A Novel Approach to Assessing Abundance and Behavior in Summer Populations of Little Brown Myotis in Yellowstone National Park

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Austin G. Waag

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This thesis titled
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by

AUSTIN G. WAAG

has been approved for
the Department of Biological Sciences
and the College of Arts and Sciences by

Joseph S. Johnson
Assistant Professor of Biological Sciences

Joseph Shields
Interim Dean, College of Arts and Sciences
ABSTRACT

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A Novel Approach to Assessing Abundance and Behavior in Summer Populations of Little Brown Myotis in Yellowstone National Park

Director of Thesis: Joseph S. Johnson

Currently, there is a dearth of information regarding the status and ecology of bat populations in the Rocky Mountain region of North America due to the scarcity of known hibernacula, which are the primary location for performing population counts in eastern North America. The lack of knowledge and traditional tools required to monitor these populations presents barriers for biologists and land managers tasked with conserving bat species believed to be at risk of extinction or extirpation. Unfortunately, there are no estimates of abundance available for many populations of bats and the absence of locations to monitor populations leaves biologists in many regions without the techniques required to create such estimates. To provide much-needed population ecology data, we designed and built a long-term passive monitoring system capable of continuously monitoring the abundance of little brown myotis (*Myotis lucifugus*) residing in distinct populations within Yellowstone National Park. To advance our understanding of bat ecology for this region, our monitoring system also assessed roost fidelity, connectivity, and seasonal movements. We subcutaneously implanted high-frequency passive integrated transponders (HF-PIT) into 297 female little brown myotis and installed 8 continuously scanning readers and 45 antennas inside 3 maternity roosts used by distinct populations in the Lamar Valley, Tower Junction, and Mammoth Hot Springs regions of
Yellowstone National Park. We recorded 2,929,742 detections of 196 HF-PIT tagged bats (66.0%) between June 2017 and August 2018. We used a mark-resight analysis to quantify abundance based on detections of individually marked bats recorded by high-frequency radio-frequency identification (RFID) readers and counts of bats exiting monitored roosts. From these mark-resight analyses, we estimated the pre-parturition size of the Mammoth population to be 847 (95% CI = 749-987; SE 59.8) in 2017, and 836 (95% CI = 722-989; SE 67.3) in 2018. The Lamar Valley population was estimated to consist of 208 (95% CI = 198-222; SE 6.2) bats in 2018. Population estimates were not possible at Tower Junction, but RFID readers showed that only a single HF-PIT tagged bat moved among these three roosts. Population estimates at Mammoth and Lamar reflect roost availability, as 258 and 25 potential roosts were available within 1 km of RFID-monitored in Mammoth and Lamar Valley, respectively. Although roost availability and populations were larger in Mammoth, roost fidelity was similar between the two sites, with bats detected in the monitored roosts on 36.5% of days in Mammoth and 31.4% of days in Lamar Valley. Combining traditional emergence counts with RFID technology and mark-resight analyses presents a novel approach to estimating bat populations during summer, which has long vexed researchers. While especially useful for regions lacking caves, and thus any traditional method to census populations, these techniques provide a vital opportunity to advance knowledge of bat ecology nationwide during a season rarely studied.
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This research showcases collaborations from many incredible people who spent countless hours of work in the field, lab, and office to make this research a success.

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All animal capture and handling procedures were approved by the Institutional Animal Care and Use Committee of Ohio University (16-H-023).
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CHAPTER 1: INTRODUCTION

The purpose of this introductory chapter is to describe and link our efforts to advance methodology currently used to monitor wildlife populations (Chapter 2) and detail our creation of novel techniques and analyses used to perform a long-term monitoring study on little brown myotis (Myotis lucifugus) in Yellowstone National Park (Chapter 3). Chapter 2, “Anti-collision technology improves data collected from studies using PIT tags” is written as a stand-alone short communication intended for submission to the journal Mammal Review. It describes the innovative technology that I created to study population dynamics of bats in Yellowstone, which was previously beyond our capabilities. Chapter 3, “Mark-resight analyses provide precise estimates of Myotis lucifugus populations during summer” is written as a stand-alone publication for the Journal of Animal Ecology. It showcases how we estimated abundance, roost fidelity, roost connectivity, and seasonal movements for three distinct populations of little brown myotis during 2017 and 2018. This thesis concludes (Chapter 4) with information about how these tools can be used to enhance the monitoring of wildlife species across many taxa, and how these methods can assist in the management and conservation of their populations.

Traditionally, bat population estimates are performed at winter hibernacula, such as caves, where bats congregate in large numbers, allowing biologists to simply count the number of bats occupying the roost. Unfortunately, while this is a reliable technique in eastern North America, there are very few known hibernacula in the Rocky Mountain region of North America, precluding the use of this method (Kunz et al. 2009, Hendricks...
It is currently believed that bats in the Rocky Mountains instead overwinter in non-traditional hibernacula, such as rock crevices, trees, and buildings due to the lack of caves in the area, which is a result of the arid climate and geology of the region (Weary and Doctor 2014, Johnson et al. 2017, Neubaum 2018, Weller et al. 2018). Thus, there is currently a major need for novel methods for biologists in this area to monitor populations, in addition to enhancing their knowledge about the ecology of bats inhabiting this region.

This need for new methods to track populations of bats across time and space is all the more urgent due to the many threats that affect populations of bats today, such as climate change (Adams and Hayes 2008, Adams 2010), habitat loss (Willig et al. 2007), and an emerging fungal disease known as white-nose syndrome (WNS) (Frick et al. 2010a). WNS, which is caused by the cold-loving fungus (*Pseudogymnoascus destructans*), was first discovered in New York State during the winter of 2006 (Blehert et al. 2009). Most alarmingly for bats residing in Yellowstone, the fungus was recently detected during the winter of 2017-2018 in southeast Wyoming, 497 km from the eastern boundary of Yellowstone. Since 2006, WNS has been associated with the deaths of millions of bats across 11 North American species, including the little brown myotis. Little brown myotis in particular have declined by an average of 91% in areas that have been affected by the fungus for at least two years (Turner et al. 2011).

My thesis project was inspired by a desire to meet the challenge of providing biologists in Yellowstone with the ability to track populations, enhancing their ability to respond to any potential population declines. By pairing these population and ecology...
estimates to environmental monitoring tools, biologists will be able to formulate more comprehensive hypotheses to help determine what is driving changes in these populations. To accomplish this, I developed a long-term monitoring system that uses high-frequency radio-frequency (RFID) technology, which operates at 13.56 MHz. To my knowledge, this is the first wildlife study to deviate from using low-frequency PIT tags, which typically operates at 125-135 kHz. This decision is significant because all studies using low-frequency PIT tags are hindered by a limitation known as tag collision, where readers are unable to detect tags if more than one is present by an antenna at the same time (Shih et al. 2006, Klair et al. 2010). While rarely mentioned, all previous and currently performed PIT-tagging studies are therefore restricted to installing antennas across animal movement paths, such as roost entrances, to reduce the risk that multiple tags will be present within an antenna’s electromagnetic field at any one instance. However, by using high-frequency technology with anti-collision capabilities, we are, for the first time, able to install antennas within roosts to continually and unfailingly detect all present bats. By injecting female little brown myotis with high-frequency PIT tags, we are now able to record up to 100 bats at each antenna, up to 4 times per second.

This advancement to PIT-tagging methodology allowed us to expel traditional study design limitations, providing us the opportunity to design and investigate novel hypotheses to improve our knowledge of bat ecology in Yellowstone. Presented in Chapter 2, we show how this technology can be used to measure interactions and relationships between individual bats across the maternity season through various short-term and long-term examples and reveal the seasonal movements of bats from these
maternity colonies in RFID-monitored roosts. As described in Chapter 3, biologists in Yellowstone can now input detections of PIT-tagged bats into mark-resight analyses to measure abundance. Chapter 3 also describes how this technology can be used to understand connectivity between populations and fidelity to specific roosts, topics that are central to understanding bat habitat use (Kunz et al. 2003).

The little brown myotis is an ideal species to study using high-frequency RFID technology due to their reliable nature of forming large seasonal maternity colonies in buildings across Yellowstone, relatively long lifespans (>30 years), and high site fidelity (Davis and Hitchcock 1965, 1995, Fenton and Barclay 1980, Johnson et al. 2017). Female little brown myotis will also use and frequently switch between many roosts across the local landscape. This complex social behavior renders traditional methods of counting bats as they emerge from buildings at dusk a poor method for studying populations, since you are only observing a small subset of the entire population that uses the roost on any given night. Since performing these nightly emergence counts is typically the primary method used to monitor summer populations across North America, our novel techniques and analyses provide a vital opportunity to improve our ability to estimate abundance and increase our knowledge of bat ecology during the summer months.

