DIFFERENTIATING WESTERN AUSTRALIAN NYCTOPHILUS (CHIROPTERA: VESPERTILIONIDAE) ECHOLOCATION CALLS

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The ultrasound calls of the six species of Nyctophilus bat in Western Australia can be differentiated using power spectral analysis. Their search-mode call sequences show distinctive trends of variation in peak-frequency ($F_{peak}$) values call-to-call. These differences are too subtle to be separated in amplitude-time domain with the processing tools currently available. The call-to-call variations in $F_{peak}$ through time can be characterised from the call sequence recordings using Fourier analysis if care is taken to select genuine search-mode sequences. These variations can then be used to correctly identify the species emitting the call.

Key words: bats, echolocation, ultrasound, Fourier analysis, frequency division, species differentiation.

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UNTIL now, zoologists have been unable to differentiate all Australian Nyctophilus species from their calls (Adams 2000; Chick et al. 2000). Most attempts have relied on analysis in frequency-time domain (Parsons et al. 2000), but this approach has limited value for Nyctophilus species because the majority have low power calls in the 45 - 55 kHz range, with a varying frequency scanning characteristic (Fig. 1). Characteristically, even search-mode calls are not prominent above background noise levels (Fig. 1A) when compared with molossids and 'non-gleaning' vespertilionids. In this paper we compare the call sequences of Western Australian (WA) Nyctophilus to see whether species can be distinguished in power-frequency domain.

METHODS

Six species of Nyctophilus occur in Western Australia (Churchill 1998). N. g. goffroyi Leach 1821 occurs throughout WA, although a morphological variant occupies mangrove communities from Exmouth Gulf northwards as well as the sub-humid tropical regions of the Kimberley. On cranial characteristics the variant is comparable (McKenzie 1983: 48) with N. g. pallescens (Thomas 1913). N. Gouldi Tomes 1858 and N. timoriensis (Geoffroy 1806) are temperate-zone species in WA. N. Gouldi is confined to sub-humid forest regions, while N. timoriensis also extends across the southern and central Wheatbelt, and southern arid zone. N. bifax Thomas 1915 and N. arnhemensis Johnson 1959 are tropical species of semi-arid to sub-humid areas, including the western Pilbara, western Ashburton and Kimberley. N. Walkeri Thomas 1892 is confined to the sub-humid tropics.

While studying bat communities throughout W.A., capture and release techniques were used to collect a library of ultrasound calls. Most of the reference recordings were made using Anabat II (Titley Electronics, Australia) and D940 (Pettersson Elektronik, Sweden) detectors in frequency-division mode (/16 and /10, respectively). Output was stored directly onto Metal Type IV cassette tapes using a Sony Walkman Professional (WMD6C) tape recorder. Note that the Anabat ZCAIM unit (Titley Electronics) was not used in conjunction as this unit further processes and filters the data which is then only useful in time domain analyses and presentations. Species calls were characterised in terms of one ‘fundamental harmonic’ attribute, the frequency of peak power in the fundamental harmonic ($F_{peak}$). This was determined by using COOL EDIT 2000 (Syntrillium Software, USA). All of the reference calls in the library were night-time releases with a bioluminescent marker (Cyalume) attached to the bat.
Fig. 1. Example of a typical Nyctophilus search-mode call sequence, and the power spectrum of one of the calls, showing the broad band and low power nature of the calls (Anabat II signal: frequency divide by 16). Fig. 1A shows power spectrum in frequency domain of the call at time = 0.79 sec. Peak, minimum and maximum frequencies are noted as is the third harmonic. Note that the higher harmonics are artifacts of the Fourier transformation. Fig. 1B shows the call sequence in time domain lasting 0.9 sec.

When selecting reference calls for further study, sequences were excluded if they were too brief for the bat to settle into its normal search mode (Obrist and Wenstrup 1998) following hand release. Typically three seconds were required for the call sequence to normalise into search mode, and so calls were only used if they included a continuous sequence at least this long, or the bat subsequently turned and flew back within range of the detector. If a second bat was present calls were excluded because calls made by the released bat might have been confused with those made by the intruding bat.

