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Don't believe the mike: behavioural, directional, and environmental impacts on recorded bat echolocation call measures[§]

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[§] This review is one of a series of papers arising from “*Learning to Listen — Second International Symposium on Bat Echolocation Research: Tools, Techniques, and Analysis*” that was held in Tucson, Arizona, USA, 26 March – 1 April 2017. Invited speakers were encouraged to submit manuscripts based on their talks, which then went through the normal *Canadian Journal of Zoology* review process.

Abstract

Echolocation calls produced by bats in their larynges allow these flying, nocturnal mammals to orient and find food at night. The acoustic signals are not like bird song, and even individual bats exhibit great flexibility in call design and between-species overlap is common. As a result, identifying bats to species by their echolocation calls even in communities with few bat species can be difficult. Unfortunately, the situation is worse still. As a result of several factors – some to do with microphones, some with environment, some with bats and the calls themselves – acoustic information transmitted to- and transduced by microphones can be dramatically different from the actual signal produced by the bat and as would be recorded on axis, close to its mouth using ideal microphones under ideal conditions. We outline some of these pitfalls and discuss ways to make the best of a bad situation. However, overall we stress that many of these factors cannot be ignored and do impact our recordings.

Keywords: Echolocation, bats, recording, signal fidelity, microphones, directionality

51 **Background and introduction**

52 Currently, there are well over 1300 recognized species of bats making bats the
53 second most species rich mammalian order after the rodents (Fenton and Simmons
54 2014). The estimate increases each year. Some of the largest bat communities –
55 known to number more than 150 species (Fenton and Ratcliffe 2010) – are found in
56 S. America and SE Asia, relatively understudied locations that are likely inhabited by
57 even more as yet unidentified species. More than 1000 bat species are laryngeal
58 echolocators and most, if not all, consistently produce echolocation calls whenever
59 they are in flight. When foraging and in transit, bats are difficult to visually identify to
60 species given the speed of flight, low light conditions, and their generally small size.
61 However, an individual bat's recorded echolocation calls not only yield valuable
62 insight into the bat's current behavioural context, call design can potentially provide
63 reliable clues to a bat's species identity. That said bat echolocation calls have been
64 shaped by natural selection primarily to optimize perception, not as a means of
65 communicating species identity and quality as a potential mate, as in species-specific
66 bird song (Barclay 1999). As a result, even at the individual level, bats produce calls
67 of different duration, peak frequency (frequency of maximum energy), and bandwidth
68 depending on context and environment (Surlykke and Moss 2000).

69 All non-echolocating or non-laryngeal echolocating bats belong to the family
70 Pteropodidae (~200 species); all other bat species belong to one of the ~20
71 remaining chiropteran families (Fenton and Ratcliffe 2010). Based on acoustic
72 recordings, some bats can be accurately assigned to their correct family based on
73 unique suites of call characteristics and knowledge of what families are present at a
74 given geographic location. For example, across the United Kingdom there are ~20
75 bat species, belonging to either the family Vespertilionidae or the family

Rhinolophidae (two species) (ter Hofstede et al. 2013). Vespertilionids all produce frequency-modulated (FM), downward sweeping calls, at low duty cycles (<30%) (Figure 1a). Rhinolophids produce longer, essentially constant frequency (CF) calls that are often bookended by very short, steep downward sweeps (Fenton et al. 2012; Figure 1b). No matter what the context, in the United Kingdom the chances of confusing a vesper bat's echolocation calls with those emitted by a rhinolophid are vanishingly small (Figure 1) and because the two rhinolophid species found in Britain use very different peak frequencies in the CF portion of their calls (Greater horseshoe bat, *Rhinolophus ferrumequinum* (Schreber, 1774): ~80kHz; Lesser horseshoe bat, *Rhinolophus hipposideros* (Bechstein, 1800): ~110kHz) (Fenton et al. 2012), it is possible to assign each to species with confidence at any given location (ter Hofstede et al. 2013).

