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Another quantitative measure of bat species activity and sampling intensity considerations for the design of ultrasonic monitoring studies

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To date, much of the research that has used ultrasonic detectors as a tool to address questions on the spatial and temporal distribution of bat species activity have been limited by the lack of a reliable and quantifiable unit of activity, and a poor understanding of sampling intensity required to accurately assess site-specific activity levels. Here it is demonstrated that file size (i.e., bytes) of Anabat-recorded echolocation sequences of the little brown bat (*Myotis lucifugus*) was highly correlated with the number of calls, and was easily determined, and therefore represents a reliable and quantifiable unit of echolocation activity. Additionally, it is shown that accurate quantification of a site-specific magnitude of *M. lucifugus* activity may not be possible, even with a sampling intensity of up to 20 nights. As a result, ultrasonic monitoring studies must be designed to minimize the effects of the high variability in bat species activity at a site among nights.

Key words: Anabat, echolocation, index, *Myotis lucifugus*, research design, sampling intensity

INTRODUCTION

Recent developments in ultrasonic recording devices, and their increased availability has led to a considerable amount of research on the spatial and temporal activity patterns of bat species or guilds. Hayes (2000) presented a series of practical considerations and assumptions that should be taken into account when designing such studies. Two additional considerations that could limit the efficacy of such studies include: 1. whether there is a reliable, and quantifiable unit of bat species (or guild) activity; and 2. knowledge on how many nights of sampling (i.e., sampling intensity) are required to provide an accurate site-specific estimate of the magnitude of activity.

The traditional unit of bat activity has been the number of 'passes' (Thomas, 1988; Krusic and Neefus, 1996; Hayes, 1997; Hecker and Brigham, 1999; Zimmerman and Glanz, 2000; Siedman and Zabel, 2001). A bat pass is typically defined as a single sequence of two or more recorded echolocation calls (Thomas, 1988). Recently, with the increased use of ultrasonic detectors such as Anabat (Titley Electronics, Ballina, N.S.W., Australia), the number of files has been used synonymously with number of passes (e.g., Jung *et al*., 1999; Siedman and Zabel, 2001). A problem with these indices is that there is no control for

the variation in the number of calls in the pass or file. Factors that may cause variation in the number of calls may include the number of individuals flying in the reception area of the detector, activity of the bat (i.e., commuting vs. foraging), and the orientation of the bat relative to the detector microphone. It is expected that these factors may vary among sites, habitats, and different times of the night or season. The ideal unit of activity would be a species-specific number of echolocation calls recorded in a standardized recording space per unit time. Standardizing the recording space among detectors seems possible (Krusic *et al*., 1996; Jung *et al*., 1999; Larson and Hayes, 2000). Using the Anabat system, Britzke *et al*. (1999) found it was possible to account for the number of calls in a file using the percentage of the maximum possible number of data points (i.e., buffer size). Although buffer size was highly correlated with the number of calls, and was calculated by the Anabat software automatically, it needs to be recorded manually for each file.

An alternative to buffer size is the activity index (AI; Miller, 2001). The AI is calculated as the number of one-minute periods (other lengths of time may be used as well) in which a species was recorded. Miller (2001) found this to be a better index for areas of high species diversity because of the large proportion of files that contain the calls of > 1 species.

Here, I explore the suitability of another unit of bat species activity similar to, but more practical than, buffer size. This unit is the sum of the file sizes (i.e., bytes) for a species (or guild) recorded in a standardized recording space per unit time. To assess the suitability of this measure two predictions were tested using Anabat-recorded echolocation sequence files for *Myotis lucifugus*: 1. uncleaned echolocation sequence file size is linearly associated, and highly correlated, with the number of calls in a sequence; and 2. at a site there is variation in the size of files recorded at different times of the night.

Another crucial question regarding the design of ultrasonic monitoring studies is how many nights of sampling are required to determine the mean magnitude of activity at a site. Although a critical question, it has received very little attention relative to the number of published papers on habitat associations of bats using ultrasonic detectors. Hayes and Adam (1996) found that the magnitude of bat activity at a forest site varied by a factor of 6 over four nights. Work on *Myotis* spp. in Oregon suggested that inaccurate estimates of site-specific activity would likely result if fewer than 6–8 nights are sampled at a site (Hayes, 1997). Here I explore whether this 'rule-of-thumb' is appropriate for assessing the magnitude of activity of *M. lucifugus* at a river, woodland pond, and forest-clearcut edge.

