Echolocation calls of *Natalus primus* (Chiroptera: Natalidae): Implications for conservation monitoring of this species

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Echolocation calls of *Natalus primus* (Chiroptera: Natalidae): Implications for conservation monitoring of this species

Lida Sanchez¹,²*, Christian R. Moreno¹ and Emanuel C. Mora¹,³

**Abstract:** *Natalus primus* constitutes one of the most vulnerable mammalian species of Cuba. Until now, only one extant population is known to live in one single cave in the westernmost part of Cuba, within the Guanahacabibes National Park. Over multiple trips, we recorded ultrasonic vocalizations from several individuals of this species. We found short, high frequency-modulated multiharmonic calls for *N. primus*; these could be used to identify this species in acoustic inventories conducted in Cuba. Identifying *N. primus* through their echolocation calls will allow conducting passive acoustic monitoring, constituting a noninvasive approach to study this vulnerable species without causing disturbances on its roosts and foraging areas.

**Subjects:** Environment & Agriculture; Bioscience; Environmental Studies & Management

**Keywords:** acoustic monitoring; echolocation; Guanahacabibes; *Natalus primus*

1. Introduction

*Natalus primus* (Cuban greater funnel-eared bat) is the largest species within Natalidae. Few studies had addressed issues from its natural history, most of them referring taxonomy (Tejedor, Silva-Taboada, & Rodríguez-Hernández, 2004; Tejedor, Tavares, & Silva-Taboada, 2005). This bat species...
was thought to be extinct but was recently rediscovered in a cave in the westernmost part of Cuban archipelago (Tejedor, Silva-Taboada, & Rodríguez-Hernández, 2004), although fossil records suggest the species resided across the entire archipelago at one time (Silva-Taboada, 1979). This species was categorized as vulnerable (Dávalos & Mancina, 2010), regarding the unique extant population of this species in this remote area in Cuba. In spite of this, no direct conservation plans are taking over this species, neither a long- or short-term monitoring to check its population status. More studies regarding its natural history and environmental requirements are needed, in order to draw more accurate conservation plans for this bat species.

Bioacoustics, the study of sounds produced by animals, has been widely used for species identification on highly vocal species such as bats (Hughes et al., 2011), cetaceans (Oswald, Barlow, & Norris, 2003), frogs (Brandes, Naskrecki, & Figueroa, 2006), and birds (Anderson et al., 1996). Acoustic surveys constitute a good complement for traditional capturing methods (Kuenzi & Morrison, 1998), allowing the detection of otherwise elusive species and causing almost no disturbance in their habitats (Laiolo, Vögeli, Serrano, & Tella, 2007). Acoustic studies have helped to determine habitat selection (Russ & Montgomery, 2002) and identification of cryptic bat species (Davidson-Watts, Walls, & Jones, 2006). Description of species vocal repertoire opens the possibility of using this powerful tool in studies regarding ecology, population dynamics, and conservation (Laiolo, 2010). Several variables measured from these vocalizations have been used to distinguish species, such as duration, mean frequency, and inter-pulse intervals (Fukui, Agetsuma, & Hill, 2004). Recent studies also had included the frequency pattern of vocalizations to recognize species, using several methods such as cross-correlation and neural networks (Gaston & O’Neill, 2004; Mellinger & Clark, 1997). Using bioacoustics could constitute a successful way to make a noninvasive approach to study this vulnerable bat species.

We report here the first description of the echolocation calls of *N. primus*. This description together with the frequency pattern of its vocalizations will allow the identification of this species in future acoustic surveys.

2. Materials and methods

2.1. Study site and capture of individuals

We conducted our study in the site where *N. primus* was rediscovered: Cave La Barca (N 21°50′36.2″; W 084°45′57.5″) (Tejedor, Tavares, & Rodríguez-Hernández, 2005), located within Guanahacabibes National Park. This is a hot cave composed by five main galleries with 500 m of linear extension and multiple entrances (Tejedor, Tavares, & Rodríguez-Hernández, 2005). We visited this cave five times from February to September 2015, accorded to the validity of the permissions requested to enter the National Park and handle the animals (Permission number: PO2014/96 and PO2015/16). To know more about features of this cave, we measured two climatic variables, such as temperature and relative humidity, using HOBO data loggers (Onset Computer Corp. Bourne, MA) in June 2015 for three consecutive days at four locations. We took climatic measurements outside the entrance of the cave and in three galleries in the cave: in the most inner (heat Gallery), in an intermediate one and a gallery with a skylight.

