Survey Methods for Monitoring Bat Populations
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Long-term monitoring of endangered Mexican Long-nosed Bats (*Leptonycteris nivalis*) and a test of an automated census approach

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Abstract - Management strategies for bats require accurate knowledge of population size to determine population trends. We used thermal-infrared cameras to record bats, including endangered Mexican Long-nosed Bats (*Leptonycteris nivalis*), emerging from a roost between 2008 and 2023. Thirty-five videos were analyzed manually to obtain a count of emerging bats and then were analyzed using an automated program, ThruTracker. This automated method generally performed well, with overall 90.8% accuracy (range = 64–99%). Maximum annual colony size of *L. nivalis* fluctuated from 294 to 3360 bats (mean = 2156) across 16 years. There was no evidence of a significant decline. We conclude that ThruTracker software can be effective for estimating overall population size and for detecting changes in populations over time.

Introduction

Successful species recoveries under the Endangered Species Act are achieved only if regular monitoring is performed and recovery of populations can be known. *Leptonycteris nivalis* (Saussure) (Mexican Long-nosed Bat) is a phyllostomid bat recognized as endangered by the State of Texas, the United States Fish and Wildlife Service, Mexico, and the IUCN (USFWS 1988, 1994, 2018). While this migratory species occurs throughout much of Mexico, only a single maternity roost is known in the United States; the roost is occupied largely by lactating females and newly volant juveniles (young of the year) during the summer (USFWS 2018). Adult males are generally not encountered at this site, and newborn pups have never been documented. The roost, Mount Emory Cave (hereafter Emory Cave), is located at an elevation of 2140 m within the Chisos Mountains of Big Bend National Park (BBNP) in Texas.

In the northern half of its range, *L. nivalis* relies primarily on the nectar and pollen of *Agave* (century plants) and plays an important role in pollination of these plants and in the maintenance of arid-land ecosystems (Gómez-Ruiz and Lacher 2016, Kuban 1989). In 1972, Easterla proposed that Emory Cave was a "spillover" colony in years of low food supply in Mexico, and that bats did not necessarily use the cave every year. The colony and the natural history of the species is better known (USFWS 2018) since that time, but the "spillover hypothesis", which states that bats only migrate to this roost in some years, has not been tested. Migratory routes and connections among roost sites in Mexico are still largely unknown (USFWS 2018, 2024a). However, the species is regarded as a single population (Ammerman et al. 2019; USFWS 2018, 2024a), and evidence is growing to support connectivity between *L. nivalis* in Emory Cave and in the southern portion of its

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range. For example, Pourshoushtari and Ammerman (2020) demonstrated a lack of genetic distinction between roosts at the northern and southern extents of the *L. nivalis* distribution, utilizing microsatellite markers. Additionally, it seems that these bats migrate along a corridor of flowering *Agave* and other nectar producing plants. Modeling efforts support the close spatio-temporal relationship between *L. nivalis* occurrence and *Agave* nectar corridors (Burke et al. 2019, Gomez-Ruiz and Lacher 2016).

All listing and recovery documents recognize the need for accurate, long-term monitoring of this species (USFWS 1994, 2018, 2024a, 2024b), but it is challenging to establish a survey method for *L. nivalis* that can be applied range-wide. This species cannot be effectively monitored using mist nets over water or around *Agave* plants, as this method poses important logistical challenges. For example, during 25 years of netting over water sources in BBNP, only 5 individuals from over 7200 captures have been *L. nivalis* (Higginbotham and Ammerman, 2002; L. Ammerman, unpubl. data). Meanwhile, roost counts conducted by personnel at BBNP between 1988 and 2000 suggested that major fluctuations in colony size (0–6630 bats) occurred from year to year (R. Skiles, Big Bend National Park, TX, unpubl. data); Easterla (1972) had previously described a similar pattern. However, these estimates were conducted only once per year via surface-area counts, in which the number of bats per square meter on the cave ceiling was estimated and then extrapolated over the estimated area of the roost that was covered by bats (Thomas and LaVal 1988). Emory Cave has many crevices and deep cavities that are inaccessible (Veni 2016) and certainly contain roosting bats that are overlooked with a visual method (Ammerman et al. 2009). An accurate census is critical to understanding and ultimately protecting the population of *L. nivalis* in Texas.

Because thermal-infrared cameras detect heat produced by bats, it is possible to record and census bats independent of ambient light (Frank et al. 2003). This method has proven successful for numerous studies on the behavior of free-ranging bats (Hristov et al. 2008)—for censusing millions of *Tadarida brasiliensis* (I. Geoffroy) (Brazilian Free-tailed Bats) that emerge nightly from certain caves in Texas (Horn and Kunz 2008), estimating abundance at a cave in Brazil (Otálora-Ardila et al. 2020), observing bats at wind turbines (Cryan et al. 2022), and censusing *L. nivalis* at Emory Cave (Ammerman et al. 2009). Ammerman et al. (2009) used an infrared thermal-imaging camera to record and census bats that emerged nightly from Emory Cave in 2005 and compared colony estimates using this method to estimates derived from surface-area counts. Ammerman et al. (2009) concluded that thermal-imaging techniques make a more accurate census of this colony possible and have several advantages over traditional methods. A census of the Emory Cave colony in July 2005 showed the population of all bat species in the cave to be just under 3000 bats (Ammerman et al. 2009). This 2005 census was conducted in a single summer season, and a long-term approach was necessary to understand annual fluctuations in colony size.

Photos and video recordings have been an important tool in wildlife research for decades, but often the researcher becomes tasked with viewing continuous videos to locate specific activity (e.g., emergence) that can appear over long intervals of time or for brief moments throughout the survey (Evans et al. 2015). Videos obtained by thermal-infrared technology (or night vision or visible light) have typically been evaluated manually, but the development of computer-assisted tracking programs have the capability of reducing the time investment required for analysis. The use of automated software has been employed frequently in recent years for analysis of wildlife data, and automated programs also can play important roles in use of continuous videos for wildlife monitoring (Marcot et al. 2019). Programs such as ThruTracker (Corcoran et al. 2021) are open-source and can identify and track wildlife in motion, with algorithms to count bats entering or leaving a roost. This application of automated methods could assist bat researchers or resource managers with monitoring efforts once this approach has been validated. Some testing of the performance of computer-assisted methods has been conducted as applied to census counts of bat emergences (Bentley et al. 2023, Corcoran et al. 2021). Compared to manual counts, accuracy of programs, such as BatCount (Bentley et al. 2023) and ThruTracker (Corcoran et al. 2021), has ranged from 50.8 to 99.6%. Testing software performance by Corcoran et al. (2021) and Bentley et al. (2023) included videos with variable emergence rates, different cameras, different bat species, and different video durations, but none of these tests have been performed with extended videos (>2 h).

In this study, our objective was to use thermal-infrared technology to determine colony size of *L. nivalis* at Emory Cave during the peak abundance of *Agave* in early July of 2008 through 2023. A secondary objective was to use the recordings of bat emergences to test the performance and accuracy of ThruTracker (Corcoran et al. 2021) against the manual approach to determine colony size.