Materials and Methods

Data for this study were collected as part of a study on bat species ecology in and around Fundy National Park (FNP), New Brunswick (45°35'N, 65°03'W), Canada. All data were collected using automated Anabat detectors interfaced directly to laptop computers with standardized reception areas. Anabat will save an echolocation sequence of two or more calls when: there has been 5 s since the last call; or file buffer size is filled (16,384 data points); 15 s has elapsed since the start of the first call detected. Only two species were present in the study area and identification of the echolocation sequences was possible using a holographic neural network trained on known calls recorded in the study area (Broders *et al*., In press). Although not all echolocation sequences were identified, a systematically selected subsample of approximately 30% indicated that < 5% of the sequences recorded at the study sites were attributable to *M. septentrionalis*, so no attempt was made to identify all sequence files, and it is believed that these results are attributable to *M. lucifugus*.

To determine if uncleaned echolocation sequence file size was a good predictor of the number of calls in echolocation sequence files, *M. lucifugus* files of a variety of sizes that were recorded June–August

2000 within 2 h of sunset were randomly selected from all sequences recorded over a small (0.1 ha) woodland pond (depth ≈ 1 m). The forest that surrounded the pond was a mixed deciduous forest (canopy height ≈ 15 m) that consisted primarily of sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*), and to a lesser extent red spruce (*Picea rubens*). All echolocation sequences used in this part of the study were recorded using the same detection system placed at the same site, and set on the same sensitivity with the same orientation. From each file the number of echolocation calls was determined using the software Analook (v4.7j, written by Chris Corben) after striking the file-cleaning key (i.e., z) once. The Pearson correlation coefficient was calculated and used to assess the linear association of file size and number of calls (Sokal and Rohlf, 1995).

To assess whether there was temporal variation in file size among periods of a night at a site 2 analyses were performed, based on the nightly magnitude of *M. lucifugus* activity. Nights were arbitrarily assigned as high activity nights if there were > 800 Anabat files recorded over the entire night and low activity nights if there were 50–175 Anabat files recorded over the entire night. This subdivision was done because during nights of high activity there were more likely to be times when activity was continuous resulting in file sizes approaching the maximum possible by the Anabat software. In these situations there were no distinct 'passes' discernable and file sizes were expected to be larger than when bats were commuting through the reception area. So it was believed, a priori, that on such nights there might be more intra-night variability than on low activity nights. For this prediction, echolocation sequence files recorded under the same circumstances as described for prediction 1 were used. At this site, with 90% confidence in call classification accuracy, of 16,447 systematically selected sequences chosen for identification, 82.7% were classified as *M. lucifugus*, 2.9% as *M. septentrionali*s, and 14.4% were unclassified. Intra-night comparisons were made among 3 distinct periods. Periods were delineated based upon the time relative to sunset and sunrise, with 0% and 100% representing sunset and sunrise, respectively. Different period lengths were used for high and low activity nights to balance the requirements for sufficient sample sizes (number of files) within each period and to minimize the effects of within-night variability. Since no comparisons are made between high activity and low activity nights there were no statistical consequences of different period length. For high activity nights, each period lasted 10% of the night, and started at 10, 45, and 80% through the night. For low activity nights, each period lasted 20% of the night and started at 5, 40, and 75% through the night. ANOVA was used to test for differences in means of file size among periods for low activity nights and high activity nights (Sokal and Rohlf, 1995).

Estimation of Unbiased Site-Specific Activity Levels

To determine how many nights of sampling are required to determine the site-specific activity level, echolocation sequence files recorded May–August 1999–2001 at a river (39 nights), pond (72 nights), and a forest-clearcut edge (74 nights) were used. The use of data recorded over multiple years was justified since there were no changes in local landscape structure during the study period, no known disturbances at hibernacula in the region that might cause abnormal overwinter mortality rates, there were minimal differences in average temperature, relative humidity, precipitation and wind speed among years, and Jung *et al*. (1999) found no difference in the magnitude of bat activity between years. The river site was a second-order river with a boulder substrate that was 3–5 m wide and 20–40 cm deep at the sampling site in mid summer. Vegetation along the banks consisted of speckled alder (*Alnus rugosa*), red spruce and balsam fir (*Abies balsamea*) with a canopy height of ≈ 12 m. At this site, with 90% confidence in call classification accuracy, of 1,198 systematically selected sequences for identification, 80.6% were classified as *M. lucifugus*, 2.8% as *M. septentrionali*s, and 16.6% were unclassified. The pond site was the same as described earlier (see prediction 1). The forest-clearcut edge site was a shade-tolerant deciduous stand that was clearcut in 1997. The area had been scarified and planted with black spruce (*P. mariana*) in 2000. The sampling site was 18 m from the southern edge of the cut. At this site, with 90% confidence in call classification accuracy, of 1,806 systematically selected sequences for identification, 80.2% were classified as *M. lucifugus*, 4.5% as *M. septentrionali*s, and 15.3% were unclassified.

