Chapter 2

Comparison of Driving Transect Methods for Acoustic Monitoring of Bats

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Abstract

Acoustic monitoring for bats along driving transects typically involves recording call sequences (bat passes) continuously while driving. While this offers benefits over other survey techniques, it also poses challenges, including background noise on recordings. An alternative approach that may rectify this involves conducting sampling at discrete points along each transect instead. We compared these methods using the same bat detector, along with an additional higher sensitivity detector to determine which method yields the highest amount and quality of data per unit of time. Results from 26 18 km transects, each sampled continuously and at 10 point count sites indicate that, with a lower sensitivity detector, the two methods yield a similar number of passes per minute, percent of passes identified to species, and species documented. The higher sensitivity bat detector could not be used for continuous sampling due to background noise. However, at point count sites, the higher sensitivity detector recorded 17 times more passes per minute, 44 times more passes identified to species, and documented nearly twice as many species. Thus, while both sampling designs appear comparable, for most applications, a higher sensitivity detector trumps sampling design.

Keywords: acoustic monitoring, bats, continuous sampling, point count sampling, driving transects

1. Introduction

1.1. Importance and status of bats

Bats are an extremely important part of ecosystems across the globe, providing a variety of ecological services such as pollination, seed dispersal, and regulation of insect populations [1–3].

Their role in many ecosystems is so vital that some have suggested using bats as bio-indicators [4]. As most bats are insectivorous, one of their most significant contributions lies in reducing vegetation damage from insect herbivory in native ecosystems [2, 5, 6]. This has profound economic and social ramifications for human civilization as well. One of the biggest challenges faced by humanity in the coming decades will be the production of enough food to feed a growing population without dramatic losses in habitat and biodiversity [7]. While bats alone will not solve this problem, by devouring large numbers of agricultural pests, these small flying mammals reduce crop losses, thereby enhancing food production on existing agricultural lands [3, 8]. This, in turn, provides significant economic benefits by saving farmers billions of dollars (in US dollars, [9, 10]).

In light of the value bats have to ecosystems and modern civilization, it should be of great concern that they face a growing array of threats. These include persecution, hibernacula damage and disturbance, loss of foraging and roosting habitat, pesticide exposure, and many others [4, 11]. The net effect of all these threats is that roughly a quarter of all bat species are threatened [12]. In North America, aside from habitat loss, two of the biggest emerging threats are White Nose Syndrome (WNS) and wind turbine facilities. WNS is caused by a fungal infection spread among bats in their winter hibernacula [13]. First observed at a hibernaculum in New York in 2006, WNS has since spread across eastern North America, killing millions of bats and wiping out entire populations in some cases [14, 15]. Similar threats may be posed by wind power. With the recent push toward renewable energy, many countries have seen a tremendous growth in the number of wind power facilities. While wind turbines vary widely in their impact on bats depending on their geographic location, in some parts of North American, turbine facilities are estimated to be killing bats in the hundreds of thousands annually [16, 17]. With the slow rate at which bats reproduce [18], these numbers could be devastating to bat populations over the long term. For these and many other reasons, extensive monitoring of the status of bat populations in all affected areas is needed. Given the highly variable and broad geographic distribution of these threats, effective techniques for systematically surveying bats across large geographic areas are needed.

1.2. Challenges in studying bats across large geographic areas

As nocturnal, flying mammals, bats are uniquely challenging animals to study. However, a variety of survey techniques have been developed to overcome many of these challenges, including mist nets, radio telemetry, and ultrasonic detectors [19–21]. While each technique has its own benefits and drawbacks, ultrasonic detectors (also known as "bat detectors") have proven to be a powerful tool for examining insectivorous bat species composition and habitat use, and are among the most widely utilized tools for these purposes [22, 23].

Aerial-foraging insectivorous bats, which constitute the majority of species globally, use echolocation to navigate and find insect prey [24]. They do so by periodically emitting a sequence of ultrasonic calls (sounds above the limit of human hearing, roughly 20 kHz) and listening for the echo [25]. Information provided in the returning echoes of these call sequences enable bats to discern a variety of factors such as size, shape, location, and movement of objects in the environment, all of which are crucial for navigation and acquiring prey [26]. Another

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important aspect of bat calls is that they typically differ between species, likely to ensure species have their own "bandwidth" to facilitate effective communication among conspecifics [27]. These differences between species mean ultrasonic detectors are not only valuable in recording the presence of bats, but also in enabling investigators to determine which species are present. Bat detectors offer many other advantages as well. They are also easily deployed, they do not disturb bats, they can be used in areas where mist netting is difficult or marginally effective, and if financial resources permit multiple detectors, they can be used to survey many sites simultaneously with limited personnel [22, 23, 28]. For these reasons, among many others, bat detectors remain one of the most popular tools for studying bats.

