July 2013

Assessing and Analyzing Bat Activity with Acoustic Monitoring: Challenges and Interpretations

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A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

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ASSESSING AND ANALYZING BAT ACTIVITY WITH ACOUSTIC
MONITORING: CHALLENGES AND INTERPRETATIONS

(Thesis format: Integrated Article)

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

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Abstract

Acoustic monitoring is a powerful technique for learning about the ecology of bats, but understanding sources of variation in the data collected is important for unbiased interpretation. The objectives of this dissertation were to investigate sources of variation in acoustic monitoring and make recommendations for acoustic survey design and analysis. I addressed this goal in three ways: i) variation resulting from differences in bat detectors, ii) methods for objective identification of peak activity, and iii) the use of stationary transects to address within-site spatial variation.

First, I compared variation of detection of echolocation calls among commonly available bat detectors and found significant differences in distance and angle of detection. Consequently, this source of variation should be taken into account when comparing datasets obtained with different systems. Furthermore, choice of detector should be taken into account when designing new studies.

Second, I investigated two statistical methods for identifying peaks in activity, percentile thresholds and space-time scan statistic (SaTScan). Acoustic monitoring provides a relative measure of activity levels and is rarely evaluated based on objective criteria, so describing bat activity as "high" or "low" is useful only in context of the studies in question. Percentile thresholds allow for peaks to be identified relative to a larger distribution of activity levels. SaTScan identifies peaks in space and time that are significantly higher than the background expectation of the dataset. Both methods are valuable tools for replicable and objective identification of peak activity that can be applied at various temporal and spatial scales.

Third, I examine how within-site spatial variation can impact estimates of bat activity. I used a stationary transect of bat detectors to i) assess variation in patterns of activity at each detector, ii) test whether spatial or temporal factors were more important for explaining variation in activity, iii) explore what sampling effort in space and time is required for species-specific activity levels. The picture of activity differs significantly within a site depending on detector placement so it is important to use multiple detectors simultaneously to collect accurate estimates of activity.

Keywords

Co-Authorship Statement

A version of Chapter 2 was published in the *Methods in Ecology and Evolution* with Meredith Jantzen, Rachel Hamilton, and Brock Fenton as co-authors. Ms. Jantzen and Ms. Hamilton contributed to study design, data collection, and data interpretation. Dr. Fenton provided access to equipment. All authors contributed editorial comments on the manuscript.

A version of Chapter 3 has been submitted for publication to *Acta Chiropterologica* with Liam McGuire, Lauren Hooton, and Brock Fenton as co-authors. Dr. McGuire provided logistical support in Saskatchewan and contributed to interpretation of the data. Ms. Hooton helped create the discriminant function analysis for species identification of acoustic data. Dr. Fenton provided access to the acoustic equipment and funding. All authors contributed editorial comments on the manuscript.

A version of Chapter 4 has been submitted for publication to the *Journal of Applied Ecology* with Brock Fenton. Dr. Fenton provided equipment, funding, and editorial comments on the manuscript.

A version of Chapter 5 will be submitted for publication to *Ecology* with Brock Fenton. Dr. Fenton provided funding, equipment, and contributed to study design and editorial comments on the manuscript.

Acknowledgments

First and foremost, I would like to acknowledge my supervisor, Dr. Brock Fenton, for his guidance and support. His limitless enthusiasm and curiosity has motivated me as an academic. Brock's passion for teaching, science, bats, and lifelong learning goes beyond inspiring. Thank you for the opportunities you have opened and for never giving up on me. Some good.

The Department of Biology has been a wonderful environment to work in as a graduate student and I appreciate all of the faculty who took time to support me. I would like to thank the members of my advisory committee, Drs. Nusha Keyghobadi and Jeremy McNeil, for their recommendations, questions, and encouragement. Thanks to Ben Rubin for always being willing to sit and talk about bats and stats with me. I am grateful for my involvement with the Centre for Environment and Sustainability. I had the opportunity to be part of a community of diverse and intelligent people from a wide range of disciplines. Thank you to Dr. Dan Shrubsole who never doubted me and was always happy to talk about anything. Also, thanks to Holly Sanderson for her support in many of my endeavors.

I am grateful for all of my friends and colleagues at Western. Thank you to my labmates, my family while in Canada: Dr. Signe Brinkløv, Lindsey Clairemont, Dr. Beth Clare, Matthew Emrich, Dr. Erin Fraser, Rachel Hamilton, Colin Hayward, Lauren Hooton, Meredith Jantzen, Louis Lazure, Dr. Liam McGuire, Dara Orbach, Leslie Reynolds, Aline Simões, Dr. Mark Skowronski, Toby Thorne, and Nina Veselka. They have been friends, collaborators, and valuable resources. Without their support I would not have made it

conversations I've had were while working. The encouragement everyone showed for their resident "bat lady" made a difference. Being asked, "How are the bats," several times a week let me know you care and I appreciate everyone who was rooting for me to finish.

Finally, I would like to thank all of my family. Thank you especially to my parents, Emily, Grandma Barbara, and Laura Gomez, for their continued support and encouragement. While everyone else thinks I am crazy for being in school for so long their ongoing reminders of how proud they are of me has served as motivation to persevere. I am forever grateful to my parents for instilling love and respect for nature in me. I would not be where I am today without them, even if my dad still doesn't understand my obsession with bats.

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Chapter 1

1 Introduction

1.1 Conservation and ecological sampling

Preserving biodiversity is a fundamental goal of conservation, but many challenges remain, particularly because of a lack of information about most species. Insufficient knowledge about distribution and natural history are major hurdles because wildlife management and monitoring are basic requirements for the conservation of species. We have to know what is present to conserve it, but collecting realistic data on organisms can be challenging to researchers and conservation efforts. Rare, elusive, and cryptic species can be difficult to sample and while the presence of a species can be confirmed, its absence can only be inferred with a degree of probability (Kéry 2002). Field studies of organisms may be hampered by our limited ability to observe them and/or access their habitats. However, some of these obstacles have been resolved, at least in part, by technological advances (e.g., radio telemetry; Cagnacci *et al.* 2010), but at the same time these techniques may create new challenges to consider.

Ecological studies use many approaches at different spatial and temporal scales to address questions relating to species distribution and abundance. Sampling can provide an estimate rather than an exact measure of what is in the environment, and this is further limited by the trade-off between sampling effort and accuracy of the data obtained. Effort is limited by factors such as time, money, manpower, and habitat accessibility, while accuracy will depend on the sampling effort actually invested. It is important to recognize that balancing the trade-off between effort and accuracy comes from understanding of the

focal organism and complexity of the study system (Loehle 2004). Spatial and temporal complexity will impact the study scale, but in the end, there is no single scale that is appropriate for all ecological studies (Levin 1992).

The ability to address sources of bias will impact data accuracy and play a role in determining investment in sampling effort. Bias can arise from poor practical techniques or non-representative sampling (Sutherland 2006) related to the type of sampling method employed (e.g., visual, capture, acoustic). Poor technique, such as missed direct observations of individuals, can obviously impact the quality of data. Non-representative sampling can cause bias, for example if it is assumed that a single sampling technique will detect all individuals in a population or species in a community equally. Taking into account that variation in the probability of detection may differ among habitats, seasons, species, age or sex of individuals within a species, as well as the sampling techniques deployed, is essential to minimizing the chances of collecting biased data.

For example, visually conspicuous species are often overrepresented, so one must use sampling methods that account for those that cannot simply be seen when walking a transect (e.g., birds and bats in mist-nets, traps or fogging for insects). However, these specialized capture techniques are invasive, as well as time and labor intensive. Acoustic methods provide an efficient, non-intrusive way to study species that use auditory signals, although these record disproportionately more species with high-intensity calls and provide no data on actual population sizes (Flaquer *et al.* 2007). Consequently, to minimize bias, many researchers stress the importance of using multiple sampling techniques simultaneously (Kalko *et al.* 1996, O'Farrell and Gannon 1999, Duffy *et al.* 2000, Milne *et al.* 2004).

Many organisms produce auditory signals, produced intentionally for communication or orientation or unintentionally as a byproduct of activities such as feeding or movement, which researchers can use to document ecology and behavior. Conspicuous auditory signals are one means of detecting some otherwise cryptic organisms and bioacoustics research is an integral part of conservation plans for many animals (Baptista and Gaunt 1997).

1.2   Acoustic monitoring

Acoustic monitoring is fundamental for the study of many organisms traditionally sampled by visual or capture techniques. Songs or calls can be highly reliable taxonomic features, especially for anurans (Taylor *et al.* 1996), bats (Fenton and Bell 1981), birds (Parker 1991, Somervuo *et al.* 2006), cetaceans (Oswald *et al.* 2003), and insects (Riede 1998, Chesmore and Ohya 2004). Acoustic sampling is an important tool for wildlife management and conservation because it can estimate diversity and relative abundances.

Detection of species that can easily be heard but are not easily seen is the true power of acoustic monitoring. For example, high flying bats, such as *Lasiurus cinereus* and *Eurderma maculata*, are rarely included in capture inventories, but are readily detectable in acoustic surveys (O'Farrell and Gannon 1999). Acoustic surveys can provide more accurate estimates of diversity than capture techniques (Dawson and Efford 2009) and thus provide the opportunity to learn about organisms from the community to the individual level.

1.2.1   Species identification

Identification of organisms to species based on their acoustic signals allows researchers to

quickly survey the biodiversity of regions (Riede 1998), leading to more accurate species

counts and occupancy estimates (Brandes 2008). Birds are perhaps the best known group

where species identification with acoustic signals provides the most effective sampling

approach (Parker 1991, Riede 1993). For example, Parker (1991) recorded the

vocalizations of 85% of the 287 bird species present in the Bolivian Amazon in seven

days, compared with 54 days when using captures. The North American Breeding Bird

Survey (BBS) heavily relies on acoustic identification and has provided a wealth of

information on bird populations and relative abundances, which is used in several

conservation efforts (Sauer *et al.* 2003). Standardized protocols incorporating acoustic

monitoring, such as the BBS, have provided a powerful tool for management and

conservation at various geographic scales.

1.2.2   Environmental quality

Bioindicators are species or communities sensitive to identified environmental stressors

or disturbances that may be used to assess the quality of the environment and/or record

changes over time (Jones *et al.* 2009). Indicators of environmental change, such as shifts

in community structure and species diversity, should be included in conservation plans

(Lim and Engstrom 2001). Species that can be monitored acoustically are invaluable

indicators of habitat quality because of less invasive sampling methods. Birds and

invertebrates are most commonly used as bioindicators (e.g., Browder *et al.* 2002,

Mankin *et al.* 2010). For example, acoustic studies of orthopteran communities have been

useful indicators of eutrophication (Fischer *et al.* 1997) and bats are good indicators of

habitat quality (Wickramasinghe *et al.* 2003, Kalcounis-Rueppell *et al.* 2007).

1.2.3   Individuals and population structure

Sounds can be used to evaluate fine scale information about communities, including

structure of populations and identification of individuals. The low-frequency

vocalizations of elephants can vary with group size, composition, and reproductive status,

so recordings could provide valuable information on abundance and population structure,

especially when they live in densely forested areas (Payne *et al.* 2003). Similarly, blue

whale songs can be divided into regional types which can be used to characterize

population structure (McDonald *et al.* 2006). Bird dialects can also play an important role

in conservation, from obtaining demographic information (Laiolo and Tella 2008) to

impacting translocation efforts (e.g., Bradley 2012). Some animals can be identified to the

individual-level based on their acoustic signals (e.g., fallow deer, Reby *et al.* 1998; Stellar

sea lions, Campbell *et al.* 2002; African wild dog, Hartwig 2005), which can be used for

identification in place of physical marks (i.e., tags and bands; Laiolo 2010), providing

important information for population monitoring. A paucity of data on individual

variation currently precludes identification to the individual level in animals such as

insects, anurans, and bats (Obrist 1995, Chesmore 2001).

1.2.4   Technology: advancements and limitations

No matter the focal organism of an acoustic study, technological achievements strongly

influence the feasibility of using acoustic techniques. Advances in technology and

software have allowed researchers to overcome difficulties with data collection and

storage, making it possible to monitor previously inaccessible habitats and develop a

better "picture" of biodiversity. Acoustic studies are not without drawbacks. Analysis of

acoustic data can be extremely slow when done manually, requiring highly trained

personnel (Chesmore 2004) and limited by the availability of an accurate reference library

(Riede 1998). Manual identification of species relies on expert knowledge, which is

inherently subjective. Possibly the greatest drawback is the inability to count individuals

using acoustic methods (Brandes 2008), as activity does not equal abundance (Hayes

2000). It is not possible to determine whether sounds are coming from one or multiple

individuals moving past a microphone, so acoustic data can only provide a relative index

of activity levels (Hayes 2000). Interpretation of acoustic data often relies on descriptive

terminology, such as "higher" or "lower," limiting the accuracy of conclusions. There is

no framework or guideline on how to interpret specific activity levels beyond relative

differences in activity from one sample to the next (Kunz *et al.* 2007b).

1.2.5   High frequency sounds

While many sounds recorded during acoustic monitoring are audible to humans, others

are not. Toothed whales (Odontoceti; e.g., dolphins) and bats (Chiroptera) are two taxa

that use echolocation for communication, foraging, and orientation by emitting pulses of

high frequency sound and gathering information based on the returning echoes. Signals

produced by odontocetes travel through water at a speed about four times faster than if

produced in air. Dolphins are able to detect small targets from a few meters (Kastelein *et

al.* 1999) to over a hundred meters away (Au 1993). Most dolphins emit a combination of

whistles and clicks that do not change in duration or shape. The central frequency

depends on the intensity of the signal, ranging from 30-60 kHz (low intensity) to ~100

kHz (high intensity, Au 1993). Bat echolocation has a much slower rate of information

transfer than odontocetes because their signals move through air. Bat calls are much more diverse in structure than odontocete signals, varying in duration, from 0.3 to 300 ms; in frequency, from 12 to >200 kHz; and in shape, ranging from broadband to constant frequency (Neuweiler 1989). Detecting both dolphin and bat echolocation calls requires a researcher to rely on technology capable of detecting high frequency sounds. Bats provide additional challenges as they are small, nocturnal animals that cannot be sampled visually and capture success is limited to low flying species.