As I hope the proceeding chapters will show, my thesis research finally provides biologists in the Rocky Mountain region with the ability to detect population changes in the absence of known hibernacula. As a biologist interested in protecting and managing wildlife, it is my hope these methods enhance our ability to make informed conservation
and management decisions. Finally, it is also my hope that I have shown how these methods can also be used to investigate a wide variety of questions regarding ecology and behavior across many taxa.
A problematic issue known as tag collision exists when using low-frequency (125-135 kHz) passive-integrated transponder (PIT) equipment currently available. Tag collision occurs when more than one tag is within range of PIT tag antennas and results in either one tag’s transmission overpowering all other tags with weaker transmissions, or all tag transmissions blocking each other completely. This phenomenon, although rarely discussed in the literature, limits the ability to use PIT tags to study social mammals.

Here, we provide a much-needed communication on the limitations of currently used PIT tags and readers and provide examples from our research detailing the advantages that adopting newly available anti-collision technology provides. To eliminate tag collision and therefore increase flexibility of study designs, we used high-frequency (13.56 MHz) PIT tags in combination with antennas and readers equipped with anti-collision capabilities to study little brown myotis (*Myotis lucifugus*) movements and behaviors in Yellowstone National Park. Anti-collision improves PIT tag study design by enabling up to 100 tags within the electromagnetic field of a single antenna to be unfailingly, continuously, and simultaneously recorded. This novel advancement allowed us to install antennas within bat roosts and increased the effectiveness of antennas positioned over roost entryways. Thus, using PIT tag readers with anti-collision technology creates opportunities to uncover social interactions at finer scales than previously possible and increase existing detection rates. This technology can be used to improve the data
collected for any social mammal, as tag collision is mostly likely to impact study designs in these species.

Introduction

Passive integrated transponder (PIT) tags have been used by biologists to effectively redetect and track individuals across time and space (Boarman et al. 1998). Since their initial use monitoring fish movement (Prentice et al. 1983), PIT-tags now are commonly used to study numerous aspects of mammalian ecology, including behavior, physiology, and demographics (Harper and Batzli 1993, Schooley et al. 1993, Galimberti et al. 2000, Neubaum et al. 2005). Weighing just 0.1 g, PIT tags do not contain internal batteries, but instead receive power from radio waves emitted from antennas operating on the same frequency. PIT tags enable readers to continuously record their unique identity when in close proximity to an antenna, making them ideal for passively studying animal movement. These methods have greatly improved upon previous techniques for marking and redetecting animals, such as radio/satellite telemetry and external marks (i.e. collars, bands, tags, paints, tattoos, brands), by minimizing weight and battery life requirements, tag loss, and illegibility (Roussel et al. 2000, Gibbons and Andrews 2004, Silvy et al. 2005). Nevertheless, PIT tags are relatively under-used in studies of small social mammals, such as bats, despite their value being recognized more than two decades ago (Kerth and König 1999). This likely relates to a well-known, yet often undiscussed technological limitation of PIT tag readers, known as tag-collision, for which a solution is now currently available. These advancements detail a significant improvement that can revolutionize our ability to study numerous mammalian species.
PIT tags currently used in wildlife research all fall under the umbrella of low-frequency (125-135 kHz) radio-frequency identification (RFID) technology, but it should be recognized that RFID transponders are also available in high (13.56 MHz) and ultra-high (900-915 MHz) frequencies. Current technology for reading low-frequency PIT tags is hampered by tag collision, where readers fail to detect PIT tags when >1 tag is present at an antenna at the same time (Shih et al. 2006, Klair et al. 2010). Complete tag collision occurs when all present tags equally block transmissions as they return to the reader. Partial tag collision occurs when the tag with the strongest transmission, which is most frequently the closest tag positioned in an ideal orientation, overpowers and blocks all tags with weaker transmissions. Because of tag collision, researchers have been unable to study movements and interactions of animals within large social groups. Instead, studies are restricted to positioning antennas within primary access points to important habitats, such as bat roosts and water sources, in the hopes that only a single tagged animal will quickly pass by the antenna at a time (Kerth and König 1999, Garroway and Broders 2007, Adams and Hayes 2008, O’Shea et al. 2011). Although tag collision has likely influenced the design of all PIT tag studies in mammals, it is rarely mentioned in wildlife peer-reviewed literature despite being well recognized in the field of RFID technology (but see Smyth and Nebel 2013, Waag et al. in prep.). In addition to influencing study design, tag collision also affects how data is analyzed.

Although not currently used in wildlife studies previously, high-frequency RFID readers and antennas can be manufactured with anti-collision technology. In addition, high-frequency RFID readers have increased tag read speeds and data transfer rates,
allowing readers to record up to 100 tags at a single antenna, four times per second (Klain et al. 2010). These enhancements allow for antennas to be installed in areas where animals frequently assemble to monitor social interactions and associations. They also allow antennas placed at entryways of habitats to unfailingly detect and record all PIT-tagged individuals passing within range. While potentially beneficial for all species and systems, this novel ability to detect numerous tags simultaneously is particularly vital for studying mammals that form large congregations or roost in colonies, such as bats.

To communicate the benefits of using readers with anti-collision capabilities, we summarize the success of a high-frequency RFID monitoring system developed in Yellowstone National Park to study little brown myotis (Myotis lucifugus). Little brown myotis are an ideal species to study with PIT tags and anti-collision readers due to their propensity to form large colonies in buildings, which they show high fidelity to over their relatively long lifespans (Davis and Hitchcock 1965, Humphrey and Cope 1976, Keen and Hitchcock 1980, Barclay and Harder 2003). Here, we show the value of placing antennas on the ceiling inside roosts adjacent to roosting bats and in main entryways, by presenting short-term and long-term examples of our data. We use these examples to provide recommendations to identify study designs that will benefit from utilizing antennas inside congregation areas, on primary entryways, or a combination of techniques. We advocate for more researchers to adopt readers with anti-collision capabilities, as they provide new opportunities to study many species of mammals, enhancing the ability to research, manage, and conserve their populations.
Methods

Our study was conducted on the northern range of Yellowstone National Park, which encompasses 898,300 ha in northwestern Wyoming and adjacent sections of Montana and Idaho. We installed high-frequency radio-frequency readers and antennas inside the attics of three buildings, known from previous research to be primary summer maternity colonies of little brown myotis (Johnson et al. 2017). These roosts are located in the town of Mammoth, Wyoming (elevation of 1849 m), in the vicinity of Tower Junction (1937 m), and Lamar Valley (2000 m). From 2015 to 2018, a single 12 x 2.15 mm high-frequency PIT tag (HID Global, Granges-Paccot, Switzerland) was subcutaneously implanted into 297 female little brown myotis, captured in mist-nets (Avinet, Inc., Dryden, New York, USA) at these buildings or nearby small ponds. High-frequency antennas were constructed in the shape of 1.5 x 0.4 m and 1 x 0.2 m rectangles using a motherboard (Feig Electronic, Weilburg, Germany) and a RG6 18AWG coaxial cable. Forty-one antennas were collectively installed on the ceilings within the attics of the three buildings in areas containing high levels of bat sign, before maternity colonies formed in May (Fig. 1). Four antennas were also installed over primary roost entryways to detect bats accessing the roosts. While a vast majority of the bats using the Mammoth and Lamar Valley roosts emerge through these primary entryways, ceiling antennas help detect the presence of any bat that enters or leaves the roost through secondary entryways. Up to eight antennas were connected to a high-frequency reader (Oregon RFID, Portland, OR, USA) and programmed to continuously scan for tagged bats throughout the entire year. For each bat scanned, readers recorded the PIT tag’s unique
16-digit alphanumerical identification code, the antenna number which made the scan, and the date and time. From using high-frequency, readers and antennas were able to detect up to 100 tags per second as they moved through their electromagnetic field, at a speed of at least 50 km/hr, which is faster than the maximum flight speed of little brown myotis (Fenton and Barclay 1980).

