Evaluation of Methods for Monitoring Long-term Population Trends in Cave-roosting Bats

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Abstract: Because numerous cave-roosting bat species are experiencing population declines, especially those affected by the white-nose syndrome epizootic, it is essential to establish rigorous monitoring protocols to accurately track population trends over time. We tested the efficacy of low-cost visual counts to effectively monitor population trends of southeastern myotis (*Myotis austroriparius*) at a maternity-roost in southwestern Georgia. We conducted visual counts during evening emergence events using white light illumination. Visual counts were made during a 1-minute period out of every 5-minute interval throughout the entire emergence duration on three consecutive nights during late-June and early-July 2008 and 2009. We simultaneously recorded emergences using a night-vision video camera to allow direct comparison of visual counts with actual bat emergence numbers. Visual counts were inaccurate ($F = 26.57$, $P < 0.0001$) and inconsistent between years ($F = 37.50$, $P < 0.0001$) in providing estimates of total emergence numbers. However, depression of the emergence rate (number of bats leaving per minute) during white-light illuminated visual observations influenced our visual estimates. Additionally, we detected a positive relationship between emerging bat numbers and corresponding observer error ($r^2 = 0.9127$, 81 d.f., $P < 0.0001$). Although this strong relationship suggests that potential exists to calibrate observer error associated with visual observations, we conclude that visual counts, particularly with white-light illumination, offer little merit as an effective low-cost technique to monitor southeastern myotis colonies, and may be problematic for monitoring other colonial bat species as well. Future efforts should focus on video recording methodologies to establish monitoring programs for cave roosting southeastern myotis.

Key words: Southeastern myotis, *Myotis austroriparius*, cave-roosting bats, visual counts, near-infrared

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Life history strategies of colonial bat species, such as their low reproductive rates and aggregations at a limited number of hibernacula, make these populations especially vulnerable to decline (O'Shea and Bogan 2003). Because many colonial bat species in North America are considered federally threatened or endangered or are species of concern in specific states, it is important to establish rigorous monitoring protocols capable of tracking population trends over time (O'Shea and Bogan 2003). Additionally, the recent emergence and rapid spread of white-nose syndrome among bats in caves and the high mortality rates associated with this epizootic have compounded the need for efficient and accurate monitoring protocols (O'Shea and Bogan 2003, Blehert et al. 2009).

Several methods to monitor cave-roosting bat populations have been used in the past with varying levels of success. Factors that contribute to this census variance include the size and mobility of the species monitored, relative number of individuals present, access of investigators to roosting sites, and the availability and applicability of technological devices (i.e., cameras, infrared lights, and night-vision goggles) used for censusing (Kunz 2003). Four common methods previously used to estimate colony size at cave roosts have included direct internal roost counts, disturbance counts, guano deposition rate estimates, and evening emergence counts (Kunz et al. 1996). Roost counts are generally used when the species roosts within caves in small clusters that are easily accessible to human observers. Though roost counts are labor intensive and only yield estimates in order of magnitude (Kunz 2003), they have been effective in documenting large scale declines in use of winter hibernacula for endangered Indiana bat (*Myotis sodalis*) and gray bat (*Myotis grisescens*). Disturbance counts involve entering a roost, causing auditory disturbance and counting the number of flying bats. This method is generally not recommended because disturbance may cause adults to abandon dependent young and might be considered a "take" relative to state and federal regulations. Guano deposition rate estimates relate colony size to the amount of guano deposited over time and provide only an indirect index of abundance (Williams et al. 2002). Evening emergence counts are used to census bats emerging from caves and other structures and may have the most potential as a low-cost, noninvasive method for estimating numbers of cave-roosting bats.

Technologies, such as thermal infrared imagery, near-infrared imagery, or night vision imagery coupled with video recording devices, offer increased accuracy of evening emergence counts, but at increased costs (Reynolds and Mitchell 1998). Thermal infrared imaging technology makes it possible to count bats as they emerge from caves independent of ambient light (Ammerman et al. 2009), and its effectiveness has been repeatedly demonstrated (e.g., Sabol and Hudson 1995, Frank et al. 2003, Elliott et al. 2005, Ammerman et al. 2009). Furthermore, video recordings of thermal or night vision imagery allow complete emergence counts that can yield accurate estimates of total colony size (Elliott et al. 2005). In the case of thermal imagery, estimates can occur either by manual review of recordings or with automated computer software that uses frame-by-frame tracking and counting (Reynolds and Mitchell 1998). Although these techniques may yield better emergence estimates, they may also be prohibitively expensive for use by some management or conservation entities.