1.3   Monitoring bats

Our knowledge of bats and their natural history largely arises from the research of Lazaro Spallanzani in 1794, who provided the first evidence that bats use sounds for orientation and obstacle avoidance. While Spallanzani's methods of blinding and deafening bats were crude, he provided the basis for what Griffin (1944) later coined as "echolocation." Griffin (1958) was the first to use a "sonic amplifier" to investigate ultrasonic sounds. This detector was able to show pulse repetition rates, but the next development in detectors showed that these pulses were actually frequency-modulated calls (Griffin 2004). In 1951, when Griffin first made recordings in the field, bat detectors were still barely portable, very expensive, and extremely fragile. Griffin required the use of a station wagon to transport all the necessary equipment. Visual outputs from an oscilloscope were recorded by a video camera and audio output from a portable AM radio (Griffin 2004). Improvements, which led to the first "true" bat detector, allowed for a portable instrument that produced an audio signal of the ultrasonic pulses (Griffin 2004).

Specialized microphones convert high frequency sounds to electric signals, which are then recorded and transformed for storage, playback, and analysis. Bat detectors can be

heterodyne or broadband. Heterodyne detectors allow users to tune in to a specific, narrow range of frequencies. The heterodyne detector converts high frequency sounds to the audible range in real time. The narrow frequency range is limiting, but it provides a quick method to listen for bat presence. Heterodyne detectors provide no information on call structure and require extensive training for species identification in the field. More complete bat survey efforts require broadband sampling because the calls of species in a single community can cover a wide range of frequencies.

The maximum recorded frequency can be no more than half the sampling rate. Originally storage devices were not fast enough to record echolocation calls at a high enough sampling rate, so signals had to be brought down to a frequency range that could be analyzed. Frequency division and time expansion methods allow broadband recording and transformation of high frequencies for users to listen to at the audible frequency range (Parsons and Obrist 2004). Frequency division decreases an incoming signal frequency (kHz) to bring it into the audible range by dividing the frequency by a preset value. A drawback is that all amplitude information is lost from the recorded call and only a single harmonic is analyzed. Time expansion plays signals back at a slower speed by recording a broadband signal and playing it back with the call duration increased and frequencies decreased (Pettersson 2004). Slowed signals are able to be processed at a lower sampling rate, while preserving all call information. The system records and stores a signal, then plays it back, but during playback, the system cannot also be recording, limiting recording for only part of the available time (Parsons and Obrist 2004).While time expansion systems make high quality recordings, including the amplitude and spectral component information, the drawback is they are not capable of sampling continuously.

Until the 1980s, technology was the bottleneck to addressing many questions in acoustic monitoring. Field research of bats flourished once more portable detectors became available, and it was determined that bat species could be identified by their echolocation calls (Ahlén 1981, Fenton and Bell 1981). Recordings were originally made on analogue tape recorders, but by the mid-1980s they could be stored digitally. Once sound cards were capable of higher sampling rates, high frequency signals could be recorded directly without transformations.

1.3.1　Acoustic monitoring today

Today, devices primarily use one of two methods to analyze digitally recorded calls: zero-crossing and Fourier analyses. Zero-crossing analysis, used to analyze frequency division signals, is advantageous because it is simple and fast with low digital storage requirements. However, it suffers from the same limitations as frequency division: loss of amplitude information and analysis of a single harmonic. Fourier analysis, or spectral analysis, is the more common method of analysis for detectors, used for time expansion and untransformed, real-time signals. It generates two outputs: power spectrum and spectrogram, giving information on amplitude, frequency, and time aspects of echolocation calls. Fourier analysis calculates frequency information by averaging blocks of data across the call. Using more blocks of data increases the accuracy of frequency information, but decreases time resolution because of an inverse relationship between frequency and time (Parsons *et al.* 2000). Very little information is lost during spectral analysis and it is relatively insensitive to background noise, but analysis can be computationally demanding (Parsons *et al.* 2000, Parsons and Szewczak 2009).

An ongoing debate persists regarding the value of the two analysis methods. In practice, the quick, simplistic method of zero-crossing analysis detects fewer echolocation calls than the information-rich spectral analysis. Echolocation call features (i.e., lowest frequency and duration) and sensitivity also differ between the two types of analysis (Fenton 2000, Fenton *et al.* 2001). Spectral analysis requires more data storage and battery life than zero-crossing analysis. Full-spectrum devices allow analysis of an entire echolocation call, including harmonics and amplitude information; because bats probably use the entire call they produce, including harmonics, it is important to understand and include the entire call during analysis (Griffin 2004).

There is now a wide variety of commercially available, inexpensive, portable bat detectors, which allow extensive study of bats through acoustic monitoring; more than 500 studies have been published since 2011. Digital technology and miniaturization of electronic components have made many field studies possible, and new computer software is making analysis of recorded signals even more efficient (Parsons and Obrist 2004). Large quantities of data are easily collected with acoustic methods, but processing and analysis of these immense datasets are still problematic. Analysis is moving towards full automation based on statistical models and computer programming techniques, but has not been commercialized in a robust enough manner to be adopted for standardized protocols.

1.4   Variation in acoustic monitoring

There are three levels of variation that can complicate acoustic data interpretation. First, variation is created by the movement of sounds through air. Second, the degree of variation detected can be affected by the equipment used. Lastly, there is variation with

respect to the animals themselves, from echolocation behavior to community-level activity patterns. No matter what the focus of the study, it is vital to recognize all of the possible sources of variation in a dataset.

1.4.1   Variation from attenuation

The movement of sound through air is affected by several factors. All sounds transmitted through air are subject to spreading loss, where sound waves spread out as they move away from the source and thus lose intensity with distance (independent of frequency, Griffin 1971). Atmospheric attenuation occurs when sounds are absorbed by atmospheric moisture which can be affected by the frequency at which the sounds are emitted, as well as humidity, and temperature. Higher frequency sounds have shorter wavelengths, resulting in greater attenuation (Griffin 1971, Lawrence and Simmons 1982), but yield more details to bats about their targets (Griffin 1958, Simmons and Stein 1980). The frequencies dominated in calls of many species (20-60 kHz) suggest a balance between call range due to attenuation and detection resolution (Fenton *et al.* 1998). Species with higher-frequency components in their calls will have a lower effective range of echolocation due to attenuation and thus may be more difficult to detect with detectors.

1.4.2   Variation from equipment

The variation resulting from the use of different equipment is controllable but often underappreciated, and must be accounted for when developing standardized protocols. This is especially true for acoustic bat detectors, as not all systems have the same sensitivity (Forbes and Newhook 1990, Waters and Walsh 1994, Fenton 2000, Fenton *et al.* 2001) or hear the same signals in the same way. There are differences between brands (Forbes and Newhook 1990, Waters and Walsh 1994) and even between individual

detectors of the same model (Larson and Hayes 2000). Differences between time expansion and zero-crossing analysis detectors can be as high as 19 dB in sensitivity, resulting in zero-crossing systems missing quite a high proportion of bat activity, even for species with relatively high-intensity calls because the detection distance of the detector will be shorter for less sensitive microphones (Fenton *et al.* 2001).

1.4.3   Variation from bats

An individual may vary the structure of their echolocation calls in response to habitat structure (Kalko and Schnitzler 1993, Broders *et al.* 2004), insect noise (Gillam and McCracken 2007), and other bats (Obrist 1995). We are only beginning to appreciate individual variation (Masters *et al.* 1995, Betts 1998, Fenton *et al.* 2004), and it seems unlikely that one could identify individuals from echolocation calls in the field as variation in response to ecological conditions overwhelms the amount of variation at the individual level.

Bat activity and community structure are variable in both space and time. Numerous extrinsic factors affect temporal activity patterns of bats, including insect abundance (Hayes 1997, Lee and McCracken 2002), air temperature (Kunz 1973, Lacki 1984, Negraeff and Brigham 1995, Hayes 1997), rainfall (Fenton *et al.* 1977, Parsons *et al.* 2003), relative humidity (Lacki 1984, Adam *et al.* 1994), and wind (Adam *et al.* 1994). Activity levels can vary annually (Milne 2006), seasonally (Russ *et al.* 2003, Milne *et al.* 2005, Johnson *et al.* 2011), among nights (Hayes and Adam 1996, Krusic *et al.* 1996, Hayes 1997, Broders 2003), and within nights (Maier 1992, Krusic *et al.* 1996, Hayes 1997, Milne 2006). Activity also varies at both large and small spatial scales. Patchiness in activity can be driven by congregations of bats at special locations at specific times of

the year. Some locations attract high numbers of bats, such as bodies of water, where insects are abundant. Maternity colonies, roosting, hibernation, and migration sites are all locations where bats congregate in greater densities and present higher activity levels. While variation among habitats has received the most attention (e.g., Krusic *et al.* 1996, Vaughan *et al.* 1997, Sherwin *et al.* 2000, Loeb and O'Keefe 2006), spatial variation within a site due to habitat heterogeneity is also an important source of variation that must be considered during surveys (Hayes 2000). Multiple bat detectors recording within a site can be important for reliable estimates of activity levels (Britzke 2003, Fischer *et al.* 2009). Flying bats move through three dimensional space and the vertical stratification of bat activity can vary with species and habitat structural complexity (Kalcounis *et al.* 1999, Hayes and Gruver 2000). Few studies have attempted to sample into the canopy because of the logistical limitations of sampling at greater heights (Kalko and Handley 2001, Lim and Engstrom 2001).

1.5   Bat conservation

Bats are an important group of mammals, serving vital ecological roles as nocturnal insect predators, pollinators, seed dispersers, and have been recognized as significant natural resources in recent years (Gannon *et al.* 2003). Management agencies now recognize the need for practical research of bats (Barclay and Brigham 1996) and that the ecosystem services provided by bats are being quantified (Boyles *et al.* 2011, Kunz *et al.* 2011). Boyles *et al.* (2011) estimate that the United States agricultural industry receives \$22.9 billion in ecosystem services annually from insectivorous bats as predators of many crop and forest pests.

1.5.1   Bats as bioindicators

Changes in community structure and species diversity may serve as indicators of

environmental change and should be included in conservation plans (Lim and Engstrom

2001). Bats have been identified as good candidates as bioindicators because of the ability

of researchers to monitor trends in populations, occupancy by bats of high trophic levels,

and widespread distribution of bat taxa (Jones *et al.* 2009). For example, bats are

ecological indicators of ecosystem disturbance (Fenton *et al.* 1992, Medellín *et al.* 2000),

habitat quality (Kalcounis-Rueppell *et al.* 2007), and have been included as one of the

United Kingdom's Biodiversity Indicators (Bat Conservation Trust 2011). Research

conducted with bat detectors informs our understanding of bat ecology and behavior and

is frequently used to guide important wildlife management decisions (U.S. Fish and

Wildlife Service 2012).

1.5.2   Current threats to bats

Over 1200 species of bats have been described (Simmons 2005) and 172 species are

threatened (i.e., vulnerable, endangered, or critically endangered) while five are now

extinct (IUCN 2012). Bat populations are being impacted around the world, from habitat

destruction to climate change (Jones *et al.* 2009), but two current threats facing bats today

are wind-energy developments and white-nose syndrome (WNS). Wind turbines are

responsible for the death of countless bats, especially for migratory species (Kunz *et al.*

2007a), due to direct collision with turbine blades (Horn *et al.* 2008, Rollins *et al.* 2012)

or pulmonary barotrauma as a result of the rapid drops in air-pressure near moving

turbine blades (Baerwald *et al.* 2008). Wind energy development is increasing around the

world, as are efforts to find viable mitigation options. There is a growing demand for

surveys at wind energy developments to assess potential risks to bats using both acoustic and capture methods, but these efforts are currently not guided by any standardized survey design (Kunz *et al.* 2007a,b).

White-nose syndrome is causing severe declines of bat populations in eastern North America, with many colonies decreasing by 99% within two years of infection (Frick *et al.* 2010). This fungal infection is caused by a cold-adapted fungus, *Geomyces destructans,* resulting in increased arousals during hibernation, leading to dehydration and depleted fat reserves (Cryan *et al.* 2010, Frick *et al.* 2010)*.* Since being identified in upper New York State in 2006, it has spread rapidly, directly by bats as well as by anthropogenic activity (Frick *et al.* 2010) through the persistence on equipment, clothing, and shoes. Acoustic monitoring is an effective way to document population declines without the risk of transmission of WNS with traditional capture methods (Brooks 2011, Dzal *et al.* 2011, Ford *et al.* 2011).

Given the importance of bats as bioindicators and worldwide decline in populations a global monitoring program with standardized methodologies is needed (Jones *et al.* 2009, Stahlschmidt and Brühl 2012). Lack of standardized protocols hinders the establishment of clear guidelines for surveys and effective regulation of assessment efforts by government, such as for wind energy developments. There are long-term monitoring efforts with standardized protocols in the United Kingdom (Walsh *et al.* 2001), which include acoustic surveys consisting of 1 km walking transects with frequency division detectors. New York State has implemented a standardized driving transect protocol to monitor bat populations post-WNS, which is now being adopted by other regions

although there is little research into the effectiveness of moving transects for sampling bat communities (Russ *et al.* 2003, Stahlschmidt and Brühl 2012).