Using the computer package COOL EDIT 2000 (Syntrillium Software, USA), the Nyctophilus call sequences in this library were firstly converted to “.wav” or “.mp3” files from the analogue tape recordings using a mono-sampling rate of 44100 at 16-bit resolution. Secondly, power-spectral analyses were carried out for the individual calls within each search-mode sequence using a 2048-point Blackmann-Harris fast-Fourier transform.

Only the fundamental harmonic was considered because the higher harmonics are artificially generated by the Fourier power series analysis when frequency divide-down calls are analysed. From these analyses, calls were characterised, and features were sought that discriminated species. Following discovery of the call pattern of each species, a blind test was undertaken to ensure fidelity of the method. An unidentified reference call of each species
labelled only "Temperate" or "Tropical" Nyctophilus was provided and analysed. Species were correctly identified in each case.

RESULTS AND DISCUSSION

Lancaster et al. (1995) discussed the coupling of wing downstroke muscles to call production in bats. Nyctophilus have wing-beat frequencies between 9 and 12 Hz and, unlike most other genera of bat, produce from one to several calls per wingbeat (unpubl. data). When sequences involving multiple-calls per wingbeat were reviewed, we noted that few of the calls had enough power to allow their frequency-power parameters to be characterised accurately. Only sequences of regular, evenly spaced calls at a rate of one call per wing-beat (search-mode) provided consistent power-frequency patterns, call-to-call, so these were analysed. These 'single call per wing-beat' sequences typically consisted of calls of 5 to 8 msec. duration. Given that this resulted in the call containing typically 15 to 25 cycles, consistently accurate frequency-power analysis could be expected (Parsons et al. 2000). Results are presented in Table 1 and Fig. 2.

In WA's temperate regions (south of 24°S), all three species present use a typical range of $F_{\text{peak}}$ with occasional excursions above or below the typical range. The species can be discriminated on the basis of changes in the $F_{\text{peak}}$ values (at one call per wing-beat) as well as the more usual $F_{\text{peak}}$ values. From our data, the usual values of $F_{\text{peak}}$ are used for 5 to 10 calls and then an excursion normally lasting 1 to 5 calls is often inserted. Comparison of the search mode $F_{\text{peak}}$ usual values can discriminate the three candidate taxa (N. g. geoffroyi, N. timoriensis and N. gouldi) if a clean call sequence of some length is available. The occasional frequency-agile subsequences (e.g., "rising to 64 kHz for "N. gouldi" in Table 1) absolutely characterise each species. In this example, N. gouldi produces a short sub-sequence of one to five calls with peaks that rise above its typical frequency range (Fig. 2). These 'rising-peak' subsequences were noted in N. g. geoffroyi and N. gouldi, both of which forage 'air-to-air' and 'air-to-surface' (unpubl. data after Bullen and McKenzie 2001; Grant 1991), although the latter confines its foraging in WA to cluttered airspace in the south-west forests. We suppose that the bat is using these frequency-agile call sequences to scan the surface characteristics of objects it has detected.

Nyctophilus arnhemensis, N. bifax, N. g. geoffroyi and N. g. pallescens occur over significant areas of tropical W.A.; ultrasound calls of the two allopatric sub-species of N. geoffroyi are difficult to separate. N. g. geoffroyi is the bat most commonly recorded and is characterised by its regular 47 to 48 kHz $F_{\text{peak}}$ calls. This use of a steady, typical peak-frequency range, with short rising sequences of one to four calls reaching 51 kHz is indistinguishable from sequences by temperate populations described above. N. g. pallescens is separated from N. g. geoffroyi by its wider range of typical frequencies 47 to 52 kHz, and absence of any 'rising sub-sequences'. N. bifax is differentiated from the other tropical species by its slightly higher and wider range of 'typical' peak frequencies (50 to 54 kHz), in combination with its frequency-agile sub-sequences down to 42 kHz. Note the consistent call pattern with the other 'ambusher' N. timoriensis (Thompson 1991; Bullen and McKenzie 2001), N. arnhemensis

