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Echolocation call production during aerial and terrestrial locomotion by New Zealand's enigmatic lesser short-tailed bat, *Mystacina tuberculata*

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SUMMARY

Linkage of echolocation call production with contraction of flight muscles has been suggested to reduce the energetic cost of flight with echolocation, such that the overall cost is approximately equal to that of flight alone. However, the pattern of call production with limb movement in terrestrially agile bats has never been investigated. We used synchronised high-speed video and audio recordings to determine patterns of association between echolocation call production and limb motion by *Mystacina tuberculata* Gray 1843 as individuals walked and flew, respectively. Results showed that there was no apparent linkage between call production and limb motion when bats walked. When in flight, two calls were produced per wingbeat, late in the downstroke and early in the upstroke. When bats walked, calls were produced at a higher rate, but at a slightly lower intensity, compared with bats in flight. These results suggest that *M. tuberculata* do not attempt to reduce the cost of terrestrial locomotion and call production through biomechanical linkage. They also suggest that the pattern of linkage seen when bats are in flight is not universal and that energetic savings cannot necessarily be explained by contraction of muscles associated with the downstroke alone.

Key words: *Mystacina tuberculata*, echolocation, terrestrial locomotion, flight, linkage.

INTRODUCTION

Bats and birds are the only vertebrates capable of true flight, and the energetic cost of flight is roughly similar in both taxa (Rothe et al., 1987; Speakman and Racey, 1991). However, bats must also support the high energetic cost of echolocation (Speakman et al., 1989). If the energetic cost of simultaneous flight and echolocation were equal to the sum of each, it is unlikely that bats would have the energy necessary to echolocate during flight (Speakman et al., 1989). To avoid this problem, bats link the production of echolocation calls with particular kinematic portions of the wingbeat cycle, which are themselves already synchronised with respiration (Suthers et al., 1972). As a general rule, bats inspire on the downstroke and expire on the upstroke (Suthers et al., 1972). Also as a general rule, aerial-hawking bats produce a single echolocation call within each wingbeat cycle (Jones, 1994), and it is emitted around the time that the upstroke transitions to downstroke: the wing's upper reversal point (Wong and Waters, 2001). At these times, several groups of flight muscles are active, together slowing the upward motion of the wing then accelerating it downward (Hermanson and Altenbach, 1983; Altenbach and Hermanson, 1987). The contraction of these muscles presumably increases intra-thoracic pressure, and, by extension, subglottal pressure, facilitating the rapid exhalation that accompanies the production of high-intensity echolocation calls (Wong and Waters, 2001). Because of this linkage, the total cost of flight and echolocation by bats is equivalent to the cost of flight alone. Essentially, bats echolocate for free (Speakman et al., 1989; Speakman and Racey, 1991).

Shortly after the discovery of the linkage phenomenon, Speakman et al. (Speakman et al., 1989) noted:

> 'In small terrestrial animals where no large muscle system like the pectoralis and scapularis groups ventilate the respiratory system at the same high rates and depths as those observed in flying mammals and birds, echolocating might be a much more costly option.'

In the same manuscript, Speakman et al. also noted:

> 'It is significant therefore that whilst many small terrestrial mammals can produce low intensity ultrasound, are receptive to it, and often communicate with it, none has developed a system of echolocation involving high intensity ultrasound calls.'

Very few terrestrial animals use echolocation (Thomas and Jalili, 2004), and those that do tend to use lower intensity pulses than bats use [e.g. shrews (Gould et al., 1964)]. This might reflect the prohibitively high energetic cost of echolocation in the absence of flight (Speakman et al., 1989; Speakman and Racey, 1991) or simply the need for bats, which travel faster than terrestrial mammals do, to have information about more distant targets than those of terrestrial mammals. However, two species of bat that are known to echolocate in flight also spend significant proportions of their time walking on the ground like terrestrial mammals: the common vampire bat (*Desmodus rotundus*) and the New Zealand short-tailed bat (*Mystacina tuberculata*). How the structure echolocation calls produced by these bats, and their pattern of emission, differ in the air *versus* on the ground offers insight into the importance of locomotor context on echolocation call design. *Desmodus rotundus* is a phyllostomid sanguivore that feeds on domesticated livestock such as cattle and goats (Greenhall and Schmidt, 1988), while *M. tuberculata* is an omnivore endemic to New Zealand and the sole remaining member of the family Mystacinidae (Lloyd, 2001). These two lineages evolved their terrestrial abilities independently and under different selective pressures (Riskin et al., 2006).

Both *D. rotundus* and *M. tuberculata* are agile on the ground and have a quadrupedal walking gait similar to those of most other terrestrial mammals (Riskin and Hermanson, 2005; Riskin et al., 2006). While it is known that both species echolocate while moving on the ground (Jones et al., 2003), nothing is known about linkage of echolocation and flight, or while walking, for any species.

