II MATERIALS AND METHODS

2.1 Study Sites and Animals

The study area Kalakad Mundanthurai Tiger Reserve is situated in the southern Western Ghats region (Latitude 8^{o} 25' North to 8^{o} 53' North, Longitude 77^{o} 10' East and 77^{o} 35' East) and extends over 895 sq. km. Distributions of *Rhinolophid* bat species in the study area are reported on plate 2. The present research recorded echolocation sounds of Rhinolophids inhabited Kalakad Mundanthurai Tiger Reserve. To conduct the experiment, four species of Horseshoe Bats that inhabited abandoned buildings and cave roosts located in KMTR were captured by using harp traps and mist nets. Each bat's weight and length of its forearm was measured by using calipers and portable scale. The four species are *Rhinolophus rouxii, Rhinolophus lepidus, Rhinolophus pusillus* and *Rhinolophus beddomei* (Figure 1).

Figure 1 - *Rhinolophus* species of KMTR

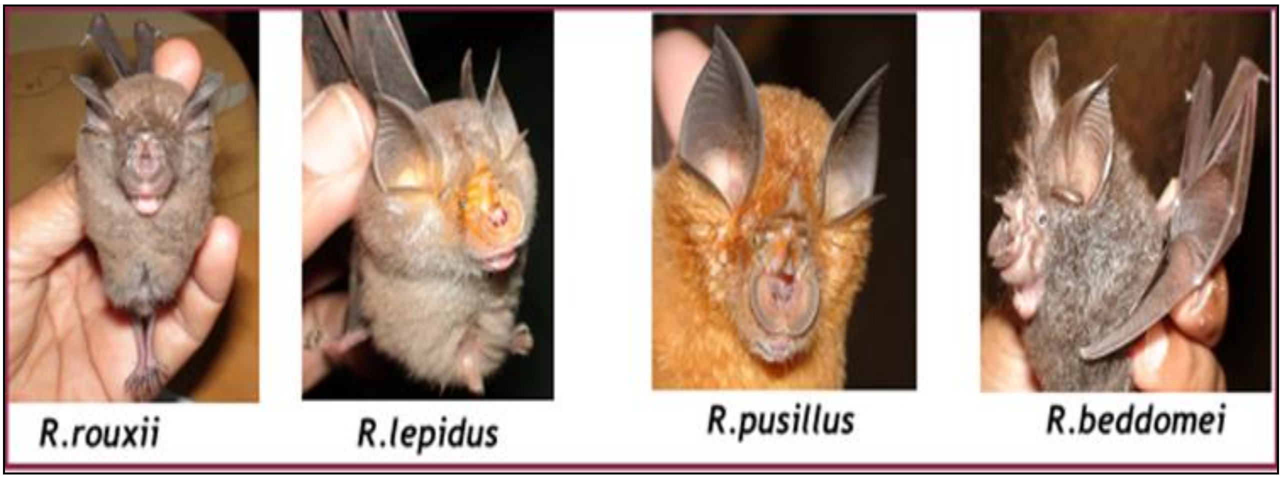
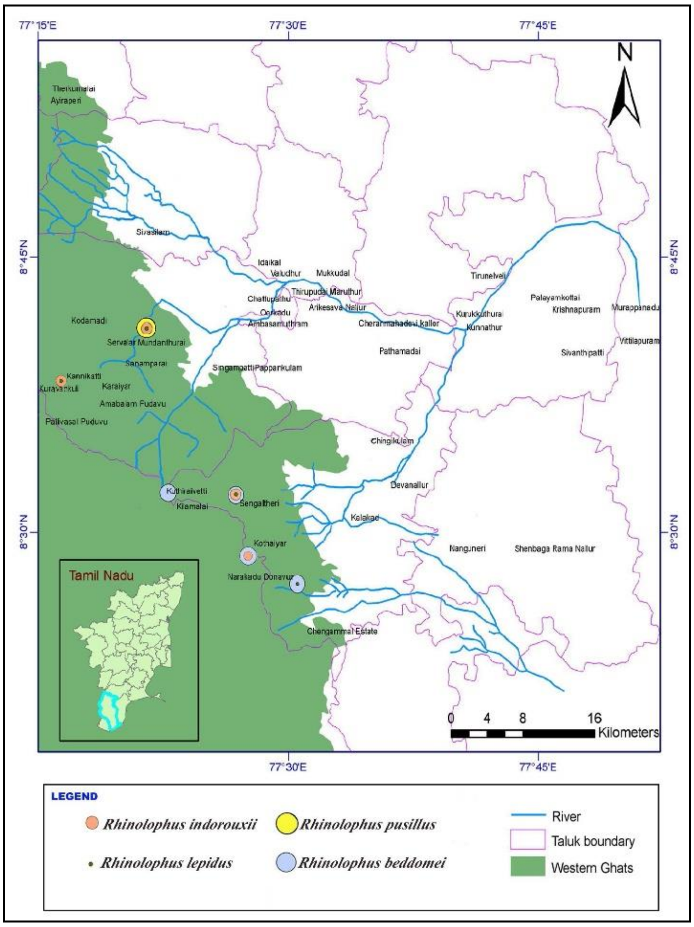