Field-site description

Emory Cave is a tectonic cave formed in rhyolite that is 86-m deep and 562-m long (Veni 2016). It is located in a pine-oak-juniper association (Easterla 1972, 1973) in the Chisos Mountains, which are surrounded by lowland Chihuahuan Desert. The main cave entrance is almost completely obstructed by a tree, *Prunus serotina* var *rufula* (Wooton and Standl) (Southwestern Chokecherry) that was originally identified incorrectly as *Ostrya chisosensis* Correll (Chisos Hophornbeam) in Ammerman et al. (2009). This cave has a continuous cool air flow that suggests other openings, but none large enough for bat emergences has been found (Veni 2016). In summer, internal ambient temperatures ranged between 15.2 and 18.3°C, and mean relative humidity was 87% (Ammerman et al. 2009). The temperature of 1 chamber used by *L. nivalis* ranged between 11.4 and 18.7°C from April to September (Brown 2008). A small number of individuals of other species, such as *Myotis thysanodes* Miller (Fringed Myotis) and *Corynorhinus townsendii* (Cooper) (Townsend's Big-eared Bat), also use the cave as a roost. *Antrozous pallidus* (LeConte) (Pallid Bat), *Eptesicus fuscus* (Palisot de Beauvois) (Big Brown Bat), *M. ciliolabrum* (Merriam) (Western Small-footed Bat), and *M. volans* (H. Allen) (Long-legged Myotis) have been captured occasionally at the site (Adams 2015).

Methods

Data collection

We recorded bats emerging from Emory Cave in 2008–2023, primarily in early July when colony size should be the largest due to the availability of agave. However, some additional censuses were conducted in May, June, and August. We used 4 models of thermal-infrared cameras (FLIR Systems, Boston, MA) over the span of 16 years. Frame rates and resolution differed among models. The P65 (used during 2008–2012) had a resolution of 640 by 480 pixels and frame rate of 60 Hz, whereas the SC660 (used 2013–2015) and the T650SC (used 2016) had resolutions of 640 by 480 pixels, but a frame rate of 30 Hz; the E60 (used 2017–2023) had a resolution of 320 by 240 pixels and a frame rate of 30 Hz. We used a 45° wide-angle lens in most years and recorded emergences directly to a laptop computer instead of an internal memory card.

The thermal camera was mounted on a tripod just inside the outer room of the cave, such that the inside of the cave was to the right and the stream of bats emerging was perpendicular to the camera (Fig. 1). The camera was positioned so that the field of view included both the roof and the floor of the cave, so as to count all bats that moved through this outer room and exited the cave (Fig. 1). With this setup, the stream of bats emerging was approximately 3.5–4 m from the camera. The camera was controlled by ResearchIR Max software (FLIR Systems). We began recording the emergence between 10 min before sunset and 10 min after sunset. Computer files were transferred to an external hard drive for storage and later analyses. We obtained environmental conditions for each emergence from a weather station (CSBT2) 2.7 km from the cave, using mesonet.agron.iastate.edu/sites/ or mesowest.utah.edu/.

Data analysis—manual census

A recording of the emergence was analyzed manually using ResearchIR Max. We tallied the number of bats exiting and entering the cave per minute by watching a slow replay of the emergence video. The colorized images corresponded to temperature values in different parts of their body (Fig. 1). The body temperature of *L. nivalis* was highest in the core of its body and the massive musculature along the humeri, resulting in a glowing "T" shaped or diamond-shaped thermal image (Ammerman et al. 2009). Because the other species in Emory Cave produced a streamlined bullet shape, and because of the large size of *L. nivalis*,

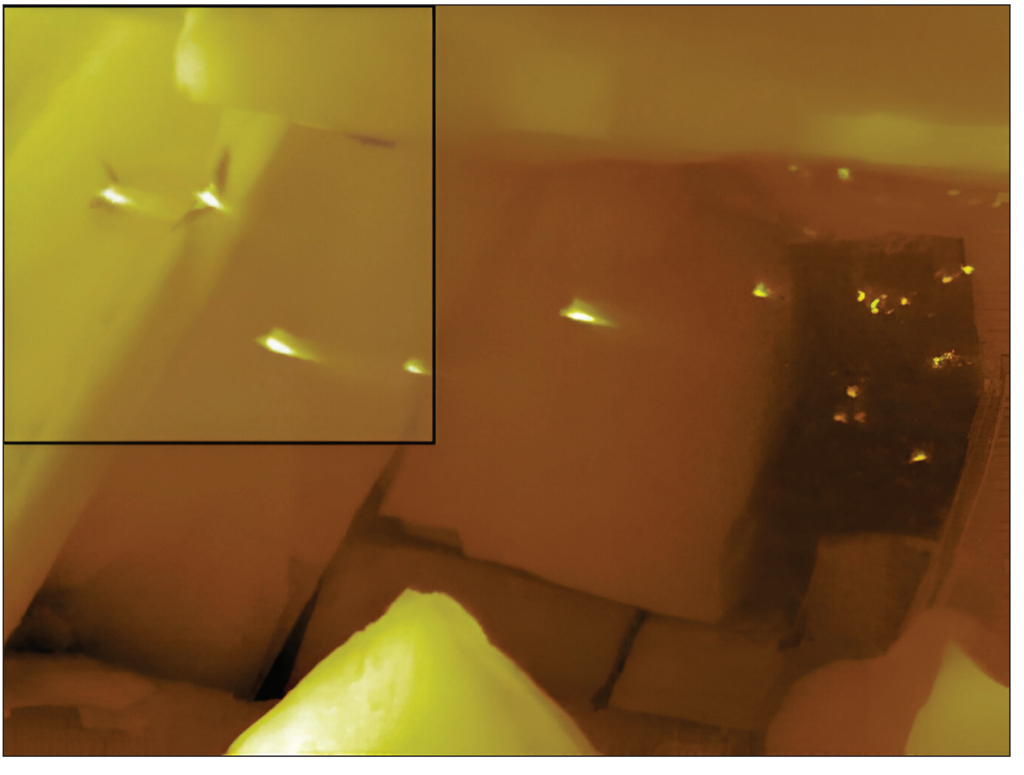

Figure 1. Edited screenshot image of field of view of camera (FLIR E60) used to record emerging *L. nivalis* from Emory Cave, with an outline of a box superimposed on the area used by ThruTracker for automated counts. The camera was supported by a tripod approximately 4 m from the perpendicular stream of emerging bats. The external opening to the cave is on the left; the inside of the cave is on the right, through the dark rectangular opening. Light colors represent warm temperatures, whereas dark colors are cooler temperatures. Note the light areas on the forearms of *L. nivalis* that can be used to distinguish this species from smaller vespertilionids.

this species could be discerned from the others (primarily *C. townsendii* and *M. thysanodes*) and counted separately. For the census, bats that were not *L. nivalis* were categorized as vespertilionids. This tally produced a conservative census of *L. nivalis*, because undoubtedly some bats were not in an ideal position or passed too close to the lens and could not be positively identified as *L. nivalis*.