The merits of less expensive and labor intensive colony estimation options such as direct visual counts are inconclusive in the literature. Some evidence suggests visual counts are subjective with accuracy highly dependent on observer skill (Sabol and Hudson 1995), whereas another study suggests the method can provide accurate estimates (Reynolds and Mitchell 1998). Counts are generally conducted for short periods of time at regular time intervals (i.e., 1-min durations at 5-min intervals) throughout an emergence event and then interpolated to estimate the entire emerging colony size. An advantage of this technique is that minimal equipment is needed, but the ability to accurately estimate colony size is unknown and warrants further investigation.

Emergence counts at cave entrances offer a non-invasive monitoring opportunity, but a lack of evidence exists as to which methods can yield adequate results for the lowest cost. Thus, the goal of this investigation was to test the capabilities of low-cost visual counts in monitoring population trends. Our specific objectives were to 1) determine if observers conducting visual counts could accurately and consistently estimate emergence numbers, 2) examine the effect of white light used to illuminate the cave entrance on bat emergence behavior and consequently on emergence estimates, and 3) assess if observer error was affected by increasing numbers of emerging bats.

Study Site

We conducted our investigation at a maternity colony of southeastern myotis (*Myotis austroriparius*) approximately 40 km northeast of Albany, Georgia, in Lee County. The maternity colony was located in a cave near Chokee creek on private land. The cave is found in the limesink region of the Dougherty Plain physiographic region of southwest Georgia (Wharton 1978). The cave entrance was constricted to a single small opening approximately 5 m wide by 1 m high. The cave opens into a bottomland hardwood forest composed of mature bald cypress (*Taxodium distichum*) and various oak species (*Quercus* sp.) with little to no understory vegetation obstructing the entrance. Use of this cave as a southeastern myotis maternity colony has been documented since 2005 (J. Ozier, Georgia Department of Natural Resources, pers. communication).

Methods

Sampling Methods

We conducted visual counts on three consecutive nights in 2008 (1–3 July) and 2009 (19–21 June) by a single observer following methodology described by Sabol and Hudson (1995). The observer was positioned 2 m from the cave creating a field of view perpendicular to the flight path of emerging bats and sat in the same place during each emergence count to ensure consistency between observations. Counts were performed during 1-min intervals spaced evenly (every 5 min) across the entire emergence period. We used white light (Brinkmann Q-Beam Max Million Rechargeable Spotlight 1-million CP, Brinkmann Corp., Dallas, Texas) to illuminate the cave entrance allowing all exiting bats to be counted. When we observed bats re-entering the cave during visual counts we deducted them from the number exiting yielding a net total emergence count.

We used a Sony digital Handycam video camera (Model DCR-DVD910) with near-infrared night vision capabilities to record bat emergences simultaneously with visual counts. We added additional near-infrared lights (Model IRLamp6 20° Beam Angle, Wildlife Engineering; www.irlight.com), powered by a 12V battery, to supplement the near-infrared lights on the video camera. The camera was attached to a tripod and positioned next to the observer approximately 2 m from the cave perpendicular to the entrance and bat flight path. Each night the camera clock was synchronized with the observer's clock. Recording began when the first bats were observed emerging, typically between 2145 hours and 2150 hours, and continued until the number of bats exiting diminished to <4 bats exiting/minute.

We viewed recordings of each emergence on a laptop computer using Windows Media Player version 11.0. We divided each video into discrete minutes with each minute receiving a complete count by a single observer. For each minute, only bats newly emerging into the field of view were recorded to ensure double counting bats from a previous minute did not occur. Minutes on the video that corresponded with minutes when visual counts occurred in the field were identified and paired with the respective visual count observation. Minutes near the beginning and end of each video when bat emergence rates were low, were watched at full speed. During minutes when the bat exit rate increased, videos were slowed down to as low as 0.056 of real time to ensure count accuracy.