It is reasonable to expect the timing of echolocation to coincide with walking kinematics. For one, breathing cycles of quadrupedal terrestrial mammals are known to correlate with the timing of footfalls. In dogs, for example, the deceleration of the center of mass that occurs early in the footfall sequence causes the organs of the abdomen to push cranially against the diaphragm and thoracic cavity, facilitating exhalation (Bramble and Carrier, 1983; Bramble and Jenkins, 1993). This might reduce the cost of call emission for crawling bats. However, slow gaits may not cause sufficient movement to effect a visceral 'pump' mechanism linking walking and echolocation. However, horses and dogs can (if they choose to) ignore footfall timings, and the importance of this mechanism for bats is not known (Ainsworth et al., 1997).

In the present study, we investigated the coupling of echolocation and locomotor kinematics of *M. tuberculata* during aerial and terrestrial locomotion. We predicted that *M. tuberculata* would couple echolocation call emission and wingbeat cycle in the manner already demonstrated for other bats, despite its divergence from all other bats some 45 million years ago. We also predicted that *M. tuberculata* would couple echolocation call emission and stride cycle when walking on the ground. Further, we compared the structure of echolocation calls made during walking to those made during flight. We expected to find more intense calls in the air, since bats in flight need information about more distant objects than crawling bats do. If call emission rates are dictated by stride frequency in flight and on the ground, we should expect calls to be emitted more frequently during flight than on the ground.

MATERIALS AND METHODS

The study was carried out in the Eglinton Valley, Fiordland, New Zealand (44°58′S, 168°1′E) in January 2007. Eight southern short-tailed bats (*Mystacina tuberculata tuberculata* Gray 1843; four adult males and four adult non-pregnant, non-lactating females) were captured using mist nets and transported back to the field-based laboratory individually in small cloth bags. When not being used in experiments, bats were kept in a dark room and fed water and mealworms. No bat was kept for more than 48 h and all were released at their point of capture after use. All manipulations were done under permits issued by the University of Auckland Animal Ethics Committee and the New Zealand Department of Conservation, with approval from the Cornell University Institutional Animal Care and Use Committee.

Kinematic and echolocation recordings

Bats were flown individually down a corridor (8.0 m length × 1.5 m width × 2.2 m height; Fig. 1). Video of the bats in flight was recorded using two phase-locked high-speed digital video cameras (Redlake Systems, Photron USA, San Diego, CA, USA), each recording at 250 frames s^{-1}. One 'on-axis' camera was placed 7.0 m down the corridor from the point of release, looking down the length of the corridor. The whole hallway was visible to the on-axis camera. The second camera was placed roughly perpendicular to the first, in a small alcove so that a ~3.4 m^3 (1.5 m×1.5 m×1.5 m) volume of the flight corridor approximately 5.0 m from the point of release was visible to both cameras simultaneously. This permitted 3-D reconstruction of a bat's position within that volume, using the Direct Linear Transformation technique (Hedrick et al., 2004). The position of the audio microphone used to record echolocation calls (see below) was within the calibrated volume, so its position was also known.

Echolocation calls produced by bats as they flew down the corridor were recorded using a Brüel and Kjær $\frac{1}{4}''$ microphone (Nærum, Denmark; model 4939, frequency response 20 Hz–100 kHz ± 0.2 dB, protection grid removed). The microphone was positioned in the middle of the corridor, 1.5 m above the ground, to ensure bats approached it on-axis. Echolocation calls were digitized directly to computer using a National Instruments DAQCard 6062E (National Instruments, Austin, TX, USA), sampling at 220 kHz with 12-bit precision. Digitization was controlled by Avisoft Recorder software (v. 3.4; Avisoft Bioacoustic, Berlin, Germany). Echolocation calls were analysed in Raven (v. 1.2; Cornell Laboratory of Ornithology, Ithaca, NY, USA) and Matlab (Release R2007a, The Mathworks, Natick, MA, USA). Peak intensity of calls was calculated relative to a Brüel and Kjær sound calibrator (type 4231; producing 92 dB SPL at 1 kHz).

Audio and video recordings were synchronized using a custom-built synchronizer. When triggered by hand, the synchronizer produced a 2-s 500 Hz square wave at 1 V, which was recorded alongside the audio by the DAQCard whilst simultaneously illuminating a small LED. The LED was visible to both video cameras, allowing synchronization of the two cameras and the audio recordings to a resolution of 4 ms.

Timing of echolocation pulses within a wingbeat cycle

To determine the timing of echolocation pulses relative to the wingbeat cycle, we divided each wingbeat into a downstroke phase and upstroke phase, based on the upper and lower reversal points of the wingtip, visible in the on-axis camera. Because the sounds emitted