Plate 2 - Distribution of studied *Rhinolophus* species

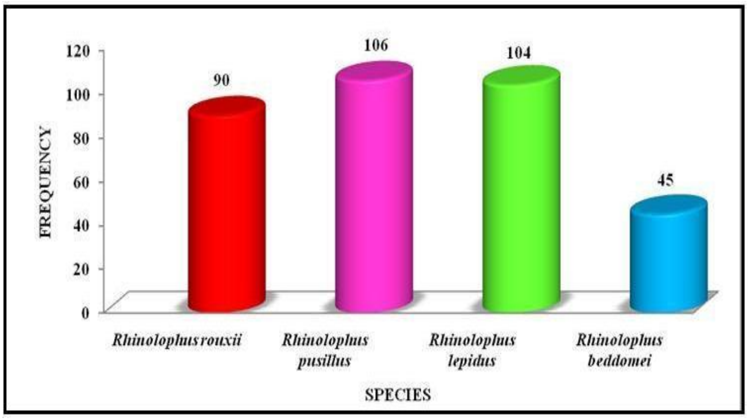


2.2 Echo call Recording

Calls were recorded by manually triggering a Pettersson D-240X bat detector (Figure 2). Reference calls were recorded from captured bats. The recordings were made from hand-released and free-flying bats. The range of recording situations was used intentionally to cover as much variability as possible within species, and thus produce a more robust and comprehensive identification key. Horseshoe bats in this pilot study had a frequency range from 44 kHz to over 106 kHz (Figure 3). Initial identifications were made in the field using taxonomic knowledge and keys of external characteristics, whilst further species determination was possible from measuring typical call characteristics such as call shape, frequencies and temporal features.

Figure 2 - Pettersson D-240X bat detector

Figure 3- Frequency range of bat pass in Rhinolophids of KMTR



2.3 Acoustic Analysis

Bat calls were analysed through acoustics softwares Bat Sound 4.1. After selectively extracting the most accurate and vivid pulse sections, PD (pulse duration), PI (pulse interval), PF (peak frequency), SF (start frequency), EF (ending frequency), NH (number of harmony) within a call, and MP (maximum power) were measured. PD was calculated by measuring the time distance from the beginning to the end of the pulse. PI was calculated by measuring the time distance between one pulse's beginning time and the successive pulse signal's beginning time. PF was measured by analyzing the frequency of the most vivid and darkest part of the pulse signal revealed in the spectrogram, in other words the frequency of the section where MP occurred.

2.4 Statistical analysis

Statistical analyses through SPSS software helped to pool and compare the recorded data of the research station. Data are expressed as mean ± SD. Student's t-test was used to analyze differences in mean. ANOVA was performed to assess overall differences between the bat activities in the study sites. A value of $P<0.05$ was considered statistically significant.