1.5.3 Standardized protocols

Standardization of survey protocols is an important step to make applied research applicable to management and government policies, as the transition of scientific research to environmental policy decisions is often challenging. Policy based on scientific research is crucial for concerted efforts addressing increased conservation needs. Lack of standardization makes it virtually impossible to compare results, making standardized experimental approaches a necessity (Hayes 1997). Techniques to make monitoring more efficient and accurate will greatly benefit science and conservation efforts. Bats are critical to ecosystem function and the need for large-scale, standardized monitoring efforts is greater than ever before in the wake of threats, such as WNS and wind energy developments. Eventually, standardized protocols based on research will outline methods, from detector model, detector deployment, species identification, to statistical methods for evaluating data.

Some research has investigated variation affecting bat monitoring surveys and made recommendations for future surveys (Hayes 1997, Fischer *et al.* 2009, Skalak *et al.* 2012), but not enough studies have been published to provide a basis for standardized methods. Sources of variation — atmospheric attenuation, bat detectors, and bats — must be considered to collect unbiased acoustic data (Hayes 2000). While atmospheric attenuation cannot be controlled, variation from detectors and bats should be. Any standardized protocol must address and account for these sources of variation. In this dissertation my goal is to investigate these two sources of variation and propose methods for replicable,

objective evaluation of bat activity levels. It was my objective to address these specific questions related to the acoustic monitoring of bats: i) how does detector choice impact acoustic monitoring results?, ii) what is peak bat activity and how is it identified?, iii) how does detector placement impact the design and interpretation of acoustic monitoring studies?

1.6   Dissertation structure

Each chapter in my dissertation was prepared for independent publication. Chapter 2 has been published, Chapters 3 and 4 are in review for publication, and Chapter 5 is pending submission for publication. The four chapters are united by a common theme of investigating sources of variation in acoustic bat surveys in order to make recommendations for standardized sampling protocols. Below is a brief outline of each chapter.

In Chapter 2 (Do you hear what I hear? Implications of detector selection for passive acoustic monitoring of bats), I examined how several brands of bat detectors provide different depictions of the same dataset. The purpose was to quantify variation in detection performance among several commercially available bat detector systems and investigate if this is an important source of variation in acoustic monitoring methods. With an unprecedented variety of detectors available, it is crucial for researchers and management officials to understand differences in the performance of each of the detectors, both while selecting detectors to use and interpreting results from studies using different detector brands. I conducted a two-part study using five passive bat detectors: first, a controlled experiment with synthetic calls recorded at fixed frequencies, distances, and angles; and second, a field experiment recording free-flying bats.

In Chapter 3 (How high is high? Using percentile thresholds to identify peak bat activity), I address the question, "What is peak activity and how is it identified?" and illustrate a method for classifying activity levels and investigate how patterns and peaks of activity vary among sites. Describing bat activity as "high" or "low" is useful only in context and is rarely evaluated based on objective criteria. I recorded acoustic bat activity at three sites, spanning a range of situations. I calculated species-specific thresholds of activity levels at six (25th - 99th) percentiles derived from a larger distribution of activity levels among all sites. I used these percentile thresholds to identify important sites for each species based on where I found high activity, defined by objective criteria. It is important to have clear definitions of "high" activity, especially when making conservation and management decisions.

In Chapter 4 (Identifying peaks in bat activity: a new application of the space-time scan statistic), I address the same question as in Chapter 3, but present an alternate solution to the problem of identifying peak bat activity. I propose a new application for the space-time scan statistic (SaTScan) as an objective statistical technique for identifying peak periods of bat activity and compared it to the use of percentile thresholds, at three scales: within nights, among nights at a site, and among sites. I then experimentally tested SaTScan by analyzing species-specific activity at three sites. SaTScan has the potential to be a valuable tool for quickly identifying activity peaks with an objective, replicable, and statistically-sound method that can be applied at many temporal and spatial scales.

In Chapter 5 (Value in variation? Stationary acoustic transects to account for spatial variation in bat activity), I examine how horizontal and vertical variation within a site can impact estimates of bat activity. I measured bat activity with linear, stationary transects of

bat detectors at four sites in Ontario. I assessed variation in patterns and levels of bat activity at each detector with percentile thresholds and SaTScan. I also tested whether spatial or temporal factors were more important for explaining variation in activity. Lastly, I explored what sampling effort is required for species-specific activity levels at each site.

In Chapter 6, I conclude my dissertation with a summary of how addressing variation in acoustic bat surveys is necessary for accurate estimates of activity and make recommendations for sampling protocols. Finally, I highlight future directions for the study of bat ecology with the intention of better conservation and management.

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Chapter 2

2   Do you hear what I hear? Implications of detector selection for acoustic monitoring of bats[1]

2.1   Introduction

Echolocation provides a window through which the behavior and ecology of bats can be evaluated. Specifically, calls used by echolocating bats can be conspicuous to bat detectors, permitting biologists to distinguish among species by their calls and to identify foraging activity. Bat detectors, instruments sensitive to the acoustic frequencies dominating bat calls, have been extensively used in a range of bat studies, from those investigating echolocation behavior, to others documenting patterns of distribution and activity levels. By 2012, the variety of commercially available bat detectors offered a spectrum of features at a range of prices (e.g., weatherproofing, temperature sensors, storage options; Table 2.1) but key features, such as microphone quality, sampling rate, and recording technology will determine the ability to detect bats. Many published articles have used data from bat detectors to address questions about the echolocation behavior of bats, as well as their patterns of activity and habitat use (e.g., Gillam 2007, Collins and Jones 2009, Müller *et al.* 2012).

Table 2.1. A comparison of the features associated with commercially available ultrasonic bat detector systems.

System and manufacturer feature	AnaBat SD2 Titley Electronics	Avisoft UltraSoundGate 116 w CM16/CMPA Avisoft Bioacoustics	Batcorder 2.0 ecoObs	Batlogger Elekon AG	Song Meter SM2BAT 192 kHz Wildlife Acoustics
Recording technology	Zero-crossing	16-bit, full-spectrum	16-bit, full-spectrum	16-bit, full-spectrum	16-bit, full-spectrum
Sampling rate	N/A	500 kHz	500 kHz	312.5 kHz	192 kHz (384 kHz available)
Sound file type	AnaBat	.wav	.raw	.wav & .xml	.wav & .wac
Storage type	Compact Flash (CF)	External through computer	SDHC	SDHC	SDHC x 4
Storage capacity	128 GB	unlimited	16 GB	32 GB	128 GB
Battery	4 AA batteries	Runs off computer	NiMH 6V 2700mA, rechargeable	LIB 3.7V 4600mAh, rechargeable	4 D batteries
Microphone type	Condenser	Condenser	Electret	Electret	Electret
Omnidirectional microphone?	No	No	Yes	Yes	Yes
Recording schedule?	Yes, through CF Reader	Yes, through Avisoft-RECORDER software	Yes	Yes	Yes
Post-process tools	AnaLook	Avisoft-SASLab Pro	None	BatExplore	Batch noise scrubber, zero-cross converter, Wac2Wav converter
Channels	1	1	1	1	2
Weatherproof enclosure?	No	No	Yes	with StrongBox	Yes
Weatherproof microphone?	No	No	No	No	Yes
GPS?	Can connect externally	No	No	Yes	optional
Temperature sensor?	Internal temperature	No	No	External temperature	Internal and external
Price (USD)	$2,200†	$5999*	$3,273*‡	$2,035*‡	$999*

Pricing from company *website or †manufacturer. ‡Price converted to USD.

Acoustic sampling is a common, powerful technique for monitoring the activity of echolocating bats. Bat detectors are widely used by researchers, including those working for government agencies, environmental consulting firms, and academics. Behavioral, presence/absence, and relative abundance data are commonly collected with these devices. The results of research relying on bat detectors inform our understanding of bat ecology and behavior and are frequently used to guide important wildlife management decisions (U.S. Fish and Wildlife Service 2012). Acoustic monitoring is non-intrusive and capable of recording large quantities of data. However, the specific combination of hardware may affect the quality, precision, and quantity of data collected.

Variation in microphone sensitivity and detection algorithms can produce data sets that differ among detectors. Both Downes (1982) and Fenton (2000) noted significant differences in detection sensitivity among brands of narrowband and broadband acoustic detectors. This variation has the potential to affect acoustic monitoring studies and their conclusions; whether the focus is curiosity-driven research or environmental assessments where low bat activity is assumed to equal low numbers of bats and therefore low risk (U.S. Fish and Wildlife Service 2012).

The acoustic nature of bats is highly variable (e.g., frequency, intensity, etc.) which can influence detectability by even the 'best' detectors. Bats using low-intensity echolocation calls dominated by higher frequencies are less detectable than those using high-intensity calls dominated by lower frequencies. Higher-frequency sounds attenuate more quickly and will be detected less frequently than higher intensity, lower-frequency calls (Lawrence and Simmons 1982), resulting in under-representation of these species in

acoustic surveys (Murray *et al.* 2009). Detection bias will be further compounded by the sensitivity and frequency response of the bat detector. Different systems vary in their performance over the range of biologically relevant frequencies. If the microphone has lower sensitivity to high frequencies, the bias caused by atmospheric attenuation will be further exaggerated. The consequences of detection bias will depend on the community being studied; the frequencies of bat calls range from ~8 kHz to > 200 kHz, and this range varies with a given bat community. Researchers must consider the community in question when choosing the most appropriate bat-detecting system for their research (Limpens and McCracken 2004).

Microphones with lower sensitivity will detect bats at shorter distances relative to more sensitive microphones. Detectors with shorter detection ranges will sample a smaller airspace and thus have a lower probability of detecting any bats present. Also, not all detectors are equal in their directionality and the orientation of the detector in relation to the bat affects detection (Britzke *et al.* 2010). When all other factors are equal, detectors with omnidirectional microphones will have a better chance of detecting a bat, compared to more directional microphones. However, a less directional microphone will be less sensitive, giving it a smaller detection range (Limpens and McCracken 2004). The smaller the microphone, the more omnidirectional it will be.

Three levels of variation can confound data acquired with bat detectors. First is the variation associated with the movement of sound through air. Second is that intrinsic to the instruments. Third is variation in echolocation behavior and call design among bats. Whether the focus of a study is echolocation behavior or documenting patterns of habitat use, it is important to distinguish between factors two and three. We presented synthetic

acoustic signals and echolocation calls of free-flying bats in the wild to compare

ultrasonic call detection by five commercially available bat detectors. Our goal was to

provide data about relative bat detector performance and bat echolocation behavior.

With an increasing number of commercially available bat detectors, it is important to

address variation in the technologies. A fundamental factor of any methodology is

addressing the capabilities and limitations of the equipment being used. It is vital to be

aware of the differences that may result from the use of different equipment even when

the same sampling method is employed. To date, no study has examined the differences

in the detection efficacy among direct high-speed bat detector models.

2.2   Methods

We simultaneously deployed five direct high-speed bat detectors for recording both

synthetic playback and free-flying bats: AnaBat SD2 (Titley Scientific, Ballina, NSW,

Australia), Avisoft UltraSoundGate 116 CM16/CMPA (Avisoft Bioacoustics, Berlin,

Germany), Batcorder 2.0 (ecoObs, Nuremberg, Germany), Batlogger (Elekon AG,

Luzern, Switzerland), and Song Meter SM2BAT (Wildlife Acoustics, Inc, Concord,

MA). There are a several other commercially available detectors that we were unable to

include in this study, for example D500X and D1000X (Pettersson Elektronik) and

AR125 (Binary Acoustic Technology). During all trials, microphones were within 10 cm

of each other, on a parallel plane. Microphone order and position were rearranged

randomly for each trial to change microphone position, but maintain consistent

microphone spacing. We avoided variation by recording with only one detector of each

model and recording with all detectors at the same time.

2.2.1   Optimizing detector recording settings

We used playback of synthetic signals to optimize detection settings for each system. Our synthetic signal file was 1478 ms in duration, and consisted of 20, 57 ms long, constant frequency (CF) signals, five signals at each of four frequencies: 25, 55, 85, and 115 kHz. For playback, we used a laptop running Avisoft RECORDER-NiDAQmx software connected to an ultrasonic playback interface with an integrated D/A power amplifier (UltraSoundGate Player 116). The interface was connected to an UltraSoundGate Dynamic Speaker ScanSpeak (hardware and software: Avisoft Bioacoustics, Berlin, Germany), which we did not calibrate. When possible, we recorded with all combinations of setting configurations for each detector. When combinations were prohibitively large (>100) we recorded in intervals spanning the full range of configurations. For each configuration, we played synthetic signals 5 m from each device. We analyzed each recording visually to find the optimum settings for recording conditions. In cases where multiple configurations were equal, we chose the settings closest to the default settings for the detector. These settings were used for the remainder of our experiments (Table 2.2).

Table 2.2. Detector settings used in this study.

AnaBat SD2	Avisoft UltraSoundGate 116	Batcorder 2.0	Batlogger	Song Meter SM2BAT
Gain: 7	Gain: 7	Critical frequency: 14 kHz	Crest	Sampling rate: 192 kHz
Data Div - 16	Trigger: permanent	Threshold: -36 dB	minCrest: 5	Compression: WAC0
	(continuous)	Post trigger: 800 ms	minRMS: 2	Gain: 36 dB
	Sampling rate: 500 kHz	Quality: 40	minPeak: 5	Dig HPF: fs/16
	Format: 16 bit		HighPass: 6	Dig LPF: Off
	Buffer: 0.050			Trigger Level: 15 SNR
	No. Buffers: 4			Trigger Win Right: 1 s
				Div Ratio: 16

See each respective detector manual (available online) for the setting description.

