Survey Methods for Monitoring Bat Populations
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Evaluating an Acoustic Method for Estimating Trends in Bat Summer Colony Counts with AudioMoth Recorders

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Abstract – Passive acoustic monitoring for bats has become a common method to determine species presence and activity levels. However, current acoustic methods are ineffective for monitoring species abundance at large summer colonies. We used synchronized acoustic and thermal-imaging data collected at 6 colonies of *Myotis grisescens* (Gray Bats) and found a significant positive relationship between acoustic energy and number of emerging bats. Our findings reinforce that acoustics have the potential to estimate population sizes of summer bat colonies. Additionally, we examined ultrasonic amplitude variance across 19 AudioMoth devices at 5 different gain settings and found significant differences among devices and settings. Further exploration into device variability and bat behavior are necessary to develop a robust model of population estimates using acoustic energy.

Introduction

Across the globe, bats are threatened by habitat loss, bushmeat trade, diseases such as white-nose syndrome, mortality from wind turbines, and climate change (Boyles et al. 2011, Festa et al. 2023, Frick et al. 2020, Furey and Racey 2016, Mickleburgh et al. 2002). The International Union for Conservation of Nature characterizes 80% of bat species as needing conservation action or research attention to assess population status (Frick et al. 2020). However, managers lack the reliable population data needed to inform conservation and management decisions for many species (Frick et al. 2020). For population estimation at roosts, conventional censusing methods such as capture and winter counts in hibernacula can be inefficient, time intensive, cost prohibitive, or invasive to the targeted species (Frick et al. 2020, Furey and Racey 2016, Walters et al. 2013).

An alternative to conventional censusing methods is passive acoustic monitoring (PAM), which has primarily been used to detect and monitor bat populations across landscapes (Sugai et al. 2019). PAM techniques can be used to infer bat abundance (Riedle and Matlack 2013), distribution (Krauel and LeBuhn 2016, McCracken et al. 2018), habitat use (Blanco and Garrie 2020, Russo and Jones 2003), activity (Adams and Fenton 2017, Kitzes and Merenlender 2014), and behavior (Jespersen et al. 2022, Schwartz et al. 2007). Despite progress in developing PAM methods for monitoring bats on the landscape, reliable PAM techniques for censusing and monitoring bats in large roosts or caves lag behind. In temperate zones, many species use caves for part of their annual life cycle, with caves often playing crucial roles during hibernation and maternity periods (Furey and Racey 2016). Reliable and efficient techniques are essential to monitor the abundance of cave-dwelling bats and assess their response to management practices, development activities (e.g., prescribed burns, installation of gates on cave openings, wind-energy installations near colonies), and introduced diseases.

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A common approach to census large summer colonies is to conduct exit counts during evening emergence, which is typically obtained via manual counts or the use of near-infrared or thermal imaging (Loeb et al. 2015). Exit counts can be complicated by dense vegetation, low light conditions, and multiple cave openings (Ammerman et al. 2009). Thermal imaging, although costly ($5000–$10,000 USD), has become the common method for censusing bats in low-light environments (Cilulko et al. 2013, Hristov et al. 2008, Loeb et al. 2015). Although automated counting software exists (Bentley et al. 2023, Corcoran et al. 2021), the adoption of thermal imaging can be limited by the training required to image emergences and analyze resulting videos (Ahlberg et al. 2024). Near-infrared (NIR) imaging presents challenges because NIR lights need to be placed within a few meters of emerging bats, which is impractical for caves with standing water or tall entrances. Furthermore, NIR lighting can create shadows, complicating counts (V. Kuczynska, US Fish and Wildlife Service, Missouri Ecological Services Field Office, MO, Pers. Comm.).

Motivated by the recent availability of affordable acoustic recording units, like the AudioMoth (Hill et al. 2018), and the need for a low-cost automated method to estimate the number of bats emerging from roosts with large populations, we aimed to estimate population sizes using acoustic energy. Prior work demonstrated that acoustic energy could estimate population size for large colonies (Kloepper et al. 2016). However, that study focused on a single species, *Tadarida brasiliensis* I. Geoffroy (Brazilian Free-tailed Bat), at a single location, leaving its suitability for other species and locations uncertain.

Here, we evaluate the use of automated PAM methods to monitor summer roost populations of *Myotis grisescens* Howell (Gray Bat). Gray Bats, which have been on the federal list of endangered species since 1976 (US Fish and Wildlife Service 1982), remain vulnerable to land-use and climate change, despite recovering from historically low population levels (Festa et al. 2023, Frick et al. 2020, LaVal et al. 1977). The US Fish and Wildlife Service (2024) estimates that 98% of the 5 million Gray Bats hibernate in only 15 caves and that these overwintering bats disperse to at least 95 summer roosts, many of which lack population estimates (V. Kuczynska, Pers. Comm.). This lack of data makes it difficult to assign biological significance and prioritize conservation actions to sites (V. Kuczynska, Pers. Comm.). To address this problem, we modeled the relationship between acoustic energy and emergence density at multiple maternity caves of Gray Bats. We hypothesized that acoustic energy, measured as root mean square (RMS) power, will increase with number of bats emerging.

Field-site description

Data were collected at 6 limestone caves hosting large maternity colonies of Gray Bats in Missouri, USA (Fig. 1). These caves are used exclusively by Gray Bats for roosting during the summer (V. Kuczynska, Pers. Comm.). At all sites except Tumbling Creek, data were obtained either inside or outside the caves. At Tumbling Creek, recordings were made both inside and outside the cave.