III RESULT

The study period was between April 2012 to March 2013. *Rhinolophus rouxii* is a medium sized rhinolophid with forearm length ranges from 44.4- 52.3 mm. Generally observed to fly around bushes and amongst tree, with a flapping flight, avoiding twigs and other obstacles (Brosset 1962). *Rhinolophus pusillus* is a small horseshoe bat which is intermediate in size with forearm ranges from 34.9-37.8 mm. The horseshoe nose leaf is relatively wide with sella constricted in the middle. The wing shape made the *R.pusillus* to adapt their foraging in cluttered environments with slow flight performance. *Rhinolophus lepidus* is a medium-sized horseshoe bat with an average forearm length of 37-41.8 mm. The peculiar noseleaf is lacking of lateral lappets, which are brightly coloured with dark orangish tinge. The connecting process is clearly notched and pointed, the sella concave. They are slow and low fliers and explores the foliage of trees. *Rhinolophus beddomei*, is larger than *R.rouxii* with an average forearm ranges from 54.9- 64.3 mm. They are fast fliers and forage in less cluttered habitat, along the paths in the jungle and jungle margins. It usually spends the day hanging by one foot, with the wings wrapped around the body.

3.1 Roost location

The *Rhinolophus species* were captured from the foraging and roosting area by setting mist nets, harp trap and hand nets in their flight pathways and were released after study. The studied rhinolophids are highly sensitive and vulnerable to threats to their roosting sites. They enjoy a diverse range of structures as diurnal roost sites in both natural and man - made structures. Table 1 shows the roost location of Rhinolophidae family members of KMTR. In the present study, *R.rouxii* were located from their roosts like tunnels and caves. *R.lepidus* and *R.pusillus* were sited in caves. *R.beddomei* prefers to roost in caves and rarely in the abandoned estate bunglow located in the forest interior. The rhinolophids prefer to select caves located near water sources like rivers, streams and water falls of the forests of KMTR.

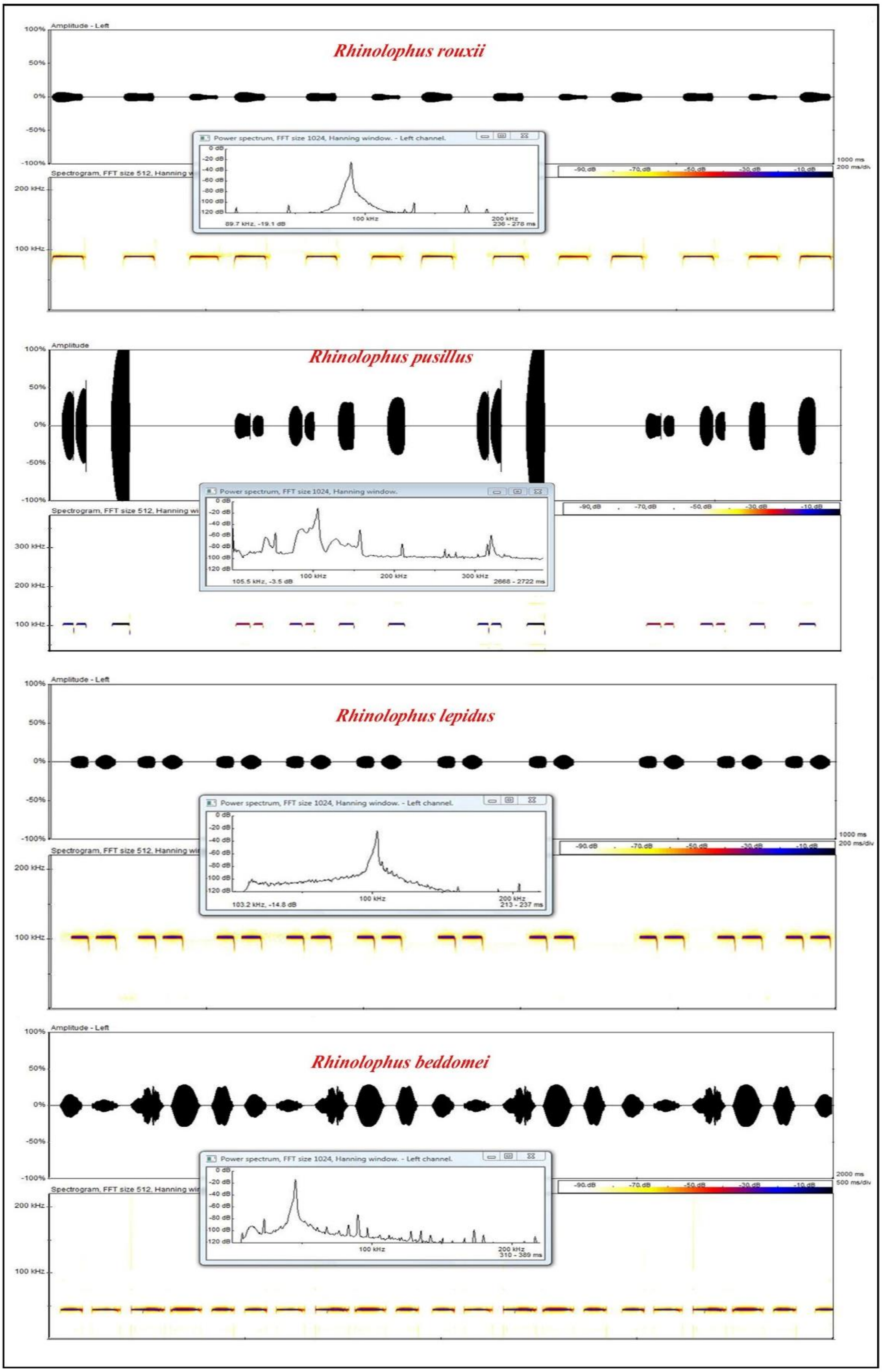
Table 1- Roost location of Rhinolophidae family members of KMTR