For the purposes of this short communication, we limited our data collected from two roosts during 2018, by which time anti-collision was enabled on our readers and an adequate sample size of tagged bats had been obtained at two of our monitored roosts. To assess the utility of antennas placed on the ceiling, and therefore the utility of anti-collision, we compared the number of daily detections of unique bats recorded on ceiling antennas to those placed at entryways and assessed the percentage of bats that would have been undetected if only one of these methods were utilized. We also assessed the utility of anti-collision and ceiling antennas for studying short-term social interactions by creating network maps for bats scanned at one of our antennas at four time points over the course of a single day. Network maps were made in UCINET (Analytic Technologies, Lexington, KY, USA), which created a link between all bats detected roosting at a single antenna in the Mammoth roost. We created these network maps across three consecutive 30-minute time intervals to show how short-term social interactions of roosting bats fluctuate throughout the day. Links were sized by the number of minutes bats spent together at the antenna during the 30-minute period. Nodes were sized by the number of minutes that bats were present at the antenna during the 30-
minute period. Finally, we quantified the percent of time that 6 bats were found roosting at specific ceiling antennas within the roost to illustrate variability in roosting behaviors.

Results

Readers recorded 1,073,843 detections of 143 bats between May 10 and August 15, 2018. Entryway antennas recorded 47,350 and 63,952 of these detections at Mammoth and Lamar Valley, respectively. Ceiling antennas recorded 390,129 and 183,653 of these detections at Mammoth and Lamar Valley, respectively. Readers at Tower Junction, connected only to ceiling antennas, recorded 388,759 of these detections.

At Mammoth, ceiling antennas detected 56 unique bats during 2018, 9 (16%) of which were never detected at entryway antennas. The entryway antennas detected 51 unique bats, 4 (8%) of which were never detected by ceiling antennas. At the Lamar Valley roost, ceiling antennas detected 63 unique bats, all of which were also detected by entryway antennas. The entryway antennas detected 85 unique bats, 22 (25.9%) of which were never detected by ceiling antennas. Thus, while more total detections of bats were recorded on the ceiling antennas than the entryway antennas, more unique bats were recorded by the entryway antennas at both the Mammoth and Lamar Valley roost (Fig. 2A, 2B). Combining data from both roosts, anti-collision allowed >1 bat (range: 2-6) to be detected simultaneously at entryway antennas on 2,360 occasions (4,996 total detections; 0.47% of total detections).

Network maps created using data from a single antenna varied substantially over three 30-minute time intervals (Fig. 3A, 3B, 3C). Bats varied on their strength of
selection for sections the attic monitored by antennas (Fig. 4). Some individuals (B330, B256, and 35F2) were found to have relatively high preference to a specific area (99.6%, 98.2%, 95.6% of total detections over the maternity season were at a single antenna, respectively). Other individuals (5584, BAF2, and 9B9C) showed relatively low preference for specific areas and were frequently detected using various antennas across the roost for short periods of time.

Discussion

To date, tag collision has shaped and limited the design and analyses of PIT tag studies involving mammals. Here, we have shown that high-frequency antennas and readers, possessing anti-collision capabilities, dramatically improve the capabilities of PIT tag methodology, allowing for innovative hypotheses to be proposed and investigated. Although we have focused on bats, an order (Chiroptera) known for their complex social behaviors, as an example in our communications, it is not difficult to envision the effectiveness in using anti-collision to study other taxa. For example, anti-collision provides the opportunity to position antennas within the dens of mammals and under the nests of birds or detect fish congregating in a body of water. By permitting all present individuals with PIT tags to be continuously recorded, this technology has the potential to intimately investigate numerous aspects of animal ecology, such as behavior, fidelity, and movements.

Our primary objective was to assess the benefit of technologies capable of eliminating tag collision, which occurs when using PIT tags and readers operating at low-frequency (Shih et al. 2006, Klair et al. 2010). We found that using high-frequency
equipment possessing inherent anti-collision capabilities allowed us to detect all PIT tagged bats present by each antenna, greatly increasing both the number of scanned bats and the number of locations where antennas could be placed. To our knowledge, we are the first bat researchers to continuously detect roosting bats by positioning antennas adjacent to areas of high congregations of animals, such as on the ceiling of attics. This allowed us to record 864% more detections of bats than the entryway antennas alone. This increase is far more meaningful than simply detecting a small number of individuals repeatedly, but instead represents an important advance. At the Mammoth roost in 2018, ceiling antennas increased the number of unique individuals detected by 16%, over what was detected on the primary entryway antennas. The ceiling antennas allowed us to improve our ability to detect the presence of marked bats, because there were more entryways to the attics than could be monitored with readers, including many that were difficult or impossible to find. Thus, we would have never detected a significant portion of our tagged individuals without the ceiling antennas and anti-collision capabilities. These unique detections suggest that when attempting to study bats, or similar mammals with multiple entrances to their den or roost, antennas should be placed directly in the social roosting space, provided anti-collision is available.

However, it is essential to emphasize that entryway antennas should not be considered obsolete. These antennas proved more effective at detecting movements of bats, and we therefore recommend their use when attempting to gather daily presence and absence data. This is especially true when monitoring large roosts with complex interiors that are difficult to cover with antennas or when it is unclear of exactly where bats are
roosting. While anti-collision is not required to use entryway antennas, we found that it improved the readers’ ability to detect bats entering and exiting roosts. An increased ability to detect bats at these antennas is vital for accurate estimates of daily use or roosts as well as using more advanced analyses, such as estimating survival (Maslo et al. 2015) or population sizes (Waag et al. 2018 in review). For example, 4,996 detections of bats entering and exiting the attic at the same time through these primary entryway antennas would have not been recorded without anti-collision on our entrance antennas during 2018. This is a considerable number given the populations sizes at our two monitored roosts (847 and 208), and the average number of bats observed passing through entryway antennas during emergence counts (227 and 140) (Waag et al. 2018 in review). While only 0.47% of our total detections at entryway antennas included >1 bat passing through at once, this still represents a noteworthy improvement. Currently, studies using entryway antennas without anti-collision must either assume that >1 tagged individual pass by antennas so infrequently as to not significantly affect any subsequent analyses. However, our results show that studies with larger populations or heavier flows of bats passing through antennas would miss a higher percentage of detections. Thus, we recommend utilizing anti-collision technology when using entryway antennas whenever possible.

In addition to using entryway antennas with anti-collision, ceiling antennas expanded the utility of PIT tags in our study beyond simple documentation of presence at the roost to include movements within the roost at any temporal scale of interest. Stated differently, this allowed PIT tags to be used to quantify not only dyadic association rates on a daily scale but also social interactions within the roost. Previous studies of dyadic
relationships within bat social groups, have contributed significantly to our understanding of social ties between two individuals based on the assumption that two bats cohabitating a roost interact and have a positive association (Chaverri 2010, Patriquin et al. 2010, Johnson et al. 2013). However, we report frequent movements and shifting interactions among bats within roosts. These data demonstrate how bats cohabitating a roost on any given day utilize different areas of the roost, resulting in some pairs of bats having stronger ties than others (Ortega and Maldonado 2006). Anti-collision technology provides opportunities to investigate dyadic interactions, such as these, and improve our certainty in quantifying association between bats and estimating their relationships.

Through showcasing various short-term social interactions between bats at a single ceiling antenna and long-term preference of several bats across an entire maternity season, we have shown that associations and relationships can be much more complex than previously considered. Our primary purpose in presenting these examples and writing this communication is to demonstrate that techniques now exist that are capable of recording data necessary to further investigate these relationships and interactions. Advanced methods, such as network analyses, can be performed using these data to provide further information about these associations (Garroway and Broders 2007, Chaverri 2010, Patriquin et al. 2010, Johnson et al. 2012a, 2013).