2.2.2   Synthetic call playback

We played the synthetic CF signals three times at 5 m intervals (5 – 40 m) and three

angles (0°, 45°, 90°) in an open field. This resulted in 15 calls of each frequency played

at each distance and angle (24 combinations). We used the automated detection feature

(Table 2.3) of callViewer (v. 18, Skowronski and Fenton 2008), to count the number of

calls detected by each system and manually inspected each recording to ensure that there

were no false positives. CallViewer is a custom echolocation sound analysis program

written with MATLAB software (The MathWorks, Natick, Massachusetts). Because

AnaBat file formats are not compatible with callViewer software, we visually inspected

these recordings in AnaLook (v. 3.8, Titley Electronics, Ballina, Australia). We used

general linear models to analyze the number of signals detected (considering each

frequency separately) with angle, detector, distance and all two-way interactions. To

compare among detectors we generated pair-wise comparisons of the estimated marginal

means, controlling for the effect of distance and angle. We used a similar approach to

compare the effect among the three angles. We estimated the detection range by

modeling the probability of detection of each signal frequency at each angle by all

detectors with a logistic regression in PASW18 (SPSS Inc., Chicago, IL). From the fitted

logistic regression we determined the distance corresponding to a detection probability of

0.50 as our estimate of detection range (i.e., beyond this distance there is less than a 50%

chance that the signal would be detected).

Table 2.3. Automated detection parameter settings used for call analysis in callViewer.

Parameter	Setting
Minimum link length	10
Window length (ms)	0.3
Frame rate (fps)	10000
Chunk size (sec)	1
Minimum energy (dB)	14
Echo filter threshold (dB)	10
UPPER cutoff freq. (kHz)	Inf
LOWER cutoff freq. (kHz)	15
Window type	Blackman
Delta size (+/- frames)	1

2.2.3   Recording free-flying bats

Free-flying bats produce complex, frequency-modulated calls that vary in intensity in contrast to the simple, constant-frequency signals we used for the synthetic playback experiment. To introduce the variability that is present in natural settings we recorded free-flying bats. We deployed the detectors for two hours per night on three separate nights in a suburban area in London, Ontario, Canada. The Avisoft system detected more bat echolocation calls than any of the other detectors so we used the data from it as a baseline. We chose 26 easily identifiable passes (minimum seven consecutive calls), from hoary bats (*Lasiurus cinereus*), and counted the number of calls in each pass. We manually counted all calls recorded regardless of call quality or completeness. We used callViewer to analyze the full spectrum system calls and AnaLook to analyze calls from AnaBat. We calculated the proportion of calls detected per pass relative to Avisoft, arcsine-square root transformed the data, and compared detector performance with ANOVA and Tukey's post hoc test in PASW18 after finding no effect of recording night.

2.3 Results

2.3.1   Synthetic call playback

Overall, Avisoft detected the most signals (1067 signals, 25% of all signals presented), and AnaBat detected the fewest (240 signals, 5% of all presented). Avisoft was the only system that detected the 115 kHz signal and only at 5 m (Fig. 2.1A). AnaBat did not detect CF signals at 85 kHz and 115 kHz (Fig. 2.1E). The other detectors only recorded 85 kHz signals at 5 m, except Avisoft which recorded these signals at 10 m (Fig. 2.1). All

systems detected the 55 kHz signals, but detection range varied from 7 m to 16 m at 0°
(Fig. 2.2). Song Meter did not detect 115 kHz signals because the frequency is outside of
this model's detection capabilities. A model with a higher sampling frequency is
available and would likely have detected higher frequency signals.

The number of signals detected at 25 kHz varied significantly among detectors ($F_{4,348}$ =
21.32, $p < 0.001$; Fig. 2.3), except Batcorder and Song Meter. AnaBat recorded the
fewest 25 kHz CF signals. There were also differences among detectors in the number of
55 kHz signals detected ($F_{4,346}$ = 22.74, $p < 0.001$; Fig. 2.3); Avisoft recorded more than
Song Meter and AnaBat, while Batcorder recorded more than AnaBat. Batlogger
recorded significantly more signals than any other detector for at 25 kHz and 55 kHz.

There was a significant interaction between detector and distance for both 25 kHz and 55
kHz signals ($F_{4,348}$ = 9.42, $p < 0.001$; $F_{4,346}$ = 13.63, $p < 0.001$; Fig. 2.1). For 25 kHz,
Batcorder and Song Meter detections reflected a greater rate of attenuation with distance
than AnaBat, Avisoft, and Batlogger. For 55 kHz, AnaBat had the greatest rate of
attenuation with distance and Batlogger had the lowest (Fig. 2.1).

Overall, there was an effect of angle for both 25 kHz and 55 kHz signals ($F_{2,348}$=24.92,
$p<0.001$; $F_{2,346}$=21.06, $p<0.001$; Fig. 2.1); the number of signals detected declined as the
angle increased. The effect of angle was the same among all detectors ($p > 0.05$). There
was no interaction between angle and distance for 25 kHz signals ($p > 0.05$), but there
was an interaction for 55 kHz signals ($F_{2,346}$ = 12.62, $p < 0.001$). For 55 kHz signals,
there was no difference between 0° and 45°, but these two angles had a greater rate of
decline in number of signals over distance than 90°.
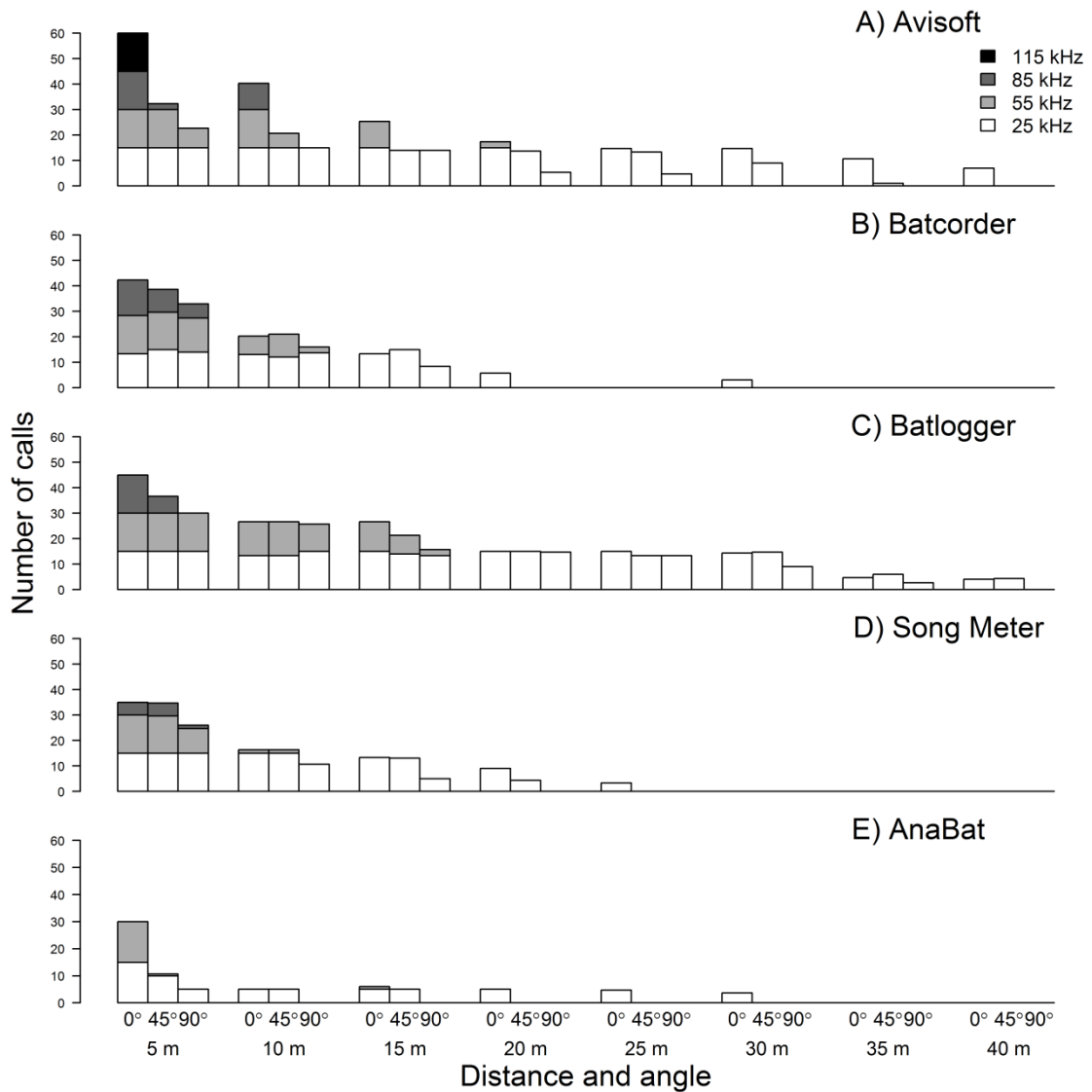
Figure 2.1. Mean number of calls detected by each bat detector system at four frequencies at each distance and angle during the synthetic playback experiment. There were 15 calls played for each frequency/distance/angle combination.
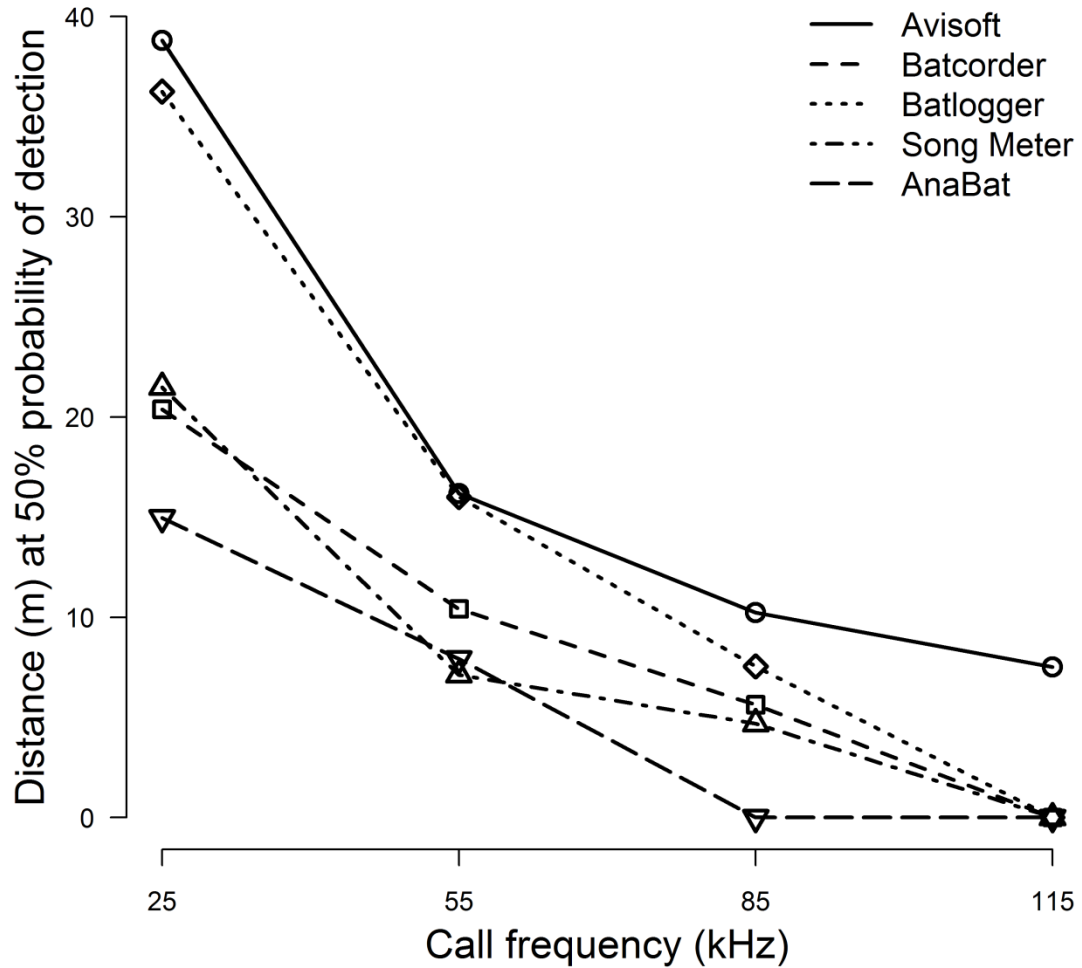
Figure 2.2. Distance of 50% probability of detection calculated with a logistic regression for each frequency at 0° by each bat detector system during the synthetic playback experiment. Patterns were similar for all detectors at 45° and 90°, but with lower overall probability of detection.
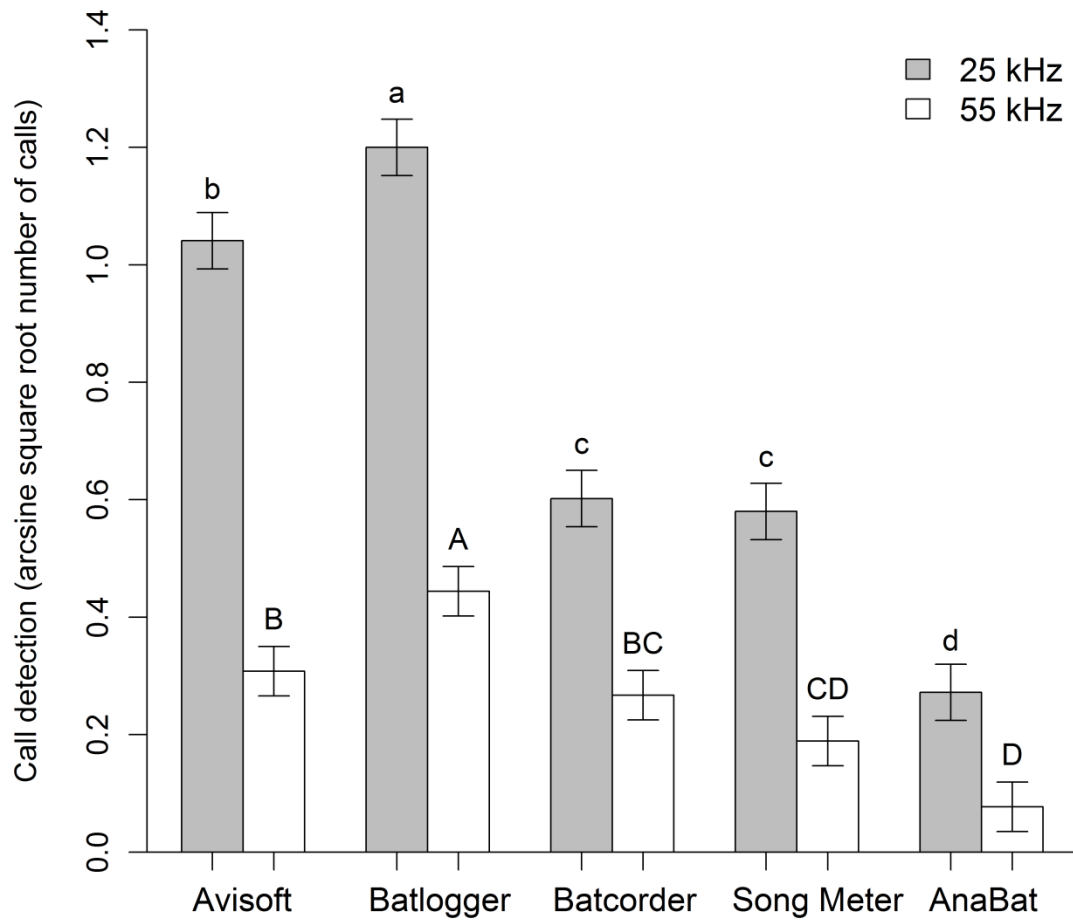
Figure 2.3. Performance varied among detectors with a strong effect of frequency. Call detection (arcsine square root transformed number of calls) ± SE by call frequency evaluated at a distance of 22.5 m. Detectors with the same letter superscript were not significantly different from each other within each frequency.