S.No.	Name of the bat species	Location of the roost	Type of Roost	Name of roost	Colony size (Approximate)
1	*Rhinolophus rouxii*	Shengaltheri (Kalakad hills) Ele: 2814 ft, N: 8°31.589' E: 77°26.759'	Cave	View point cave	550
		Mundanthurai (Pothigai hills) Ele: 1648 ft, N: 8°37.55', E: 77°17.53'	cave	Inchikuzhi cave	600
		Kodayar (Manimuthar hills) Ele: 3879ft, N: 8°41.283' E: 77°41.098'	Rocky tunnel	Kodayar Damsite tunnel	500
		Kannikatti (Pothigai hills) Ele: 2634 ft, N: 8°37.922', E: 77°16.411'	Cave	Kuravankuzhi cave	450
2	*Rhinolophus pusillus*	Mundanthurai (Pothigai hills) Ele: 1267 ft, N: 8°37.59' E: 77°19.46'	Cave	Karadi Pudavu	450
3	*Rhinolophus lepidus*	Mundanthurai (Pothigai hills) Ele: 1267 ft, N: 8°37.59' E: 77°19.46'	Cave	Karadi Pudavu	300
		Inchikuzhi (Pothigai hills) Ele: 1645 ft, N: 8°37.438', E: 77°17.636'	Cave	Kuravankuzhi cave	500
		Kodamadi (Servalar hills) Ele: 1380 ft, N: 8°48.083', E: 77°46.354'	Cave	Eluthukal pudavu	150
		Shengaltheri(Kalakad hills) Ele: 3282 ft, N: 8°31.932' E: 77°26.886'	Cave	View point cave	400
4	*Rhinolophus beddomei*	Shengaltheri (Kalakad hills) Ele: 3103 ft, N: 8°31.932' E: 77°26.932'	Man made structure	Abandoned bunglow	2
		Muthaliruppan parai (Kalakad hills) Ele: 1350 ft, N: 8°32.129' E: 77°28.539'	Cave	Muthaliruppan parai cave	1
		Kuthiraivetti (Kodayar hills) Ele: 3609 ft, N: 8°41.567' E: 77°44.208'	Cave	Kuthiraivetti Cave	2
		Kannikatti (Pothigai hills) Ele: 2634 ft, N: 8°37.922', E: 77°16.411'	Cave	Kuravankuzhi cave	2