Outside sites (Mauss, Tumbling Creek-Outside, and Mary Lawson) often had vegetation near the openings where bats emerged. At Tumbling Creek-Outside, bats exited through a chute gate ~3.5 m above the ground. The opening within the chute is ~10 m wide and 1.5 m tall. During the May surveys, water, with a water depth of ~0.5 m, flowed from inside the cave, beneath the gate and directly below where the bats were recorded inside the cave. Mauss Cave has a gated entrance ~8 m wide and 5 m tall at its peak, narrowing to ~1 m at the sides. Bats primarily emerged through a 1-m gap between the gate and cave ceiling. Mary Lawson's cave opening also has a gate that is ~7.5 m wide and 6 m tall. Bats mostly exited through a 1.5-m gap between the top of the gate and the cave ceiling.

Inside sites (Rocheport, Tumbling Creek-Inside, Devil's Icebox, and Bat Cave Oregon) featured cave formations and muddy ground. At Rocheport, recordings were conducted inside a large hall ~6 m tall and 20 m wide. At all sites except Bat Cave Oregon and Devil's Icebox, bats emerged through a single opening. At Bat Cave Oregon and Devil's Icebox, 2 potential emergence sites were present, but recordings were conducted inside the cave where bats were exiting in a single stream before the passage split. The hall where recordings were made at Bat Cave Oregon was ~3.5 m high and 5.5 m wide, while the chamber at Devil's Icebox was ~1.8 m tall and 3 m wide.

Methods

Data collection

We collected synchronized ultrasonic acoustic and thermal video data of emerging Gray Bats on 27 occasions from May 2022 to July 2023, including simultaneous inside and outside recordings at 1 location (Tumbling Creek Cave) for 2 emergences. Ultrasonic acoustic data were recorded using AudioMoths (v. 1.2.0, Open Acoustic Devices, Southampton, UK), which were housed in polycarbonate weatherproof cases (AudioMoth IPX, Open Acoustic Devices), which were acquired through the GroupGets crowdfunding platform (https://groupgets.com). AudioMoths were selected due to their low cost ($150 USD for the device and weatherproof case, but electronic designs are freely available). In addition, we chose AudioMoths because of their rugged housing, ability to record continuously, and because we were able to adjust gain values lower than those of commercially available bat recorders such as Song Meter (Wildlife Acoustics, Maynard, MA) or Ranger (Titley Scientific, Columbia, MO). Lowering the gain helps minimize clipping, which is a distortion that occurs when the recorded audio exceeds the maximum amplitude of the recording system; this distortion was a common issue at sites where bats flew close to our acoustic recorders.

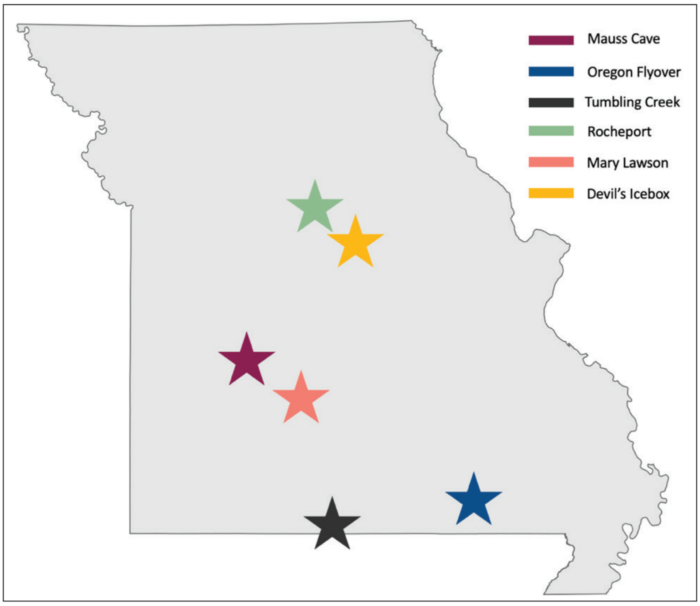

Figure 1. General location of the 6 study sites across Missouri, USA.

In 2022, all devices were programmed with the standard AudioMoth firmware (v. 1.8.0). For 2023, we programmed devices with custom low-gain firmware designed by Open Acoustic Devices specifically for this project, which allowed a further decrease in gain and reduction in clipping (firmware available upon request from the author). Low-gain range was enabled for devices programmed with the custom firmware. All AudioMoths were programmed with a sampling rate of 250 kHz, recording duration of 595 seconds and sleep duration of 5 seconds.

Thermal video was captured with a thermal-imaging monocular (Scion OTM266, Teledyne FLIR, Wilsonville, OR) at 30 frames per second and a resolution of 640 by 480 pixels. Data collection typically began within 30 minutes of sunset, when bats became active near the roost opening, until the rate of emergence decreased to <5 bats per each minute for 5 minutes, with recording duration lasting between 25 and 80 minutes across the dataset. Temperature (°C) and relative humidity (%) were recorded at the start and end of data collection for each emergence by a datalogger (HOBO MX1101, Onset Computer Corporation, Bourne, MA). Starting and ending measurements were averaged for analysis, except on nights with only 1 data point (i.e., either start or end measurement). There were 3 additional occasions (Bat Cave Oregon on 8 June 2022, and Tumbling Creek on 13 July 2022 and 18 May 2023) when the environmental data were lost during data transfer or were not collected due to equipment malfunction.

Acoustic data acquisition

We extracted all acoustic data in RStudio (R Core Team 2021), using the seewave (Sueur et al. 2008) and tuneR (Borg 2016) packages (R script available upon request from the author). These packages convert sample values to 16-bit linear pulse-code modulation (PCM) format (−32,768 and 32,768), which we standardized to double floating-point values (−1 to 1), to be consistent with approaches used in other bioacoustics analysis software, including MATLAB (MathWorks, Natick, MA) and Raven Pro (Cornell Lab of Ornithology, Ithaca, NY). A first-order Butterworth bandpass filter was applied around 35 and 70 kHz, encompassing the frequency range of the fundamental harmonic of Gray Bat calls (Decher and Choate 1995).