Bats had to leave the screen view on the edge farthest from the interior of the cave to be counted as an "exit". Because some bats circled at the entrance, the net number of bats that emerged each minute was calculated by subtracting the number that entered the cave from the number that exited. These data were used both to generate an emergence profile (net number emerging/min) and to determine colony size for each night of recording by plotting a cumulative curve. We used the highest bat count as the size of the colony for each recording. A linear regression in Excel was used to evaluate population trends over time. To compare cumulative curves across all 16 years, we compiled yearly emergence data into a series of cumulative emergence plots, using the ggplot2 (v3.4.2; Wickham 2016) and grid (v4.2.1; R Core Team 2024) packages in R Studio (v4.2.1; R Core Team 2024). A subset of 6 files was analyzed twice by 2 different people, using manual methods. All files of the emergences are available from L. Ammerman, Angelo State University. Emergence count data are publicly available in the North American Bat Monitoring Program database (NABat, sciencebase.usgs.gov/nabat/#/data/inventory).

Data analysis—automated census

To determine the efficacy of ThruTracker, we converted all .seq files generated by the FLIR cameras to .wmv files with ResearchIR Max. We analyzed the resulting video files in ThruTracker (v2.0.3 and v2.0.5, Corcoran et al. 2021) using the following parameters: min object pixels, 20; max object pixels, 5e+04; sensitivity, 10; background frames, 200; image smoothing, 5; max tracks, 20; min track length, 5; max gap length, 5; and match threshold, 30. We established these parameters by running 2–5-sec long clips (<200 frames) from emergence recordings through ThruTracker and adjusting values in the options menu, until the correct number of bats was counted. The same parameters were applied to every full-length video.

To classify tracks as either exits or re-entries, we used the Track Counter option to draw a rectangle around the area in which bats were emerging. To ensure sufficient contrast between the warm bodies of bats and the background, we extended the boundaries of the selected area just beyond the warmer rocks near the exit area. Once the exit area was selected, we used the pop-up menu to select boundaries for counting. The Track Counter tool assumes that an exiting bat will be moving out of the selected area and an entering bat will be moving into the selected area (Fig. 1). However, because our selected area is along the left edge of the frame, the program was only able to count bats entering and exiting the selected area from the right side. In this case, a bat entering the selected area was exiting the cave, and a bat exiting the selected area was re-entering the cave (Fig. 1). Typically, one would get a count for total bat exits by subtracting the re-entries from exits, but due to this reversed directionality into and out of the selected area, we obtained our counts by subtracting the exits from the re-entries. Because a user might draw the rectangle slightly differently from another user when analyzing each video file, this process was completed by 2 individuals (1 used ThruTracker v2.0.3 and 1 used v2.0.5), and the mean was taken to represent the automated count value for each census. We determined congruence of results between individual operators using Spearman's correlation (Hollander and Wolfe 1973) in R.

Although we were initially interested in optimizing settings for counting only *L. nivalis* leaving the cave, we found that there were too many factors affecting the relative size and appearance of bats in our thermal recordings and that the software could not differentiate between *L. nivalis* and other species. Therefore, the exit counts produced by the automated method were compared for relative accuracy against the total number of bats of any species recorded emerging from the cave. Percent accuracy was calculated by [(observed value - true value)/true value] x100 and subtracting this absolute value from 100. Manual counts were considered the true colony size for each video. A paired *t*-test was conducted to determine if census videos were significantly overcounted or undercounted by ThruTracker. A linear regression in Excel was performed to investigate the relationship between manual counts and the mean of the 2 automated trials conducted by different operators. Further, we ran a test for association between paired samples in R software (v4.2.1; R Core Team 2024) using the Spearman method (Hollander and Wolfe 1973).

Results

Manual census of all bats

Between 2008 and 2023, we collected 35 videos of emerging bats at Emory Cave. Most videos (n = 27) were collected in early July of each year, but others were from May, June, and August. Duration of the video files was 46–196 min (mean: 107 min). These 35 files were used to test performance of ThruTracker, but a subset of 14 files was selected to analyze annual changes in colony size of *L. nivalis*. These 14 files were selected because they represented the highest colony count in July of each year.

The total number of bats (*L. nivalis* and vespertilionids combined) emerging from Emory Cave, based on the manual census, ranged from 103 bats in May 2018 to 3586 bats in July 2009 (Fig. 2). The maximum number of vespertilionids using the cave was 591 in 2008, and the minimum number was 39 in 2011. Since 2011, the number of vespertilionids typically has remained below 220 bats (Table 1). Of the 6 video files that were analyzed twice using manual methods, the difference in number of bats counted ranged from 1 to 8 bats (mean = 4.5 bats) and was never more than 0.9% of the total count. The smallest percent difference in manual counts was 0.07% (2715 versus 2717 bats) and the highest percent difference was 0.9% (102 versus 103 bats). In some years (2017 and 2018) there were multiple censuses conducted from May to August (Fig. 2); in those years, the seasonal increase and decrease in colony size was evident due to the arrival and eventual departure of migratory *L. nivalis*. In years with more than 1 census in July, the total census counts were similar. Five censuses (3 July 2009, 3 July 2012, 3 July 2016, 9 July 2022, and 9 July 2023) were not complete because of battery failures and, therefore, were not representative of total colony size (Fig. 2) on those dates. However, these videos were included for testing the automated method.

Manual census for *L. nivalis*

Manual counts over all months and years indicated that the lowest number of *L. nivalis* that emerged was 5 bats in May 2018 (generally before the arrival of *L. nivalis*). In July, when colony size was expected to be highest, number of bats varied from as low as 1151 to as high as 3360 (Table 2; Fig. 2), except 1 anomalous year (only 294 *L. nivalis* in 2008). The following results were based only on the single most complete census from each of the 14 years in July (no census was conducted in 2019 or 2020). The video from nights that were used for evaluating population trends were collected under various environmental conditions and are summarized in Table 2.