| Tropical regions | N. g. pallescens | 3$^a$ | 14 | 50.7±2.4 | 47 - 53 | 50.8±1.2 | 50 - 52 | dropping to 35 kHz |
| N. arnhemensis | 3$^b$ | 24 | 50.4±1.6 | 50 - 54 | dropping to 42 kHz |
| N. bifax | 5$^b$ | 29 | 52.4±1.6 | 50 - 54 | dropping to 42 kHz |
| N. walkeri | 7$^c$ | 37 | 56.0±1.5 | 54 - 58 | rising to 64 kHz |

| Temperate regions | N. timoriensis | 6$^d$ | 30 | 44.4±2.0 | 43 - 47 | dropping to 35 kHz |
| N. gouldi | 8$^c$ | 55 | 51.8±1.6 | 50 - 53 | rising to 51 kHz |

| Both regions | N. g. geoffroyi | 8$^f$ | 38 | 47.7±0.6 | 47 - 51 | rising to 51 kHz |

Table 1. Typical frequency of peak power ($F_{\text{peak}}$) in Western Australian Nyctophilus search mode call sequences (10 - 11 calls per sec). a, Pilbara and Kimberley; b Pilbara, Ashburton and Kimberley; c, Kimberley; d, southern temperate; e, south-west forests; f, all.
Fig. 2. Search-mode call sequence data for six *Nyctophilus* species. Individual bat sequences are separated by spaces.
can be differentiated from all other WA *Nyctophilus* by a combination of its typical call sequence (50 to 52 kHz), the narrowness of their range of values (cf. *N. bifax*), and its occasional frequency-agile subsequences of 1 to 5 calls stepping down to 35 kHz (cf. *N. g. pallescens*). *N. walkerii* can be readily distinguished by its high typical peak frequency range of 54 – 58 kHz.

Large portions of the original emitted signal information may be lost during the recording of the sequence using zero crossing analysis techniques (Parsons et al. 2000). This technique is extremely susceptible to the presence of noise in the signal. It instantaneously presents that part of the signal (frequency) that has the most energy associated with it and eliminates the relative power spectrum. Also ZCA removes all higher harmonic information contained in the original signal. It is apparent from the results that our application of Fourier analysis does provide accurate discrimination of the highest power frequency being emitted by the bat. By using Cool Edit software we can obtain more information from Anabat II recorded calls than previously thought. It is the pattern of the variation in the $F_{\text{peak}}$ of the fundamental harmonic from call to call for single call per wing beat sequences that is the discriminating feature, and this pattern can be used with confidence.

Given Fourier transformation of call sequences at one call per wing beat, we have shown that consistent results are obtained both between individual sequences of con-specifics and in comparison between sequences by different species. It is clear that the Fourier transform method tracks the fundamental harmonic of these *Nyctophilus* calls.

The observed differences between WA *Nyctophilus* species appear to be too subtle to be separated in amplitude-time domain with the processing tools currently discussed in the literature. WA *Nyctophilus* species can be reliably identified from their call characteristics using frequency-amplitude domain power spectral techniques. The call sequences should be stored as analogue information on tape, or as .wav or .mp3 files. The variations in the $F_{\text{peak}}$ with time can be characterised from the call sequence recordings using Fourier analysis if care is taken to select genuine search-mode sequences. These variations can then be used to identify correctly the species emitting the call.

**ACKNOWLEDGEMENTS**

We wish to thank C.L. Bullen, M.H. McKenzie and A.N. Start for field assistance. The Western Australian Department of Conservation and Land Management contributed to the cost of the project. We also wish to thank the referees who provided valuable comments on an early draft.

**REFERENCES**