3.2 Acoustic call structure of Horseshoe bats of KMTR

Horseshoe bats are high-duty cycle echolocators (long call duration relative to the interval between consecutive calls) and have calls that are dominated by a long constant-frequency (CF) component. Call sounds like a prolonged warbling or whistling. Horseshoe bat calls consist of prolonged (up to 50 msec or more) whistling sounds at a near constant frequency. The recorded echo calls of all the four studied rhinolophids are shown in plate 3**.**

Plate 3 – Echo calls of studied *Rhinolophus* species

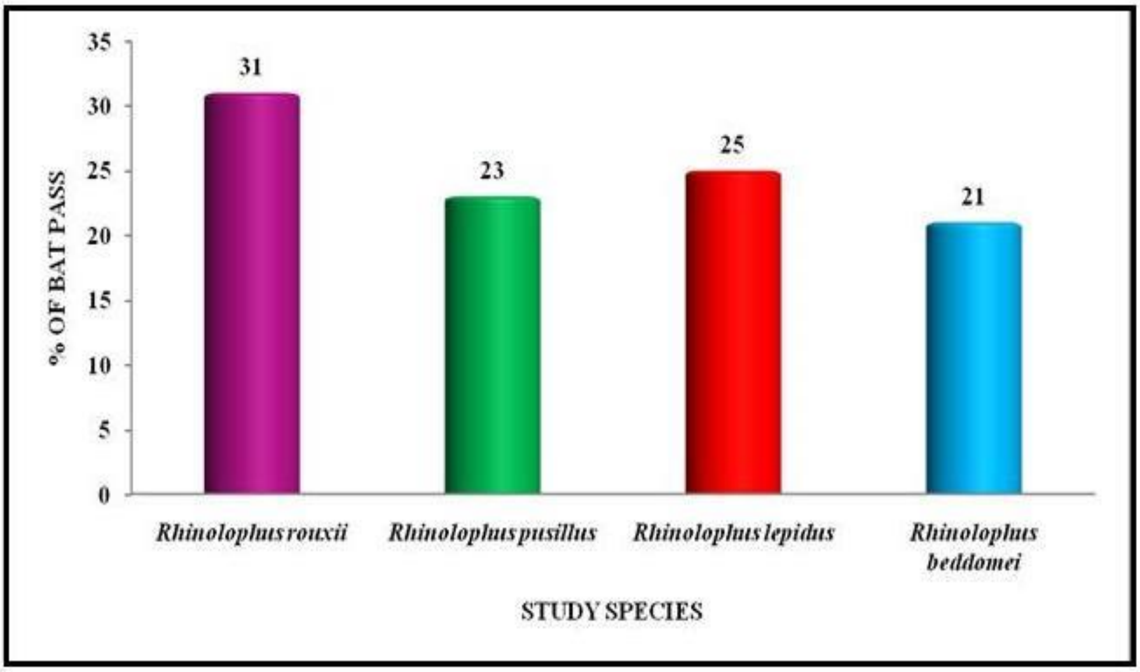


Differences in call duration, call interval and frequency values are outlined in Table 2. The activity richness of Rhinolophids of KMTR is documented in Figure 4. Among the four species, *R. rouxii* shows higher activity richness in the study station.

Table 2. Measurements of Echo calls through Bat sound software
Value: Mean ± Standard deviation

Species name	Pulse Duration - PD (ms)	Pulse Interval - PI (ms)	Maximum frequency-FMAXE (kHz)
Rhinolophus indorouxii	40.14±5.51	70.57±18.20	90.23±1.08
Rhinolophus pusillus	39.2±13.10	51.6±19.49	105.83±1.39
Rhinolophus lepidus	23.39±3.02	33.57±4.26	103.7±0.62
Rhinolophus beddomei	70.82±9.65	89.31±6.11	45.17±0.43

Figure 4 - Activity richness of Rhinolophids of KMTR



IV DISCUSSION

4.1 Acoustic monitoring

Acoustic monitoring is a valuable method to assess presence and activity of species which rarely get caught in ground or canopy mist nets (Flaquer *et al* 2009). The use of ultrasonic detectors to record echolocation calls has become an important part of studying bat ecology. With the presence of endangered species of bats, and the increased awareness of bat activity in industries such as wind energy, mines, road construction, power lines, and timber, accurate identification of local bat fauna is imperative. Increasingly, the use of bat detectors to passively monitor these sites has become the preferred manner in which these surveys are conducted.