We defined the beginning of an emergence as the time when 10 cumulative bats had left the cave. Emergence of *L. nivalis* began a mean of 30 min after sunset (range = 16–41 min)

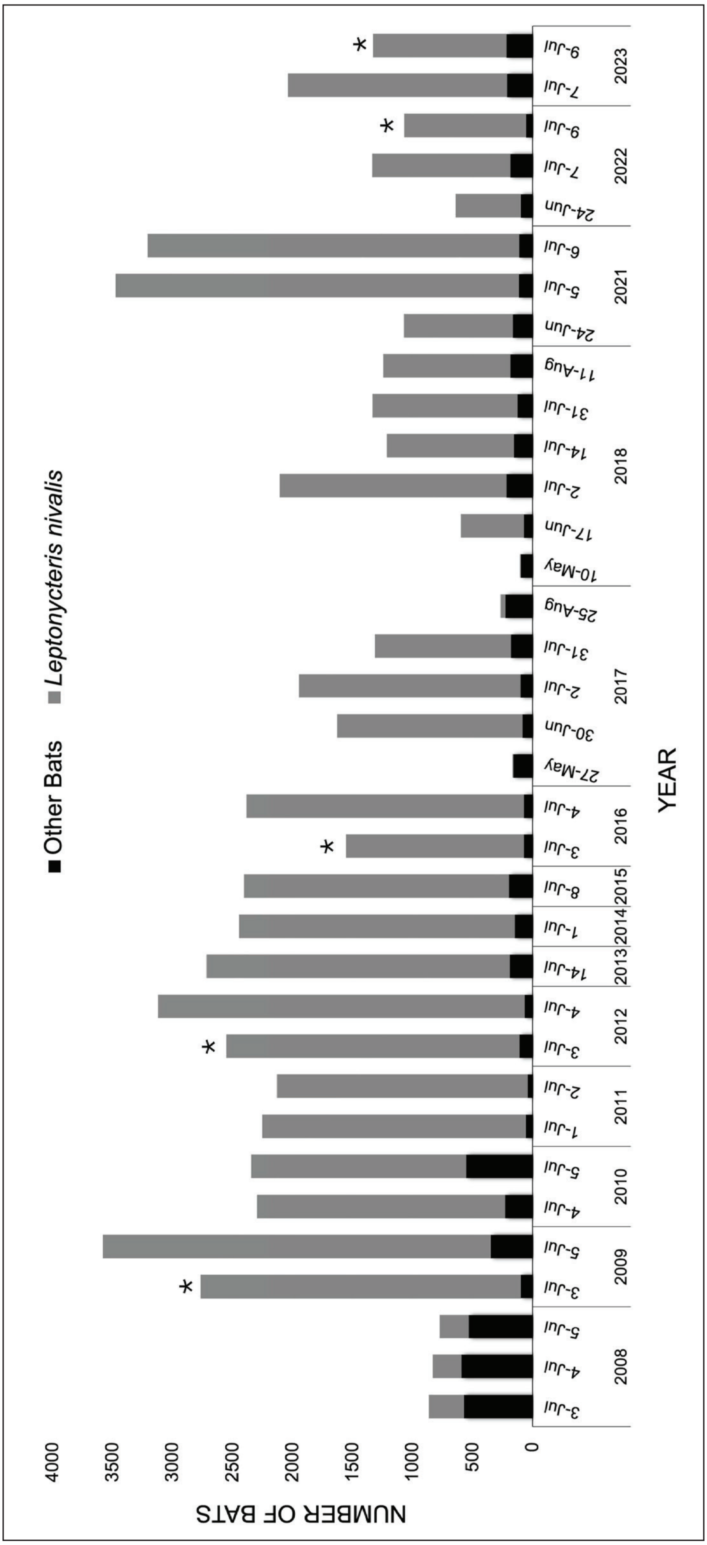

Figure 2. Manual census results for *L. nivalis* (gray) and other species (black) that use Emory Cave. Note fluctuation among species within the same census year and throughout the span of this study. Five census nights (asterisks) were shortened and not representative of total colony size, but were used to test ThruTracker performance. Census events were extended from May to August in 2017 and 2018, to gauge seasonal cave occupancy. The census was not conducted in 2019 or 2020.

Table 1. Summary of 35 videos used for analysis of population trends of *L. nivalis* (LENI) and to evaluate performance of automated census counts using ThruTracker (Corcoran et al. 2021). Cameras used were all from FLIR. TT= ThruTracker. Asterisks (*) indicate short, partial censuses on these dates (due to battery failure, camera problem, or late start time) that were used for testing ThruTracker, but not for population trend analysis.

Year	Date	Camera model	LENI	Other bats	Total bats	% LENI	TT count test 1	Test 1 % accuracy	TT count test 2	Test 2 % accuracy	Mean ThruTracker count (test 1 and 2)	Mean% accuracy
2008	3 July	P65	294	572	866	34	828	95.6	825	95.3	826.5	95.4
2008	4 July	P65	240	591	831	29	825	99.3	801	96.4	813.0	97.8
2008	5 July	P65	244	532	776	31	794	97.7	773	99.6	783.5	98.6
2009	*3 July	P65	2669	98	2767	96	2699	97.5	2448	88.5	2573.5	93.0
2009	5 July	P65	3238	348	3586	90	2663	74.3	2697	75.2	2680.0	74.7
2010	4 July	P65	2066	230	2296	90	2004	87.3	1926	83.9	1965.0	85.6
2010	5 July	P65	1790	554	2344	76	2575	90.1	2683	85.5	2629.0	87.8
2011	1 July	P65	2197	57	2254	97	2115	93.8	2064	91.6	2089.5	92.7
2011	2 July	P65	2091	39	2130	98	2024	95.0	2032	95.4	2028.0	95.2
2012	*3 July	P65	2443	108	2551	96	1975	77.4	1976	77.5	1975.5	77.4
2012	4 July	P65	2979	132	3111	96	2663	85.6	2705	86.9	2684.0	86.3
2013	14 July	SC660	2525	192	2717	93	2742	99.1	2919	92.6	2830.5	95.8
2014	1 July	SC660	2297	149	2446	94	2456	99.6	2407	98.4	2431.5	99.0
2015	8 July	SC660	2210	195	2405	92	2344	97.5	2286	95.1	2315.0	96.3
2016	*3 July	T650sc	1483	72	1555	95	1534	98.6	1521	97.8	1527.5	98.2
2016	4 July	T650sc	2309	74	2383	97	2252	94.5	2297	96.4	2274.5	95.4
2017	27 May	E60	6	160	166	4	262	42.2	190	85.5	226.0	63.9
2017	30 June	E60	1543	85	1628	95	1521	93.4	1759	92.0	1640.0	92.7

Table 1, continued. Summary of 35 videos used for analysis of population trends of *L. nivalis* (LENI) and to evaluate performance of automated census counts using ThruTracker (Corcoran et al. 2021). Cameras used were all from FLIR. TT= ThruTracker. Asterisks (*) indicate short, partial censuses on these dates (due to battery failure, camera problem, or late start time) that were used for testing ThruTracker, but not for population trend analysis.