Because AudioMoths are not calibrated by the manufacturer, we cannot calculate amplitude values in sound pressure level, which is typically used to quantify amplitude (Lynch et al. 2011; Shannon et al. 2016). Instead, we used a process to determine relative RMS power of the signal values, which are comparable to the absolute value of the waveform. To calculate RMS power (relative dB) per second, we converted RMS signal power values to RMS relative power in relative decibels, using the following formula:

$$\mathrm{RMS_{rel}} = 10\log_{10}\left(\frac{\mathrm{RMS_{act}}}{\mathrm{RMS_{max}}}\right) + \text{gain offset},$$

where $\mathrm{RMS_{rel}}$ = the RMS power measurement in relative decibel units, $\mathrm{RMS_{act}}$ = the recorded signal RMS power measurement, and $\mathrm{RMS_{max}}$ = the RMS power of the maximum signal energy measurement possible (i.e., a 1-second simulated floating-point file consisting of alternating −1 and +1 values). Additionally, to compare changes in acoustic energy across nights at Mary Lawson, we normalized our acoustic data relative to the noise floor (background noise) by adding the absolute value of the quietest signal to all $\mathrm{RMS_{rel}}$ values for each night. This measurement is termed normalized $\mathrm{RMS_{rel}}$.

Ultrasonic amplitude variance testing and gain-offset calculations

We performed a series of simple tests to determine how gain affects ultrasonic sensitivity among devices (see Supplemental Fig. 1 in Supplemental File, available online at http://

www.eaglehill.us/NABRonline/suppl-files/nabr-010i-s1-Eddington). We tested 19 AudioMoths (9 new devices and 10 devices that had undergone varying levels of field use) using a low-cost ultrasonic calibrator ($199; Wildlife Acoustics, Maynard, MA), which emits square-wave pulses at 40 kHz. AudioMoths were turned on and placed in an assigned case 45 minutes prior to testing to allow the pressure within the polycarbonate case to equalize. The calibrator and microphones were each attached to a tripod 1 m above ground, 4 m apart, and facing each other. Each AudioMoth recorded ~20 chirps from the calibrator for 5 unique combinations of firmware and gain. These combinations were standard firmware with gain set to low and medium low, and custom firmware with gain set to medium, medium high, and high. We manually extracted 20 milliseconds of each pulse and extracted the $\mathrm{RMS}_{\mathrm{rel}}$ in RStudio (R Core Team 2021). We then calculated the mean $\mathrm{RMS}_{\mathrm{rel}}$ across devices for each combination of gain setting and firmware.

Each combination of gain setting and firmware produces different mean $\mathrm{RMS}_{\mathrm{rel}}$ values (Fig. 2a, Supplemental Table 1, Supplemental Figs. 2–6), and we used different combinations of settings in our dataset; hence, we performed a gain-offset on our acoustic data. To do so, we calculated the difference between our selected baseline of the standard firmware at low-gain setting and mean $\mathrm{RMS}_{\mathrm{rel}}$ for all combinations of firmware and gain. We then transformed all our field measurements with the appropriate conversion.

Additionally, we compared 2 new AudioMoths that demonstrated high inter-unit variability in initial trials. We compared the 2 devices when housed in the polycarbonate cases and when unhoused, to determine whether the cases were contributing to variability.

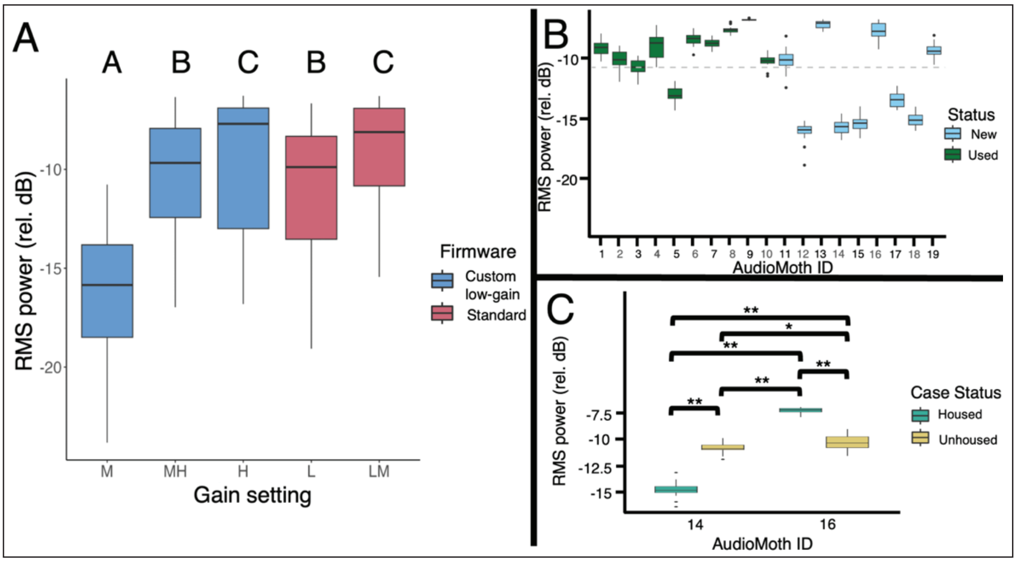

Figure 2. (A) Differences in mean $\mathrm{RMS}_{\mathrm{rel}}$ across firmware and gain settings for all AudioMoths from the analysis of ultrasonic amplitude variance. Groups with different letters differed significantly ($P < 0.05$). (B) Differences in $\mathrm{RMS}_{\mathrm{rel}}$ across 19 AudioMoths in the ultrasonic amplitude variance trial using standard firmware, low-gain. Dashed gray line represents the mean $\mathrm{RMS}_{\mathrm{rel}}$. (C) Significant differences in $\mathrm{RMS}_{\mathrm{rel}}$ for 2 new AudioMoths, when housed in polycarbonate cases versus unhoused. One asterisk indicates $P = 0.03$, whereas 2 asterisks indicate $P < 0.0001$. In all panels, bold line indicates the median, boxes represent the interquartile range, whiskers represent the first and fourth quartiles, and dots represent statistical outliers.