Roughly half of laryngeal echolocating bats belong to the superfamily Vespertilionoidea. Approximately 425 of these are vespertilionids, ~100 of them are molossids, with the remaining 30 or so species found in the families Natalidae and Miniopteridae (Fenton and Simmons 2014). With respect to echolocation call design, bats from the superfamily Vespertilionoidea exhibit the greatest flexibility (Simmons et al. 1979; Neuweiler 1990). For a given individual of a given species, as the bat moves from open space to more cluttered space call duration decreases, peak frequency and bandwidth increases (Jensen and Miller 1999; Surlykke and Moss 2000). As a result of this vast number of species and the incredible extents of individual flexibility, sympatric species of similar size and foraging ecology often overlap in call design when in the same habitat (Obrist 1995; Biscardi et al. 2005). Worse still for species identification the calls of a larger vespertilionid species in clutter may look like those of a smaller species (e.g., Jakobsen et al. 2013). As these

bats approach airborne prey, they also decrease call duration and increase peak frequency and bandwidth (Griffin et al. 1960; Surlykke and Moss 2000), however at the terminal phase of attack this pattern ends: bats instead drop peak frequency by an octave (Ratcliffe et al. 2013). These terminal buzz calls are highly conserved across species and thus especially poorly suited for species identification (Ratcliffe et al. 2013). These challenges are given fuller treatment in Russo et al. (this issue).

In addition to the above issues, all associated with the difficulty of assigning a given call or series of calls to one species or another, the recording scenario (i.e. the recording equipment and the bats location relative to the recording equipment) will more often than not severely impact the recorded structure of the call, adding substantially to the uncertainty involved in species recognition. Our purpose here in this short paper is twofold: first to provide guidelines for call description that - while not silver bullets - we believe will make the best of a bad situation and, second, to make new researchers aware of the limits imposed on recorded call fidelity. We essentially focus on a single echolocation call from the vespertilionid *Myotis daubentoni* (Kuhl, 1817) to illustrate that even a single call, produced by a single bat can be transmitted and transduced under different conditions in very different ways, and that this fact makes even describing a single call accurately with respect to emitted structure difficult. Given this reality, assigning calls recorded in multi-species bat communities to species automatically is a risky business.

Quantifying common call parameters

Laryngeal echolocating bats tend to produce calls well above our hearing range; that is, most bats use call frequencies above 20 kHz. To listen to them they must be artificially transposed to frequencies roughly 10 times lower than in reality

(Griffin 1958). For these and other reasons many bat researchers tend to inspect echolocation call design and call emission rates visually. Ideally, using a combination of spectrograms, oscillograms and power spectra (FFTs, fast Fourier Transforms) (Figure 2). Spectrograms allow for rough visualization of call shape and frequency content and an even rougher estimate of signal duration and, using a colour or grey scale bar, energy distribution among the included frequencies. While they are often used for much more, our recommendation is that spectrograms only be used to distinguish echolocation calls from echoes and non-echolocation call sounds and for species identification in bat communities where the time-frequency structure clearly separates species, as in the previous example of the horseshoe bats in the United Kingdom. With respect to environmental influence, a spectrogram of a vespertilionid bat echolocation call that has travelled along a single path to the microphone (i.e. not containing any reflections) looks like a smooth downward sweep (Figure 1a, Figure 2a). Calls that have instead travelled along multiple paths as a result of reflections off vegetation and/or water surfaces will appear distorted (Figure 2b), often containing nulls (i.e. wave cancellations) that are specific to the time-delay between the direct path and the longer path of the reflection(s).