We estimated colony size by compiling the net total number of bats observed exiting the roost on each video (complete count). We then estimated colony size for each emergence event from the 1-min interval visual counts (visual count) and the corresponding 1-min interval video counts (video count). Each 1-min estimation procedure used an interpolation algorithm to approximate the number of bats exiting during the other four minutes by assuming a constant rate of change between subsequent visual or video observations that occurred during white light illumination (Rudolph et al. 2005). Complete counts were assumed to accurately represent true emergence numbers for comparisons to derived estimates.

Analysis

We conducted a two-factor analysis of variance (ANOVA) to test whether observers could accurately and consistently predict true colony size using visual observations and to determine if an observation effect occurred during white light illumination of the cave entrance (Dowdy et al. 2004). The two factors examined in the ANOVA were colony size estimation method and year. To minimize impacts on our analysis from between year colony size variation, we made pair-wise comparisons treating the complete count as a baseline setting it equal to zero. The corresponding visual and video counts were then set relative to the zero baseline by subtracting the complete count from the derived visual and video count estimates. This procedure forced overestimates by visual or video counts to register as positive and underestimates to register as negative. Specifically, we tested three null hypotheses (H_o) using an additive fixed effects model. Our first H_o predicted no difference among bat observation methods on emergence population estimates. Our second H_o predicted no difference between years on emergence population estimates. Our last H_o predicted that effects from bat observation methods acted independent of year effects (i.e., no interaction effect). When differences were observed, Tukey's Studentized Range Honestly Significant Differences (HSD) test was conducted to determine how the bat observation methods differed from the complete count and from each other, and whether estimates were consistent across years.

We also used linear regression to examine whether a relationship existed between increasing bat emergence numbers and error associated with visual counts during 1-min intervals (Chatterjee and Hadi 2006). For this analysis, we combined observations across years. Error was calculated as the difference between the 1-min visual count and the corresponding 1-min video count. Error was then regressed against the 1-min video count which was assumed to represent the true number of bats emerging during that time period. All statistical procedures were conducted using SAS statistical program 9.2 (SAS Institute, Inc. 2008).

Results

The duration of the recorded evening emergences lasted between 60 and 90 minutes. Based on complete counts, the mean emerging population size was 11,326 (± 135; 95% CL) bats and 3,588 (± 158; 95% CL) bats in 2008 and 2009, respectively. Our ANOVA indicated that bat emergence estimation method ($F_{2,12}$ = 26.57; $P < 0.0001$) and year ($F_{1,12}$ = 37.50; $P < 0.0001$) influenced estimation accuracy, but not in an additive fashion. The interaction between bat emergence estimation method and year was significant ($F_{2,12}$ = 33.14; $P < 0.0001$) necessitating further investigation about the interaction. Tukey's test indicated that both interpolated estimation methods (visual and video counts) differed from complete counts and from each other, and that results were inconsistent between years (Figure 1). In 2008, our visual and video counts differed from each other with visual counts overestimating and video counts underestimating the complete count. In 2009, when mean emerging population size was less than ⅓ of that observed in 2008, visual and video counts both underestimated the complete count, but did not differ from each other (Figure 1). Linear regression analysis indicated a significant positive relationship ($r^2 = 0.9127$, 81 d.f., $P < 0.0001$) between emergence counts during 1-min intervals ($n = 83$) and error associated with the corresponding visual observations (Figure 2). At the same time, bat

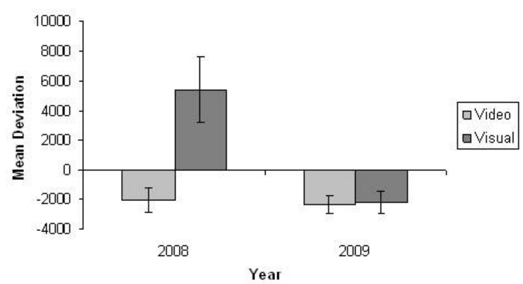

Figure 1. Mean deviation of interpolated visual and video count estimates from corresponding complete counts set equal to zero recorded over three nights of emergence at a southeastern myotis (Myotis austroriparius) maternity colony in Lee County, Georgia, 2008–2009.