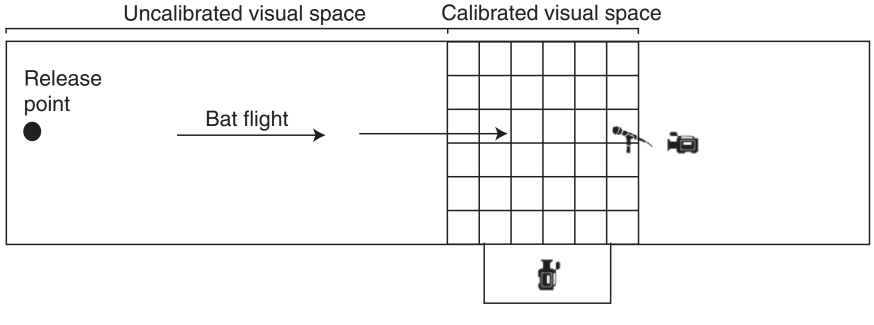

Fig. 1. Diagrammatic representation (overhead) of the corridor down which the bats flew. The calibrated space represents the area where the visual fields of both high-speed cameras overlapped, and so the position of the bats relative to the microphone could be determined. The intensity of calls was calculated as bats flew through this space. The uncalibrated space was visible only to the camera looking down the corridor. Information on wingbeat and all other echolocation call parameters were measured as the bats flew in this space.

by bats took time to reach the microphone, the apparent timing of calls relative to the wingbeat cycle was delayed, with the offset increasing with distance to the microphone. We corrected for this by shifting call recordings backward in time by the distance from the bat's mouth to the microphone, divided by the speed of sound ($340\,\mathrm{m\,s^{-1}}$). In the calibrated space, we used the actual distance between the bat's mouth and the microphone. To estimate the position of the bat's mouth before it entered the calibrated space, we used its flight trajectory in the calibrated volume to extrapolate its distance to the microphone before reaching the calibrated volume. The maximum offset resulting from the distance between bat and microphone was 0.01 s, approximately 1/68 s. This equates to a maximum potential error of approximately 16% of a wingbeat cycle.

The timing of echolocation call production in the wingbeat cycle was calculated by first assigning each part of the wingbeat cycle a degree of phase. Each wingbeat was divided into 360 deg. of phase, with 360 deg. (=0 deg.) as the transition point from upstroke to downstroke (upper reversal point) and 180 deg. as the transition point from the downstroke to the upstroke (lower reversal point). The points at which the wings extended laterally were assigned 90 deg. and 270 deg., respectively. This permitted comparisons of timing in multiple trials despite changes in the portion of the wingbeat cycle that consisted of downstroke [called downstroke ratio (Norberg, 1990)]. We measured the timing of each call, relative to wingbeat cycle, and binned calls to one of 24 bins of 15 deg. each for statistical analyses (e.g. 0–14 deg., 15–29 deg., etc.).

Walking bats

Bats were encouraged to walk along a length of treated timber ($2\,\mathrm{m}\times0.1\,\mathrm{m}\times0.025\,\mathrm{m}$), raised approximately 0.05 m off the ground. We verified that the timber was sufficiently wide to allow normal stride kinematics by comparing videos of walking bats with those recorded previously on a flat surface (Riskin et al., 2006). Video of the bats' walking was recorded using one camera looking vertically down on the timber. The same high-frequency microphone used for flight experiments was placed at one end of the timber, on-axis with the bats as they walked towards it. The microphone and bat were both visible to the camera at all times. The position of the bat's mouth relative to the microphone was calculated based on its horizontal position, with changes in height neglected. Audio and video sequences were fully synchronized, and errors associated with distance of the bat from the microphone (video *versus* audio) were corrected in the same manner as that described above.

The timing of echolocation call production relative to the terrestrial stride cycle was recorded by measuring the timing of pulse emission relative to left and right forelimb kinematics. Footfall patterns of the hindlimbs are correlated with those of the forelimbs (Riskin et al., 2006) and were thus inferred based on our recordings of the forelimbs. Each stride cycle was split into 360 deg. of phase, with 0 deg. being the point when the left forelimb was lifted from the timber and 180 deg. being the point where the left forelimb was planted back on the timber. The same method was used to classify the movement of the right forelimb, but this was represented by 180 deg. to 360 deg. The timing of echolocation call production during each stride was assigned in the same way as for when the bats were flying. All calls produced by each bat as it walked along the timber were assigned to one of the 15 deg. phase categories.

Multiple wing angles were recorded from each bat, thus potentially pseudoreplicating the data. To reduce the effect of this increased sample size, when Raleigh tests were carried out the degrees of freedom were adjusted to be 7 (one less than the total number of bats used in the study).

Call intensity and structure

Information on the intensity of echolocation calls was determined from recordings made while the bats flew in the calibrated space. Intensity is expressed in dB peSPL at a distance of 10 cm from the source. Loss of intensity due to spherical spreading was assumed to be 6.02 dB per doubling of distance while atmospheric attenuation was calculated from Lawrence and Simmons (Lawrence and Simmons, 1982) based on the peak frequency of *M. tuberculata* calls (28 kHz). Parameters measured from echolocation calls of flying and walking bats were: duration (ms), start frequency of the fundamental (kHz), end frequency of the fundamental (kHz), intensity (dB peSPL), duty cycle (%) and pulse repetition rate (Hz). Wingbeat frequency (Hz) was measured from flying bats, and stride frequency (Hz) was measured from terrestrial locomotion. To avoid pseudoreplication, comparisons between bats were based on the mean values for each individual. No differences were found between male and female bats and so data were pooled for all subsequent analyses. Where possible, paired statistical tests were carried out. All statistical tests were carried out using JMP software (v. 7; SAS Institute, Cary, NC, USA). Raleigh tests were calculated by hand according to Batschelet (Batschelet, 1981) and Fisher (Fisher, 1993).