Table 1. Summary of locations, dates, estimated population size of Gray Bats from thermal videos, temperature (°C), relative humidity (%), firmware, and gain setting. The last column indicates the coefficient of determination (R^2) for the relationship between $\mathrm{RMS}_{\mathrm{rel}}$ and estimates of emerging bats using thermal video.

Location	Date	Estimated population size	Temp (°C)	Rel. humidity (%)	Firmware	Gain setting	R^2
Mauss	13 June 2022	27,539	21.9	88.5	Standard	Low	0.62
Mauss	23 July 2022	20,270	24.7	68.5	Standard	Low	0.58
Mauss	9 August 2022	4184	22.6	83.5	Standard	Low	0.34
Mauss	23 September 2022	27,056	19	84	Standard	Low-Med	0.63
Mauss	24 July 2023	2615	23.7	90.4	Custom	Med-High	0.10
Bat Cave Oregon	7 June 2022	8166	21	93.3	Standard	Low	0.87
Bat Cave Oregon	8 June 2022	21,877	NA	NA	Standard	Low	0.71
Tumbling Creek-Inside	20 June 2022	21,333	18.1	72	Standard	Low-Med	0.58
Tumbling Creek-Inside	13 July 2022	24,818	NA	NA	Standard	Low-Med	0.72
Tumbling Creek-Inside	18 May 2023	15,339	NA	NA	Custom	Med	0.37
Tumbling Creek-Inside	1 June 2023	12,056	18.4	78.5	Custom	Med	0.07
Tumbling Creek-Inside	23 June 2023	12,923	16.9	88.7	Custom	Med	0.04
Tumbling Creek-Inside	20 July 2023	27,779	19	95.1	Custom	Med	0.31
Tumbling Creek-Inside	23 July 2023	35,684	18.1	93.7	Custom	Med	0.62
Tumbling Creek-Outside	17 May 2023	16,297	17.4	87.1	Custom	Med	0.15
Tumbling Creek-Outside	18 May 2023	13,687	NA	NA	Custom	Med	0.41
Tumbling Creek-Outside	17 July 2023	37,872	22.2	93.1	Custom	Med	0.006

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Location	Date	Estimated population size	Temp (°C)	Rel. humidity (%)	Firmware	Gain setting	R^2
Tumbling Creek-Outside	20 July 2023	25,723	25.1	99.6	Custom	Med	0.43
Rocheport	22 May 2022	21,586	18.3	74	Standard	Low-Med	0.91
Rocheport	5 August 2022	32,825	24.1	64.5	Standard	Low-Med	0.63
Rocheport	4 September 2022	37,721	19.9	77.5	Standard	Low	0.77
Rocheport	5 September 2022	63,963	20.2	80	Standard	Low	0.63
Rocheport	9 September 2022	44,720	22	80	Standard	Low	0.76
Mary Lawson	25 July 2023	11,384	23.3	75.6	Custom	Med-High	0.41
Mary Lawson	28 July 2023	7141	23.5	76.5	Custom	Med-High	0.47
Mary Lawson	29 July 2023	5723	21.7	89.5	Custom	Med-High	0.44
Mary Lawson	30 July 2023	9823	25.6	83.4	Custom	Med-High	0.23
Devil's Icebox	29 July 2022	4337	14.3	74	Standard	Low	0.46
Devil's Icebox	6 August 2022	8872	15.4	79.5	Standard	Low-Med	0.69

Thermal data acquisition

We analyzed thermal videos in BatCount (Bentley et al. 2023) to obtain a frame-by-frame count of individuals as they passed over the acoustic recording device. We summed the frame-by-frame counts over 30 frames to produce a new variable that we termed "bats-per-second". BatCount provided an estimate of population size for each emergence by summing the individual bats passing through the region of interest over the recording duration (Table 1).

Statistical analyses

All statistical analyses were performed in R (R Core Team 2021). For our primary model, assessing the relationship between bats-per-second and $\mathrm{RMS}_{\mathrm{rel}}$, we used a generalized linear mixed-effects model with a negative binomial error term and several environmental covariates. We used bats-per-second as the response variable and RMS_{rel}, location, date, temperature, and humidity as the predictor variables. RMS_{rel}, temperature, and humidity were included as fixed effects, while cave and date were each included as a random effect (for details of statistical test see Supplemental File).

For the ultrasonic amplitude variance analysis, a series of 1-way ANOVAs were used to assess statistical differences in sensitivity across combinations of firmware and gain setting (for details of statistical tests see Supplemental File), as well as individual AudioMoths within each group (i.e., 19 AudioMoths each tested with 5 combinations of firmware and gain settings). A Tukey's post-hoc test was used to assess pairwise variation in RMS_{rel} among combinations of firmware and gain settings. Lastly, to assess statistical differences in the housed versus unhoused AudioMoths, we performed a 2-way ANOVA.