2.3.2　Recording free-flying bats

Batlogger recorded significantly more hoary bat echolocation calls (relative to Avisoft) than any other system ($F_{3, 100} = 45.26$, $p < 0.001$; Fig. 2.4), while AnaBat, Batcorder, and Song Meter did not differ significantly from each other. Only AnaBat and Batcorder failed to detect all 26 passes; both of these systems did not record any calls from two passes. One of the 26 passes included a feeding buzz that was recorded by all of the detectors. Avisoft, Batcorder, Batlogger, and Song Meter recorded more calls (23 – 25 calls) in the feeding buzz than AnaBat (11 calls).
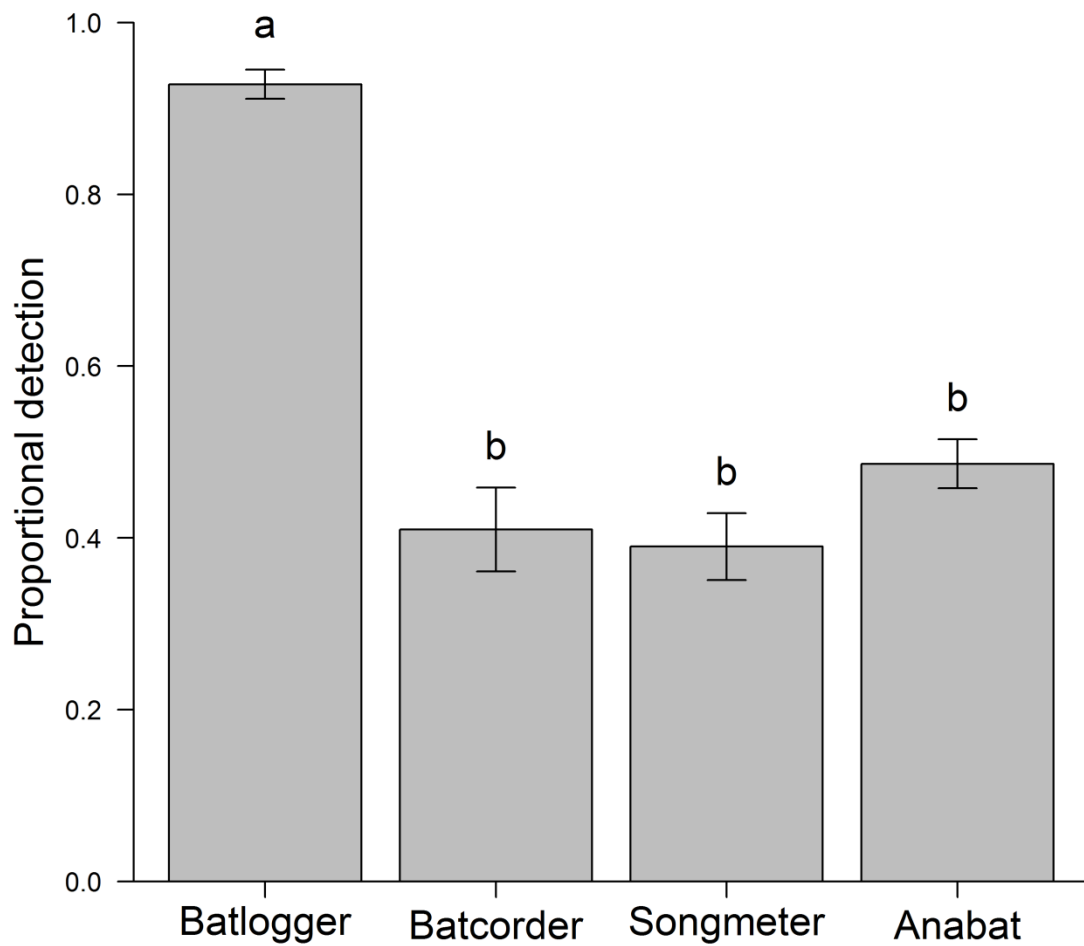
Figure 2.4. Mean number of calls ± SE per pass relative to Avisoft for each bat detector from recordings of free-flying *Lasiurus cinereus* on three nights. Batlogger detected more calls than any of the other systems (detectors with the same letter superscript were not significantly different from each other).

2.4 Discussion

Our results demonstrate that there is significant variation in detection efficacy among commercially available bat detectors. The differences in the detection abilities of these microphones, particularly in relation to differing frequency sensitivity, illustrate the hazards of comparing data collected by different detecting systems. Our results show that detection of different frequencies varied among detector systems and was affected by the distance and angle of the signal from the detector. Avisoft and Batlogger detected more of the highest frequency signals we tested than the other detectors, but as expected, these signals were detected at much shorter ranges. Detection distance for the 55 kHz synthetic signals (detected by all systems) is particularly relevant because this frequency is in the range of most species of bats that occur in temperate regions. In Hawaii, where only one species of bat occurs (*L. cinereus semotus*), any of the systems we used would suffice, although each would provide quite a different view of bat activity. In Newfoundland, where two species occur (*Myotis lucifugus, M. septentrionalis*) any of the systems we tested would suffice for *M. lucifugus* (echolocation call frequency of most energy ~40 kHz, maximum frequency ~81 kHz), but only some would accurately document activity by *M. septentrionalis,* which uses calls dominated by higher frequencies (frequency of most energy ~60 kHz, maximum frequency ~126 kHz; (Faure *et al.* 1993, Ratcliffe and Dawson 2003). In Newfoundland, some systems would be better than others. In other parts of the world, some bat species use echolocation calls dominated by frequencies >85 kHz. For these bat communities, the detection distance of the 85 kHz synthetic signals in our study is important to consider. Monitoring the activity of vespertilionid bats in the

subfamilies Kerivoulinae and Murininae would be difficult with any of the systems we tested because these species produce high frequency (80 – 200 kHz), frequency-modulated sweeps.

Variation in detection distance among detectors has important practical implications. For many studies, it is particularly important to understand the volume of airspace being sampled, such as when interpreting the results of pre-construction acoustic surveys conducted at potential wind energy facility sites where high bat mortality is a concern (Kunz *et al.* 2007). On modern wind turbines, the lower edge of the blade swept area is ~20 m above-ground (Barclay *et al.* 2007). Our data demonstrate detection ranges of 7 – 16 m, and therefore, none of the ground-based microphone systems we tested can detect bats flying in the area swept by the blades of wind turbines. Even a detector placed on the nacelle of a turbine (in the center of the blade swept area) would sample no more than one-third of the area swept by 50 m long blades (Kunz *et al.* 2007).

When we focus on detection of echolocation calls from free-flying bats, bat detectors fell into one of two performance groups. AnaBat, Batcorder, and Song Meter did not differ significantly in the number of hoary bat echolocation calls detected. These bats produce high intensity echolocation calls with a minimum frequency which is typically ~17 kHz (Obrist 1995). The minimum frequency of hoary bat calls is lower than the lowest frequency of our synthetic calls. Consequently, our free-flying bat results represent a best-case scenario; we used only high intensity, low-frequency calls and our sampling method, counting all calls regardless of quality, presented the most optimistic view of activity. In reality, many species are much less detectable and the quality of many recorded calls is too poor to be identified to species or counted as a bat call. Using

automated detection algorithms with recording quality standards will provide more objective call counts when measuring activity. If we had looked at passes from any of the Ontario *Myotis* species (calls with a minimum frequency range of ~34 - 40 kHz; Thomas *et al.* 1987), it is likely that the results from our free-flying passes would have mirrored the results from our synthetic call trials.

Among the detectors we tested, AnaBat is unique in that it is the only detector to use zero-crossing analysis which may (Corben and Fellers 2001) or may not (Fenton 2000) provide an adequate picture of bat activity. Our data contributes to this discussion, demonstrating that AnaBat is capable of performing similarly to a full-spectrum detector (Fig. 2.4), but in most cases it detects fewer calls (Fig. 2.3). Therefore, we emphasize the importance of considering the research questions and local bat fauna. While our results from the synthetic-call trials agree that full-spectrum detectors are more sensitive, our free-flying bat trial showed that there are circumstances where the differences are not substantial. Ultimately, the specific hypotheses and objectives of a study will dictate the suitability of various detectors (Limpens and McCracken 2004). No one recording system is ideal for all situations and thus it is the responsibility of the researcher (and the reader) to consider how the performance of the recording system will impact the results and conclusions of the study.

It is important to note that regardless of recording system, all microphones detect only a subset of the calls present in the environment (e.g. in our playback experiment the best system detected only 25% of the calls we played). However, our findings show that some subsets are significantly larger than others. This discrepancy is essential to remember when attempting to compare datasets collected with different detecting systems. Even

when comparing multiple detectors of the same model, the microphones must be calibrated to ensure comparable performance (Larson and Hayes 2000). With an increasing number of threats to bat populations (e.g., wind turbines, white-nose syndrome) there may be a drive to develop more rigorous monitoring programs with standardized protocols for bat surveys. Our results highlight the importance of considering the specific detector used, and the variation that may arise from different microphones.

As technology continues to evolve, the number of commercially available detectors will increase. As with the current proliferation in detectors on the market, many brands will persist (e.g., AnaBat, Avisoft) and new brands will emerge (e.g., Batlogger). In such a specialized market there will probably be few dramatic changes in the technology; we would expect to see increases in microphone sensitivity, battery life, and storage capacity, along with continued software upgrades to improve detection algorithms. With a high diversity of detectors, each with a wide range of settings and technical capabilities, it is now necessary to report not only the type of detector used, but also the settings chosen (e.g., Table 2.2) and as many hardware details as possible. The extent that detector-specific settings have on performance and accuracy between detectors of the same brand remains to be seen. Finally, it comes to the issue of comparability of results; different detectors will give different results, which must be taken into account.

Whether the bat-detecting system you are using hears the same signals as the one I am using depends upon the echolocation calls. There are numerous factors that contribute to variation in datasets from acoustic monitoring; our results demonstrate that the detector plays a role in this variation. Ultimately, it is crucial that differences in detector

performance be considered when designing studies and comparing results from different detectors, whether among models included in our study, other extant models, or those yet to be invented. No detector is ideal for all research questions and methods, and conversely, not all detectors are appropriate for a given question or methodology.

2.5   Acknowledgments

2.6   References

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Chapter 3

3 How high is high? Using percentile thresholds to identify peak bat activity[2]

3.1 Introduction

Researchers passively monitor bats by eavesdropping on their echolocation calls (Hooper 1966), providing insight into many aspects of ecology and behavior (e.g., Fenton 2003, Neuweiler 1989). Determining the timing and location of peak activity levels is important for an understanding of ecology and behavior, and has management implications. Activity levels of bats can vary dramatically temporally with increased activity during certain life history events or at ecologically relevant sites, such as riparian foraging areas (e.g., Rautenbach *et al.* 1996, Hayes 1997), maternity colonies (e.g., Murray and Kurta 2004), migration stopover sites (e.g., Barclay 1984, Dzal *et al.* 2009), or pre-hibernation swarming sites (Parsons *et al.* 2003). Conversely, areas used for commuting or dispersed foraging are likely to have lower activity levels.

There is no reliable way to convert the number of echolocation calls or passes recorded into the number of bats present, so acoustic monitoring only provides relative indications of low or high bat activity. However, these classifications are subjective and there is no framework or guideline with respect to interpreting activity levels, beyond activity in one sample being relatively higher than another (Kunz *et al.* 2007b). Previously, several

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[2] A version of this chapter is in review.