With respect to computational influence, obtaining quantitative measurements from spectrograms such as call duration, bandwidth and peak frequency is also prone to errors because of the inherent trade-off between accuracy in the time and frequency domains of the spectrogram. That is, call duration will be overestimated on most if not all spectrograms (Figure 3), while call peak frequency and bandwidth obtained from spectrograms are typically just as unreliable. Our strong recommendation is to always measure call duration on the oscillogram and frequency parameters on the power spectrum using standardized repeatable measures. For

further details on the pitfalls of spectrogram measurements we recommend Zollinger et al. (2012) and Brumm et al. (2017). Commonly measured frequency parameters are peak frequency (pF), minimum frequency ($F_{\min}$), maximum frequency ($F_{\max}$), and bandwidth (BW). Peak frequency is the frequency containing the most energy, i.e. the highest value on the power spectrum. Bandwidth, $F_{\min}$ and $F_{\max}$ are less straightforward parameters, as simply measuring where the call appears to start and end is highly subjective and very sensitive to the signal-to-noise ratio of the call. Instead, bandwidth, $F_{\min}$ and $F_{\max}$ are usually defined relative to the peak-level of the power spectrum as XdB below the peak level where XdB is commonly 6dB, 10dB, 15dB, or 20dB below pF (Figure 3). Bandwidth is then simply $F_{\max}$ minus $F_{\min}$. The selected bandwidth dB-value varies between studies and is usually chosen based on the signal-to-noise ratio of the recordings. This measure of bandwidth based on the overall signal tells us much about sensory ecology of the bat. However, measuring bandwidth from the power spectrum can make it difficult to measure the frequency content of the individual harmonics and, if taking such measures is also desired, there are options available to separate the harmonics and then still use a power spectrum to quantify the frequency content of individual harmonics (see Surlykke et al. 2009 for more information). Signal duration appears as an easy parameter to measure and is in fact often eyeballed based on an observer's judgement, but standardized measures should be applied here as well. Common methods for duration measures are energy content of a given time section containing the call or, similar to the bandwidth measures, X dB down from the peak of the signal's envelope (see Madsen and Wahlberg 2007 for more information).

So in summary, we recommend that spectrograms only be used to verify the signal as a clean, clear bat echolocation call, and in large bat communities to assign

the call to one or a subset of plausible bat species or bat families. Oscillograms should be used to measure signal duration and other time parameter such as the time elapsed between one signal and the next. Power spectra should be used to measure frequency parameters such as peak frequency and bandwidth, and for all measures one should use standardized methods for all recordings to ensure reliability and repeatability. Although uncommonly used in studies of bat echolocation, an alternative means of visualizing the time-frequency structure of echolocation calls is with sparse time analysis, which produces a visualization of call design much more accurately with respect to both time and frequency than do spectrograms (for further information see Gardner et al. 2006, for examples see Elemans et al. 2011).

Implications of the recording scenario

The recording scenario will have profound implications for the recorded call, such that a recording of a given call will vary dramatically depending on the specific microphone, the direction of the microphone relative to the bat, the distance between the microphone and the bat and the direction the bat is calling relative to the microphone. Three microphone sizes are typically used for ultrasonic recordings, large ~1/2 inch microphones (such as Avisoft CM16 and Peterson DX1000), medium ~1/4 inch microphones (such as GRAS and B&K) and small microphones ~1/8 inch (such as Knowles FG series). As a general rule, microphone sensitivity increases with size, which means that a large microphone like the Avisoft CM16 is inherently much more sensitive than the much smaller Knowles microphone and that the larger microphone also has a lower noise-floor than the small microphone, meaning it can

200 record quieter sounds. However, larger microphones are more directional than
201 smaller microphones.

202 Directionality refers to the fact that a microphone has a varied response to
203 incoming sound depending on the angular incidence upon the membrane (Figure 4).
204 In other words, the measured sound pressure will decrease as the off-axis angle
205 increases. Directionality depends on the relationship between the size of the
206 microphone and the frequency of the incoming signal. This means that a large
207 microphone is more directional than a small microphone at any given frequency, and
208 any microphone becomes more directional with increasing frequencies. This also
209 means that even the small Knowles microphones are directional at high enough
210 frequencies, although for most bat echolocation calls they are essentially omni-
211 directional. The gold standard is B&K or GRAS 1/4 inch condenser free-field
212 microphones (but note also the ~1/8 inch versions, which are seldom used due to
213 their low overall sensitivity). These ~1/4 inch microphones have a flat frequency
214 response between 4 Hz and 100 kHz (± 2 dB GRAS 46BF-1), meaning that they are
215 equally sensitive to all frequencies within this range along the acoustic axis. This is
216 extraordinary. Most microphones do not have a flat frequency response, which also
217 needs to be accounted for when reporting call frequency content even when calls are
218 recorded on axis (for an example see Ratcliffe et al. 2005).