A simple linear regression was used to assess the relationships between overall population estimate and R^2 values from the bats-per-second to RMS_{rel} model outputs, and between total emergence duration and R^2 values from the bats-per-second to RMS_{rel} model outputs. Additionally, a simple linear regression was used to examine the relationship between bats-per-second and total normalized RMS_{rel} across single nights of recording at Mary Lawson.

Results

Ultrasonic amplitude variance results

We found a mean difference of 7.3 dB between the most sensitive (standard firmware, low-medium gain) and least sensitive (custom low-gain firmware, medium gain) gain settings (Fig. 2A; supporting statistical information for ultrasonic amplitude variance testing is in Supplemental File). Sensitivity differences were significant ($P < 0.001$) among most firmware and gain settings, except between custom low-gain firmware with high gain setting and standard firmware with low-medium gain setting ($P = 0.051$), and custom low-gain firmware with medium-high gain setting and standard firmware with low-gain setting ($P = 0.13$; Fig. 2A; Supplemental Tables 2–3; Supplemental File). We found significant sensitivity differences ($P < 0.0001$) among individual AudioMoths within each firmware and gain combination (Fig. 2B; Supplemental Tables 4–8; Supplemental Figs. 2–5; Supplemental File). Follow-up tests comparing 2 new devices showed that polycarbonate cases significantly affected device sensitivity ($P = 0.001$; Fig. 2C; Supplemental Table 9; Supplemental File).

Model results

Across the 6 locations, RMS_{rel} increased significantly with the number of bats-per-second ($P < 0.001$; Fig. 3; Supplemental Table 10; for model details see Supplemental File), with R^2 ranging from 0.006 to 0.91 across sites and population estimates ranging between 2615 and 63,963 individuals (Table 1). For each increase of 1 relative dB, the model predicted a 17.6% increase in bats-per-second. Temperature and humidity had no significant effects ($P > 0.05$).

Emergence profiles differed among nights at the same site for some populations, including Mauss Cave, Bat Cave Oregon, Tumbling Creek-Inside, and Tumbling Creek-Outside (Fig. 4). There was a general trend of shorter, denser emergence periods producing lower RMS_{rel}, compared to evenings with longer, less dense emergence periods. For sites with similar emergence patterns across consecutive nights of recording, such as Mary Lawson, the relationship between RMS_{rel} and bats-per-second remained constant (Figs. 4 and 5).

Overall, we found that population size of a roost did not significantly affect the bat-acoustic relationship (P = 0.09; r^2 = 0.1; Fig. 6) nor did emergence duration (P = 0.91; r^2 = 0.0004; Fig. 7). However, this was site-specific. At Mauss Cave, for example, on nights when emergence totaled more than 20,000 individuals, R^2 values ranged from 0.58 to 0.63, while on nights with less than 5000 individuals, R^2 values ranged from 0.10 to 0.34 (Table 1). Larger populations did not always yield higher R^2 values. At Bat Cave Oregon R^2 values of the model of bats-per-second to $\mathrm{RMS}_{\mathrm{rel}}$ remained high across 2 consecutive nights (Table 1; Fig. 5), despite high variance in emergence size (8166 individuals the first night; 21,877 the second night; Fig. 4). Interestingly, emergence density was higher for the night with low population size, with an average of 84 bats-per-second and lower for the night with higher population size, with a mean of 26.2 bats-per-second.

Discussion

Overall, we found that acoustic energy increased with higher emergence density. In addition, for a given location, the rate at which acoustic energy changed with bat density was consistent when emergence profiles were similar. This relationship, however, varied across nights with different emergence behaviors, highlighting the complexity of factors that may influence bat emergence, such as weather, phenology, and reproductive state (Frick et al. 2012). Our findings build on prior work linking acoustic energy to emergence density in *T. brasiliensis* (Kloepper et al. 2016) and suggest that, on a coarse level, acoustic-energy metrics may help document changes in phenology and monitor relative population trends throughout a season. We observed variation in the relationship between $\mathrm{RMS}_{\mathrm{rel}}$ and bats-per-second across different nights at some locations, hence averaging across multiple nights may improve the accuracy of population estimates, as reported in Kloepper et al. (2016).

AudioMoths are rapidly growing in popularity due largely to their low cost and user-friendly interface. For this study, we found it necessary to collect data with different gain settings across and within sites to avoid clipping in our acoustic recordings. Although applying a gain-offset enabled more accurate comparisons across surveys, the results of the AudioMoth ultrasonic amplitude variance tests reveal a concerning confounding variable.

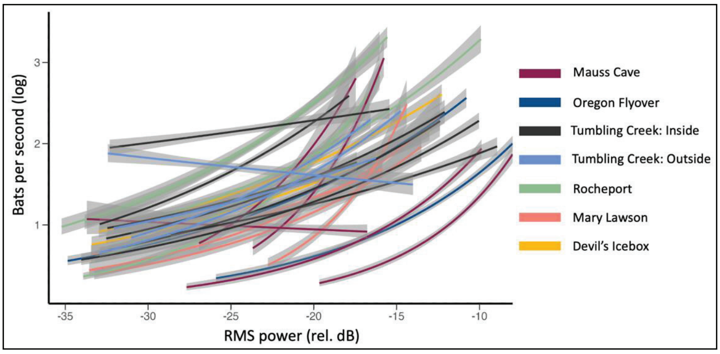

Figure 3. Relationship between acoustic energy ($\mathrm{RMS}_{\mathrm{rel}}$) and thermal video determination of bats-per-second for 27 emergences of Gray Bats at roosts in Missouri, USA. The curves represent the line of best fit from a generalized linear model with a negative binomial error term. The colors represent different sites, while each line is a unique emergence.