Citation: Adams. A. M., L. P. McGuire, L. A. Hooton, and M. B. Fenton. How high is high? Using percentile thresholds to identify peak bat activity. Acta Chiropterologica: in review.

methods have been used to identify peaks of activity, including visual identification with activity plots (Hayes 1997), choosing an arbitrary level of activity as 'high' (Broders 2003, Brooks and Ford 2005), or calculating the number of calls that are above a certain threshold (Gorresen *et al.* 2009, Hamilton 2012). Since acoustic monitoring provides a relative indication of bat activity it is important to define objective criteria for making comparisons among sites or time periods.

Our goal was to determine a method for objectively identifying peak bat activity with the purpose of examining the use of percentile thresholds for this task. Percentile thresholds have been used to identify high occupancy sites and make inferences about habitat attributes (Gorresen *et al.* 2009). Percentiles are simply 100 regular intervals in any cumulative distribution with the median at the 50th percentile where half of all observations fall below this threshold. Percentile thresholds are not affected when data differs in dispersion pattern, and are less susceptible to outliers than traditional statistics, such as analysis of variance (Sokal and Rohlf 1981). When applied to acoustic bat activity, the 99th percentile represents the most infrequent activity levels, with the greatest number of calls, where these periods of high activity only occur 1% of the time (e.g., Fig. 1). Ecological data are often overdispersed (O'Hara and Kotze 2010), with many observations with few calls and few with many calls, which violates key assumptions of common statistical methods. To illustrate the use of percentile thresholds, we used acoustic recordings from several sites in Canada. The aims of this paper are: (1) explore the suitability of percentile thresholds for identifying peak bat activity within- and among-nights and (2) compare the use of percentile thresholds to traditional statistical procedures.

3.2   Methods

3.2.1   Study areas

Data were recorded at (i) Long Point, Ontario, a 35 km long peninsula extending from the

north shore of Lake Erie, for six nights during both spring (June 2008) and autumn

migration (August/September 2008); (ii) an abandoned mine near Renfrew, Ontario for

five nights during both spring (May/June 2008) and autumn swarming (August 2008);

and (iii) along the Battle Creek in Cypress Hills Interprovincial Park, Saskatchewan for

five nights during summer (July 2009).

We considered three species in our analysis, *Lasiurus borealis, L. cinereus,* and *Myotis*

lucifugus. Given the migratory nature of *L. borealis* and *L. cinereus* we predicted that we

would observe high activity in autumn at Long Point as it is an important site for bats

during migratory periods (Dzal *et al.* 2009, McGuire *et al.* 2012). Cypress Hills is a

forested region where all three species occur (Willis and Brigham 2003, 2005), although

L. borealis is rare (Willis and Brigham 2003) and we expected this site to be the least

important to *L. borealis*. *Myotis lucifugus* is known to swarm and hibernate at Renfrew

(Fenton 1969), therefore we expected to observe high activity only during the autumn

swarming period.

3.2.2   Acoustic monitoring and analysis

We recorded continuously from dusk until dawn at all locations, on nights with no rain,

using externally polarized condenser microphones (Avisoft CM16/CMPA) connected to

an Avisoft UltraSoundGate 416-200 or UltraSoundGate 116 (Avisoft Bioacoustics,

Berlin, Germany) at 8 bit with a 250 kHz sampling rate, and gain at seven. The system was operated with Avisoft Recorder USG software.

We identified echolocation calls in all files using the automated detection feature in callViewer (v. 18; Skowronski 2008), a custom sound analysis program written with MATLAB software (The MathWorks, Natick, Massachusetts). We filtered the data to eliminate noise and weak or fragmented calls, only including detections with duration 0.99 – 30 ms and minimum frequency (Fmin) 15 - 60 kHz. The filter parameters were selected based on conservative estimates of the echolocation call structure of the bat species present at our recording sites. We identified calls to species using quadratic discriminant function analysis (DFA, Appendix A), which compared our unidentified data to a training dataset that included seven species: *Eptesicus fuscus, Lasionycteris noctivagans, L. borealis, L. cinereus, M. lucifugus, M. septentrionalis,* and *Perimyotis subflavus*. All species were weighted equally in the classification analysis. Classification of each call was based on 11 call parameters extracted by the automated detection feature of callViewer (Skowronski and Fenton 2008). Cross-validation indicated the species classification accuracy was greater than 88% for the three species included in our analysis (Table A1). To further improve classification accuracy, and because DFA does not assign calls to an "unknown" category, we applied a post-hoc, species-specific filter to remove any data that were above or below typical durations and minimum frequencies for each species (Table A2).

3.2.3   Statistical analysis

The sampling unit in our analyses was the number of calls of a given species recorded each hour. We also summed the calls per hour, of the seven species post-DFA and

filtering, for total number of calls per hour for all species combined. We calculated

percentile thresholds of activity for *L. borealis, L. cinereus, M. lucifugus,* and all species

combined based on the distribution of the number of calls per hour for all nights

regardless of site. We considered six thresholds, the 25th, 50th (median), 70th, 90th, 95th,

and 99th percentiles, for each species and all species combined. Passive acoustic

monitoring often includes many time periods with no echolocation calls recorded,

resulting in issues of zero-inflation in statistical analysis (McCullagh and Nelder 1989);

to avoid these issues, we excluded time periods when no bats were recorded, thus framing

our analysis in terms of ranking activity given the presence of bats. We recognize that

zero calls per hour is informative about activity levels at a site and differs from non-data,

but since thresholds are used as a measure of how high activity is it is not necessary to

have a descriptor when they are not present. The absence of echolocation calls in acoustic

recordings is unambiguous and therefore, does not need to be included in the definition of

relative activity level thresholds. By creating a large dataset of recordings from a wide

range of ecological situations we created a null distribution to generate percentile

thresholds. We then compared results from a particular site to the percentile thresholds of

the larger distribution. We counted the number of nights with at least one hour above

each percentile threshold. We also calculated differences in number of calls per hour,

including time periods with zero activity, among sites with Kruskal-Wallis ($\alpha = 0.05$)

using *kruskal.test* in R (v. 2.13.1; R Development Core Team 2011) and a pair-wise post-

hoc test (*kruskalmc* in pgirmess package in R; Giraudoux 2011) because data were

positively skewed, where many hours contained few echolocation calls.

We used the previously calculated percentile thresholds to evaluate patterns of within-night activity, when peaks occurred and the degree of variation within a night, specifically for *L. borealis* at Long Point and *M. lucifugus* at Renfrew. We identified when activity was over the median to see if peaks occurred at dusk (first hour of the night), in the middle of the night, or at dawn (within one hour of sunrise), looking for patterns of unimodal, bimodal, constant, or irregular activity. To measure the degree of variation of activity levels within-nights, we calculated the proportion of the night with activity above or below the median, where constant activity would always be above or below the median.

3.3 Results

3.3.1 Among sites

We recorded for a total of 258 hours and for all seven species combined, after excluding samples when no calls were detected, the resulting samples size was 219 hours. Half of the time which contained fewer than 265 calls per hour (50th percentile), while the top 10% of activity ranged from 4504 to 28358 calls per hour (above the 90th percentile, Table 3.1, Fig. 3.1). All sites had activity levels above the 50th percentile, but Renfrew was the only site to have activity above the 90th percentile threshold and even exceeded the 99th percentile threshold (Table 3.2A) during the swarming period, when activity was significantly higher than all other sites ($H_4 = 32.01$, $p < 0.001$; Table. 3.2A).

Table 3.1. Number of calls per hour at each percentile threshold for each species of bat and all species combined for a distribution of activity from three sites in Canada. Half of the activity falls below the 50th percentile (median) and 10% of activity is above the 90th percentile threshold.

	Number of calls at percentile threshold					
	25th	50th	70th	90th	95th	99th
Species combined	100	265	493	4504	10616	19510
Lasiurus borealis	7	15	31	66	81	158
L. cinereus	20	63	115	266	396	534
Myotis lucifugus	25	88	314	5818	11865	19404
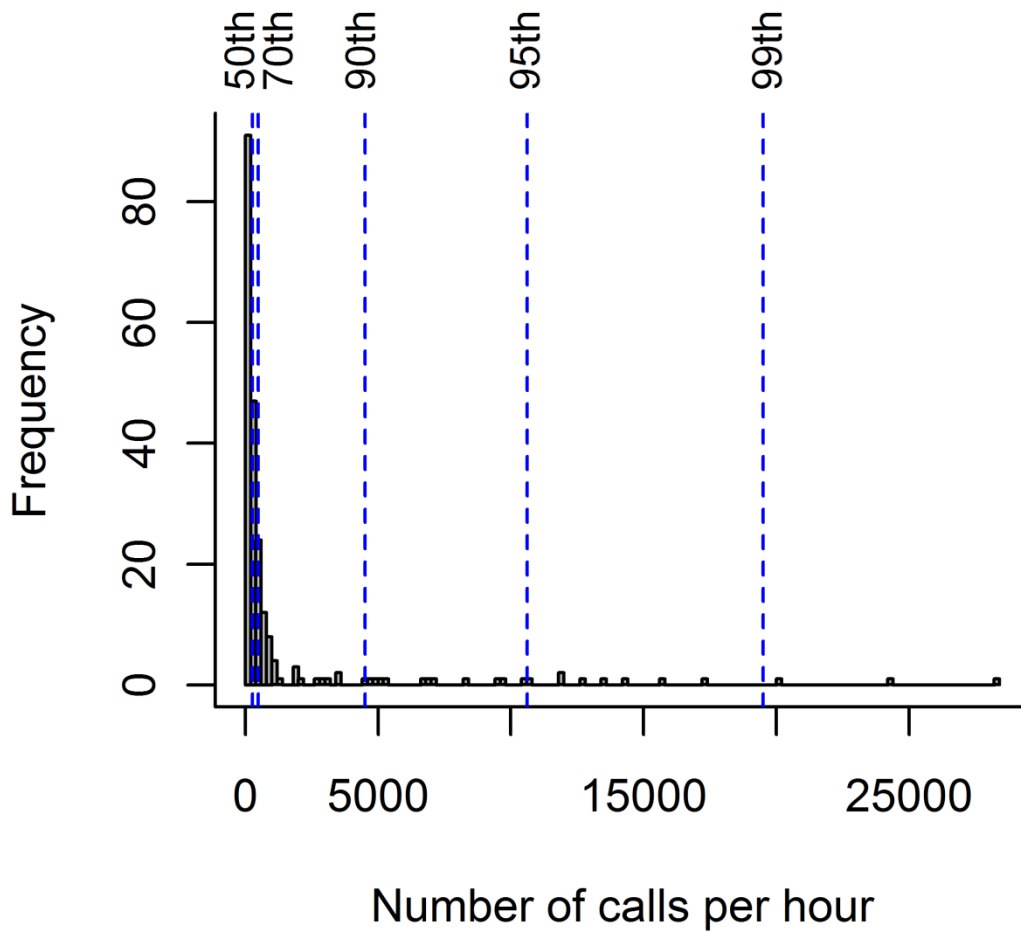
Figure 3.1. Frequency of activity levels for all bat species combined at three sites in Canada. Dashed, vertical lines are thresholds of the number of calls per hour based on the 50th, 70th, 90th, 95th, and 99th percentiles of the distribution. Activity over the 50th percentile threshold (median) is considered high activity because half of all detections had fewer than 265 calls per hour.

Lasiurus borealis was detected in 109 hours of recording, and half of the time these were brief passes with less than seven calls per hour (Table 3.1). Activity exceeded the 90th percentile at Long Point and Renfrew (swarming), while Long Point (migration) was the only site with activity levels that exceeded the 99th percentile threshold (Table 3.2B). Nightly activity was not significantly different among sites ($H_4 = 45.85$, p = <0.001, Table 3.2B).

Lasiurus cinereus was detected during 147 hours of recording. Long Point was the only site with activity above the 70th percentile threshold (Table 3.2C). Nightly activity varied among sites ($H_4 = 149.78$, $p < 0.001$) and was significantly higher at Long Point (migration) than Renfrew and Cypress Hills (Table 3.2C).

Myotis lucifugus was detected during 177 hours of recording. Activity was above the median at all sites, but only above the 90th percentile threshold at Renfrew (swarming). Activity was significantly higher at Renfrew (swarming; $H_4 = 75.02$, $p < 0.001$; Table. 3.2D).

Table 3.2. Summary of bat activity, for A) all species combined, B) *Lasiurus borealis*, C) *L. cinereus*, and D) *Myotis lucifugus*, at three sites in Canada in 2008 and 2009. Activity over the 50th percentile threshold was considered high and the relative importance of a site was based on the threshold activity exceeded.