It is important to note that our research would not have been possible without anti-collision, rather than high-frequency itself. High-frequency PIT tags have approximately half the read range of similar low-frequency tags, with lower tag power consumption requirements (Klair et al. 2010). This decreased read range required us to
carefully design and place antennas surrounding areas of high bat use within the roosts. All of our antennas were tested in the office and field to ensure that the detection distances allowed for all present tags to be read. Thus, high-frequency should only be used when antennas can be placed relatively close to bats. However, when used appropriately, high-frequency holds other advantages beyond anti-collision, such as transfer rates approximately 1000% that of low-frequency devices (10 kB/s vs 100 kB/s), faster tag read speeds, and is typically less expensive while requiring smaller passive tag sizes (Klair et al. 2010). Nevertheless, it is likely that using low-frequency technology may be the preferred option for studies where increasing read range is paramount. While not currently available, future innovation could enable anti-collision capabilities for low-frequency readers and tags. Until then, our research shows that using high-frequency technology to gain anti-collision capabilities can improve PIT tagging methodology and create opportunities to study novel questions, across a multitude of mammalian species.
Figure 1. Photograph of eight ceiling (solid arrow) and two entryway (dashed arrow) antennas installed inside an attic of an abandoned building, which is home to a maternity colony of little brown myotis in Yellowstone National Park, USA.
Figure 2. Comparison of daily detections of unique bats recorded by ceiling and entryway antennas from first detection to end of 2018 field season (May 10, 2018 to August 14, 2018) at the Mammoth roost (A) and (June 19, 2018 to August 14, 2018) at the Lamar Valley roost (B).
Figure 3. Network map displaying fluctuations in short-term social interactions between 11 bats at a single ceiling antenna in the Mammoth roost across three consecutive 30-minute intervals [16:27:00-16:56:00 (A); 16:57:00-17:26:00 (B); 17:27:00-17:56:00 (C)]. Links are sized by the number of minutes bats were spent together at the antenna during the 30-minute period. Nodes are sized by the number of minutes that bats were present at the antenna during the 30-minute period.
Figure 4. Antenna preference of 6 bats across the 2018 maternity season at a single array of 8 antennas attached to a reader in the Mammoth roost. Bats B330, B256, and 35F2 displayed high preference (across 22, 17, and 2 days, respectively) for space monitored by a single antenna. Conversely, bats 5584, BAF2, and 9B9C displayed low preference for any specific space within the roost (across 24, 5, and 9 days, respectively) and were often detected moving throughout the building during the day.
CHAPTER 3: MARK-RESIGHT ANALYSES PROVIDE PRECISE ESTIMATES OF MYOTIS LUCIFUGUS POPULATIONS DURING SUMMER

Abstract

The precipitous decline of bats in North America has created an urgent need to better understand their population ecology. Unfortunately, there are no estimates of abundance available for many populations of bats believed to be at risk of extinction or extirpation, and the absence of winter hibernacula in many regions leaves biologists without the traditional tools required to create such estimates. To provide much-needed population ecology data, we used a mark-resight analysis to quantify abundance based on detections of individually marked bats recorded by high-frequency radio-frequency identification (RFID) readers and counts of bats exiting monitored roosts. To mark bats, we subcutaneously implanted high-frequency passive integrated transponders (HF-PIT) into 297 female little brown myotis (Myotis lucifugus). To detect marked bats, we collectively installed 8 continuously scanning RFID readers and 45 antennas inside 3 maternity roosts used by distinct populations in the Lamar Valley, Tower Junction, and Mammoth Hot Springs regions of Yellowstone National Park. We recorded 2,929,742 detections of 196 HF-PIT tagged bats (66.0%) between June 2017 and August 2018. Using mark-resight analyses, we estimated the pre-parturition size of the Mammoth population to be 847 (95% CI = 749-987) in 2017, and 836 (95% CI = 722-989) in 2018. The Lamar Valley population was estimated to consist of 208 (95% CI = 198-222) bats in 2018. Population estimates were not possible at Tower Junction, but RFID readers installed there showed that only a single HF-PIT tagged bat moved among these three
roosts. Population estimates at Mammoth and Lamar reflect roost availability, as 258 and 25 potential roosts were available within 1 km of RFID-monitored roosts in Mammoth and Lamar Valley, respectively. Although roost availability and populations were larger in Mammoth, roost fidelity was similar between the two sites, with bats detected in RFID-monitored roosts on 36.5% of days in Mammoth and 31.4% of days in Lamar Valley. Combining traditional emergence counts with RFID technology and mark-resight analyses presents a novel approach to estimating bat populations during summer, which has long vexed researchers. While especially useful for regions lacking caves, and thus any traditional method to census populations, this novel approach provides a vital opportunity to advance knowledge of bat ecology worldwide during a season rarely studied.

Introduction

How animal populations interact with their environment and fluctuate over time are central concepts in animal ecology (Elton 1927). Studies of animal populations not only provide knowledge of abundance and distribution, but can also yield valuable data on how species respond to environmental changes (Walther et al. 2002, Adams and Hayes 2008). Such knowledge is vital for land managers facing challenges from invasive species (Sakai et al. 2001), climate change (Parmesan 2006), and emerging infectious diseases (Frick et al. 2010a). For organizations tasked with conservation and management, distinguishing between natural population dynamics and abnormal declines is imperative, but often requires estimates of abundance prior to perceived population declines (Weller et al. 2009, Lindenmayer et al. 2012)
It is believed that bat populations in western North America are about to experience significant declines due to the recent spread of white-nose syndrome (WNS) to the region (Knudsen, Dixon, & Amelon 2013; Lorch et al., 2016; Vonhof, Russell, & Miller-Butterworth, 2015). Estimates of abundance for bats inhabiting western North America are rare, and far less is known about bat ecology in this region when compared to eastern North America. Unfortunately, this lack of knowledge, combined with the unique life-histories of many bat species, creates difficulties for utilizing traditional techniques to monitor these populations (Barclay and Harder 2003, O'Shea et al. 2003, O'Shea et al. 2004). This is especially difficult for areas with a dearth of known hibernacula where bats traditionally roost in large congregations (O'Shea et al. 2003). One such area is the Rocky Mountain region of North America (Perkins et al. 1990, O'Shea et al. 2003, Hendricks 2012, Weary and Doctor 2014, Neubaum 2018, Whiting et al. 2018). The arid climate and paucity of limestone in this region hinders the formation of caves, which are the primary locations for performing population counts on *Myotis* species in eastern North America (Kunz et al. 2009). This lack of caves and winter habitat where bats assemble in large numbers, precludes the ability to estimate bat populations in much of the Rocky Mountain region, creating a critical gap in knowledge (Keinath 2005, Weary and Doctor 2014, Tinsley 2016, Neubaum 2018). In these areas, recording demographics of summer populations may be the only strategy to describe and monitor these populations (Loeb et al. 2015). However, such attempts face many challenges; in fact, while the literature explaining the shortcomings of these efforts is
extensive, studies attempting to move the field forward are rare (O’Shea et al. 2003, Neubaum et al. 2005, Kunz et al. 2009, Neubaum 2018).

For decades, studies monitoring summer populations of bats have been limited to counting bats as they exit maternity roosts, which are typically trees or buildings (Hayes et al. 2009). However, for many bat species, emergence counts performed at a single roost alone are not suitable for estimating populations because of their propensity to form fission-fusion social groups (Kerth and König 1999). Bats in these social groups inhabit numerous roosts dispersed across the local landscape and are typically found in highly variable numbers within any given roost on any given day (Kerth and König 1999). Thus, attempting to perform emergence counts at all known roosts simultaneously is often impractical and time-consuming (Hayes et al. 2009), while also leaving an unknown proportion of the population unaccounted on any given night.

While by themselves, emergence counts are unsuitable for estimating bat populations, performing these counts at well-used roosts could create powerful measures if paired with the proportion of known and unknown individuals that emerge from the roost. Similar to a mark-recapture study design, this proportion acts as the foundation needed to construct robust estimates of population size (Otis et al. 1978). In classic mark-recapture studies, animals are initially marked, returned to the population, and then subsequently recaptured and reidentified. However recapturing marked bats through capture efforts is challenging and time consuming, typically resulting in a relatively low percentage of recaptures (O’Shea et al. 2004, Kunz et al. 2009). An alternative approach to identifying marked bats can be accomplished through using radio-frequency
identification (RFID) readers and passive integrated transponders (PIT). Once tagged, bats can be redetected by installing RFID antennas at entryways to their roosts (Kerth and Reckardt 2003) or on the ceilings inside the roost (Waag et al. in prep.). Despite the obvious benefit of applying this technology to address gaps in bat population ecology, to our knowledge, no study has attempted to utilize analytic methods using detections of PIT tagged bats in this way.

Immigration-emigration mark-resight analyses are an analytical approach ideally suited to estimating abundance using the type of data described above. Through mark-resight models, detections from RFID antennas (resightings of marked individuals) can be combined with emergence counts (sightings of marked and unmarked individuals) to estimate demographic parameters, such as population size, over a specific duration (McClintock et al. 2009). While similar to conventional mark-recapture methods, mark-resight models are better equipped to estimate abundance, rather than survival and detection probabilities, by accounting for imperfect detection from resighting bats rather than physically recapturing them (McClintock and White 2012). Mark-resight analyses therefore have the potential to be a significant value to biologists and land managers tasked with conserving and managing bat populations, especially in regions where recapturing or counting bats at hibernacula is impractical or impossible.