Given the significant differences in sensitivity across both new and used devices, our findings highlight the risk of using uncalibrated devices for amplitude measurements or to determine detection range. Our testing of housed and unhoused AudioMoth devices suggests that variation is likely driven by the housing rather than the recording devices themselves. This finding aligns with previous studies that have identified AudioMoth housing as the primary cause for variability among devices (Lapp et al. 2023, Osborne et al. 2023). However, this variation remains problematic as these units require weatherproof housing for use in the field. The variation in sensitivity for ultrasonic frequencies may be due to the placement of devices within housing units, which could result in differences in recorded amplitudes if the recording unit is not consistently aligned with the acoustic membrane. Further testing of housed and unhoused devices, as well as repeated testing of individual units, will help identify the mechanism driving the observed variation and reveal whether improved housing could lead to more consistent results. Despite the complex variation in sensitivity across devices in our study, we still found an overall significant relationship between $\mathrm{RMS}_{\mathrm{rel}}$ and bats-per-second, and this relationship likely will strengthen once device variation is accounted for.

Although comparing data across sites in our study was challenging, we found promising evidence for the acoustic censusing methods, after accounting for differences among indi-

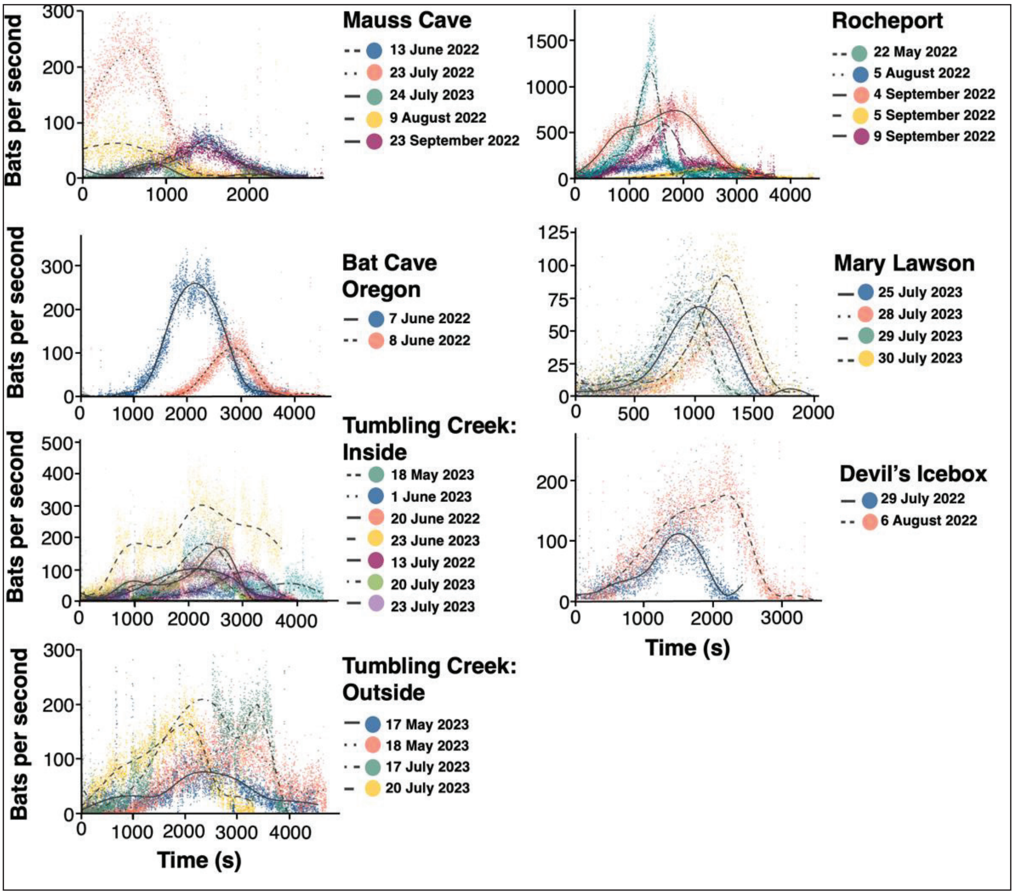

Figure 4. Emergence profiles, bats-per-second over time (s), for each site and emergence from the video counts (right side).

vidual AudioMoths. At Mary Lawson we collected 4 nights of data within a single week using the same AudioMoth, with 3 of those 4 emergences recorded on consecutive days (Figs. 4–5). We found a positive linear relationship in the total number of bats versus total RMS_{rel} (Fig. 8). Calculating the slope of the trend line for changes in acoustic energy over multiple consecutive nights minimized the impact of nightly variation in emergence conditions. Thus, monitoring relative changes in acoustic energy at a site, as a proxy for relative population size, may offer valuable insights into site-specific phenology and indicate peak occupancy. These acoustic population trends can help managers determine ideal censusing periods without the need for expensive ($1000+ USD) bat detectors. However, we recommend that users ground-truth these acoustic recordings with thermal or manual counts for at least 1 night at each location, until the acoustic censusing method is more thoroughly validated.