Bats were captured with hand and mist nets; these were located very close to the walls at low heights (<1.5 m above the floor) in the heat gallery of this cave. Individuals were captured and placed in cloth bags to prevent bite and injuries among individuals. This is a very delicate species with broad wings and long tail membranes and becomes stressed very easily, thus, gentle handling is required. For each individual, we recorded morphological variables such as forearm length and body weight, sex, and reproductive condition.

2.2. Acoustic recordings

We recorded their echolocation calls by allowing the bats to freely fly within a small gallery in the cave where no other bats were already roosting. This gallery was similar to a narrow hall (rectangular shape), allowing to close the entrance of it with long cloth sheets to avoid the animals to escape.
Each individual was allowed to fly in this gallery and when two flight paths (full sequence of calls approaching the microphones) were obtained, individuals we allowed to fly out of the gallery by moving the cloth sheets. We used an array of microphones to obtain good quality recordings (obtaining vocalizations from different angles) and the construction of flight trajectories for this species. The array was arranged in a t-shape form, using four ultrasonic condenser microphones (Avisoft CMPA/CM16) coupled to an amplifier, connected to a laptop running Avisoft USGH software (Avisoft Bioacoustics, Germany). Microphone’s gain was set in order to have a good signal to noise ratio in our recordings. We selected the channel containing calls with the highest amplitude to build the flight trajectories. We did not have flight paths containing echolocation calls from more than one individual, due that each individual was released inside the recording gallery after the previous one flew out. Flight trajectories were built using the software Bat3D (González, 2012) based on the time delays from the arrival of every emitted sound to each microphone, taking as reference the microphone located in the center of the array.

2.3. Call analysis

We conducted call analysis using two different softwares in order to characterize differently the echolocation calls. We intended to have a characterization from the whole call (all harmonics included) and from each harmonic separately. The channel containing calls with the highest amplitude was selected to make the call analysis, and only those calls above 20 dB relative to background noise, were analyzed. We used the automated detection feature of callViewer18 (Skowronski & Fenton, 2008) to characterize each harmonic independently from the echolocation calls. This is a custom echolocation sound analysis program written with MATLAB software (The MathWorks, Nadick, MA, USA). Detection parameter settings used with this software were: minimum link length = 10, Window size = 0.3, frame rate = 10,000, chunk size = 1, minimum energy = 12 dB, Echo filter hold = 6 dB, and window type = Blackman.

Call variables measured for each harmonic from every call were: duration (ms), minimum and maximum frequencies (kHz), and frequency of most energy (kHz). With these values, we calculated other variables such as bandwidth (kHz) (the difference between maximum and minimum frequencies) and slope rate (kHz/ms) (bandwidth divided by duration). We also used automatic measurement feature from Avisoft SASLab Pro software (Avisoft Bioacoustics, Germany) to characterize entire call. Here, signals were characterized—10 dB from the maximum amplitude peak in the power spectrum of each signal. Call variables measured were the same as in callViewer18.

3. Results

Fifteen adult individuals from *N. primus* were captured in the innermost parts of La Barca cave (e.g. Figure 1(A)). Their unique flight pattern resembling a butterfly at low heights bordering the walls of the caves allowed to distinguish this species from the rest of the bats inhabiting this cave. Forearm length ranged from 46.9 to 50 mm and body weight ranged from 7 to 9.5 g. The two pelage-color morphs were also observed from these captured individuals. Females were found lactating in September (Figure 1(B)). To confirm the precise period in which this species gave birth their
newborns was difficult. The inner gallery of cave La Barca in which most of the species concentrate is a guano swamp, making very difficult to explore it by foot. None of the captures females were carrying their babies.

We successfully recorded a total of 349 echolocation calls from 13 individuals. Two individuals were released after taking their morphological measurements because they were very stressed and we decided not to record their vocalizations. Flight trajectories were very similar among individuals (Figure 2(A)), each one of it contained more than 30 echolocation calls. These flight trajectories allowed to make a good selection of the flight sequences to be analyzed in the acoustic characterization, we show waveforms of some of these (Figure 2(B)). Those trajectories in which the animals were bordering the walls or flying in a direction to the end of the gallery were not analyzed.