219 The distance between the bat and the microphone has important implications
220 as well. Sound attenuates with distance as a product of spherical spreading loss and
221 atmospheric attenuation. On the one hand, spherical spreading loss is frequency
222 independent and reduces sound pressure by $20 \times \log_{10}(R/R_0)$ where R is the distance
223 between the bat and the microphone and R_0 is the reference distance (0.1 meter is
224 commonly used for bats, but 1 meter is the standard distance in acoustics). As a rule

of thumb, this equates to a halving of the sound pressure (-6dB) per doubling of the distance from the sound source. Atmospheric attenuation, on the other hand, is highly frequency dependent, and higher frequencies will attenuate more so than lower frequencies. Thus, a given call's maximum frequency will appear to be lower when recorded at a microphone further away from the bat than when recorded at a microphone closer to the bat (Figure 5). In addition atmospheric attenuation increases as a linear function of distance (X dB/m), so that while the effects of spherical spreading diminish with distance, those of atmospheric attenuation do not (Griffin 1958, 1971; Lawrence and Simmons 1982).

The direction the bat emits its call and the breadth of the sonar beam also impact recordings. Bats emit their echolocation calls directionally, that is they emit the highest sound pressure in the forward direction (the acoustic axis) and sound pressure reduces with increasing off-axis angle (Figure 4). As discussed for microphones, echolocation call directionality depends on the relationship between the size of the emitter (likely the size of the open mouth for mouth-emitting bats) and the frequency emitted. For example, in the downward sweeping, frequency modulated echolocation calls of a typical vesper bat the biosonar beam produced at the start of the call i.e. at high frequencies is much narrower than the beam produced at the tail end of the call i.e. the lower frequencies. This means that unless the bat is directing its echolocation call directly at the microphone, the recorded call will be effectively low-pass filtered relative to the directly emitted call meaning that the recorded call will have much less energy in the higher frequencies than the emitted call had (compare traces on Figure 5).

Taken together the limitations discussed above mean that most bat recordings are low-pass filtered to a lesser or greater degree, which will be reflected in the

measured parameters irrespective of the selected measurement criteria. Even at relatively short distances the combination of microphone directionality, bat directionality, atmospheric attenuation and spherical spreading loss can result in a profound difference between the recorded call and what the bat actually emitted (Figure 5). Even by utilizing systematic repeatable measures for call duration, peak frequency and bandwidth, the distance and angle between bat and microphone can have dramatic effects on call characteristics as transduced by even the most flat and almost omnidirectional microphones available. Add to this the fact that the bats under some conditions may well detect the microphones themselves and as a result will decrease call duration and call intensity (Nørum et al. 2012). Thus it is clear that even under controlled conditions call design as emitted by the bat is not reliably transduced by microphones. And the situation only deteriorates in the wild. While multi microphone arrays and computational calibration and correction can go a long way in reconstructing echolocation calls including emitted intensity, these technologies are neither economically viable nor practical for most researchers. This does not mean we should throw our hands in the air and give up, but we should be aware of the limits of single microphone recordings and the environmental impacts of long appreciated acoustic principles of sound.

In closing, the above is both brief and conservative and we by no means suggest you should “just take our word for it”. For those new to the bat echolocation, the “magic well” has long been and will always be Donald Griffin’s (1958) *Listening in the Dark*. We cannot recommend this classic book enough. Some of our favourite papers on bat echolocation call design and bat behaviour include Obrist (1995), Schnitzler and Kalko (2001), and Surlykke and Moss (2000). Pye’s (1993) short paper on similar topics to those we consider above is excellent; the chapters on

275 sound and hearing in Bradbury and Vehrencamp's (1998) *Animal Communication*
276 cover a lot of ground, providing a thorough introduction to bioacoustics. Finally, while
277 there are more recent reviews on bat echolocation and neurobiology, in our opinion
278 Neuweiler's (1990) publication in *Physiological Reviews* remains among the best.