Mark-resight methods are particularly useful for the study of species that form large social groups during summer, but are difficult to recapture, such as the little brown myotis (Myotis lucifugus) (Humphrey and Cope 1976, Barclay and Harder 2003, Kunz et al. 2003, Tinsley 2016). Little brown myotis are found throughout North America and
females are well-known for forming large fission-fusion seasonal maternity colonies in buildings (Fenton and Barclay 1980, Kerth and König 1999, Olson and Barclay 2013). Little brown myotis are also long-lived (>30 years) and have low fecundity (no more than 1 young per year), which allows biologists to reasonably mark a significant proportion of the population and track these individuals for a long duration (Keen and Hitchcock 1980, Davis and Hitchcock 1995, Barclay and Harder 2003). Furthermore, little brown myotis are one of the species most heavily impacted by WNS, making the development of tools for studying this species in regions without hibernacula a priority for conservation (Frick et al. 2010a, Turner et al. 2011). Once considered to be one of the most abundant bat species in North America (Fenton and Barclay 1980), the little brown myotis has experienced average population declines of 91% in areas where bats have been affected by WNS for at least two years (Turner et al. 2011). Since little brown myotis aid in suppressing populations of herbivorous insects, an ecosystem service valued at billions of dollars, a precipitous population decline due to WNS may drastically influence ecological structure and function for many species and agricultural systems (Jones et al. 2009, Maine and Boyles 2015).

The goal of our study was to input continuous detections of HF-PIT tagged bats and periodic emergence count data into mark-resight models to yield the first estimates of little brown myotis abundance in a region lacking cave roosts, Yellowstone National Park. We also aimed to correlate population sizes with roost availability, assess roost fidelity, examine trends in seasonal use of building roosts, and investigate the degree of connectivity between several distinct populations in a relatively small section of
Yellowstone. Finally, we assessed the relative importance of specific roosts to little brown myotis through measures of roost fidelity and roost connectivity. Because it is thought that bat populations are limited by the availability of roosts (Kunz et al. 2003), we hypothesized that populations would be larger, and roost fidelity would be lower, in areas of Yellowstone with more buildings compared to an area with fewer buildings. We also hypothesized that since WNS has not yet arrived in Yellowstone that estimates of abundance for the same population would be statistically indistinguishable over two consecutive years (Keen and Hitchcock 1980). In addition, because we know that little brown myotis are present in Yellowstone at least from April to November (Johnson et al. 2017), we hypothesize that detections of HF-PIT-tagged bats at RFID-monitored roosts would begin in April and end in November.

Materials and Methods

Study Area

Our study was conducted in Yellowstone National Park, which encompasses 898,300 ha in northwestern Wyoming and adjacent sections of Montana and Idaho. Within this area, we focused our study on three buildings known from previous research to house large maternity colonies of little brown myotis (Johnson et al. in prep.). These roosts were located in the town of Mammoth Hot Springs, Wyoming (elevation of 1849 m), as well as in the vicinity of Tower Junction (1937 m), and Lamar Valley (2000 m) (Fig. 5). Previous radio-telemetry work has shown that these three buildings represent distinct colonies where movement of bats among roosts is likely to be rare (Johnson et al. in prep.). These buildings are located in the 155,000 ha area of Yellowstone known as the
Northern Range. The Northern Range is composed of non-forested grasslands (primarily *Festuca idahoensis*) and sagebrush steppe (primarily *Artemisia tridentata*), bordered by forests composed of lodgepole pine (*Pinus contorta*), douglas fir (*Pseudotsuga menziesii*), engelmann spruce (*Picea engelmannii*), Rocky Mountain juniper (*Juniperus scopulorum*), and quaking aspen (*Populus tremuloides*). A National Oceanic and Atmospheric Administration weather station on the Northern Range, located at an elevation of 1,912 m, recorded an average annual precipitation of 41.71 cm from 1980-2010 (Arguez et al. 2012). Average minimum temperatures on the Northern Range are below freezing from October through April, the months bordering the summer maternity season.

**Bat Capture and Tagging**

All methods were approved by the Institutional Animal Care and Use Committee of Ohio University (16-H-023) and followed the American Society of Mammalogists guidelines for the use of wild mammals in research (Sikes et al. 2011). From 2015 to 2018, we captured little brown myotis in mist-nets (Avinet Inc., Dryden, NY, USA) positioned outside RFID-monitored building roosts and nearby small ponds. In a few rare instances, bats were captured by hand inside of their roosts. Morphological characteristics, such as species, sex, and age were recorded for all captured bats. All bats were identified as juveniles or adults by the degree of epiphyseal-diaphyseal fusion on the joints of the phalanges (Brunet-Rossinni 2009). A single 0.1 g 12 mm x 2.15 mm high-frequency (13.56 MHz) PIT tag (HID Global, Granges-Paccot, Switzerland), was subcutaneously implanted in female bats parallel to the vertebrate on the left mid-dorsal
side. Before and after PIT tagging, all bats were scanned with a handheld high-frequency RFID device (ISC.PRH102; Feig Electronic, Weilburg, Germany) to ensure that previously tagged bats were recorded as a recapture and all newly injected tags were operating properly. We used a sterile syringe (Avid Identification Systems, Inc. Norco, CA, USA) to inject one HF-PIT tag under the skin of each bat, and sealed injection points with Vet-Bond (3M, St. Paul, MN, USA) to help prevent infection and reduce the probability of tag loss. Unscented baby powder (Johnson & Johnson, New Brunswick, NJ, USA) was applied to quickly dry the Vet-Bond and reduce handling time.

**RFID Monitoring and Emergence Counts**

To redetect HF-PIT tagged bats following capture, we constructed high-frequency RFID antennas, we used RG6 18AWG coaxial cable linked through a motherboard (Feig Electronic, Weilburg, Germany). Antennas were designed in the shape of 1.5 x 0.4 m and 1 x 0.2 m rectangles and installed in high traffic areas within attics on the ceiling (Waag et al. 2018 in prep.). Antennas were also installed around primary entryways to the roosts to detect a majority of the colony as they exit the buildings during their evening emergence. Installation occurred during early May 2017 and 2018, when bats were not present in the attics, to avoid disturbance. Areas that possessed high levels of bat sign, such as guano and scratch marks, were prioritized for antenna placement.

Up to eight antennas were connected to high-frequency RFID readers (Oregon RFID, Portland, OR, USA) and programmed to continuously scan for HF-PIT tagged bats throughout the entire year. For each tagged bat that was scanned by the antennas, RFID readers recorded the HF-PIT tag’s unique 16-digit alphanumeric identification code,
antenna location, and the date and time. High-frequency RFID readers possess innate anti-collision capabilities to prevent a complication known as tag collision from occurring, which prohibits tags by an antenna from being detected if more than one tag is present (Shih et al. 2006, Klair et al. 2010). Complete tag collision occurs when multiple tags present by an antenna block all transmissions as they return to the RFID reader. Partial tag collision occurs when the tag with the strongest transmission, which is typically the closest tag in a proper orientation, overpowers and blocks all tags with weaker transmissions. Using high-frequency RFID readers possessing innate anti-collision capabilities allowed for up to 100 HF-PIT tags to be detected at each antenna, while recording HF-PIT tags up to 4 times per second. RFID readers were also able to detect HF-PIT tags moving through the electromagnetic field of antennas at a speed of at least 50 km/h, which is faster than the maximum flight speed of little brown myotis (Fenton and Barclay 1980). This was confirmed by attaching HF-PIT tags to projectile darts (NERF; Hasbro, Pawtucket, RI) and repeatedly launching them through antennas to ensure all HF-PIT tags passing through the antenna’s electromagnetic field would be read.

We recorded evening emergences of bats leaving primary entryways of RFID-monitored roosts approximately once a week during the maternity season using FLIR Scout III 640 thermal monoculars (FLIR Systems, Inc., Wilsonville, OR, USA), which recorded footage in 640 x 480 resolution at 30 frames per second. Emergence counts began at civil twilight and concluded 15 minutes after the last bat was observed. Bats were observed emerging using the FLIR monocular and tallied using a handheld clicker.
While using the FLIR’s black and white infrared mode, emerging individuals could be readily identified independent of available ambient light by their warm, and consequently, black wing membranes (Fig. 6). After bats have been outside for approximately 10 seconds, varying slightly with ambient temperature, their wing membranes appear white. These tools increased the accuracy of our emergence counts by allowing observers to safely avoid recounting bats that have already emerged or counting bats that emerged from an opening where no antennas are positioned. Emergence counts on nights where precipitation exceeded 10 minutes or continued sporadically were excluded from population analyses. Likewise, emergence counts where sustained winds were greater than 28 km/h during the survey period were also disregarded.