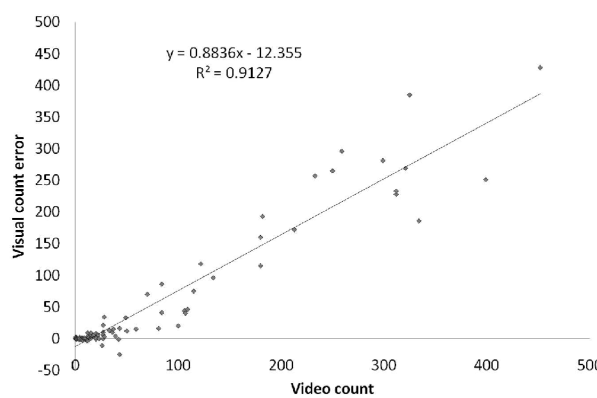

Figure 2. Relationship between magnitude of visual count observer error during individual 1 min observations ($n = 83$) and increases in actual bat emergence numbers obtained from simultaneous night vision video recordings of the 1-min interval observation periods over three nights of emergence at a southeastern myotis (Myotis austroriparius) maternity colony in Lee County, Georgia, 2008–2009.

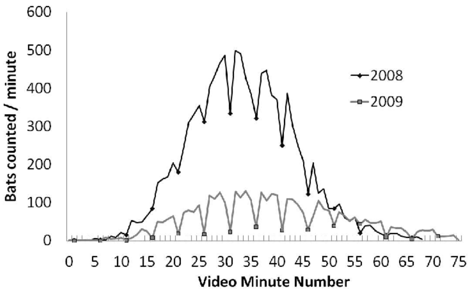

Figure 3. Bat emergence rate per minute documented from videos recorded over the duration of evening emergence events from a southeastern myotis (Myotis austroriparius) maternity colony in Lee County, Georgia, 2008–2009. Each symbol on the 2008 and 2009 lines correspond to 1-min intervals with white light illumination.

emergence rate during discrete minutes of observation on videos during both years declined during minutes while the cave entrance was illuminated with white light (Figure 3).

Discussion

Results from our study indicate that visual evening emergence counts likely are not viable as a low-cost alternative for monitoring colonial cave-roosting bat populations. Observers overestimated the emerging numbers of bats when the emergence population was large (i.e., 2008), but underestimated when the population was small (i.e., 2009), indicating complexity in the relationship between derived colony estimates from visual observations and true colony size. A strong predictable relationship between an index and the population parameter being monitored is important (Williams et al. 2002). Visual evening emergence counts offer neither a strong nor a consistent relationship to the actual colony size.

The inconsistency of population estimates obtained from visual counts relative to corresponding complete counts likely were due to an interaction between bat emergence rate and an observation effect caused by our methodology. Our linear regression showed a general tendency of the observer to overestimate the number of bats emerging, and that the amount of overestimation was positively correlated with the number of bats emerging (Figure 2). In contrast, we observed that the presence of white light tended to depress emergence rate. In the videos we observed that when the cave entrance was illuminated by white light the bat emergence rate was low, but immediately increased when the white light was turned off (Figure 3). However, because we observed inconsistency between years with different population sizes these caves apparently are not equal. The observer overestimation tendency during large emergence population years exceeded the depression effect of white light on bat emergence behavior (Figure 1). But during small emergence population years the observer tendency to overestimate the emergence rate did not exceed the depression of the emergence caused by the white light resulting in an underestimation of the true emergence population.

Visual counts have had limited success in a number of previous studies (Sabol and Hudson 1995, Reynolds and Mitchell 1998, Kunz 2003, Elliott et al. 2005), but none of these studies directly evaluated visual counts against video-recorded evidence. For instance, Sabol and Hudson (1995), studying large gray bat colonies (40,000 – 189,000 individuals) in Alabama, used a visual counting method in which the observer counted every other minute of emergences, with total emergence counts obtained by doubling visual counts. They suggested that visual counts can produce adequate estimates if the observer is well trained, but they did not demonstrate that the estimates were more accurate or consistent across a wide range of emergence rates. They concluded, however, that visual counts were subjective and less consistent than more technologically-advanced techniques such as infrared videography