RESULTS

Flying and walking bats produced short, frequency-modulated echolocation calls, with up to four harmonics present within the bandwidth of the recording equipment (Fig. 2). Most energy was contained in either the first or second harmonics. When flying, individual bats produced calls that were significantly longer, started at a higher frequency and had a higher frequency with most energy compared with when the bat was walking (Table 1). Although not statistically different, calls produced when flying were on average 6 dB peSPL louder than calls produced when walking, but calls were produced at a lower rate (Table 1). This trend was mirrored in the lower duty cycle of flying *versus* walking bats.

When in flight, an average of 1.9 calls was produced per wingbeat. Bats produced calls on both the upstroke and the downstroke, with no significant preference shown ($P>0.05$, paired-$t=1.15$; Fig. 3). During a significant proportion of up- or downstrokes, only a single call was produced ($P<0.0001$, $F=25.27$, *post-hoc* Tukey-Kramer HSD, $P<0.05$ $q=2.98$; Fig. 4), when compared with either no calls or two calls. Calls were on average 1.5 dB peSPL more intense on the upstroke when compared with the downstroke, but the difference was not significant ($P>0.05$, paired-$t=2.10$). When the bats were walking, there was no significant difference between the number of calls produced per unit time when either limb was in motion or both were simultaneously planted on the substrate ($P>0.05$, $F_{2,23}=0.03$).

Analysis of linkage between wingbeat and echolocation call production showed that the distribution of calls with phase of wingbeat was not random ($P<0.001$, $\alpha=10.37\pm93.75$; Fig. 5). The majority of calls were produced when the wings were below horizontal; the bats called during the second half of the downbeat and the first half of the upbeat. When bats were walking, the distribution of echolocation calls showed no association with movement of the forelimbs ($P>0.05$, $\alpha=307.28\pm119.44$; Fig. 6).

DISCUSSION

Based on the guiding hypotheses suggested by Speakman et al. (Speakman et al., 1989), we predicted that *M. tuberculata* would

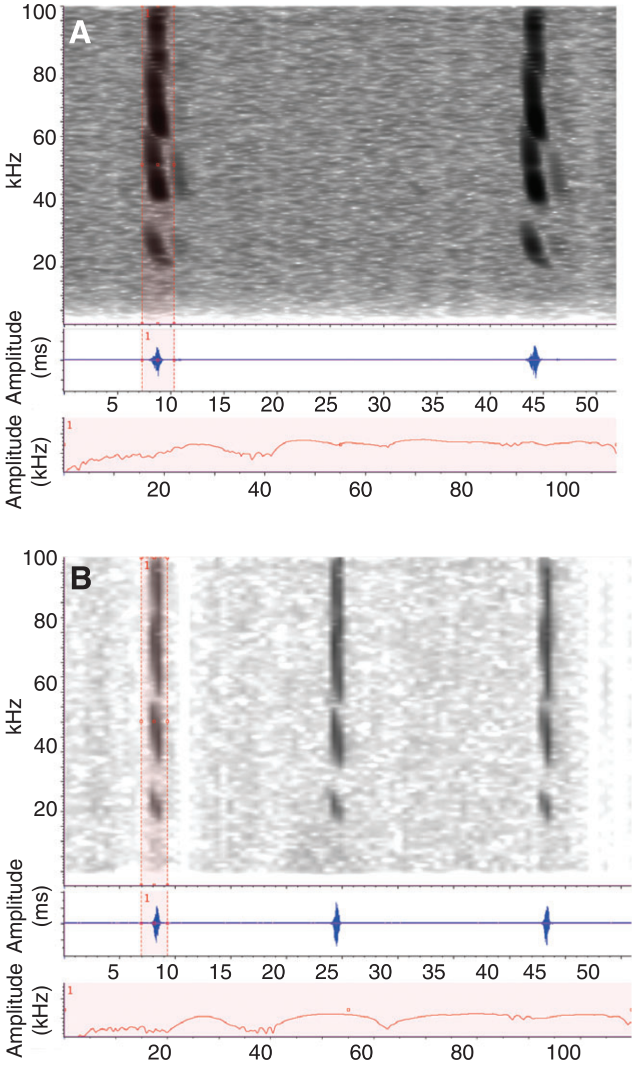

Fig. 2. Spectrogram (top), waveform (middle) and power spectrum (bottom) of echolocation calls produced while the bats were (A) in flight and (B) walking. Spectra were produced using a 512-point FFT with 85% overlap and a Hanning window. The spectrum is derived from the first call in each sequence.