However, these devices are not without limitations. For instance, an individual detector placed at a single location can only provide data for one site. If one wishes to survey a large area or multiple habitats each night, numerous detectors would be needed. This can dramatically increase the cost of a project, placing it out of reach for many investigators [29].

One technique that may help overcome this problem is the use of transects [19, 30, 31]. By moving a single detector through different habitats, a larger area can be sampled each night compared to stationary approaches. While most transect studies have employed walking transects, they are constrained in the amount of area that can be sampled by the slow pace of walking. As bats can typically fly faster than a walking observer, no two call sequences recorded along a given transect can be viewed as independent of each other. Additionally, randomly and systematically sampling across numerous habitat types on a large geographic scale becomes exceedingly difficult in areas where most properties are privately owned and require permission to sample. Driving transects solve these problems [31–33].

While driving transects represent an important addition to the tools available for studying bats in the wild, several questions remain. Most previous studies have used continuous sampling. Continuous sampling involves leaving the detector recording while driving along the transect. Although this allows for data collection along the entire length of each transect, there are potential problems. For example, if habitat types vary along each transect (which is often the case in many modern mosaic landscapes), the types of statistical techniques that can be used to test predictions about habitat use with data collected continuously are limited. In addition, sounds from vehicle movements, including airflow over the microphone, may cause significant problems with the resulting audio files. These include constantly triggering the detector to record in the absence of bats or producing extensive background noise that prevents call sequences from being detectable or making it impossible for call analysis software to identify the species emitting the calls. One possible solution to these problems would be to restrict sampling to specific points along each transect at established intervals (point count sampling). While the latter have been used extensively in bird research [34, 35], they are rare for bats. Moreover, the absence of direct comparisons of these two methods makes it difficult to determine which sampling methodology is superior. The purpose of this study was to fill this void by comparing continuous versus point count sampling along the same driving transects using two detectors varying in microphone sensitivity. In particular, we assess whether the two types of detectors and methods are comparable in number of bat passes recorded per unit of time, percent of recorded bat passes able to be identified to species, and total number of species identified.

2. Methods

2.1. Bat detectors

We recorded bats using an EM3 EchoMeter (Wildlife Acoustics Inc., Maynard, MA, USA) fitted with a Garmin GPS device that stamps all call sequence recordings with the coordinates. Since the calls of most aerial foraging insectivorous bats are above 20 kHz [36], we set the minimum frequency to begin recording (trigger threshold) to 20 kHz. This minimizes false triggers by insects, road noise and other sounds. As bat call sequences typically last only few seconds, maximum time length for individual recordings was set to 3 s to ensure file sizes of recordings were easily managed by the call-analysis software (see below). To minimize triggering by indiscernible, distant, low intensity sounds, we set the threshold amplitude to 18 db. Lastly, to determine if detector microphone sensitivity influences whether, and to what extent, background noise during driving adversely impacts the number and quality of bat passes recorded, we decided to add a second detector known for being highly sensitive. We selected the miniMIC ultrasonic microphone (Binary Acoustic Technology Inc. Tucson, Arizona, USA). The miniMIC was connected to a Dell Venue tablet via USB and call sequences were recorded using Spectral Analysis, digital Tuning and Recording Software (SPECT'R, Binary Acoustic Technology Inc. Tucson, Arizona, USA). Settings were as described for the EM3.

2.2. Sampling location

The study was conducted in the states of Maryland and Delaware on the Delmarva Peninsula, which is located along the mid-Atlantic coast of the United States between the Atlantic Ocean and the Chesapeake Bay. The peninsula consists primarily of a mosaic of agriculture (48%) and forests (37%, mostly mixed hardwood-pine and loblolly pine—*Pinus taeda*—plantations) [37]. The remainder is comprised of coastal marshes and scattered suburban and urban developments [37].