In addition to understanding site-specific relationships affecting RMS_{rel} and bats-per-second, further investigation is needed to understand how temporal variations in emergence density and acoustic behavior influence this relationship. Our data suggest

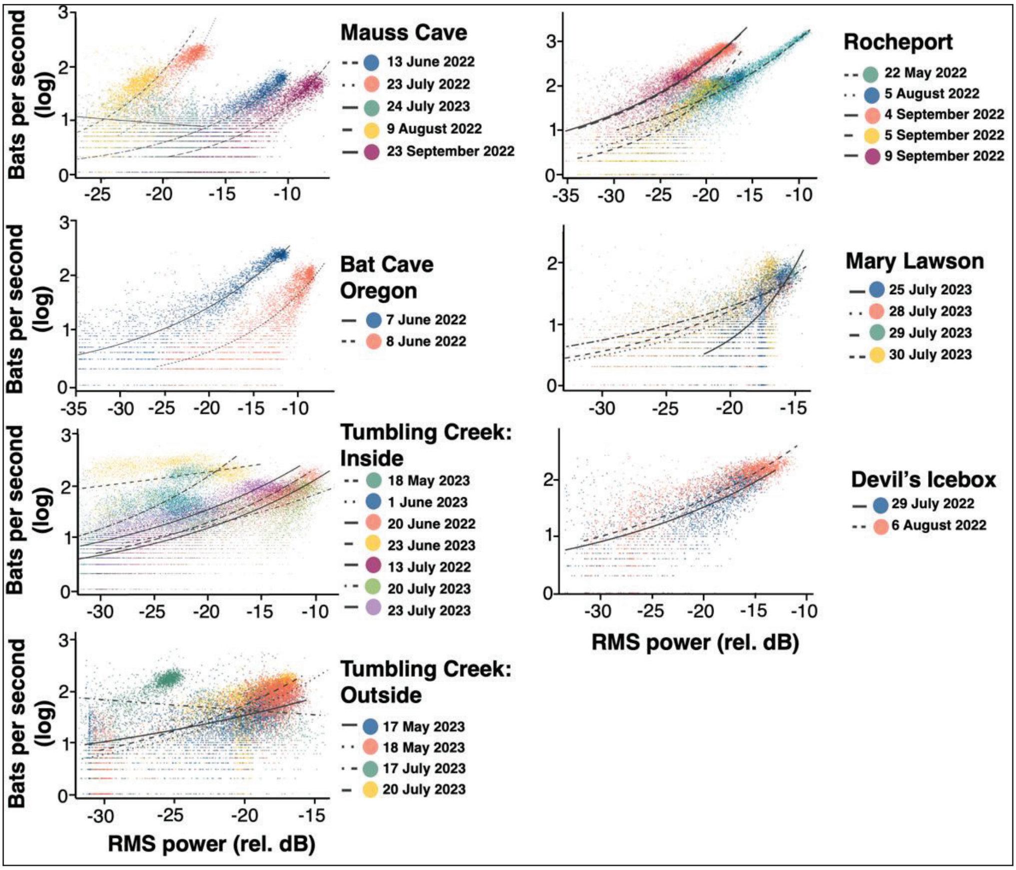

Figure 5. Thermal video estimates of bats-per-second ($\log_{10}$) versus RMS_{rel} for each night of emergence. The horizontal lines below 1 bat per second ($\log_{10}$) is an artifact of the logarithmic transformation of low-value integers. Overlap in the points for Mary Lawson makes it difficult to distinguish the orange and green points.

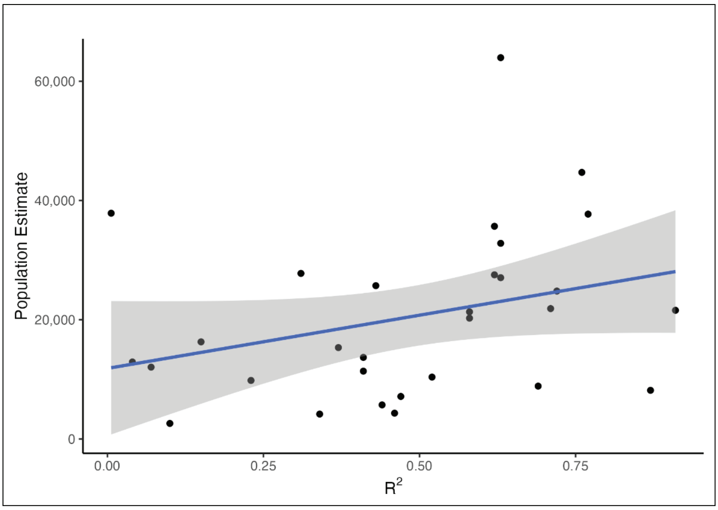

Figure 6. Population estimates of Gray Bat colonies from thermal videos versus the coefficient of determination (R^2; Table 1) for the relationship between bats-per-second and RMS_{rel}, for each night of emergence. Gray shading represents the standard error.

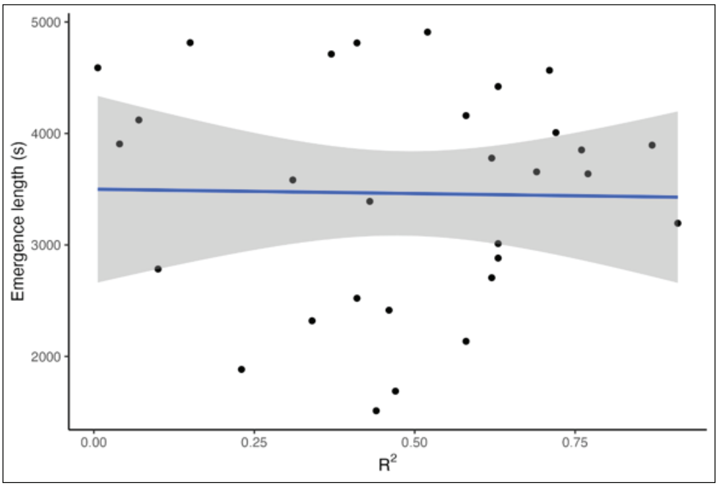

Figure 7. Total duration of emergence versus the coefficient of determination (R^2; Table 1) for the relationship between bats-per-second and RMS_{rel}, for each night of emergence. Gray shading represents the standard error.