No attempt was made to control for any exogenous (e.g., prey, temperature, humidity, etc.) or endogenous (e.g., phenology) variables because a measure that was representative of the site under typical local conditions was desired and it was not known which variables were most important. The total number of bytes of Anabat echolocation files per night was used to quantify the nightly magnitude of activity at a site. The mean number of bytes per night at a site, over all sampling nights, was used as an estimate of the unbiased site-specific activity. SPLUS

2000 (Mathsoft, 1999) was used to randomly select 1,000 independent subsets of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 nights, and calculated the mean number of bytes per night for each subset. This resulted in 1000 estimates of site-specific activity for each of 10 different sampling intensities at each site. The proportion of the 1,000 samples that were within 10, 30, and 50% of the estimated unbiased site-specific activity (i.e., using all the samples) were calculated for each site to assess the reliability of the varying sampling intensities.

Results

Anabat-recorded echolocation sequence file size was linearly associated and highly correlated ($r = 0.964$, $P < 0.001$) with the number of calls for 149 files, and therefore was a good predictor of the magnitude of *M. lucifugus* echolocation activity. For prediction 2, from 72 complete nights of echolocation sampling there were nine nights for each of low and high activity nights. There were differences in the echolocation sequence file size on nights of low ($F_{2, 24} = 4.01$, $P = 0.03$) and high ($F_{2, 24} = 2.85$, $P = 0.08$) activity, although the differences on high activity nights were not statistically significant (Fig. 1).

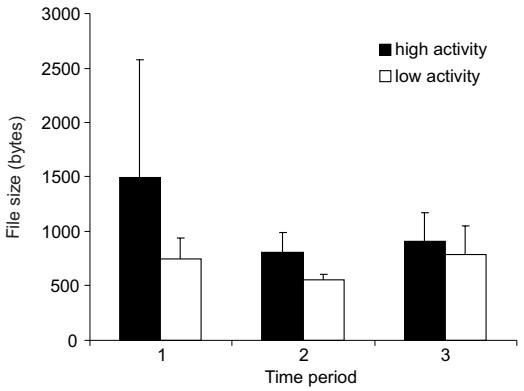

FIG. 1. Mean (+1 SD) Anabat file size during nights of low and high activity. Low activity nights were those when there were 50–175 Anabat files recorded over the entire night and high activity nights were those when there were > 800 Anabat files recorded over the entire night

The mean (SD) number of bytes per night was 140,090 (153,832) for the river, 532,792 (480,334) for the pond, and 61,428 (104,814) for the forest-clearcut edge. There was high variability in the magnitude of *M. lucifugus* activity among consecutive nights at a site. At the pond site, the magnitude of such activity differed by an average factor of 1.89 (±1.20 SD, range 1.02–6.93, $n = 60$) between consecutive nights (following removal of three outliers where the activity differed by a factor > 80 from one night to the next night). Variability was higher at the forest-clearcut edge and the river with the magnitude of activity varying by a factor of 2.66 (±1.83 SD, range 1.01–8.18, $n = 33$) and 6.04 (±7.31 SD, range 1.07–18.18, $n = 11$) between consecutive nights, respectively. Using a subset of the entire set of sampling nights it was not possible to accurately estimate the site-specific activity at any of the 3 sites. Although results were more promising for aquatic sites than the terrestrial site they were still relatively poor. Even with a sampling intensity of 20 nights, < 45% of the samples were within 10% of the mean of the entire set of nights for the river and lake and < 20% for the edge (Table 1).