2.3. Sampling protocol

We established 28 transects that were evenly spaced across the Delmarva Peninsula as described by McGowan and Hogue [38]. Each transect contained 10 sampling points spaced 2 km apart (in straight line distance) for a total of 280 sites. We restricted transects to 2 lane roads, and sampling points to the nearest safe roadside location to stop for sampling. We sampled each transect once between June and August of 2014, yielding a total of 28 sampling nights. Transects were selected randomly for sampling without replacement using the random number generator in R Statistical Software [39]. The direction of travel along each transect was also randomly chosen. Unfortunately, due to equipment failure, two transects had to be excluded from analyses, dropping our total sampling nights (and transects) to 26.

We sampled each transect for bats using two approaches: point count and continuous sampling. Point count sampling occurred for 12 min at each of the 10 sampling points along each transect. Continuous sampling was carried out by leaving the detectors to operate as we drove the vehicle at speeds of 32–48 km/h along the transect between point count sites. We sampled each transect during peak bat activity, beginning 30 min after sunset and continuing until the transect was completed several hours later. For the continuous approach, we allowed the EM3 and miniMIC to operate atop a telescoping pole connected to the vehicle at a height of 2 m while we drove between sampling points. This allowed the detectors to be at a moderately elevated height while preventing damage from overlying bridges and road signs. Upon arriving at sampling points, we stopped the vehicle and extended the pole to 4 m and recorded for 12 min. We then collapsed the pole and drove the transect until reaching the next sampling point, repeating the process until all 10 points were sampled. In all cases, the detectors were pointed toward the immediately adjacent habitat to the right the road. Following recommendations of previous studies, we restricted sampling to nights without rain, temperatures above 10°C, and wind speeds less than 20 km/h [21, 40]. Call sequences recorded while driving were allocated to the continuous sampling data pool. Those recorded within the 12 min at each site were allocated to the point count sample. In total, we logged 52 h of recording time at stationary sampling points and just over 27 h from continuous sampling.

2.4. Analyses

We defined a bat pass as a sequence of one or more echolocation calls with <1 s between sequential calls [24]. Based on currently available technology, researchers are not able to distinguish individual bats of the same species from their calls. As a result, it is not possible to determine the absolute number of bats at a given location with bat detectors [19, 41]. Instead, the number of bat passes may be viewed as a measure of overall bat activity rather than number of individuals [19, 41]. We attempted to identify all bat passes to species using Sonobat 3.2 automated classifier (SonoBat, Arcata, CA, USA). As recommended by official Sonobat Guidelines, a probability threshold of 90% was set for accurate species identification.

For comparisons between continuous versus point count methods (EM3 detector only, see Section 3), we tallied the total number of bat passes recorded along each transect while continuously sampling and separately for point count sampling. We then divided these numbers by the amount of time spent recording using each method to yield passes per minute. Since the data were not normally distributed, we compared passes per minute between the two methods at the 26 transects using a two-tailed Wilcoxon signed-rank test ($N = 26$, $\alpha = 0.05$) in R Statistical Software [39].

For reasons discussed below (Section 3), comparisons between the two detectors were not possible using continuously sampled data. We therefore limited analyses to the point count data. Since these data were recorded at 260 discrete sampling points, each sampled simultaneously by both detectors for 12 min, we were able to treat each site as a separate data point. Specifically, we compared total bat passes recorded at each site between the two detectors. Since the data were not normally distributed, we used a two-tailed Wilcoxon signed-rank test ($N = 260$, $\alpha = 0.05$) in R Statistical Software [39] to test for statistically significant differences. We also compared data on percent of bat passes identified to species and total number of species identified between the different detectors and sampling methodologies.

3. Results

The concern that the more sensitive bat detector (miniMIC) would be more adversely impacted by road noise or airflow was fully realized. The detector was sensitive to wind resistance at speeds over 10 km/h, recording tens of thousands of audio files, all obscured with background noise. This made analysis of these data impossible. Therefore, comparisons of continuous and point count sampling results could only be performed with data obtained from the EM3 detector.

Average passes per minute recorded along the 26 transects was not significantly different between continuous sampling versus point count sampling (0.076 vs. 0.067 passes/min, respectively, P = 0.097, **Table 1**). Comparisons of the proportion of bat passes identified to species and total number of species documented using the two approaches revealed largely similar results as well. Of all the passes recorded for the entire sample during continuous sampling, 20% were able to be identified to species, yielding an overall rate of 0.015 passes per minute identified to species (**Table 1**). At point count sites, 24.5% of passes were able to be identified to species, yielding a rate of 0.016 passes per minute identified to species (**Table 1**). Both approaches also documented the same four species: big brown bat (*Eptesicus fuscus*), red bat (*Lasiurus borealis*), evening bat (*Nycticeius humeralis*), and silver-haired bat (*Lasionycteris noctivagans*).