Table 1. Descriptive statistics of parameters measured from calls of flying and walking bats

	Flying	Walking	*P* (*t*)
Duration (ms)	2.2±0.25	1.6±0.14	* (2.51)
F_{start} (kHz)	35.0±0.28	33.8±0.24	* (2.92)
F_{end} (kHz)	20.9±0.26	20.9±0.24	ns (0.23)
F_{MaxE} (kHz)	27.1±0.18	26.6±0.29	* (2.86)
Duty cycle (%)	7.5±1.7	13.1±2.1	ns (2.2)
Intensity (dB peSPL)	94.1±1.9	88.2±1.1	ns (2.13)
Wingbeat rate (Hz)	11.1±0.2	–	*
Call rate (Hz)	21.1±1.8	25.4±2.8	ns (1.10)

Wingbeat rate for flying bats is also included. Values given are means±1 s.e.m. Statistics comparisons are all based on paired *t*-tests with N=8. F_{start}, start frequency of the fundamental; F_{end}, end frequency of the fundamental, F_{MaxE}, frequency with most energy. *$P<0.05$; ns, not significant at the 5% level.

(1) link echolocation call production with wingbeat during flight and (2) link echolocation with forelimb movement whilst walking. Results showed that the bats produced echolocation calls either late in the downstroke or early in the upstroke when in flight. However, calls were produced at all points in the stride cycle of the forelimbs during terrestrial locomotion. Walking bats produced calls at a higher rate but at a lower intensity compared with bats in flight.

The bats used in this series of experiments produced echolocation calls typical of the species (Jones et al., 2003; Parsons, 2001). However, bats in flight produced calls that were longer and with a fundamental frequency that was slightly higher than calls produced while walking. Bats in flight produced equal numbers of mainly single calls of similar intensity on both the upstroke and downstroke of the wingbeat. While walking, bats produced a significantly higher proportion of calls when the limbs were in motion than when they were stationary. Although not statistically significant, calls of flying bats were more intense, but produced at a lower rate, than calls of walking bats. Although calls produced during flight were associated with particular phases of wingbeat, no association was found with phase of stride when bats were walking.

Echolocation call production by *M. tuberculata* is not coupled with limb movement during terrestrial locomotion. While walking on the ground, there appears to be no relationship between the timing of call production and movement of the forelimbs (wings). To our knowledge, this is the first study to describe this relationship in bats. This result suggests that the bats receive no assistance from muscle contraction associated with terrestrial locomotion when echolocating and so must bear the full energetic cost of call production in addition to the cost of walking. Speakman et al. (Speakman et al., 1989) estimated the cost of call production by *Pipistrellus pipistrellus* (a 6 g bat) at rest to be 2.42 kJ h^{-1} or 9.5× basal metabolic rate (BMR) when calling at a rate of 10 Hz. Although the energetic cost of terrestrial locomotion by bats is unknown, an equivalent-sized mammal expends an estimated 6–8× BMR when running (Hart, 1950; Pasquis et al., 1970; Segrem and Hart, 1967). Assuming a similar cost for a bat, and the absence of linkage, the summed cost of echolocation and terrestrial locomotion may be as high as 17.5× BMR. This is far below the estimated 34× increase in BMR for *Phyllostomus hastatus* in flight [c.f. a bat resting in a chamber (Thomas and Suthers, 1972)], but greater than the 4× BMR for *P. hastatus* whist alert and perching. Speakman and Racey (Speakman and Racey, 1991) suggested that terrestrial mammals call at relatively low intensities due to the relatively high energetic cost. Calls produced by *M. tuberculata* while walking are approximately 8700 times more intense than those produced by shrews (Gould et al., 1964), suggesting that energetic cost may not limit call intensity for terrestrial vertebrates. Results from this study indicate that *M. tuberculata* calls at approximately 6 dB SPL less (a reduction of approximately 6.4%) than when flying. However, calls are produced at a rate approximately 120% greater than when bats are flying, thus more than offsetting the energetic savings of calling at a lower intensity.

It is interesting to speculate about the functional significance of echolocation when *M. tuberculata* is on the ground. Jones et al. (Jones et al., 2003) showed that, whilst foraging amongst leaf litter, *M. tuberculata* relies on olfaction and, to a lesser extent, prey-generated sounds to locate invertebrate prey. This is unsurprising given the acoustic clutter associated with the forest floor. However, despite this high degree of clutter, Jones et al. (Jones et al., 2003) and the present study both recorded echolocation calls from *M. tuberculata* while walking. Interestingly, Jones et al. (Jones et al., 2003) reported a call rate of only 5 Hz, while this study found a

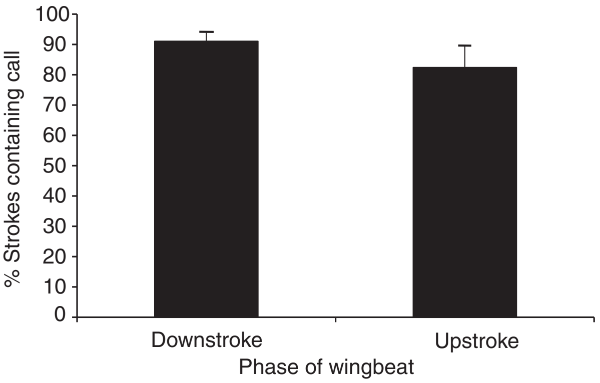

Fig. 3. Mean percentage of down- and upstrokes that coincided with the production of at least one echolocation call. Values shown are the means across the eight bats tested. Error bars represent 1 s.e.m. There was no significant difference in the proportion of calls produced during the down- and upstrokes. Details of statistical tests are given in the text.