4.2 Roosting ecology

KMTR , a valuable repository of biodiversity, in the southern end of the Western Ghats, covers nearly 63% of the India's arborescent evergreen endemic taxa. Because of the occurrence of numerous rivers and streams, this area is also called river sanctuary. Numerous studies have proved evidence that insectivorous bats prefer such kinds of ecosystem as roost sites (Kalcounis-Ruppell *et al* 2005, Ciechanowski *et al* 2007). Earlier reports also confirms their roost selection frequently lies in proximity to water (Kunz 1982, Bringham *et al* 1992).

4.3 Species identification

The species of rhinolophoid bats in KMTR can be identified from their echolocation calls with varying degrees of success. The four species, *R.indorouxii*, *R. pusillus*, *R. lepidus* and *R. beddomei* all emitted typical CF echolocation calls, strictly constant-frequency component (CF) except *R.beddomei* preceded and followed by a brief, frequency-modulated sweep. Echocall analysis made by Schnitzler (1998) on *Rhinolophus* species also confirm that the echolocation pulse of these bats has short FM component signals and long CF component signals in order to determine the flight paths and detect subtle movements of insect preys in forest habitats. Early research using bat detectors indicated that some bat species may be distinguished by unique echolocation call characteristics (Fenton and Bell 1981; Simmons *et al* 1979). Recently, several studies have identified bats with various levels of success (Fenton and Bell 1981; O'Farrell *et al* 1999; Parsons and Jones 2000; Vaughan *et al* 1997). However, other studies have been unable to effectively discriminate species with similar echolocation calls, thereby prompting the grouping of calls into multi-species groups (Krusic and Neefus 1996; Kuenzi and Morrison 1998).

Frequency distributions of rhinolophid echolocation calls show a clear species specific variation in their call frequency [*R.rouxii*- 91.27kHz (90.6-91.9 kHz), *R.pusillus*- 103.5 kHz (101.7-105.4 kHz), *R.lepidus*- 104.3kHz (103.0-106.3 kHz) *R.beddomei*- 45.1kHz (44.7-46 kHz). There is an inverse relationship between call frequency and body size. This is the major factor affecting FMAXE across species. According to Jones (1999), higher the body size, lower the frequency production. In the present study also, *R.beddomei* with greater body size (12.03±1.22 g) produces lesser frequency level (45.17±0.43982 kHz) and *R.pusillus* with lesser body size (4.67±0.28) produces higher call frequencies (105.837±1.39639 kHz) although peak frequency of the CF component in rhinolophid bats can vary with geography, sex or age. Differences in echolocation call frequencies potentially facilitate diet differences between species, or between different sex and age classes within the same species (Belwood and Fenton 1976). Therefore acoustic divergence (variation in peak frequency) can be associated with speciation in *Rhinolophus.*

V CONCLUSION AND SUGGESTION

The rich diversity of flora of Mundanthurai, the core area of Agasthyamalai Biosphere has provided bat diversity and bat activity pattern with a wide range of roosting habitats as well as foraging habitats. The majority of bats were found foraging in forested wetlands and they often move between different types of habitats, like day roosts and nocturnal foraging sites. Differences in the habitat quality like the density of resources may significantly influence the fitness of individuals. The use of ultrasonic detectors to record echolocation calls has become an important part of studying bat ecology. The research on horseshoe bats' echolocation signal patterns was carried out to contribute to future studies on identification through echolocation calls as well as other similar bat species' echolocation patterns. These bats also play key functional role in ecosystems, acting as predators of insects, including harmful forest pests. Therefore, it is concluded that further research on bats' various echolocation patterns related to their ecological habitats, morphological features and geological differences other than basic echolocation patterns is necessary.

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