Year	Date	Camera model	LENI	Other bats	Total bats	% LENI	TT count test 1	Test 1 % accuracy	TT count test 2	Test 2 % accuracy	Mean ThruTracker count (test 1 and 2)	Mean% accuracy
2017	2 July	E60	1844	101	1945	95	2184	87.7	2414	75.9	2299.0	81.8
2017	30 July	E60	1132	180	1312	86	1247	95.0	1238	94.4	1242.5	94.7
2017	25 Aug	E60	41	226	267	15	245	91.8	260	97.4	252.5	94.6
2018	10 May	E60	5	98	103	5	119	84.5	106	97.1	112.5	90.8
2018	17 June	E60	524	72	596	88	621	95.8	611	97.5	616.0	96.6
2018	2 July	E60	1889	219	2108	90	2045	97.0	1955	92.7	2000.0	94.9
2018	14 July	E60	1059	155	1214	87	1192	98.2	1267	95.6	1229.5	96.9
2018	31 July	E60	1207	126	1333	91	1378	96.6	1307	98.0	1342.5	97.3
2018	11 Aug	E60	1061	184	1245	85	1106	88.8	1126	90.4	1116.0	89.6
2021	24 June	E60	909	162	1071	85	1056	98.6	1154	92.3	1105.0	95.4
2021	5 July	E60	3360	113	3473	97	3033	87.3	3094	89.1	3063.5	88.2
2021	6 July	E60	3095	110	3205	97	2968	92.6	3032	94.6	3000.0	93.6
2022	24 June	E60	544	98	642	85	542	84.4	523	81.5	532.5	82.9
2022	7 July	E60	1151	185	1336	86	1293	96.8	1282	96.0	1287.5	96.4
2022	*9 July	E60	1016	54	1070	95	909	85.0	898	83.9	903.5	84.4
2023	7 July	E60	1827	212	2039	90	1903	93.3	2051	99.4	1977.0	96.4
2023	*9 July	E60	1111	219	1330	84	1033	77.7	1043	78.4	1038.0	78.0
				Mean	90.6		91.1		90.8			

Table 2. Environmental conditions in Big Bend National Park, during July censuses of bats emerging from Emory Cave. Data from CSBT2 weather station in Chisos Basin, Texas.

Year	Date	Total number of bats	Number of Mexican Long-nosed Bats	Moon phase	Notable conditions	TA (°C)	Precipitation in previous 24 h (cm)	Wind speed (km/h)	Wind gust (km/h)
2008	3 July	866	294	new moon	-	20.0	0.33	5.6	15.3
2009	5 July	3586	3238	waxing gibbous	cave never fully empty	23.3	0	10.5	23.3
2010	4 July	2296	2066	waxing crescent	possibly affected by Hurricane Alex	22.2	0	8.8	24.9
2011	1 July	2252	2195	new moon	extreme drought conditions	23.3	0	16.1	34.6
2012	4 July	3111	2979	waning gibbous	-	22.2	0	22.5	38.6
2013	14 July	2715	2525	waxing crescent	-	28.3	0	4.8	19.3
2014	1 July	2446	2297	waxing crescent	rained early in day, water in cave	26.1	0.20	12.9	20.9
2015	8 July	2405	2210	third quarter	-	22.2	0.05	12.9	27.4
2016	4 July	2383	2309	new moon	-	29.4	0.03	14.5	25.7
2017	2 July	1945	1844	waxing gibbous	cave never fully empty	26.1	0.03	6.4	17.7
2018	2 July	2108	1889	waning gibbous	condensation on camera	23.9	0	3.2	24.1
2021	5 July	3473	3360	waning crescent	-	20.0	1.65	6.4	14.5
2022	7 July	1336	1151	waxing gibbous	-	29.4	0	4.8	20.9
2023	7 July	2039	1827	waning gibbous	-	27.8	0	8.0	19.3

and peaked a mean of 48 min after sunset (30–58 min). The highest emergence rate was 146 bats/min in 2011, and the lowest was 25 bats/min in 2008 (mean = 100 bats/min). The emergence profile varied from year to year (Fig. 3) but typically bats finished emerging from the cave within 2 h after sunset (Fig. 4). Some emergences were early and fast (2011), and others, such as 2017, were late in the night with bats emerging slowly (Fig. 4). In addition, in some years, bats that appeared very hot on thermal video began returning to the cave before the emergence was complete (e.g., 2009), resulting in a downward trend in some cumulative curves (Fig. 4).

Population trends for *L. nivalis*

Manual census data were necessary to evaluate population trends for endangered *L. nivalis* because the automated approach could not distinguish among species. Analysis of the maximum colony size for each year (14 censuses over 16 years) showed large fluctuations despite being conducted in the first week of July each year (Fig. 5). Although maximum colony size fluctuated from year to year, there was no significant positive or negative population trend (Fig. 5). Colony size was >3000 bats in 2 years (2009 and 2021; Table 1, Fig. 5). Mean colony size over 14 census years was 2156 (*SD* = 796) *L. nivalis*.

Census results using automated approach

Manual and automated counts were highly correlated (Fig. 6; $r_s = 0.97$, $P < 0.001$). Overall accuracy was 90.8% (Table 1), with the lowest mean accuracy of 63.9% and the highest of 99%. Twenty-four of 35 videos (69%) resulted in lower counts using the automated approach, while only 11 of 35 videos (31%) had higher automated counts (Table 1). We rejected the null hypothesis of no difference between the automated and manual counts,

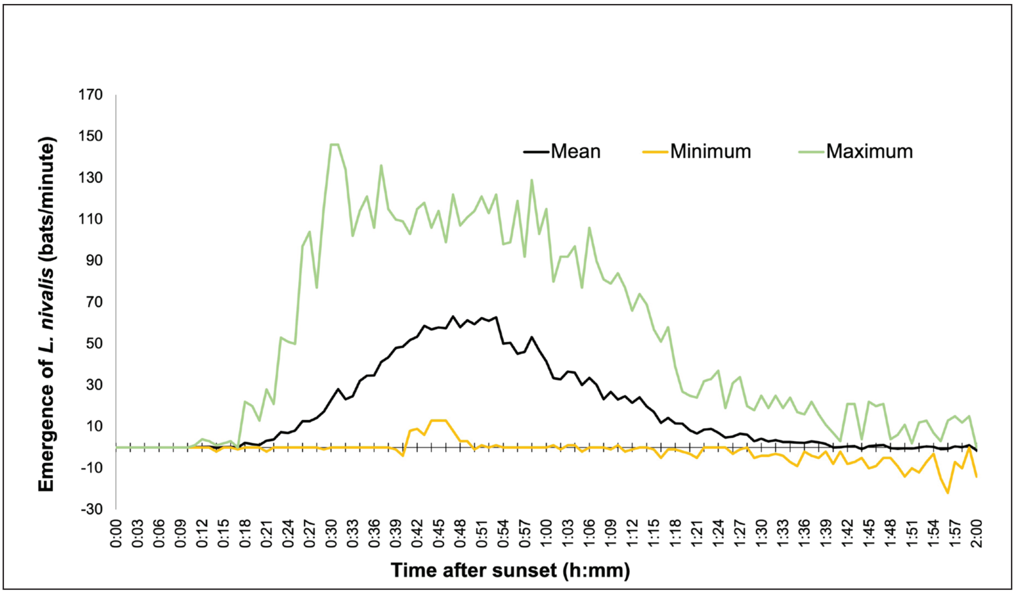

Figure 3. Emergence rate profile for *L. nivalis* leaving Emory Cave, calculated from manual census results from a single survey per year in early July (n = 14). The mean emergence rate per minute after sunset (black) is compared to the minimum (yellow) and maximum (green) emergence rates that were observed during annual census days. Negative values reflect times of night when more bats were entering the cave than were leaving.