Echolocation calls were downward frequency modulated (FM) of short durations, composed of three harmonics (Figure 2(C)). The first harmonic had in most of the cases a short constant frequency (CF) component located around 73 kHz (maximum frequency from the first harmonic) (Table 1), the second and third harmonic were completely frequency modulated with a major bandwidth, although the third harmonic was very faint in most of our recordings and no acoustic measurements could be done for it. The first harmonic was always detected, but the second harmonic was more evident, with the greatest amplitude, especially during the approach to microphone’s array. Spectral overlap among harmonics was clear on some calls but was absent in others. Almost no difference was found in the mean value for duration from the whole call in relation to each harmonic (Table 1). However, for the other call variables, means differed and is understandable, due to the specificities in the location of measurement made on each sound analysis software.

Temperature and relative humidity profiles revealed stable values for several galleries of cave La Barca, especially regarding the relative humidity (Figure 3(B)–(D)), compared to the values outside the cave (Figure 3(A)). Although, a slight temperature fluctuation was observed (±1°C) in the gallery containing skylights (Figure 3(C)). Most of the captured individuals of *N. primus* were in the heat trap, where both of these variables were constant (Figure 3(D)).

4. Discussion

Articles referring the echolocation behavior from the bat species within Natalidae are not numerous. Most of them are reflecting the description of the species vocalizations (Murray, Fraser, Davy, Fleming, & Fenton, 2009), or their presence in different localities (García-Rivera, Montes Espín, Hernández Hernándes, Borroto, & Mancina, 2015). This study presents the description of the
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Echolocation calls from the largest species of Natalidae, as the first step to undertake more studies about the ecology of this species. In Cuba, specifically most of the bioacoustics and ecological studies had focused on Molossidae (Mora et al., 2011; Mora & Torres, 2008), Phyllostomidae (Macías & Mora, 2006; Macías, Mora, García, & Macías, 2006) and Vespertilionidae (Mora, Rodríguez, Macías, Quiñonez, & Mellado, 2005; Rodríguez & Mora, 2005). The butterfly bat (Nyctiellus lepidus), the smallest bat in Cuba is quite numerous in several caves along the archipelago (Silva-Taboada, 1979) and their colonies are of a considerable size (Borroto-Páez & Mancina, 2011). Although, almost no studies regarding aspects of their natural history have been published so far.

N. lepidus inhabits cave La Barca as well as N. primus, within the cave these two species can be easily identified by their flight maneuvers and body size. Echolocation calls from N. lepidus are quite distinctive in their frequency pattern from those of N. primus, presenting non-overlapped multiharmonic FM calls (Murray et al., 2009) and they lack this short CF component found for N. primus. From the 15 captured individuals of N. primus, their forearm length and body weight measures concur with those obtained previously for the species (Tejedor et al., 2004). This species presents FM calls, while the CF component of the first harmonic in these (Figure 2) could be a feature that, together with call variables measured, will allow distinguishing this species from the rest of FM bat species coexisting in the same area.

With data presented here, acoustic surveys could be implemented in the surrounding areas from cave La Barca, trying to locate the foraging zones of N. primus and what kind of environment they prefer to develop this activity: open, edge or narrow environments. This information may contribute as well to give a right estimation of the species extend of occurrence and offer new areas of occupancy. Still though, more acoustic recordings should be conducted on this species, in order to obtain a more accurate identification of this species on the field by considering possible modifications in

Table 1. Echolocation call variables measured for 13 individuals of N. primus

| N = 349 Harmonics | Duration (ms) | Minimum frequency (kHz) | Maximum frequency (kHz) | Peak frequency (kHz) | Bandwidth (kHz) | Slope modulation (kHz/ms) |
|-------------------|---------------|-------------------------|-------------------------|-----------------------|----------------|--------------------------|
| 1st (n = 343)     | 1.9 ± 0.5     | 38.8 ± 10.0             | 73.0 ± 13.1             | 60.2 ± 10.6           | 34.1 ± 6.6     | 34.1 ± 5.6               |
| 2nd (n = 200)     | 1.7 ± 0.5     | 64.0 ± 8.0              | 114.4 ± 5.5             | 85.3 ± 6.9            | 50.3 ± 9.8     | 29.5 ± 6.0               |
| Whole call        | 1.7 ± 0.5     | 46.1 ± 8.9              | 103.8 ± 13.9            | 78.5 ± 15.6           | 57.7 ± 13.9    | –                        |

Notes: Arithmetic means, variation coefficient, and standard deviations are given to each call variable in each harmonic and for the entire call. N represents the number of echolocation calls obtained from all recordings and n represents the number of call harmonics.