**Population Estimates**

We used an immigration-emigration logit-normal mark-resight estimator (IELNE) (McClintock and White 2012) in Program MARK (White and Burnham 1999) to estimate the population of female little brown myotis occupying monitored roosts in Mammoth during 2017 and 2018, and Lamar Valley during 2018. The IELNE explicitly accounts for imperfect detection and produces estimates of the “superpopulation” ($N^*$), which represents the total number of females (both marked and unmarked) that use each monitored roost over the course of a maternity season. Thus, superpopulations are equivalent to the size of an entire fission-fusion social group that is associated with each RFID-monitored roost. Parameters modeled were capture probability ($p$), individual heterogeneity ($\sigma$), mean population size ($\bar{N}$), offset of $\bar{N}$ ($\alpha$), and superpopulation size ($N^*$). AICc scores were lowest for the 2017 and 2018 Mammoth population estimates.
when \( p \) and \( \alpha \) were time-varying \((t)\) and \( N^*, \bar{N} \), and \( \sigma \) were constant(.). While the Lamar Valley population was modeled under the same fit, other models are being currently explored and may be considered more appropriate in the near future. This analysis avoids the requirement of geographic closure, meaning that bats may leave or enter the study area between secondary occasions (i.e., individual emergence counts) within primary sampling intervals (i.e., the maternity season) (McClintock and White 2012). Another unique advantage to using this estimator is that new marks are allowed to be introduced into the superpopulation at any time, except during an emergence count (McClintock and White 2012).

This estimator used the number of unique RFID detections of bats (without replacement) that traversed through antennas placed at roost entryways during emergence counts to determine the number of tagged, and thus, resighted individuals. The emergence counts provided the total number of individuals, both tagged and untagged, that exited the building through areas covered by antennas. The number of untagged bats observed was estimated by subtracting the total number of individuals by the number of tagged individuals. Each emergence count was paired with RFID detections over the same duration at the same roost to create a separate secondary occasion. Each annual maternity season was analyzed as a separate primary interval, composed of all secondary occasions performed during that year at the roost. Because tagged bats were always identified at the individual-level, no unidentified marks were recorded and factored into these analyses. Because internal PIT tags do not increase sightability, we assumed that tagged and untagged individuals have the same probability to be observed leaving a
RFID-monitored roost. Additionally, we assumed all individuals possessing tags exiting through entryway antennas will be detected during an emergence because of the anti-collision technology. Thus, there was no individual heterogeneity in observation, or detection probability.

Only antennas positioned on primary entryways, rather than the ceiling, were used to determine the number of resighted and untagged individuals needed to estimate populations, because only these data could be paired directly with emergence count data. However, both ceiling and entryway antennas were used to estimate which individuals were assumed to be present in the superpopulation by the first population estimate (19 June in 2017 and 6 June in 2018) and reside within the superpopulation until at least volant juveniles were observed (6 August in 2017 and 8 August in 2018). Because of the short duration of the maternity season and expected low probability of tag loss (O ’Shea et al. 2004), this study also assumed that HF-PIT tags continued operating and remained within bats for any individual newly HF-PIT tagged or detected during the current season. Because our goal was to estimate pre-parturition population sizes, we did not attempt to estimate abundance after juveniles became volant because during these juveniles cause the number of unmarked bats in the superpopulation to sharply increase. In addition, adult females began to disperse from the maternity colonies for the season during this time, causing the number of tagged bats in the study area to decline. To assess our hypothesis that populations would be similar between two consecutive years where we did not expect significant change, we compared estimates of abundance made in 2017 to 2018. Populations at the same roost were considered to be different between years if
95% confidence intervals did not overlap. To test our hypothesis regarding the effect of roost availability on population estimates, we compared the population in Mammoth, where approximately 258 available buildings exist within a 1 km radius extending out from the RFID-monitored roost, to Lamar Valley, which has 25 available buildings within a 1 km radius. We did not use data from the Tower Junction RFID readers for population estimates because bats using this building exited the roost through numerous holes that we were unable to effectively surround with antennas.

**Roost Connectivity and Fidelity**

Data from both ceiling antennas and entryway antennas from Mammoth, Lamar Valley, and Tower Junction were utilized to provide information on the connectivity between roosts, specifically how often and far bats would move between RFID-monitored roosts. Roost fidelity was estimated by averaging the percentage of days that each tagged bat, captured from the same RFID-monitored roost, was detected using the roost during the maternity season. We also assessed monthly averages of roost fidelity at both Mammoth and Lamar Valley to determine if roost fidelity fluctuates over the maternity season. To assess our hypothesis that Mammoth and Lamar Valley would have significantly different measures of roost fidelity, a heteroscedastic one-tailed t-test was performed on the combined roost fidelity observed at Mammoth during 2017-2018 and Lamar Valley during 2017-2018.
Results

Emergence Counts and RFID Monitoring

During 2017 and 2018, we conducted 44 emergence counts across our 3 RFID-monitored roosts. At the Mammoth roost, primary exit counts ranged from 59-498 ($\bar{x} = 216.1; n = 23$). At the Lamar Valley roost, primary exit counts ranged from 5-383 ($\bar{x} = 100.8; n = 13$). RFID readers installed in these roosts recorded 2,929,742 detections of tagged bats between 19 June, 2017 and 15 August, 2018. While 65.99% (196/297) of bats tagged during 2015-2018 were redetected in 2017 and 2018, the percentage varied among roosts and capture years (Fig. 7).

Contrary to our hypothesis that bats would be detected occupying RFID-monitored roosts from April to November, the first bat of the season in 2018 was detected on 10 May in Mammoth, and 9 June in Lamar Valley. The last day of the 2017 season that bats were detected at both Lamar Valley and Mammoth roosts was 25 August. RFID reader failure prevented data collection before 19 June in 2017, precluding us from identifying the first date that bats occupied RFID-monitored roosts during 2017. While bats were observed emerging from the Lamar Valley roost until at least 1 September in 2018, we have not yet had the opportunity to download the data from both of these roosts to determine the last day that RFID-monitored roosts were used during the 2018 maternity season. However, both roosts were relatively active, with multiple individuals being detected daily until 25 August in 2017. Despite RFID readers running continuously throughout the winter, bats were not detected using the buildings until 10 May, 2018 in Mammoth and 9 June, 2018 at Lamar Valley.
Roost Connectivity and Fidelity

Only one bat (0.33%) was observed using both the Tower Junction and Lamar Valley roosts (separated by 16 km). No bats moved between Tower Junction or Lamar Valley and Mammoth (distance of 22.5 km and 37 km, respectively). Roost fidelity varied across months and populations (Fig. 8). In Lamar Valley, average fidelity was 36.8% ($n = 101$; range: 1.9-95.5%) of the 115 days monitored during the 2017 and 2018 maternity season. At Mammoth, average fidelity was 33.2% ($n = 91$; range: 1.4-81.7%) of the 123 days monitored during the 2017 and 2018 maternity season. Roost fidelity in Lamar Valley, where there is a lower availability of buildings, was not significantly different ($p = 0.15$). Roost fidelity varied throughout the year and was highest during July at Mammoth (41.1%, 44.3%) and Lamar Valley (43.3%, 55.7%) during 2017 and 2018, respectively (Fig. 8). Roost fidelity was lowest in June at Mammoth (23.4%) during 2018 and Lamar Valley (16.7%, 13.9%) during 2017 and 2018, while roost fidelity was lowest in August at Mammoth (14.5%) during 2017.

Population Estimates

We estimated the Mammoth superpopulation to consist of 847 (95% CI: 749-987; SE 59.8) bats in 2017 based on 10 nights of emergence count data and RFID detections (Fig. 9). We estimated the same superpopulation to consist of 836 (95% CI: 722-989; SE 67.3) bats in 2018 based on 7 nights of emergence count data and RFID detections. We estimated the Lamar Valley superpopulation to consist of 208 (95% CI 198 – 222; SE 6.2) bats in 2018 using 6 nights of emergence count data paired with RFID detections. As hypothesized, Mammoth superpopulation estimates were relatively similar and well
within each other’s 95% confidence intervals. Also as hypothesized, the population size in Mammoth, where a greater availability of buildings is found, was notably higher than in Lamar Valley.