Since data obtained with the more sensitive miniMIC detector during continuous sampling could not be analyzed, comparisons of the two detectors were restricted to point count sampling. Here, considerable differences were uncovered. The average number of bat passes recorded at each site were significantly higher using the miniMIC detector compared to the less sensitive EM3 detector (mean = 13.17 vs. 0.812 bat passes per site, respectively, N = 260, P < 0.001, **Table 2**). This translates to an average of 1.098 passes per minute for the miniMIC versus 0.067 for the EM3 (**Table 2**). Magnified over 52 hours of recording at the 260 sites, this resulted in a considerably higher number of total bat passes recorded with the miniMIC (3550) compared to the EM3 (211) (**Table 2**). Furthermore, due to the superior resolution of the audio files obtained with the miniMIC, a considerably higher proportion of bat passes were able to be identified to species (64.1% vs. 24.5%, **Table 2**). The combination of a higher number of calls recorded with a higher proportion identified to species meant that the miniMIC

	Continuous sampling	Point count sampling
Mean (SD) passes/minute	0.076 (0.073)	0.067 (0.128)
Percent passes identified to species	20.0%	24.5%
Passes/minute identified to species	0.015	0.016
Total number of species identified	4	4

Passes per minute were not statistically different between the two approaches (Wilcoxon test, N = 26, P = 0.097).

Table 1. Comparison of bat detection rates between continuous versus point count sampling along 26 transects using the EM3 bat detector.

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	EM3 detector	miniMIC detector
Mean (SD) passes at each site	0.812 (5.15)	13.17 (24.24)
Mean passes/minute	0.067	1.098
Total passes recorded	211	3550
Percent passes identified to species	24.5%	64.1%
Passes/minute identified to species	0.016	0.724
Total passes identified to species	52	2276
Total number of species identified	4	7

The miniMIC documented significantly more calls than the EM3 (Wilcoxon test, N = 260, P < 0.001).

Table 2. Comparison of bat detection rates between the two different bat detectors at 260 point count sampling sites.

obtained vastly more calls identified to species throughout the study compared to the EM3 (2276 vs. 52, respectively, **Table 2**). Lastly, the miniMIC not only documented the four species found with the EM3 (see above), but it also uncovered three additional species: hoary bat (*Lasiurus cinereus*), tricolored bat (*Perimyotis subflavus*), and one or more species in the genus *Myotis* (we were unable to confidently identify the specific species).

4. Discussion

Bats face a growing array of threats. Many of these threats have complex and overlapping geographic distributions. Given the uncertainty of how these threats interact and impact bats across the landscape, it is becoming increasingly important to monitor populations across large geographic areas. Driving transects offer one the most cost effective and least labor-intensive tools for doing this. However, driving transects can be implemented in different ways and it is important to determine which approach is superior in terms of the amount and quality of data obtained.

When comparing results from a single detector capable of yielding analyzable audio files from both continuous and point count sampling, these two methods appear comparable. Specifically, mean number of passes per minute, percent of passes identified to species, passes per minute identified to species, and number of species identified were similar between the two approaches (**Table 1**). They also documented the same four species. If this holds with other detectors that are similarly unaffected by airflow or driving noises, we conclude that either driving transect technique can be a viable option. With such detectors, the needs of the particular project should dictate which option is selected. For example, if one seeks to test hypotheses about habitat use or other factors, the ability to use a variety of standard statistical techniques such as ANOVA (or nonparametric equivalents) for data from discrete sampling points may indicate the point count method is preferable. If, on the other hand, one simply seeks to document the bat fauna of an area, particularly in places it may not be safe to stop and record for extended periods, continuous sampling might be preferable.

The above conclusions are based on the use of a detector capable of operating while driving at speeds above 10 km/h without significant airflow or driving noise interference. We recommend testing any detectors intended for continuous sampling on driving transects to ensure they yield audio files of adequate quality for extracting bat passes and identifying them to species. Data obtained from the miniMIC suggests not all bat detectors may be capable of this. It remains unclear whether other high-sensitivity detectors are similarly affected, or whether accessory devices such as wind screens can mitigate these issues. Future work should test a variety of high sensitivity bat detectors with different types of wind screens to determine if it is possible to use these devices for continuous sampling. If not, our data suggest overall detector sensitivity is vastly more important than driving transect sampling design.