much higher rate of 25.4 Hz. In the present study, there was little or no acoustic clutter surrounding the bats as they walked along the plank of wood, and so echolocation would have provided the animals with information on their surroundings. Suthers et al. (Suthers et al., 1972) recorded call rates of up to approximately 16 Hz from *P. hastatus* scanning their clutter-free laboratory environment while resting or crawling. By contrast, little information would have been available to the bats within the leaf litter of Jones et al.'s study (Jones et al., 2003). However, this difference in clutter still does not explain why *M. tuberculata* continues to echolocate whilst amongst the leaf litter, an environment where echolocation is unlikely to provide any useful information on the surrounding environment, and yet costs a significant amount of energy.

Mystacina tuberculata couples wingbeat with echolocation call production in a manner typical of a gleaning bat. The majority of aerial-hawking bats produce between 1 and 1.5 echolocation calls per wingbeat, with clutter-foraging and gleaning species producing <2 calls per wingbeat (Britton et al., 1997; Grinnell and Griffin, 1958; Holderied and von Helverson, 2003; Jones, 1994; Kalko, 1994; Suthers et al., 1972; Waters and Wong, 2003). Whilst in flight, *M. tuberculata* produced on average 2 calls per wingbeat. These results disagree with those of Jones et al. (Jones et al., 2003), who found that *M. tuberculata* called approximately once per wingbeat. The conclusions of Jones et al. were based on an assumed wingbeat rate of approximately 10 Hz (modified from Jones, 1994), an assumption that agrees with our measured rate of 11.1 Hz. However, call production rates measured in this study are significantly higher than those of Jones et al., who obtained values of 12.4–13.8 Hz from free-flying bats. Interestingly, the results of Jones et al. (Jones et al., 2003) disagree with the predictions of Jones (Jones, 1994) that gleaning bats such as *M. tuberculata* will produce <2 calls per wingbeat. The results of the present study agree with the predictions of Jones (Jones, 1994), and we suggest that the higher call rate may be due to the unfamiliarity of the bats with the flight room. Suthers et al. found that as *P. hastatus* became familiar with their flight room, calling rates decreased and bats tended to produce only one call per wingbeat (Suthers et al., 1972). As the call rates published by Jones et al. (Jones et al., 2003) were derived from recordings made near roosts, it is possible that these bats relied more on spatial memory and so called at a lower rate. Unfortunately, Jones et al. (Jones et al., 2003) do not provide call rates for their bats when recorded in a flight room within a similar space to that used in this study.

Echolocating bats couple call production with wingbeat cycle, thus minimizing energetic cost. This tactic is so successful that echolocation in flight incurs little additional cost above that of flight alone (Racey and Speakman, 1987; Speakman et al., 1989; Speakman and Racey, 1991; Thomas and Suthers, 1972). Studying *P. hastatus*, Suthers et al. showed that respiration, wingbeat and echolocation call production were linked when the animals were in flight, but not when resting or crawling (Suthers et al., 1972). They showed that, in flight, inspiration was associated with the downstroke of the wing and that single calls were generally emitted at the end of the downstroke, i.e. at the end of inspiration. When more than one call was produced per wingbeat cycle, they occurred at the beginning and end of expiration, or during early inspiration. At these points in the respiratory cycle, the wings were rising from their downward-most position or were near the peak of their upward-most position. Wong and Waters found that *Pipistrellus pygmaeus* tended to produce single and double pulses in the second half of the upstroke or the first half of the downstroke (Wong and Waters, 2001). Britton et al. found a similar result for *Myotis dasycneme* (Britton et al., 1997). In the case of double pulses produced by *P. hastatus* and all pulses produced by *P. pygmaeus* and *M. dasycneme*, call production occurs during contraction of the pectoralis and serratus ventralis muscles as they decelerate the wing during the