Discussion

We found that immigration emigration mark-recapture analyses are an effective way to analyze detections of PIT tagged bats and emergence count data to produce relatively precise population estimates of little brown myotis during the summer. Although a population estimate alone is less valuable than estimates that are paired with informative hypotheses to determine what is actually driving the observed changes in populations, our use of these analyses is of significant value because they provide animal ecologists using PIT tags with a framework from which to leverage their data to study populations (Boarman et al. 1998, Gibbons and Andrews 2004). However, by using these techniques alongside other tools, such as environmental monitoring, cause and effect can be connected to proactively determine if management action is needed or if observed fluctuations are naturally occurring (e.g. due to changing environmental conditions). Biologists can then determine where and how to use limited resource funds or utilize advanced supplementary statistical methods, such as population viability and population sensitivity analyses (Maslo et al. 2017).

By having a better understanding of the factor(s) that cause population change in the system and continually monitoring abundance, biologists will be better equipped to evaluate the effectiveness of current population management actions (conservation, control, or manipulation) and examine how factors causing population change are
affected across different spatial and temporal scales. This is essential for researching, managing, and conserving bat species in North America. We contend this is especially true of bats in the Rocky Mountain region of North America, which are currently flanked by the presence of WNS located in populations to the east and west, but for which few population studies are available (but see Whiting et al. 2018). However, knowledge of populations and their movements in this region prior to the arrival of WNS will be important to help develop informative population thresholds required to effectively alert biologists to population declines, so that they may provide a timely and appropriate response.

Producing estimates of summer populations of bats has long frustrated biologists due to their nocturnal and often highly cryptic behaviors, along with the inherent difficulties in capturing and counting them (Gannon and Willig 1998, O’Shea et al. 2003). Due to a previous absence of tools available for studying summer populations, the majority of demographic information used to describe region-wide population sizes is based on counts at winter hibernacula (Clawson 2002, Thogmartin et al. 2012, 2013, Maslo et al. 2015, Whiting et al. 2018). This bias towards winter studies is problematic because they tend to underestimate population sizes and are not possible in areas lacking large hibernating populations (Tuttle 1979, 2003, Whiting et al. 2018). Furthermore, the migratory behavior of many bat species creates a population structure where overwintering populations in most regions consist of different individuals at dissimilar abundances than are present during summer (Davis and Hitchcock 1965). Thus, precise, repeatable estimates of populations during summer are sorely needed. At Mammoth,
where our data allowed us to estimate populations over two years, we generated estimates with confidence intervals that were notably narrow and represented a standard error of 60 and 67 bats in 2017 and 2018, respectively. This represents the magnitude of population change we would be able to observe with this technique and is a significant improvement over studies using recaptures of banded bats at hibernacula to estimate abundance over time (Whiting et al. 2018). The narrowness of our confidence intervals stem from our ability to resight a high proportion of tagged bats, which itself stemmed from the high fidelity of bats to monitored roosts.

Our study adds new insights to a significant body of literature describing aspects of little brown myotis population dynamics. These studies provide a portrait of a long-lived species with reported apparent adult female survival rates of little brown myotis ranging from 0.63 (95% CI = 0.56-0.68) to 0.90 (95% CI = 0.77-0.94) and juvenile survival rates of 0.23 (95% CI = 0.14-0.35) to 0.46 (95% CI = 0.34-0.57) (Frick et al. 2010b). Importantly for our study, Frick et al. (2010b) also found that juvenile survival rates declined as juveniles are born progressively later. In Yellowstone, the short growing season leaves juvenile bats with little time to prepare for migration and hibernation prior to onset of winter (Johnson et al. 2017). Thus, we postulate that survival would be lower in Yellowstone compared to areas with longer summers and earlier parturition dates. Buildings, which provide temperatures closer to the thermoneutral zone of little brown myotis and ample space for social groups to use social thermoregulation, may provide some protection from this environmental and reproductive challenge (Lausen and Barclay 2006, Shively and Barboza 2017). How often and which buildings are used by bats is
particularly valuable to biologists when quantifying the importance of managing specific buildings, as it is believed that female little brown myotis in Yellowstone may be dependent on benefits that buildings provide to help offset costs of reproduction and thermoregulation (Johnson et al. 2017).

Buildings have long been recognized as valuable roosts for little brown myotis, and the increase in human structures on the landscape has been hypothesized to have led to range expansion for this species prior to the arrival of WNS (Lewis 1995, Kunz and Reynolds 2003, Shively and Barboza 2017). It is therefore no surprise that we documented a much larger population of little brown myotis in Mammoth, an area with a greater availability of susceptible roosts. Unlike our prediction, however, roost fidelity did not differ among these colonies. Thus, although availability to potential roosts influenced the size of the populations, it implies that the rate bats switch roosts in Yellowstone is constant and independent of the number of available roosts. This behavior for little brown myotis to frequently switch roost sites asynchronously, creating a dynamic social group known as a fission-fusion colony, is well documented in the literature (Kerth and König 1999). As observed in other systems, roost switching in Yellowstone is likely performed to take advantage of fluctuating weather, foraging opportunities, and social interactions (Lewis 1995). It is also speculated that it is done to reduce interaction with predators, disturbance, and ectoparasites (Lewis 1995).

Average roost fidelity was also observed to be highly variable depending on the month (13.9%-55.7%), with July always reporting the highest fidelity and June typically reporting the lowest fidelity (Fig. 8). Over the several million detections recorded by our
RFID readers in 2017 and 2018, a majority of bats tagged from 2015-2018 were frequently redetected at RFID-monitored roosts, with this percentage differing between roosts and capture years (Fig. 7). Together, these results display an effective technique and analysis used to intimately and discreetly study the behavioral ecology of a declining species, while also monitoring long-term population dynamics. As observed in other studies, little brown myotis at the Mammoth and Lamar Valley roost possessed high fidelity to their maternity roosts and often used the same roost across years (Humphrey & Cope 1976).

In addition to a single bat being observed using both the Lamar Valley and Tower Junction roosts during this study, a female little brown myotis was also observed moving between these same roosts during the summer of 2014 through radio-telemetry. These measures of roost connectivity indicate that movement typically happens at a smaller-scale and is likely limited to nearby buildings. While still a significantly rare event, both of these movements occurred directly following a capture event, so it is possible that this behavior may not naturally occur in the absence of disturbance. While we have detected a high proportion of individuals using RFID-monitored roosts over multiple years, bats have been observed to only use the same maternity colony across years. Similar site fidelity, or “site attachment”, has been observed in Indiana and Kentucky by (Humphrey and Cope 1976) where none of the 2841 banded adult and juvenile females were ever observed at another location than their original maternity colony. They estimated a site attachment value of 78% for adult and juvenile females based on occupancy and capture efficiency. Our findings are also consistent with research performed by (Wilder et al.
that found western populations of little brown myotis are more genetically isolated than eastern populations, possibly due to a lack of long-distance migrations and reduced movement between roosts.

Despite buildings being vital habitat in Yellowstone for female little brown myotis to form summer maternity colonies, no individuals were detected using roosts during winter months (September to April). Acoustic detectors positioned across Yellowstone detected 35-40 kHz frequency *Myotis* (*M. lucifugus, M. volans, or M. ciliolabrum*) during all months, except December, January, February (Johnson et al. 2017), suggesting that at least some individuals of these species remain in Yellowstone during the winter. This timing parallels observations seen in northeastern United States where little brown myotis typically emerge from hibernation around early April and approximately two weeks later in areas of central Manitoba (Norquay and Willis 2014). Activity of previously radio-tagged little brown myotis throughout October also implies that some individuals are remaining in Yellowstone for the winter and are forgoing long-distance migration (Johnson et al. 2017). Since bats are known from acoustic and radiometry research to be present within Yellowstone from April to November and not utilize RFID-monitored roosts in April, October, or November, we reject our hypothesis. These findings imply that bats are instead using non-traditional hibernacula in the area, such as scree or rock-crevices. This prediction is reinforced by research that presents bats roost in similar habitats in regions where cave hibernacula are also scarce or non-existent (Lausen and Barclay 2006, Neubaum et al. 2006, Hendricks 2012, Neubaum 2018, Weller et al. 2018).
We chose an immigration-emigration mark resight model to analyze our data in part because it does not require the assumption of geographic closure, which is typically required when estimating superpopulations (McClintock and White 2012). This is highly beneficial when studying bats or any animal with a large activity area because it means that no assumptions will be violated if animals leave or enter the study area. Despite little brown myotis possessing relatively high roost fidelity during the maternity season, the lack of data on their behavior in the Rocky Mountain region of North America made it difficult to say with certainty that this assumption would not be violated had it been made (Humphrey and Cope 1976, Lewis 1995). That said, other estimators, such as the logit-normal estimator and zero-truncated Poisson log-normal estimator, should be considered as viable options when studying other species and systems (McClintock et al. 2009).