Figure 3. Temperature and relative humidity profiles from different galleries of La Barca cave, Guanahacabibes, Pinar del Rio, Cuba; on 3 days of continuous sampling (X axis), reporting 1 value every 30 min. Surroundings outside the cave (A).

Notes: Inner galleries of this cave (B and C). Heat trap (D).
their frequency pattern and values of their call variables, as have been detected in other bat species (Mora, Macías, Vater, Coro, & Kössl, 2004; Mora & Macías, 2007).

According to the spectro-temporal call pattern and pulse intervals, this species can be classified as a low duty cycle (LDC) bat. LDC bats can separate their call and echo in time, to avoid self-deafening (Fenton, 2013; Lazure & Fenton, 2011). Frequency-modulated signals of broad bandwidth, as the ones emitted by this species, are well suited for exact target location where range and angle must be precisely measured (Schnitzler & Kalko, 2001). This spectral pattern might be convenient for this species moving at low heights within vegetation (field observations). Individuals of N. lepidus were observed to forage at sunset, very close to the beach, at the vegetation edge. Based on the acoustic recordings made in the entrance of cave La Barca, N. lepidus is the first species to go out of the cave to forage (data not shown in this article). No individuals from N. primus were seen at this time in these locations.

Further studies are needed to better understand the ecology of this species. Priority areas include improving our knowledge about their roosting needs, foraging strategies, and habitat use. Previous studies point that bats from Natalididae prefer roosts (caves and abandoned mines) with stable values of temperature and relative humidity, and with water reservoirs in some galleries, such as the case of N. stramineus (Torres-Flores & López-Wilchis, 2010). In our study, we present temperature and relative humidity profiles from three consecutive days, obtained from different galleries in this cave (Figure 3), they show relatively constant values of relative humidity among the three galleries for those sampled days, but temperature remained constant only in the heat trap (Figure 3(D)) which also presents a guano swamp. This might by one of the features preferred for this species in the selection of its roost, but the distance to the seashore could constitute another important one, due that most of the fossil records from this species in Cuba were caves with these characteristics (Silva-Taboada, 1979). Recording temperature and relative humidity in multiple galleries within the cave for a long-term period will be necessary, to make a more conclusive argument regarding these climatic roosting requirements for the species. This species had been seen roosting together with Mormoops blainvillei (Tejedor et al., 2004), suggesting that maybe could have similar preferences for roosting sites within the cave. M. blainvillei in general sense is a quite fragile species, compared to other cave-dwelling bats species, during handling or conducting experiments outside cave environments, individuals from M. blainvillei can dehydrate very easily and even die (author’s field observations). This could be one of the reasons explaining the similarity in the roosting sites within the cave among M. blainvillei and N. primus. Thermal conductance and metabolic rates measurements jointly with long-term measurements of cave temperatures, similar to the ones conducted for M. blainvillei and other cave-dwelling bats (Rodríguez-Durán, 1995), will be necessary for N. primus to confirm this.

Cave La Barca has been recognized as one of the most diverse caves in Cuba, housing up to 13 bat species (Tejedor, Tavares, Silva-Taboada, 2005). For almost all of them, the echolocation behavior has been addressed in several publications (Macías & Mora, 2006; Macías, Mora, & García, 2006; Maclas, Mora, Koch, & Von Helversen, 2005; Mora & Macías, 2011). Our description of N. primus echolocation calls (regarding call measurements and frequency pattern) will be useful to identify this species among the rest of the bat species inhabiting Guanahacabibes National Park. Our future intentions are record vocalizations in different areas within this National Park and conduct automated detection to detect the presence of N. primus in these locations using their call measurements presented here or spectrogram templates, similar to what have been done already in other species (Bardeli et al., 2010; Mellinger & Clark, 1997).

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Corrigendum
This article was originally published with errors. This version has been corrected/amended as follows: Competing Interests was not included. Now this has been included.

Correction
This article was originally published with errors. This version has been corrected. Please see Corrigendum (https://doi.org/10.1080/23312025.2017.1374910).

Cover image
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