Overall, the more sensitive miniMIC recorded nearly 17 times more bat passes than the EM3 (**Table 2**). Factoring in that nearly 3 times as many of the miniMIC passes could be identified to species, this yielded nearly 44 times more calls identified to species and nearly twice as many bat species identified (**Table 2**). These differences are substantial and have profound implications for the types of conclusions that can be drawn from comparably designed studies. The failure of the less sensitive detector to record numerous bat passes at each site lowers the power of a study. It means any differences that may exist in activity among species or habitats may fail to be detected or may not be identified as significantly different due to the small amount of resulting data. Perhaps even more importantly, the fact that nearly half the species present were effectively missed by the less sensitive detector could alter conclusions about species presence, distribution, habitat associations, and many other ecological questions. The findings from the lower sensitivity detector are particularly troubling for research related to species conservation, as the very species typically of greatest concern (rare and threatened species) are the ones most likely to be missed. All three of the additional species recorded with the miniMIC are uncommon or rare in the sampled area [38]. This is especially true of the genus *Myotis*. Most *Myotis* species in eastern North America have been devastated by White Nose Syndrome, with concerns that at least one species is in danger of becoming regionally extinct in the coming decades [42]. Failing to detect these species in areas where they persist could adversely impact conservation efforts. For example, the presumed absence of such species in a given area may fail to trigger recovery measures normally implemented by governmental and nongovernmental organizations when rare or threatened species are detected. It could also lead to the diversion of much needed conservation resources away from areas where the species persist because they are presumed absent. Given these concerns, if future research confirms that higher sensitivity detectors are not viable options for continuous sampling, the greater amount and quality of data obtained from such detectors strongly suggests priority should be given to using these types of detectors at point count sites rather than using lower sensitivity detectors for continuous sampling.

It is important to note that even with a high sensitivity detector operated at point count sites, driving transects have limitations. Some areas or habitats may lack adequate road access. Depending on how limited road access is, this may put analysis of certain habitats off limits, or cause them to be significantly underrepresented in the sample. In such cases, the use of other techniques such as walking transects, mist nets, or unmanned stationary bat detectors may be indicated. Roads are also, by definition, human-altered environments. Their presence and usage can have a variety of impacts on adjacent environments [43]. Even if much of the surrounding habitat is largely intact, the presence of roads effectively creates a habitat edge. Some species are adapted to interior habitat conditions and avoid or are otherwise negatively impacted by edge conditions [44]. While this is often not a significant problem with insectivorous bats, since many species prefer edges like forest edges [30, 45–47], if there is reason to believe research questions about focal species in the study area might be adversely impacted by sampling at habitat edges, driving transects may not be appropriate. For the region sampled in the present study, driving transects have proven comparable in documenting the bat fauna to unmanned stationary bat detectors placed in both interior and edge conditions of different habitats [38].

5. Conclusions

Like many mammals, bats across the globe face a variety of threats that imperil their very existence. In North America, many of these threats are both increasing and span large geographic areas. The growing and expansive nature of these threats requires the urgent development and deployment of sampling techniques capable of effectively and efficiently documenting changes in the status of bat populations across large areas. Driving transects have been proposed and implemented as a tool for doing precisely that. Unfortunately, previous studies failed to examine the implications of using different sampling methodologies or detectors on the results obtained.

In this study we showed that, with a lower sensitivity detector that is unaffected by wind and driving noise, sampling continuously while driving yields similar results to sampling at discrete sampling points. However, detector sensitivity proved to be much more important than sampling technique in terms of the amount and quality of data obtained. That is, the higher sensitivity detector documented substantially higher numbers of bat passes and species than the lower sensitivity detector. The downside to the former is that data obtained while driving could not be analyzed due to significant interference from driving noise and airflow over the microphone at speeds above 10 km/h. Based on our findings, for most studies using driving transects to study bat populations, we suggest detector sensitivity should take priority over sampling design. If future studies are unable to resolve the problems of using high sensitivity detectors while continuously sampling along driving transects, this would necessitate using point count sampling instead. We recommend selecting the detector capable of obtaining the greatest amount and quality of call sequence recordings under a given research design, then conducting preliminary trials with continuous and point count sampling. If airflow or driving noises significantly diminish the data available with continuous sampling, as in the current study, point count sampling would be the more appropriate sampling regime to use for most applications.

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