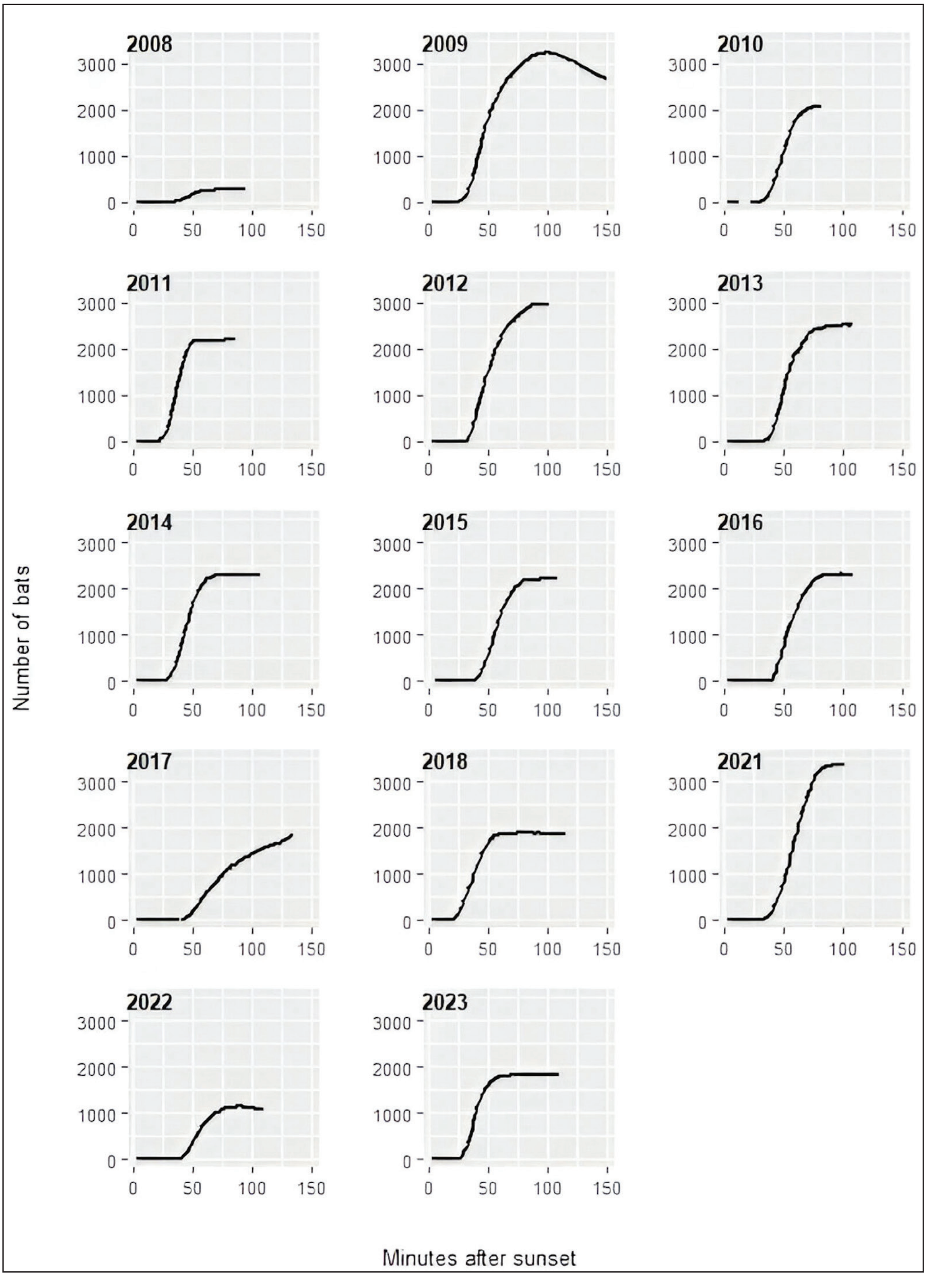

Figure 4. Cumulative curve of net number of *L. nivalis* that emerged over time (min after sunset) from manual censuses of Emory Cave each year in July. The curve shows a decrease (e.g., in 2009) when more bats began entering the cave instead of exiting.

showing that the automated approach significantly lowered the number of bats counted in the videos overall (t_{34} = -2.75, P = 0.005). Apparently, more variation occurred between the 2 methods (Fig. 6) when comparisons involved higher numbers of bats (>2500).

The 2 automated tests were highly correlated (r_s = 0.98, P < 0.001), suggesting that user differences had little effect on the automated counts. The mean accuracy of automated counts for both users was similar (User 1 = 90.6%, User 2 = 91.1%; Fig. 7, Table 1). Typically, the time spent actively using ThruTracker, excluding waiting time for video conversion and ThruTracker processing, was about 10 min per recording. The time for video conversion and ThruTracker processing was 4–6 h combined; however, these processes did not involve operator effort, only computer processing time. This represented a substantial time savings compared to manually counting census videos, which could require 8–10 h of labor to process a recording lasting 2–3 h.

Discussion

Our study showed that automated methods, such as ThruTracker, can generate results that are consistent with manual methods, while also saving time. The process of converting files and counting bats with the software did not require constant attention by the user, and thus, person-hours were saved compared to manual methods. We estimate that the person-hours involved for manual methods were 48–60 times longer than the person-hours required for automated methods. Due to the time savings, researchers are increasingly turning to automated methods to monitor colonies of bats. For example, Koger et al. (2023) recently used 10 cameras and developed a computer-vision pipeline to census a large colony (>700,000) of *Eidolon helvum* (Kerr) (Straw-colored Fruit Bat) in Zambia with a complex emergence pattern.

The Recovery Implementation Strategy (USFWS 2024b) for *L. nivalis* states the need for a standardized monitoring program for all roosts and foraging grounds, and our work describes progress toward addressing that recovery action. Overall, we found ThruTracker

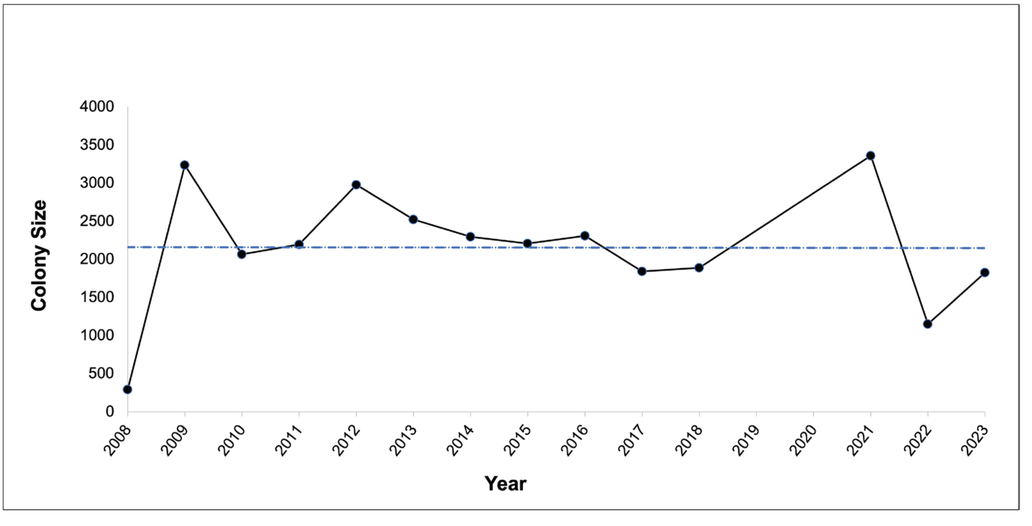

Figure 5. Population trend for *L. nivalis* at Emory Cave from 2008 to 2023. The official annual census count was obtained from the census day with the highest manual count for early July in that year. Additional data for each census are in Table 2. Blue dashed line is regression line with r^2 <0.001.