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287 **References**

288 Barclay, R.M.R. 1999. Bats are not birds: a cautionary note on using echolocation
289 calls to identify bats: a comment. *J. Mammal.* **80**: 290-296.

291 Biscardi, S., Orprecio, J., Fenton, M.B., Tsoar, A., and Ratcliffe, J.M. 2004. Data,
292 sample sizes and statistics affect the recognition of species of bats by their
293 echolocation calls. *Acta Chiropt.* **6**: 347-363.

295 Bradbury, J.W., and Vehrencamp, S.L. 1998. Principles of animal communication.
296 Sinauer Associates, Inc., Sunderland, MA.

- 298 Brumm, H., Zollinger, S.A., Niemelä, P.T., Sprau, P., and Schielzeth, H. 2017.
299 Measurement artefacts lead to false positives in the study of birdsong in noise.
300 *Methods Ecol. Evol.* **8**: 1617-1625.
301
- 302 Elemans, C.P.H., Mead, A.F., Jakobsen, L., and Ratcliffe, J.M. 2011. Superfast
303 muscles set maximum call rate in echolocating bats. *Science*, **333**: 1885-1888
304
- 305 Fenton, M.B., and Ratcliffe, J.M. 2010. Bats. *Curr. Biol.* **20**: R1060-R1062
306
- 307 Fenton, M.B., and Simmons, N.B. 2014. Bats: A world of science and mystery.
308 University of Chicago Press, Chicago, IL.
309
- 310 Fenton, M.B., Faure, P.A., and Ratcliffe, J.M. 2012. Evolution of high duty cycle
311 echolocation in bats. *J. Exp. Biol.* **215**: 2935-2944
312
- 313 Gardner, T.J., and Magnasco, M.O. 2006. Sparse time-frequency representations.
314 *Proc. Natl. Acad. Sci. U.S.A.* **103**: 6094-9.
315
- 316 Griffin, D.R. 1958. *Listening in the dark*, 2nd edn. Yale University Press, New Haven,
317 CT.
318
- 319 Griffin, D.R. 1971. The importance of atmospheric attenuation for the echolocation of
320 bats (Chiroptera). *Anim. Behav.* **19**: 55-61.
321

Griffin, D.R., Webster, F.A., and Michael, C.R. 1960. The echolocation of flying insects by bats. *Anim. Behav.* **8**: 141–154.

ter Hofstede, H.M., Goerlitz, H.R., Ratcliffe, J.M., Holderied, M.W. and Surlykke, A. 2013. The simple ears of noctuid moths are tuned to the calls of their sympatric bat community. *J. Exp. Biol.* **216**: 3954–3962.

Jakobsen, L., Ratcliffe, J.M., and Surlykke, A. 2013. Convergent acoustic field of view in echolocating bats. *Nature* **493**: 93–96.

Jensen, M.E., and Miller, L.A. 1999. Echolocation signals of the bat *Eptesicus serotinus* recorded using a vertical microphone array: effect of flight altitude on searching signals. *Behav. Ecol. Sociobiol.* **47**: 60–69.

Lawrence, B.D., and Simmons, J.A. 1982. Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *J. Acoust. Soc. Am.* **71**: 585–590.

Madsen, P.T., and Wahlberg, M. 2007. Recording and quantification of ultrasonic echolocation clicks from free-ranging toothed whales. *Deep Sea Res. Part I: Ocean. Res. Pap.* **54**: 1421–1444.

Neuweiler, G. 1990. Auditory adaptations for prey capture in echolocating bats. *Physiol. Rev.* **70**: 615–641.