A) All species combined

Site	No. nights	Mean calls per hour ± SD	Peak time of night (hour after sunset) ± SD	Number of nights with at least one hour above percentile threshold					
				25th	50th	70th	90th	95th	99th
Cypress Hills	5	231.2 ± 309.9 b	1.6 ± 0.5	3	3	3	0	0	0
Long Point spring	6	277.2 ± 224.7 b	3.6 ± 1.8	6	6	4	0	0	0
Long Point migration	6	246.1 ± 237.5 b	2.8 ± 1.9	6	4	3	0	0	0
Renfrew spring	5	484.3 ± 1019.3 b	3.6 ± 0.5	5	4	2	1	0	0
Renfrew swarming	5	5503.5 ± 6910.9 a	4.0 ± 0	5	5	5	5	4	2

B) *Lasiurus borealis*

Site	No. nights	Mean calls per hour ± SD	Peak time of night (hour after sunset) ± SD	Number of nights with at least one hour above percentile threshold					
				25th	50th	70th	90th	95th	99th
Cypress Hills	5	4.6 ± 12.6 b	2.8 ± 1.3	4	3	3	0	0	0
Long Point spring	6	9.7 ± 17.0 b	4.5 ± 1.9	6	6	4	1	0	0
Long Point migration	6	25.4 ± 39.9 a	4.0 ± 3.0	6	6	5	3	3	1
Renfrew spring	5	6.3 ± 12.8 b	3.2 ± 0.8	6	4	2	0	0	0
Renfrew swarming	5	5.2 ± 18.7 b	9.5 ± 1.0	4	4	1	1	1	0

C) *L. cinereus*

Site	No. nights	Mean calls per hour ± SD	Peak time of night (hour after sunset) ± SD	Number of nights with at least one hour above percentile threshold					
				25th	50th	70th	90th	95th	99th
Cypress Hills	5	45.5 ± 59.5 b	2.2 ± 1.1	5	3	2	0	0	0
Long Point spring	6	149.6 ± 148.3 a	3.3 ± 1.9	6	6	6	5	4	0
Long Point migration	6	78.0 ± 111.3 ab	3.5 ± 2.3	6	5	5	2	1	1
Renfrew spring	5	0.4 ± 1.5 c	--	0	0	0	0	0	0
Renfrew swarming	5	0.2 ± 0.2 c	--	0	0	0	0	0	0

D) *Myotis lucifugus*

Site	No. nights	Mean calls per hour ± SD	Peak time of night (hour after sunset) ± SD	Number of nights with at least one hour above percentile threshold					
				25th	50th	70th	90th	95th	99th
Cypress Hills	5	139.5 ± 239.3 b	1.6 ± 0.5	5	3	3	0	0	0
Long Point spring	6	7.5 ± 19.9 c	4.7 ± 1.9	3	1	0	0	0	0
Long Point migration	6	87.8 ± 109.5 b	5.7 ± 3.4	6	4	2	0	0	0
Renfrew spring	5	424.4 ± 1007.7 b	3.6 ± 0.5	5	5	3	0	0	0
Renfrew swarming	5	5409.4 ± 6826.4 a	4.0 ± 0	5	5	5	5	4	1

Note: Means in second column followed by the same letter are not significantly different (p<0.05) according to Kruskal-Wallis pair-wise post-hoc test.

3.3.2   Within nights

Lasiurus borealis within-night activity at Long Point was less variable during spring than during autumn migration, with the majority of activity below the median ($75 \pm 11\%$ below median, Fig. 3.2A) and bimodal peaks above the median during spring. During migration, activity fluctuated more above and below the median ($53 \pm 31\%$ below median) and peaks of activity, above the median, were consistently in the middle of the night and at dawn (Fig. 3.2B).

Myotis lucifugus had activity above the 50th percentile four hours after sunset on every night during swarming at Renfrew (Table 3.2D) and the majority of activity was above the median ($74 \pm 18\%$ above median), all in the middle of the night lasting until dawn (Fig. 3.2D). During spring, activity fluctuated more ($38 \pm 33\%$ above median), with unimodal peaks of activity in the middle of the night (Fig. 3.2C).
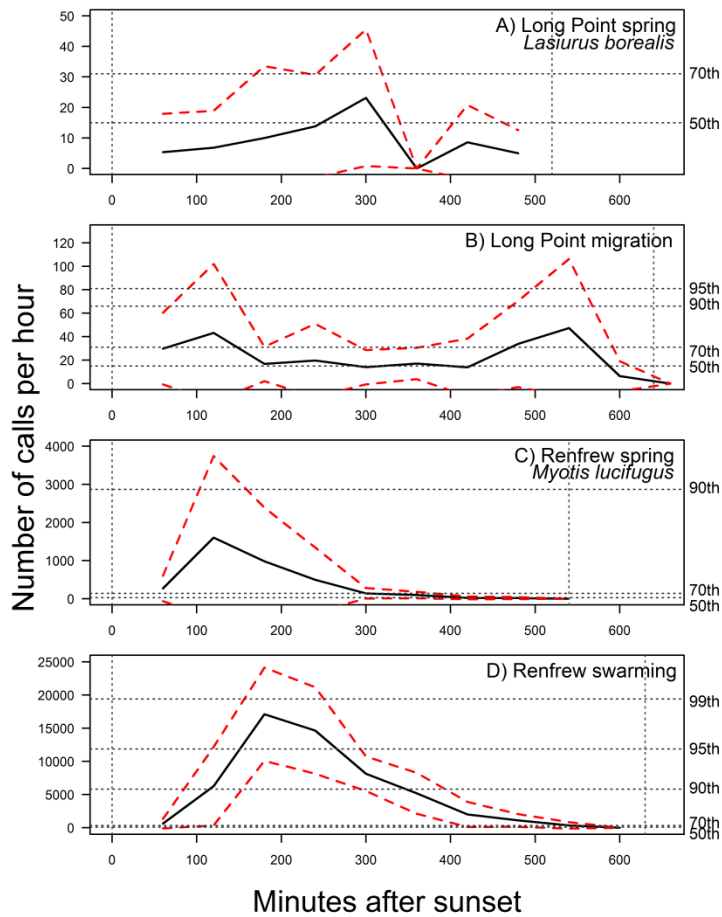
Figure 3.2. Comparison of species-specific, within-night bat activity patterns between seasons at sites in Ontario, Canada, highlighting the periods when activity is above certain thresholds for two species. Mean hourly activity for *Lasiurus borealis* at Long Point during A) spring (n = 6 nights) and B) autumn migration (n = 6) and *Myotis lucifugus* activity at Renfrew during C) spring (n = 5) and D) swarming (n = 5). Solid lines are rolling averages (20 increments across each night) of the mean nightly activity among all nights at a site ± SD (dashed red); horizontal dashed lines (grey) are percentile thresholds; vertical dashed lines (light grey) are sunrise and sunset.

3.4   Discussion

Percentile thresholds allowed for objective identification of peak activity levels at sites, while taking into account among-site and within-night variation of activity. Any activity above the median could be considered 'high' activity since it is higher than the majority of activity levels among all sampling units (Sokal and Rohlf 1981). All thresholds above the median are simply establishing 'how high' activity is. One way to interpret this scale is to identify sites based on the highest percentile threshold activity levels exceeded. For example, during swarming at Renfrew *M. lucifugus* had activity levels regularly exceeding the 95th percentile, while no other site exceeded the 70th percentile (Table 3.2D). It could be interpreted that sites with activity levels exceeding the highest percentile thresholds are important sites to bats. An international program identifies important bird areas based on criteria: significant numbers of threatened, range-restricted, migratory, or congregatory species. By the same logic, sites with activity over the highest thresholds could be defined as important areas for bats, with the assumption that high acoustic activity links to high abundance.

Examining overall bat activity, data for all species combined, Long Point does not appear to be a particularly important site. However when evaluating at the species-level it is apparent that Long Point is very important to migratory species, *L. borealis* and *L. cinereus,* especially during migration (Table 3.2B). Activity for all species combined (Tables 3.1,3.2A) was most influenced by *M. lucifugus* because of the nature of its echolocation, having shorter call duration and pulse interval (Miller 2001), resulting in more calls per individual. Because species differ in detectability and frequency of their

echolocation calls it is important to conduct species-specific analyses or use activity indices for more accurate comparisons among species (Miller 2001).

Traditional statistical methods, such as Kruskal-Wallis, do not go to a level of detail necessary for further identification of degrees of activity levels. Kruskal-Wallis results showed that sites with activity exceeding the 95th percentile had significantly higher activity, but did not provide as much information about differences of importance among sites, such as the magnitude of species-specific activity at each site. Percentile thresholds allowed us to evaluate skewed distributions and draw conclusions objectively, while looking at a finer scale.

Using percentile thresholds to define high activity is a replicable method of describing within-night activity patterns, including important times of night and degree of variation, which allows for interpretation of how a site is used by bats and its potential significance to them. From an applied perspective, it is crucial for mitigation and management decisions that methods be clearly defined when identifying times and locations with high or low activity. While a bimodal distribution of activity is typical of many insectivorous bat species, with a peak at dusk during initial foraging and a smaller peak at dawn (Kunz 1973, Hayes 1997), it is important to specify how these peaks in activity are identified. Percentile thresholds are a method to not only identify these peaks, but also describe the magnitude of activity levels relative to a larger dataset.

Environmental assessment surveys represent one area where using acoustic recordings to monitor bat activity is of particular importance. With increasing development of wind energy facilities and associated bat mortality (Kunz *et al.* 2007a), there is a growing

demand for environmental consultants to conduct pre- and/or post-construction acoustic surveys of local bat communities. There is an impetus for increasing knowledge and data about variation in activity levels, which could lead to viable mitigation options. Most pre- and post-construction surveys are not guided by a standardized survey design (Kunz *et al.* 2007a). Consequently, it is difficult to compare among acoustic monitoring surveys conducted by different groups at different times in different locations. The principle behind conducting pre-construction surveys is to assess potential risks of wind turbines to bats in an area (Kunz *et al.* 2007b). Essentially, environmental consulting agencies make recommendations based on the activity levels at a site, aiming to not erect turbines in areas of high bat activity. The lack of an agreed upon definition of 'high' bat activity and a lack of standardization in survey methodology make this a futile expectation. Assessment recommendations are typically made by comparing site activity levels to sites in a region. Unless these comparisons are made based on clearly defined thresholds they run the risk of being subjective, leading to unsubstantiated conclusions.

The practice of establishing a definition of 'high' activity for a given site (e.g., Broders 2003) is worthwhile, but one must clearly define which criteria are being used to measure peak activity relative to a baseline. It is a difficult practice to remove subjectivity when making decisions based on relative data, such as activity levels from acoustic recordings. Communicating the relative activity level based on percentile thresholds is an objective method and allows us to move away from subjective practices.

We have demonstrated the concept of applying percentile thresholds for identifying sites important for bats at a relatively small scale. Our analysis is effective because our dataset included a wide range of activity levels. However, percentile thresholds depend entirely

on the dataset, and underlying distribution of activity levels they are based on. If generating percentile thresholds for a species is based on a limited range of activity levels, such as *M. lucifugus* from Renfrew during swarming, no other site would be considered to have 'high' activity. Increasing the number of sampling points contributing to the overall distribution of activity levels increases the power of this analysis. The next step is to move towards a null distribution to generate percentile thresholds. Our ability to place a given survey in the broader context will continue to improve as the database increases to include more natural variation (i.e., seasonal, annual, geographic, meteorological). Such an endeavor is far too extensive for any one group to undertake, but through collaboration and technological advancements, it is possible that such a database could be realized. Creating a public repository of acoustic datasets in order to evaluate activity of a species in the context of its entire range would allow us to standardize terms such as 'high' activity in an objective manner.

3.5 Acknowledgements

3.6 References

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Chapter 4

4 Identifying peaks in bat activity: a new application of the space-time scan statistic[3]

4.1 Introduction

An important first step in identifying peak bat activity is establishing the range of local

variation in activity as this may vary temporally within or among nights, as well as

seasonally or annually. Such scales of variability are the result of numerous factors,

including insect abundance (Taylor and O'Neill 1988, Hayes 1997, Lee and McCracken

2002), air temperature (Kunz 1973, Lacki 1984, Negraeff and Brigham 1995, Hayes

1997), rainfall (Fenton *et al.* 1977, Parsons *et al.* 2003), relative humidity (Lacki 1984,

Adam *et al.* 1994), wind (Adam *et al.* 1994), and species-specific life history factors such

as reproductive timing (e.g., Maier 1992, Johnson *et al.* 2011) or seasonal movements

(e.g., Barclay 1984, Parsons *et al.* 2003). By understanding patterns and variation in

activity we can better understand the behavior of bats.

Understanding bat activity patterns and identifying periods of peak activity at various

time scales, within-night to annual patterns can be important for basic research,

monitoring or management. Acoustic monitoring of echolocation calls is a commonly

used method of measuring bat activity. Although there is no demonstrated quantitative

relationship between the numbers of calls and number of bats, the data indicate relative

[3] A version of this chapter is in review.

Citation: Adams. A. M. and M. B. Fenton. Identifying peaks in bat activity: a new application of the space-time scan statistic. Journal of Applied Ecology: in review.

levels of bat activity. However, there is no currently no definition of high activity or objective means of identifying periods of high activity.

Activity patterns may permit inference on how bats use a site. For example, activity at a roost may produce two clear peaks of activity corresponding to emergence and return at dusk and dawn (Kunz 1973, Hayes 1997), while monitoring the same site when young are nursing may show a unimodal peak of activity while females are pregnant (Maier 1992). At a swarming site, when bats congregate in August and September for pre-hibernation mating, activity may peak several hours after sunset reflecting the times of arrival of bats that have travelled long distances to the site (Fenton 1969). Migration stopover sites could have nightly unimodal peaks of activity at dawn during migratory periods as species arrive at sites along migration routes (McGuire *et al.* 2012). Previously, peaks in activity have been identified visually with activity plots (Hayes 1997), with selection of an arbitrary level of activity as 'high' (Broders 2003, Brooks and Ford 2005), or by the number of calls above percentile thresholds (Adams *et al.* submitted, Gorresen *et al.* 2009). Percentile thresholds identify peak acoustic bat activity (Adams *et al.* submitted) by comparing when activity exceeds thresholds based on a larger distribution of activity from a range of sites, placing activity levels in a larger context.