White light has been successfully used by many wildlife field studies, but most nocturnal mammals alter their behavior in the presence of white light (Finley 1959). White light has been shown to specifically affect bat behavior in a number of studies (Laidlaw and Fenton 1971, Usman et al. 1980, Downs et al. 2003). In Europe, Downs et al. (2003) studied the effects of different light colors and intensity on small colonies of soprano pipistrelle bats (*Pipistrellus pygmaeus*) roosting in manmade structures and ranging in size from 51 to 536. Relying solely on visual counts they found that most bats emerged during the no light treatment and fewest during white light treatment. Laidlaw and Fenton (1971) observed that white light illumination of attic colonies of little brown bats (*Myotis lucifugus*) and big brown bats (*Eptesicus fuscus*) in Ontario, Canada, resulted in significantly earlier evening departures suggesting a possible behavioral avoidance of the white light by individual bats. Number of bats in colonies exposed to light also decreased between 53% and 89% for little brown bats and between 41% and 96% for big brown bats. It is possible bats avoid emerging during bright conditions to avoid predators (Usman et al. 1980).

There are several alternatives to white lights that have shown promise in aiding visual counts. In some situations red lights may provide sufficient illumination for accurate emergence counts without obstructing normal bat behavior (Finley 1959, White and Seginak 1987, Ludlow and Gore 2000, Downs et al. 2003). Downs et al. (2003) found that bat emergence estimates under red light conditions were intermediate to no light and white light conditions, suggesting that red light may be an option to improve emergence counts in some situations. However, in situations where bats emerge at full speed or a large number of bats emerge at one time, red lights may not provide enough illumination for accurate counts. Use of infrared night vision is another alternative to provide better visibility of bats without altering bat emergence behavior (Sabol and Hudson 1995). Martin et al. (2003) successfully used infrared lights and night vision goggles to evaluate the initiation of emergence of gray bat. However, night vision equipment is more expensive than light filters used to produce red light, and the use of night vision equipment lacks an assessment of accuracy against simultaneous video recording.

Even when using an alternative illumination method (e.g., night vision or red light) the tendency to overestimate counts at higher emergence numbers likely still exists. Thus, the need to calibrate error associated with visual observations to better estimate actual emergence numbers will still be needed. Accuracy of emergence counts depends on numerous factors including the size, configuration, and spatial distribution of roost openings, the number of openings from which bats depart, the number of bats that emerge, the visibility of the bats at that time, and the experience of the observer (Kunz 2003). As a result, each observer should be calibrated to each cave using simultaneous video recordings. Therefore, even if visual counts are used and calibrated, a video camera capable of night vision would still be necessary, thus eliminating or at least reducing any low-cost benefits of visual counts.

Near infrared video recordings of bat emergence events from cave roosts may be a reliable method for establishing long-term monitoring protocols. Near-infrared video cameras and supplementary near-infrared lighting systems are considerably less expensive (approximately US$600–$1,000 combined cost) than thermal imagers used in other bat emergence studies (that cost anywhere from $8,000 to $60,000; e.g., Sabol and Hudson 1995, Frank et al. 2003, Elliott et al. 2005, Ammerman et al. 2009). Furthermore, our research, along with research by Elliott et al. (2005), suggests that watching the entire emergence video may not be necessary to accurately estimate the true emerging population. Elliott et al. (2005) obtained accurate emergence estimates by counting total number of emerging bats on 40% of each video and interpolating the remaining 60% of the emergence. Additionally, they found that counting the emergence on as little as 17% of each video may be accurate as long as colony size was >2,000 bats. At caves where long-term population trend estimates are needed, the amount of video to be counted to obtain a desired level of accuracy could be optimized to make this method more efficient and cost-effective.

Management Implications

Our results suggest that visual counts using white light illumination are not a viable option for monitoring long-term population trends of cave-roosting southeastern myotis. Other illumination methods, such as red lights or night vision equipment, are less likely to alter bat behavior but the tendency of observers to overestimate counts at higher bat numbers requires calibration of error using actual emergence numbers. We suggest that the most cost effective method of providing accurate bat emergence counts is to sub-sample recordings obtained with near-infrared video cameras and a supplemental lighting system. However, the intensity of subsampling required to obtain a desired level of accuracy is likely cave-specific and may vary with seasonal or annual fluctuations in bat numbers.

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