- 347 Nørum, U., Brinkløv, S., and Surlykke, A. 2012. New model for gain control of signal
348 intensity to object distance in echolocating bats. *J. Exp. Biol.* **215**: 3045-3054.
349
- 350 Obrist, M.K. 1995. Flexible bat echolocation: the influence of individual, habitat and
351 conspecifics on sonar signal design. *Behav. Ecol. Sociobiol.* **36**: 207–219.
352
- 353 Pye, J.D. 1993. Is fidelity futile? The ‘true’ signal is illusory, especially with
354 ultrasound. *Bioacoustics*, **4**: 271–286.
355
- 356 Ratcliffe, J.M., Raghuram, H., Marimuthu, G., Fullard, J.H., and Fenton, M.B. 2005.
357 Hunting in unfamiliar space: echolocation in the Indian false vampire bat, *Megaderma*
358 *lyra*, when gleaning prey. *Behav. Ecol. Sociobiol.* **58**: 157-164.
359
- 360 Ratcliffe, J.M., Elemans, C.P.H., Jakobsen, L., and Surlykke, A. 2013. How the bat
361 got its buzz. *Biol. Lett.* **9**: 20121031.
362
- 363 Schnitzler, H.-U., and Kalko, E.K.V. 2001. Echolocation by insect-eating bats.
364 *BioScience* **51**: 557–569.
365
- 366 Simmons, J.A., Fenton, M.B., and O’Farrell, M.J. 1979. Echolocation and pursuit of
367 prey by bats. *Science* **203**: 16–21.
368
- 369 Strother, G.K., and Mogus, M. 1970. Acoustical beam patterns for bats: some
370 theoretical considerations. *J. Acoust. Soc. Am.* **48**: 1430–1432.
371

Surlykke, A., and Moss, C.F. 2000. Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory. J. Acoust. Soc. Am. **108**: 2419-2429.

Surlykke, A., Boel Pedersen, S., and Jakobsen, L. 2009. Echolocating bats emit a highly directional sonar sound beam in the field. Proc. R. Soc. Lond. B Biol. Sci. No. **276**: 853-860.

Zollinger, S.A., Podos, J., Nemeth, E., Goller, F. , and Brumm, H. 2012. On the relationship between, and measurement of, amplitude and frequency in birdsong. Anim. Behav. **84**: E1-E9.

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Figures and figure legends

Figure1. Spectrograms of bat echolocation calls. (a) Daubenton's bat *Myotis daubentonii* (family Vespertilionidae) and (b) the lesser horseshoe bat, *Rhinolophus hipposideros* (family Rhinolophidae). Rhinolophid echolocation call file courtesy of M. Brock Fenton.

Figure 2. Oscillogram, spectrogram and power spectrum of an echolocation call from *Myotis daubentonii*. a shows the call recorded directly in front of the bat with no reflections from nearby surfaces. b shows the same call with a reflection generated artificially by addition of the same call with a delay of 80 μ sec, the delay can be computed by the nulls in the power spectrum, frequency separation = 12.5 kHz, time separation = $1/12.5 \text{ kHz} = 80 \mu\text{sec}$.

Figure 3. Oscillogram, spectrogram and power spectrum of the same echolocation call (*M. daubentonii*) visualized using different FFT-sizes for the spectrograms. The duration estimate is based on the 99% energy content of the shown time segment measured from the oscillogram and the bandwidth is the -20 dB bandwidth measured from the spectrum. The indicated duration and bandwidth (dashed line) illustrates the trade-off between time and frequency accuracy of the spectrogram depending on the window size.

Figure 4. Directionality of a GRAS 40 BF ¼" microphone (left panel) and of a bat emitting sound as a piston with a 3.5 mm radius (right panel, see Strother and Mogus 1970 for details). Top right panel shows the emitted directionality at the listed frequencies, bottom right panels shows the relative spectrum of the emitted sound at the indicated angles.

Figure 5. Combined effects of distance and echolocation beam-direction for the frequency content of the recorded echolocation call (*M. daubentonii*), the illustrated off-axis angle (red and purple) is 40°. The illustrated scenario has no effect of microphone directionality, i.e. the microphone is pointing directly at the bat.

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