When using the IELNE model, it is necessary to estimate or know the number of marks present in the superpopulation or select a date after which individuals are assumed to be present in the superpopulation. This assumption can lead to over-inflating the population estimate if bats arrive in the superpopulation after this date. However, if individuals are present in the superpopulation before this date and have not yet been detected at a RFID-monitored roost, they will be assumed to be absent when they are actually present in the area, potentially leading to an underestimating of the population estimate. Thus, we suggest that techniques are consistently selected across years, based on what is most appropriate for the species and system. If potential unintended increases or decreases exist in the population estimates due to selecting an inappropriate start date,
it is important to ensure that they affect population trends similarly over time to compare and track populations across these years. Our study assumed that all bats detected during each maternity season were present within the superpopulation at the time of the first mark-resight estimate, since this is the most conservative and likely option. This assumption was selected based on previous acoustic and capture data, showing that bats awake from hibernation and form maternity colonies by at least the first mark-resight estimate (Johnson et al. 2017).

Since 1989, biologists have used PIT tags to perform research on various aspects of bat ecology, often displaying higher redetection rates than traditional mark-recapture techniques with lower long-term project costs (Barnard 1989, Kerth and König 1999, Ellison et al. 2007, Garroway and Broders 2007, Kerth et al. 2011, O’Shea et al. 2011, Johnson et al. 2012b). Our research differs from previous PIT tagging studies in that it is the first wildlife study, to our knowledge, to utilize high-frequency technology, enabling the ability to record up to 100 tags at each antenna. Handheld thermal monoculars allowed us to observe and record bats independent of ambient light. While (Kunz et al. 2009) recommended the potential of using handheld thermal monoculars to count emerging bats, he also cautioned that technology suitable for bat research costs anywhere from $60,000 to $100,000. Fortunately, recent technological advancements have lowered the cost of devices ideal for performing such research to a manageable $3,000. The loss or destruction of tags, although relatively rare (1.6% in O’Shea et al. 2004), is a concern for most PIT tagging studies. Since we did not double-mark captured bats during this study, we are unfortunately unable to assess the proportion of bats in our population that
have lost tags. From thoroughly scanning all roosts in 2018 with a handheld RFID scanner, we have discovered a single rejected HF-PIT tag in an attic. This HF-PIT tag was regularly detected at antennas for 8 days after the bat was tagged and was presumably lost around this time.

Anthropogenic or natural spreads of invasive pathogens, such as WNS, possess a substantial risk to biodiversity and ecosystem integrity, which may result in unpredictable changes to ecosystem and function in a multitude of communities within Yellowstone (Frick et al. 2010a). With the recent detections of *Pseudogymnoascus destructans*, the fungus that causes WNS, in Washington, Wyoming, and South Dakota, an increased risk of WNS soon affecting little brown myotis in Yellowstone makes documenting current population sizes and behaviors all the more important. Should WNS enter Yellowstone, biologists are now equipped with a proven long-term monitoring system, and new techniques to create population benchmarks, enhancing their ability to determine when population metrics have deviated significantly from the norm. In addition, the continuous tracking of populations, movements, and roost fidelity will lead to a better understanding of life histories, social strategies, and community processes for little brown myotis in Yellowstone, further enhancing our ability to make informed conservation and management decisions (Schradin and Hayes 2017).
Figure 5. Map of three distinct monitoring sites of female little brown myotis within Yellowstone National Park (denoted by black stars). In each of these areas, RFID-monitoring equipment is installed in the primary roost most used by bats in the area.
Figure 6. Screen capture of bats emerging from Lamar Valley RFID-monitored roost at sunset using a FLIR Scout III 640 thermal monocular. Footage was recorded in the black and white infrared mode at 640 x 480 resolution, at 30 frames per second. Bats emerging for the first time in the night are identified by their warm, and consequently, black wing membranes. Bats that have been exposed to night temperatures for approximately 10 seconds or more, varying slightly with ambient temperature, are identified by their cold, and consequently, white membranes.
Figure 7. Percentage of bats redetected in 2017 and 2018 based on the year that they were captured at the A) Mammoth and B) Lamar Valley roosts.
Figure 8. Roost fidelity during the 2017 and 2018 maternity season of bats captured and detected at the Mammoth and Lamar Valley roosts. In Lamar Valley, average fidelity was 36.8% \((n = 101; \text{range} = 1.9\% - 95.5\%)\) of the 115 days monitored. At Mammoth, average fidelity was 33.2% \((n = 91; \text{range} = 1.4\% - 81.7\%)\) of the 123 days monitored.
A

Emergence Counts | Population Estimate | 95% Confidence Interval

B

Emergence Counts | Population Estimate | 95% Confidence Interval
Figure 9. Pre-parturition population estimates and corresponding emergence counts at A) Mammoth in 2017, B) Mammoth in 2018, and C) Lamar Valley in 2018. Population estimates and associated 95% confidence intervals represent a single estimate of the population over the entire maternity season (1 primary occasion).
CHAPTER 4: CONCLUSION

As presented in detail throughout Chapters 2 and 3, my thesis research resulted in the successful designed and construction of a long-term monitoring system used to study little brown myotis (Myotis lucifugus) in Yellowstone National Park. I used this system to estimate population sizes and answer several ecological questions, creating a framework for future studies. Being able to effectively estimate summer populations of bats is especially vital for biologists working in regions that lack known hibernacula or any reliable method to monitor their bat populations, such as Yellowstone. However, even biologists working in regions where monitoring winter populations is possible can benefit from studying summer populations, as my thesis describes. This is because winter and summer populations of bats are often composed of different individuals. Thus, the ability to better study bats during summer provides a more comprehensive picture of the ecology of bats in a system. As a result, management decisions regarding summer populations of bats can now be formed using summer data from these same populations.

While this thesis shares my findings regarding population sizes, roost fidelity, roost connectivity, and seasonal movements, this is truly just the beginning of questions that these novel techniques can help answer. An important contribution of my research is that our long-term monitoring system was designed to operate continuously, ever second of every day, without any human interaction for the foreseeable future. This longevity and efficiency make these tools relatively cost-effective, especially when compared to currently used mark-recapture techniques. In addition, because the methods I have used only require that each individual bat is captured a single time, these methods produce
lower levels of disturbance while dramatically increasing the probability that a bat will be redetected. While both Chapters focused on applying our new technology to study bats, these methods can be applied to investigate of a wide variety of questions across many taxa. In particular, these techniques can be used to greatly enhance research performed on small gregarious mammals that form large congregations.

I look forward to monitoring how aspects of bat ecology will change across time and space in Yellowstone. By continuing to collect data on tagged bats, we will soon be able to estimate apparent survival rates and improve our understanding about the population structure at each of our study locations. Since bats older than several months old are difficult to be reliably differentiated from adults, I began tagging juveniles in the summer of 2018. Monitoring these juveniles, and future cohorts will allow for estimation of age-specific mortality annually. These data are of considerable interest due to the long lifespans of bats, which have long been recognized, but poorly understood. Plans are in progress to improve our ability to estimate population sizes by installing infrared video cameras inside the attics of our RFID-monitored roosts to record and monitor the daily emergence of bats. These cameras, used in combination with the RFID readers, will provide biologists with the number and proportion of marked and unmarked bats that emerge through primary entryway antenna every day of the maternity season. Aside from removing the human effort previously required to perform emergence counts, this increase in data available for our mark-resight analyses should increase the accuracy of our population estimates and decrease the width of our 95% confidence intervals.
In addition to assisting Yellowstone biologists monitor and study bat populations, this monitoring system will also help ameliorate human-wildlife conflicts. Quantifying the importance of specific roosts to bats is valuable because the presence of bats in Yellowstone buildings is often a contentious issue. Thus, our findings regarding the importance of roosts to population sizes provides Yellowstone biologists with concrete knowledge of what will happen to little brown myotis if they are evicted from specific buildings. Finally, by pairing these population estimates to other environmental monitoring research performed in Yellowstone, biologists will be able to test specific hypotheses to help determine the variables driving any future changes observed in these populations. If white-nose syndrome (WNS) or any population-decreasing threat affects Yellowstone, biologists will now receive early warning of declines of little brown myotis, enhancing their ability to make informed conservation and management decisions for all of Yellowstone’s 13 species of bats. Since this monitoring system was installed before the emergence of WNS, the abundance and ecology data recorded from this study would also be critical for comparing little brown myotis populations and behaviors in a pre- and post-WNS landscape.
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