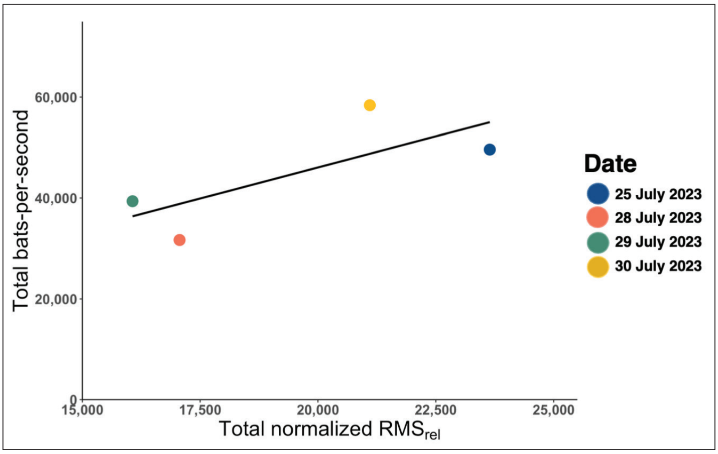

Figure 8. The relationship between the total number of bats-per-second for a single night and the total normalized RMS_{rel} for that night at Mary Lawson ($r^2 = 0.56$, $P = 0.29$).

decreased overall amplitude during high-density emergences, such as those at Bat Cave Oregon (Fig. 5). While these phenomena may be due to variation among recording devices, some bats also go silent at high densities (Chiu et al. 2008). Continued research into the vocal adaptations of bats in groups will be crucial to understanding the impact of high-density emergences on the effectiveness of acoustic censusing methods. Variation in the relationship between amplitude and bats-per-second may also result from changes in colony demographics across the season. In May emergences primarily consist of adults, while by late July emergences likely contain a mix of adults and newly volant juveniles (Brack et al. 2021). Gray Bats typically become volant at 21–33 days of age (US Fish and Wildlife Service 2024). Although vocal ontogeny in Gray Bats has not been documented, *Eptesicus fuscus* (Palisot de Beauvois, Big Brown Bat) shows differences in vocal characteristics between pups and adults up to 28 days after birth (Moss 1988, Moss et al. 1997). Therefore, juvenile Gray Bats may exhibit a different relationship between RMS_{rel} and bats-per-second, compared to adults. Further investigation into how amplitude and density trends vary over time and across sites will be crucial to the development of a robust model that can estimate population size of cave-dwelling bats from acoustic energy.

In general, the results of this study suggest that using acoustic energy to predict trends in bat emergence counts is best suited for emergences of moderate density (~50–150 bats-per-second). However, these results may be specific to Gray Bats, as Kloepper et al. (2016) found the method was most effective for high-density emergences of *T. brasiliensis*. Similar to Kloepper et al. (2016), we noted poorer fit at low densities, although it was not statistically significant (Fig. 6). At low densities, RMS_{act} is more likely influenced by non-emerging bats flying in the recording area, due to the low noise floor of low-density emergences. Use of acoustic energy to predict emergence counts relies on identifying a relationship between individuals within view of the thermal camera and the echolocation calls they produce during that time. Ultrasonic noise from individuals within range of the acoustic recorder, but not actively emerging, cannot be distinguished from emerging bats. This issue is particularly problematic for low-density emergences, during which a few nearby individuals could disproportionately affect the RMS_{act}.

For biologists interested in monitoring a single location over an extended period, there are steps to improve the precision of relative population estimates. First, averaging acoustic energy across multiple nights will result in better population estimates (Kloepper et al. 2016). Second, using a designated AudioMoth with an assigned polycarbonate case deployed at a fixed location for each site may help reduce variation in RMS_{rel} attributed to the case itself. Another option is to use a more expensive recording units such as Song Meter (Wildlife Acoustics, Maynard, MA) or Anabat (Titley Scientific, Columbia, MO). However, this introduces the risk of signals clipping. Most commercial recording units are designed to detect bats over large distances, incorporating electronic amplifiers and user-adjustable gain settings. In areas where bats pass close to the microphone, even no gain (zero amplification) can result in signals that exceed the recorder's capacity, causing distortion of the signal (Metcalf et al. 2023), although physical modifications to the microphones (e.g., attenuating foam) can help reduce clipping (Gentry-Grace 1998).

Regardless of the recording unit, we strongly recommend conducting calibration measurements at the beginning of and periodically throughout the study. Although true acoustic calibration requires specialized, expensive microphones and testing chambers, we developed a low-cost alternative to assess variation in response to ultrasonic stimuli across acoustic recorders (Supplemental File). The method provides an affordable option for researchers with limited resources to calibrate sensors.

Although acoustic methods will likely never match the accuracy of video counting, monitoring trends in acoustic activity over time could reveal important phenological events, such as peak occupancy, which could help determine optimal timing of thermal-censusing efforts. Understanding the relationship between emergence density and acoustic energy is fundamental for evaluating the efficacy of this method for monitoring population trends of colonial bats. Although we found no significant effect of temperature or humidity, these factors affect sound propagation (Goerlitz 2018) and bat behavior (Koch et al. 2023) and should, therefore, continue to be used as covariates in future studies. In conclusion, our results demonstrate the potential for acoustic-energy measurements to monitor trends in the number of bats emerging from a roost. However, for this method to infer population trends reliably across roosts, a better understanding of variation in ultrasonic performance across acoustic sensors is needed. Furthermore, validating these methods with additional species would advance the integration of acoustic estimates of the number of bats emerging from a roost into larger-scale monitoring programs such as NABat (Loeb et al. 2015).

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