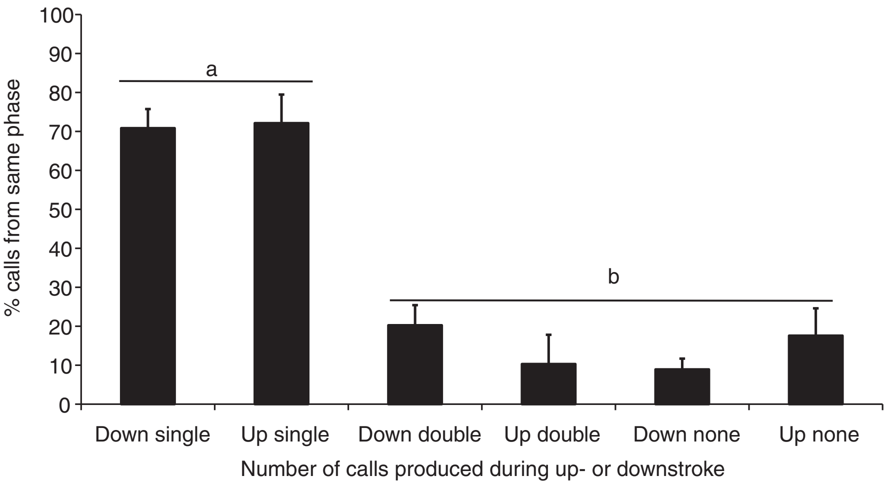

Fig. 4. Mean percentage of down- and upstrokes that coincided with the production of one, two or no echolocation calls. Categories connected by a line and lowercase letter are not significantly different at the 5% level according to a *post-hoc* Tukey-Kramer HSD test, following an analysis of variance. During a significant proportion of up- or downstrokes, only a single call was produced. Details of statistical tests are given in the text. Values shown are the means across the eight bats tested. Error bars represent 1 s.e.m.

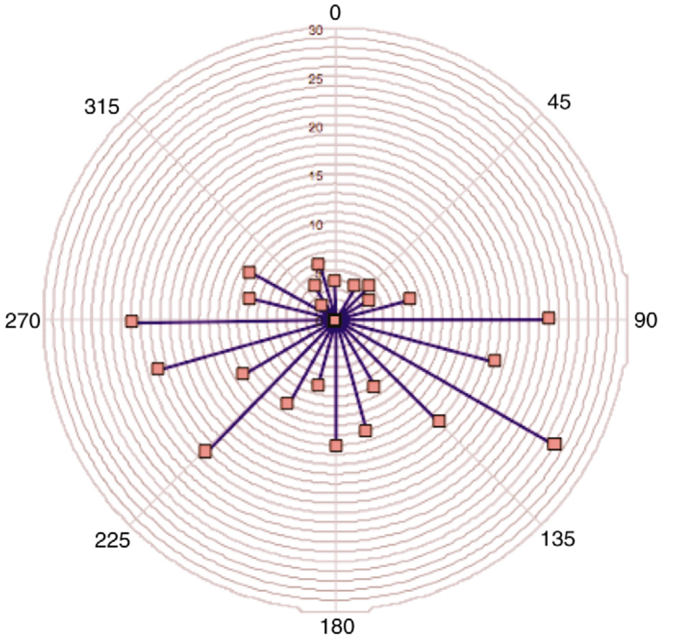

Fig. 5. Circular plot showing the timing of echolocation call production relative to phase of wingbeat (mean vector=170 deg., 95% CI=151–188 deg.). The circumference of the circle represents phase of wingbeat. Values from 0 to 180 deg. represent the downstroke while values from 180 to 360 deg. represent the upstroke. Values on the spokes of the circle are sample size. Data from all eight bats were pooled for the analysis but the degrees of freedom were set at 7 for the Raleigh tests. Echolocation calls were not randomly associated with phase of wingbeat (α=10.37±93.75, *P*<0.001); bats produced a higher number of calls during the second half of the downstroke and the first half of the upstroke.

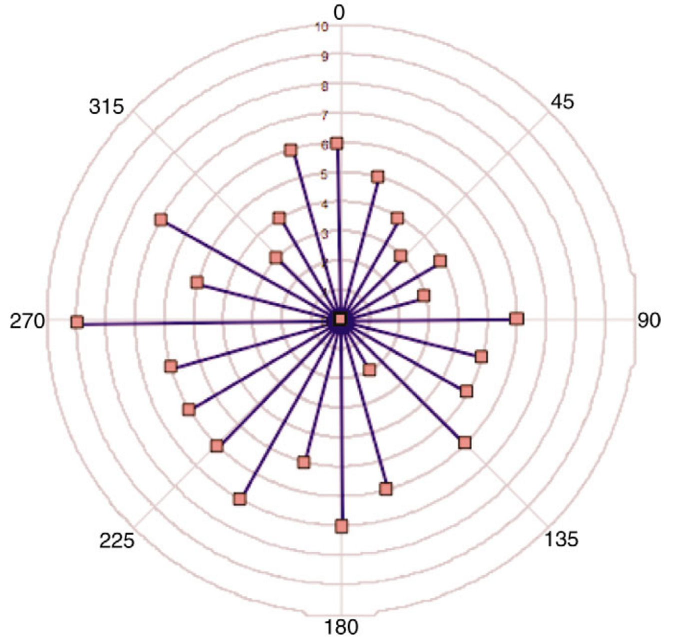

Fig. 6. Circular plot showing the timing of echolocation call production relative to phase of stride cycle (mean vector=170 deg., 95% CI=151–188 deg.). Values around the circumference of the circle represent phase of stride of the left (0–180 deg.) and right (180–360 deg.) forelimbs. Values along the spokes of each plot are sample size. Data from all eight bats were pooled for the analysis but the degrees of freedom were set at 7 for the Raleigh tests. Echolocation calls were randomly associated with phase of limb motion while the bats were walking (α=307.28±119.44, *P*>0.05).