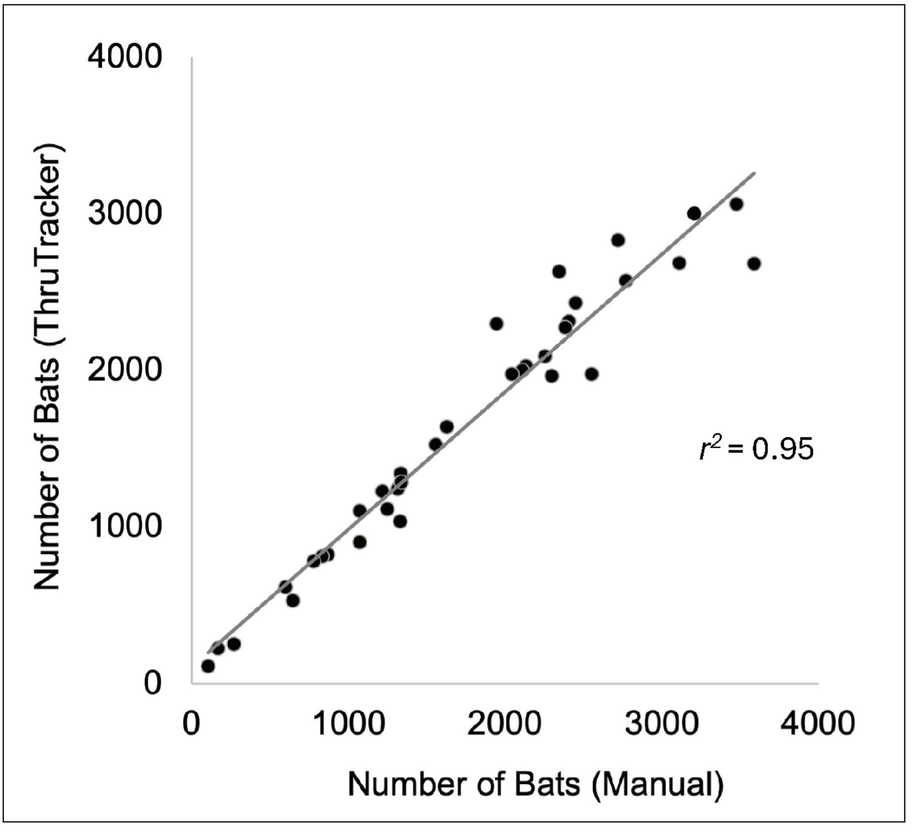

Figure 6. Manual census counts for all species emerging at Emory Cave (2008–2023), compared to the automated census (mean from 2 users of ThruTracker).

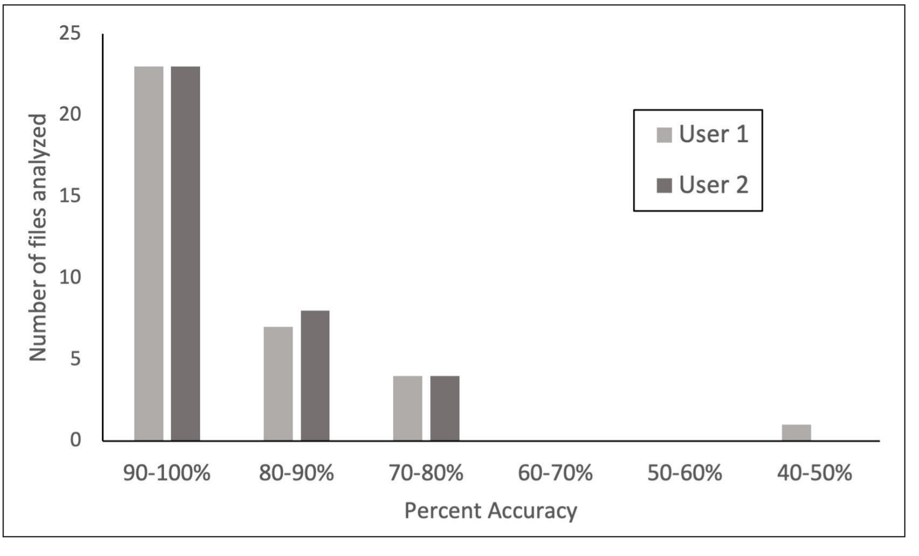

Figure 7. Accuracy of automated counts using ThruTracker, compared to manual counts of 2 different users.

to be 90.8% accurate, on average, although accuracy ranged from 63.9 to 99% (Table 1), and the software was more likely to undercount than overcount the number of emerging bats compared to manual methods. Still, our results showed a high correlation between manual and automated methods, with most tests having >90% accuracy (Fig. 7).

There was 1 census recording with a much lower accuracy than the other recordings (mean of 63.9%) which was also an overcount compared to the manual census. It is not clear to us why the automated method performed so poorly on this night in May. There are very few *L. nivalis* present in May, so most bats emerging were small vespertilionids that might not have been as easy for ThruTracker to detect. Perhaps their body temperature was similar to the rock walls and this lack of contrast was a challenge for the automated method, and returning bats were missed or the bats that circled near the entrance were overcounted. Another explanation for the discrepancy could be individual user decisions about defining the count area.

In tests of a similar tracking program, BatCount, Bentley et al. (2023) also found a range of accuracy when applied to real-world situations. In their tests with multiple camera types and different species, the accuracy compared to manual counts ranged from 50% to 94.8%. In agreement with our results (Fig. 6), they also found their recordings with high densities of bats (>800 bats per 30-sec segment) led to lower accuracy, although their emergence rates were generally higher than we observed for *L. nivalis* (mean emergence rate of 100 bats/min). Similar to our study, they found that the software regularly underestimated counts (Bentley et al. 2023). We found strong correlations between automated and manual counts at Emory Cave, perhaps because conditions were ideal for testing performance of the automated method (uncluttered background, good thermal contrast, restricted emergence area, relatively slow emergence rate). Clearly, these conditions will not occur at all roosts, and this method might not be suitable for all applications; however, the technique is worth further investigation.

There were some limitations of the automated method. The most likely explanation for the occasional inconsistency in results between different users is the way the count area was delineated. To be able to make accurate comparisons and monitor population changes across time at a single site, the count area should be standardized among users. However, the results generated by 2 users largely agreed, which suggests that other issues we identified as possible challenges for the software were playing a larger role.