Discussion

The file size accounts for the variability in the lengths of echolocation sequences (i.e., passes) recorded in a standardized recording area and is a practical alternative to the activity index. File size should be appropriate where species-specific information is not required, or where it is possible to identify the study species from all other local species and the incidence of recording multiple species in a file is low. Filenames and the associated file size of each file in a directory (e.g., a survey session) may easily be saved as a text file from DOS (from the directory of files use the command

TABLE 1. Proportion of the 1,000 random sample replicates with mean magnitude of activity within 10, 30, and 50% of the estimated site-specific activity (using all sampling nights) at a river, pond, and forest-clearcut edge

Number of nights in random sample	Percentage deviation from the mean of all samples available								
	≤ 10%			≤ 30%			≤ 50%		
	River	Pond	Edge	River	Pond	Edge	River	Pond	Edge
2	7.4	10.1	9.5	23.2	30.3	29.0	39.3	51.0	44.1
4	16.4	16.8	9.5	42.9	49.7	30.7	66.2	76.6	54.7
6	19.2	22.6	12.0	52.0	58.6	36.7	77.2	85.8	63.6
8	23.2	25.0	11.8	59.9	70.0	39.3	84.6	92.0	69.5
10	26.2	30.5	13.6	69.2	71.6	43.4	90.5	95.1	74.0
12	28.6	31.6	16.2	74.2	78.0	49.4	94.7	96.8	77.3
14	32.7	34.4	16.9	81.4	83.5	49.3	96.5	97.3	80.6
16	35.0	38.3	17.9	83.6	87.0	53.8	98.6	99.5	83.1
18	39.3	41.0	19.7	88.6	89.2	58.2	99.3	99.4	84.7
20	43.6	42.8	19.8	92.7	90.0	61.1	99.8	99.5	89.0

'dir > *filename*.txt'), and imported into a spreadsheet program where further details can be extracted from each file (e.g., date and time).

The high intra-night variation in file size probably results from temporal variation in foraging activity. Generally speaking, activity in this study area peaks shortly after sunset, therefore at a foraging area there should be a greater density of bats during this time. High bat density could lead to longer echolocation sequences, or Anabat files, in at least two ways: calls of multiple individuals may be saved into the same file, or foraging bats might partition the foraging space and an individual bat might be more likely to stay in the reception area of the detector longer. Higher intra-night variation in file size on low activity nights was somewhat surprising and probably indicates that bat foraging activity was temporally 'clumped' on all nights. This further stresses the importance of using an index of echolocation activity that accounts for the variability in the lengths of echolocation sequences, and of using the amount of activity recorded over the entire night as a dependent variable in a statistical analysis (as opposed to the amount of activity over a much smaller portion of the night).

Although not examined here, it would also seem logical to expect that there might also be variation in Anabat-recorded file sizes among habitat types (or even replicates of a habitat type), as different habitats may be used for different reasons (i.e., commuting vs. foraging). For example, in this area *M. lucifugus* activity is concentrated over water where most of their foraging occurs. However, this species also commutes and probably forages along forested trails. Because commuting passes are likely shorter than foraging passes, file sizes may differ between these habitat types.

The inability to reliably predict the mean activity at a site using all sampling nights may indicate an accurate estimate of a site-specific level of activity may not exist in southern New Brunswick, Canada (and likely elsewhere). Activity at a site was highly variable, and likely dependent upon many variables including weather conditions, phenology, proximity to roosting sites, and temporal and spatial variability in available prey. Hayes (1997) suggested that sampling a site 6–8 nights should be sufficient to reliably estimate a sites unbiased activity level (i.e., 80% of the subsamples ≤ 30% of the estimate using all sampling nights). Unfortunately, that study extrapolated the total nights activity from partial

nights, and the species being recorded were not identified. As a result, any species-specific patterns in activity may have been overlooked. In this area, it took 14 nights of sampling to achieve the same reliability at the river and pond, and even 20 nights of sampling could not achieve this reliability at the forest-clearcut edge. Although likely inadequate at all sites for most research questions, the results from both aquatic sites in this study were comparable and better than the terrestrial site. This may indicate that variation in activity at aquatic sites was less than the activity at terrestrial sites. Although it is not known if these results are typical of bat species in other areas, the lack of data to the contrary suggests researchers should carefully design studies to account for the potentially high inter-night variability at a site. Further, it is not known whether the effects of including multi-species activity in a one study increases or decreases the variability in the measure of activity.

To control for the high variability there are at least two possible remedies. If it were possible to simultaneously sample all sites of interest, this would control for all confounding variables (see also Hayes, 1997). Otherwise, each night of sampling may have to be considered independent samples and all potentially important exogenous (e.g., weather, prey, site characteristics and landscape metrics), and endogenous (e.g., phenology) factors should be incorporated into a modelling exercise to help explain the temporal and spatial variation of species activity.

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