latter part of the upstroke and accelerate the wing into the powerful downstroke (modified from Hermanson and Altenbach, 1983; Altenbach and Hermanson, 1987). According to Lancaster et al. (Lancaster et al., 1995), abdominal wall musculature may be more important in powering vocalizaton than are the flight adductor muscles during flight and at rest. They reported a consistent electromyographic (EMG) activity during inspiration in *Pteronotus parnellii,* which occurred during the late downstroke and early upstroke and which did not overlap with activity in the pectoralis or serratus ventralis muscles. Although there was a correlation of EMG activity pulses in pectoralis and serratus ventralis muscles with some pulses, there was a better fit between EMG surface recordings from the lateral abdominal wall muscles. Additionally, Lancaster et al. reported a consistent pattern of abdominal and flank movements (Lancaster et al., 1995). Inspiration was correlated with diaphragmatic activity and expansion of the abdominal wall and not expansion of the thorax. Expiration was consistent with ‘collapse’ of the abdominal wall rather than thoracic movement. However, this pattern of linkage does not explain the production of single pulses – late in inspiration or early during expiration by *P. hastatus*, early in expiration by *Myotis lucifugus*, *Pteronotus rubiginosa*, *Carollia perspillicata* and *Rhinolophus ferrumequinum* (Schnitzler, 1971) – or the production of double pulses late in inspiration and early in expiration by *M. tuberculata*.

Echolocation calls produced by *M. tuberculata* when in flight are of intermediate intensity. Griffin defined bats as being either loud (calls produced with an intensity of <110 dB SPL at 10 cm) or whispering (>75 dB SPL at 10 cm) (Griffin, 1958). Fenton and Bell further refined this classification with the introduction of an intermediate category of intensity for bats calling between 75 dB and 90 dB (both at 10 cm) (Fenton and Bell, 1981). The majority of studies investigating call intensity have been carried out in the laboratory. Results from these studies have shown that aerial-hawking bats tend to produce the most intense calls while those foraging in and amongst clutter or gleaning prey from surfaces produce the quietest calls (Fenton and Bell, 1981; Griffin, 1958). However, it has long been recognized that captive studies may underestimate the intensity at which bats produce calls [e.g. by 15–25 dB SPL for *Craseonycteris thonglongyai* and *Myotis siligorensis* (Surlykke et al., 1993)]. Some field-based studies may also underestimate call intensity, as the highly directional echolocation calls are often recorded off-axis from equally directional microphones (Ghose and Moss, 2003; Hartley and Suthers, 1989; Pye, 1993; Surlykke et al., 1993). More recent field-based studies have shown that aerial-hawking bats call at higher intensities than previously thought. Holderied and von Helverson showed that 11 species of bat in Europe could all call at intensities above 124 dB peSPL at 10 cm, with *Nyctalus lasiopterus* calling at up to 133 dB peSPL (Holderied and von Helverson, 2003). Surlykke and Kalko calculated source levels varying from 122 dB SPL to over 140 dB SPL from 11 species in Panama; calls from *Noctilio albiventris* were loudest (Surlykke and Kalko, 2008). Finally, the intensity of calls produced by *Eptesicus bottae* and *Eptesicus serotinus* have been estimated to be between 121 and 124 dB SPL at 10 cm (Jensen and Miller, 1999; Holderied et al., 2005).

The intensity of calls produced by bats that typically forage in clutter or glean prey from surfaces is less likely to be underestimated by laboratory studies. The flight rooms used to study bats in captivity likely present similar, or perhaps less-cluttered, environments than the ones encountered under natural conditions. The calls recorded here for *M. tuberculata* match search-phase calls recorded in the field in terms of temporal and spectral structure (Jones et al., 2003; Parsons, 2001), indicating that the bats were not adjusting the duration or pitch of their calls to the captive environment. Therefore, the intensity of calls recorded in this study agree with published evidence that *M. tuberculata* is a maneuverable flyer that forages *via* echolocation in areas of high clutter either by aerial hawking or gleaning (Jones et al., 2003; O'Donnell et al., 1999; Parsons, 2001; Webb et al., 1998). The relatively low intensity of *M. tuberculata* calls may be due to the increased energetic cost of echolocating at higher rates compared with aerial-hawking species (Jones, 1994) (see discussion below). These results also suggest that clutter foraging/gleaning bats may call at higher intensities than previously thought and that the original categorizations of Griffin (Griffin, 1958) and Fenton and Bell (Fenton and Bell, 1981) may require revision.

Further research is required to determine the energetic cost of terrestrial locomotion in bats, and comparative studies with other quadrupedal species such as *D. rotundus* (Riskin and Hermanson, 2005) and possibly *Diaemus youngi*, *Diphylla ecaudata* and *Cheiromeles torquatus* (Riskin et al., 2005; Schutt and Simmons, 2001) are clearly required. Riskin et al. suggest that the bounding gait of *D. rotundus* may be more energetically efficient than similar gaits in terrestrial mammals (Riskin et al., 2006). However, given that the energetic cost of echolocation during terrestrial locomotion may be half that of flight and that *M. tuberculata* is able to sustain echolocation whilst on the ground (at rates of 5 or 25 Hz), it is surprising that echolocation is not more widespread in terrestrial mammals.

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