The exact reason for disagreement between manual and automated counts will require further research. Most likely the challenges in automated tracking of bats occur during high emergence rates (Fig. 3), due to occlusion of bodies, or possibly faint thermal profiles of small-sized bats that might be missed by the software. Emergence rates varied greatly across years (Fig. 3), and understanding and anticipating factors that affect when bats will emerge at a high rate is challenging. During manual counts, the entire field of view can be used to determine if what appears as 1 bat in an image is actually more than 1 by watching the flight pattern. Automated methods are limited in this situation because they track a moving image and cannot interpret wing flaps of 2 or more bats that are superimposed.

In addition, we noticed an unusual emergence pattern in 2009, when the bats started returning before emergence was complete (Fig. 4). As a result, the cumulative curve did not plateau as in other census nights (Fig. 4). Because of this pattern, the automated count, which used the entire video, was lower than the manual count. We used the maximum number of bats as the manual count. Therefore, on this night (5 July 2009) the low accuracy can be explained by this discrepancy (Table 1). We suggest that this problem could be overcome by plotting cumulative counts obtained by the automated method to identify the number of bats counted at the point at which the net number of bats start re-entering rather than exiting. Furthermore, we observed skipping of frames in some videos that made tracking difficult for manual users, and we expect it was also an issue for the software. Camera issues did not seem to affect the results, because we noticed no obvious relationship between accuracy and type of camera (resolution, frame rate). We expected that focus issues occasionally caused by condensation on the lens might have contributed to low accuracy compared to manual methods, but mean accuracy for 1 such night (2 July 2018) was 95%.

Automated computer-assisted tools could be a welcome alternative to manual counts for roosts that are being monitored regularly. In our tests, the software rarely overestimated colony size and thus appeared more likely to provide a conservative count. Applying the automated approach consistently among video files could assist researchers in detecting population changes, using far less effort than manual review. As such, automation could provide increased precision on estimates, because it could be repeated over multiple nights without incurring the large costs of manual review. While evidence shows some inaccuracy in automation that still must be considered, in certain cases it could be worthwhile to trade a single more accurate count that requires several hours of labor with several automated counts repeated over time.

Our testing showed that ThruTracker can be an effective method for estimating overall population size and for detecting changes in populations over time, but that some situations will continue to benefit from manual counts, especially when distinguishing among species is important. If the proportion of *L. nivalis* compared to vespertilionids was similar each year at the time of the census, then perhaps the colony size could be extracted from automated census data. It is clear, though, that the percentage of all bats that were *L. nivalis* at Emory Cave each year in July is not consistent (Table 1) and ranges from 86 to 98%; therefore, we are unlikely to be able to rely solely on the automated method at this site in the future. However, calculating the number of *L. nivalis* from a proportion of the automated count of all bat species might be a useful method for obtaining a rough census or a range to assess general trends. This approach could be risky because the proportion of *L. nivalis* roosting in Emory Cave in 2008 was much lower (ca. 30%) and the automated census approach would not have provided accurate data if we had assumed that *L. nivalis* made up 86–98% of all bats that year. Further, in 2011 during a severe drought, the number of vespertilionids at Emory Cave decreased substantially, leading to a higher proportion of *L. nivalis* than would have been detected without using manual approaches. We propose that the automated approach will be most useful at sites with a single bat species or at sites with stable communities.

Our analysis suggests that the population of *L. nivalis* using Emory Cave has not declined significantly in the past 16 years, although fluctuations occurred. Accurately measuring population size for a highly mobile, migratory species, such as *L. nivalis*, presents considerable challenges. Before the use of thermal imaging to perform yearly censuses, estimates of population size for Emory Cave varied widely. From 1967 to 1970, Easterla (1972) conducted yearly surface area counts, assuming 150 bats per square foot. Initially, Easterla (1972) estimated 10,650 *L. nivalis*, but by the following year, the estimate dropped to 5000. By 1970, Easterla (1972) found no *L. nivalis* present in Emory Cave, concluding that the population was declining severely. However, the accuracy of these estimates was affected by several factors unknown to Easterla, most notably the timing of his censuses; *L. nivalis* is now known to occupy Emory Cave seasonally, with most arriving in mid-June and leaving in late August (Adams 2015, Ammerman et al. 2009). Easterla (1972) performed his first census in early July, producing a very high population estimate, but in subsequent years, his census occurred in late May or June, likely before these bats had migrated from Mexico (Ammerman et al. 2009). It also is possible that, if bats were present, they were roosting in a deeper, inaccessible part of the cave at the time of his visit (Ammerman et al. 2009). Past fluctuations were considered a northern "spillover" of bats from Mexico by Easterla (1972). Our thermal-imaging data from Emory Cave alone cannot address his spillover hypothesis, but our results do show less annual fluctuation in colony sizes than reported in earlier efforts to monitor the site (Easterla 1972; R. Skiles, Big Bend National Park, TX, unpubl. data). Future work is needed to identify variables that best explain the fluctuations in colony size and how that relates to the range-wide population status for *L. nivalis*.

Pourshoushtari and Ammerman (2020) used microsatellite analysis to determine the effective population size (N_e) of *L. nivalis* at Emory Cave. Their analysis generated 2 estimates for N_e that included different sets of alleles; 1 method produced an N_e of 5723 individuals, while the other produced an N_e of 570 individuals. Because N_e is generally lower than the actual number of individuals in a population, the mean colony size measured in the current study (2156 bats) was within this range, but low. We expect the colony size at Emory Cave to be lower than the total population size based on genetic estimates because of the migratory habits of this species, and because the bats at this site are only a portion of the total range-wide population. For example, adult male *L. nivalis* do not migrate as far north as Emory Cave (Adams 2015, Pourshoushtari 2019, USFWS 2018).

Emory Cave represents an essential site in the northern extent of the range of *L. nivalis* (USFWS 2024a). Increases or decreases observed there could indicate population-wide trends toward recovery or further decline, or alternatively, might reflect changes in habitat/roost use. No other nearby roosts have been documented within nightly commuting distance of the agave resources of the Chisos Mountains. Migration of *L. nivalis* likely corresponds with availability of flowering *Agave*, especially in the northern extent of the range (Burke et al. 2019, Gómez-Ruiz and Lacher 2016), where *Agave* are at high risk of decline due to climate change (Gómez-Ruiz and Lacher 2019, USFWS 2024b).

The revised recovery plan for *L. nivalis* (USFWS 2024a) includes updated recovery criteria for monitoring the populations at known roosts in the U.S. and Mexico. These criteria establish a threshold to determine when the range-wide population of *L. nivalis* has grown to meet downlisting or delisting changes in conservation status. Before automated methods can be applied range-wide for *L. nivalis* and be considered valid substitutes for manual methods, more work must be done. Roost types used by *L. nivalis* vary, and the bat species that share their roost differ from those in Emory Cave (USFWS 2018). These factors present challenges, but future advances in imaging technology, artificial intelligence, machine learning, and software developments will inevitably result in systematic automated wildlife tracking that is both accurate and time efficient.

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