We propose the use of the space-time scan statistic (SaTScan) as an analytical tool for identifying spatial and temporal peaks of bat activity. SaTScan was originally developed for monitoring the spread of disease by detecting localized clusters of infection in space and time where the number of cases differed significantly from the background expectation (Kulldorff 1997). However, it has also been applied to other fields (see

Kulldorff 1994 for a partial bibliography), including ecology (e.g., Coulston and Riitters 2003, Rubin and MacFarlane 2008, Vadrevu 2008, Tuia *et al.* 2008).

Autocorrelation is a general statistical property of variables observed along geographic space and time-series (Koenig 1999). Spatial and temporal autocorrelation present a statistical problem because the data violate the assumption of independence inherent in most standard statistical procedures (e.g., ANOVA; Legendre 1993). As space or time intervals decrease, the dependence between successive observations usually increases (Legendre 1993, Koenig 1999). Autocorrelation can be problematic, for studies using radio telemetry (Rooney *et al.* 1998, Dray *et al.* 2010) or those examining macro-scale patterns of species diversity (Legendre 1993, Diniz-Filho *et al.* 2003), because it inflates Type I errors and could bias environmental factors with higher spatial autocorrelation (Lennon 2000). Only a few studies have accounted for autocorrelation in spatial or temporal datasets of bat activity (Audet and Fenton 1988, Gorresen and Willig 2004, Loeb and O'Keefe 2006, Stevens *et al.* 2007, Hein *et al.* 2009). Autocorrelation may be less of a concern because bats are highly mobile and able to fly substantial distances in short periods of time (Henry *et al.* 2002), but the potential for violation of independence in temporal and spatial datasets remains because much of their behavior keeps them in a restricted area for an extended period of time. Even if autocorrelation is less likely with bat data, a technique that removes this concern would make one more confident in conclusions drawn. SaTScan controls for spatial and temporal trends, whether autocorrelated or not, by adjusting for purely spatial and purely temporal variation (Kulldorff 2004).

Our goal was to examine the validity of SaTScan as a method for identifying peaks in bat activity, by comparing it with the percentile threshold approach. We also applied SaTScan to assess temporal patterns of activity of five species of bats at three sites.

4.2   Methods

4.2.1   Study areas

We recorded bat echolocation calls in 2008 at three sites in Ontario, Canada that were at least 131 km apart, on clear nights in both spring and fall: i) Long Point, an important location for bats during migratory periods (Adams *et al.* submitted, Dzal *et al.* 2009, McGuire *et al.* 2012), on six nights during both spring (June, Site 1a) and fall migration (August/September, Site 1b); ii) along the shore of Lake Opinicon at the Queen's University Biological Station (QUBS), where bats forage and roost (Barclay 1982, Arh 2009), for five nights during both spring (May/June, Site 2a) and summer (August, Site 2b); iii) an abandoned mine near Renfrew, an important swarming site and hibernaculum (Fenton 1969, 1970) housing up to 30,000 bats during the winter, for five nights during both spring (May/June, Site 3a) and fall swarming (August, Site 3b).

4.2.2   Acoustic sampling

Each night we recorded continuously from dusk until dawn using an Avisoft UltraSoundGate System (Avisoft Bioacoustics, Berlin, Germany) at 8 bit with a sampling rate of 250 kHz, trigger set to continuous recording, and gain at seven. Externally polarized condenser microphones (Avisoft CM16/CMPA) connected to an Avisoft UltraSoundGate 416-200 were operated with Avisoft Recorder USG software.

All acoustic data were analyzed using callViewer (v. 18, Skowronski 2008), a custom

echolocation sound analysis program written with MATLAB software (The MathWorks,

Natick, Massachusetts), using the automated detection feature to identify echolocation

calls in acoustic recordings. We filtered and identified the calls to species using

discriminant function analysis (DFA, following Adams *et al.* submitted, Appendix A) for

seven species in Ontario, *Eptesicus fuscus, Lasionycteris noctivagans, Lasiurus borealis,*

L. cinereus, Myotis lucifugus, M. septentrionalis, and *Perimyotis subflavus*.

4.2.3   Statistical analysis

We summed the calls per minute, post-DFA and filtering, for all species combined

(overall activity for all seven species). We also calculated nightly activity as number of

calls per minute for each of five species (*E. fuscus, L. borealis, L. cinereus, M. lucifugus,*

and *P. subflavus).* We used two methods to identify peak bat activity, SaTScan and

percentile thresholds.

We used SaTScan software (v. 9.1.1, Kulldorff *et al.* 2005, freeware available online), to

detect peaks in activity at two levels at each site, within- and among-nights. SaTScan can

identify high or low clusters, where event occurrence is significantly more likely within

the cluster than outside the cluster ($\alpha = 0.01$). In the case of acoustic bat activity,

SaTScan would identify a cluster of high activity in space and/or time where the null

hypothesis is that activity levels are always constant everywhere. Significance of a

potential high cluster is determined based on a likelihood ratio $\lambda = L/L_0$, where L is the

max likelihood and L_0 is the max likelihood constrained to a true null hypothesis. Higher

λ means greater support for the alternate hypothesis that activity is greater inside the

cluster than outside. Monte Carlo hypothesis testing assigns the degree of significance of each cluster by determining the probability of obtaining a value that is at least as high as the observed value from randomized data (Kulldorff *et al.* 2005). Purely temporal scan statistic analysis moves a scanning window over each possible time point across an entire time frame. The window size ranges from the size of a single time point (e.g., one minute in this study) up to a user-defined maximum cluster size (MCS), which limits the maximum size of the cluster to a percentage of the total sampling period, most commonly the window size includes no more than half the total time period (MCS of 50%, Kulldorff 1997). We ran SaTScan five times for each analysis, specifying the temporal MCS at 1, 5, 10, 30, and 50%. A high MCS gives more power but low specificity in describing the boundaries of clusters (Rubin and MacFarlane 2008). The ability to search for peaks with various MCS helps give a better measure of the strength of a peak because a high MCS considers both large and small clusters, while a low MCS only considers small clusters. To apply this method to both space and time simultaneously SaTScan uses cylinders rather than one-dimensional windows. The base of the scanning window represents space and the height represents time, with both the width and the height varying up to the limits of the MCS.

The scan statistic can use different probability models depending on the nature of the data. We applied both purely temporal and spatial-temporal analysis to analyze bat activity. Purely temporal analysis, for within-site analyses, used the discrete Poisson model (Kulldorff 1997), while spatial-temporal analysis, for among-site analysis, used the space-time permutation model (Kulldorff *et al.* 2005). We used the retrospective analysis option because we had data with a fixed geographic region and fixed temporal

study period. For both models, we input case files including the number of calls per minute (count data), minute after sunset (MAS), and site location. For the space-time permutation model we also included a coordinates file with the latitude and longitude of each site location. We specified a one-tailed analysis to look for clusters with unexpectedly high numbers of calls and used 999 Monte Carlo replications. The analysis output included the "most likely cluster," which described the locations and time frame(s) that were significantly higher than the background expectation with the observed and expected number of cases and p-value. We also used multivariate SaTScan, which allows for multiple datasets to be searched simultaneously for clusters (Kulldorff *et al.* 2007), with each night within a site/season as a separate dataset to detect the peak time period among all nights for a single site/season.

We calculated threshold values at five percentile thresholds (50th, 70th, 90th, 95th, and 99th percentiles, following Adams *et al.* submitted) based on the distribution of number of calls per minute for all nights, at all sites, for all species combined, excluding all minutes with no activity from the distribution. We compared peaks identified by percentile thresholds and SaTScan by calculating the proportion of minutes above the 50th percentile threshold (median) that was included in each SaTScan high cluster time period. We also compared among-night activity between the two methods. With SaTScan, we identified peak nights with spatial-temporal analysis of mean calls per minute for each night and compared it to which nights had at least 60 minutes of activity above each percentile threshold.

To demonstrate application of SaTScan we used multivariate SaTScan to identify peaks in species-specific activity. Peak time frames for each species at each site/season were

defined by the lowest MCS that included the most nights. When all nights were not included in the peak time frame we reran multivariate analysis, only including the nights that were not included in the result of the previous analysis. We compared the peak time frames among sites and seasons for each species and among species within each site/season. We excluded species at sites/seasons with fewer than two nights of activity. Comparisons of peak time frames among sites and seasons were considered different when there was no overlap in MAS.

4.3 Results

4.3.1 SaTScan description and comparison to percentile thresholds

SaTScan defined time frames that differed in length depending on the MCS (e.g. Fig. 4.1A). The high cluster time frame for a MCS of 50% ranged from 2 – 282 minutes, while a MCS of 1% ranged from 1 – 6 minutes. When there was a strong, singular peak of activity the time frame could be the same, regardless of MCS. Sometimes, the peak time frame did not include the maximum minute of activity, but the maximum five minutes of activity was always included in the peak time frame. Multivariate SaTScan identified peaks for multiple nights within a site and season (e.g. Fig. 4.1B – F). A MCS of 50% and 30% identified peak time frames that included all nights at Site 1 and Site 3, but when the MCS was reduced some nights were no longer included. Temporal variation in activity was high at Site 2, so multivariate SaTScan was not able to identify a time frame that worked for all nights at the site/season.
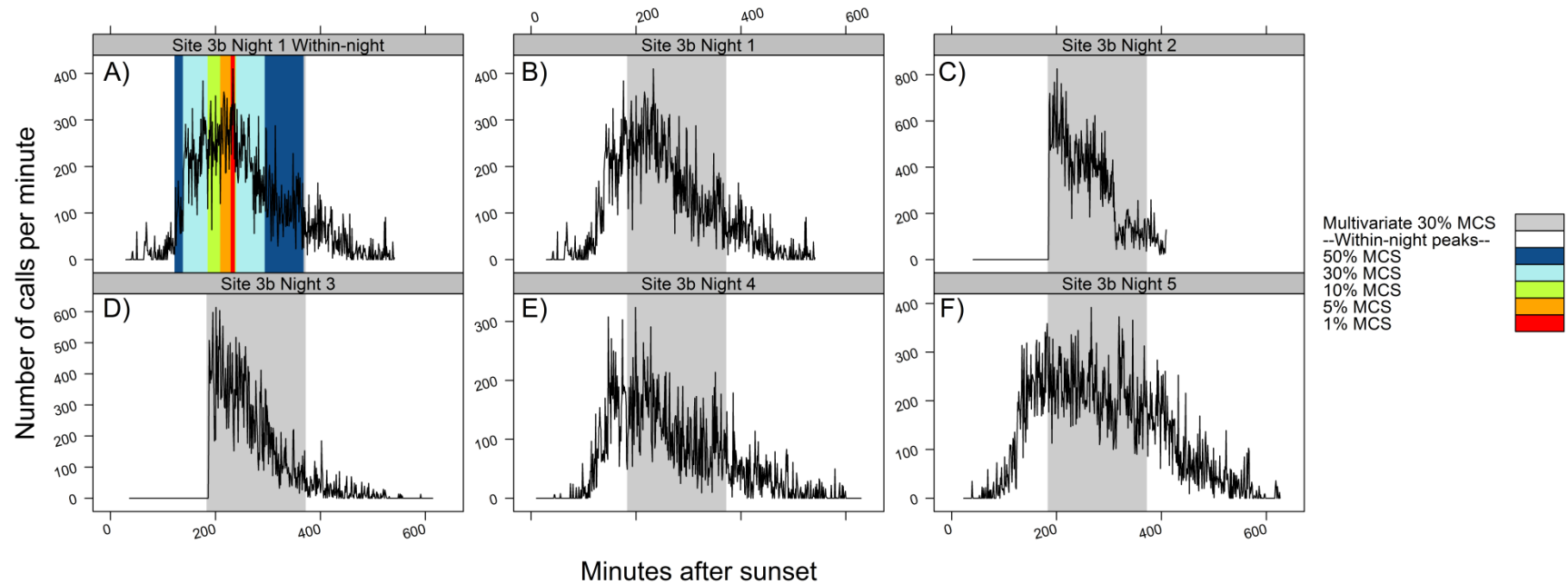
Figure 4.1. Nightly activity of bats for all nights at Renfrew during swarming (Site 3b) in 2008 with calls for all species combined. Highlighted time frames are SaTScan high cluster time frames defined by A) various maximum cluster sizes (MCS) within a single night and B-F) MCS of 30% with multivariate scan.

The number of calls per minute, for all species combined, at each percentile threshold levels were: 29 at the 50th, 70 at the 70th, 256 at the 90th, 366 at the 95th, and 592 at the 99th percentile, respectively. SaTScan and percentile thresholds differed in the number of minutes of peak activity identified (Fig. 4.2A). Percentile thresholds identified any minutes with activity over the threshold levels, while SaTScan identified a single time period that included activity that was higher than expected. SaTScan peaks were most similar to percentile thresholds at Site 3, where there was a clear, unimodal peak of activity. SaTScan was less similar to percentile thresholds at Sites 1 and 2 because activity was more evenly spread throughout the night. SaTScan with a MCS of 50% and multivariate SaTScan with a MCS of 30% included the majority of minutes above the 50th percentile at Sites 1 and 3 (Fig. 4.2A) and the majority of the total nightly activity at Sites 2 and 3 (Fig. 4.2B). The maximum minute of activity for a night was included in the SaTScan time frame on 67% of the nights at Site 1, all nights at Site 2, and 90% of the nights at Site 3.

Peak nights differed between percentile thresholds and SaTScan (Fig. 4.3). Sites 2a and 3b were the most important to bats based on percentile thresholds because they both had nights that exceeded the 90th and 95th percentile thresholds. SaTScan would identify Site 1a as an important site even though only one night had 60 minutes of activity over the 50th percentile threshold. SaTScan peak nights more closely reflect within-site peaks, rather than comparisons among sites. Peaks based on percentile thresholds do not consider space, by adding in the spatial component with SaTScan, sites that are farther apart are weighted differently.
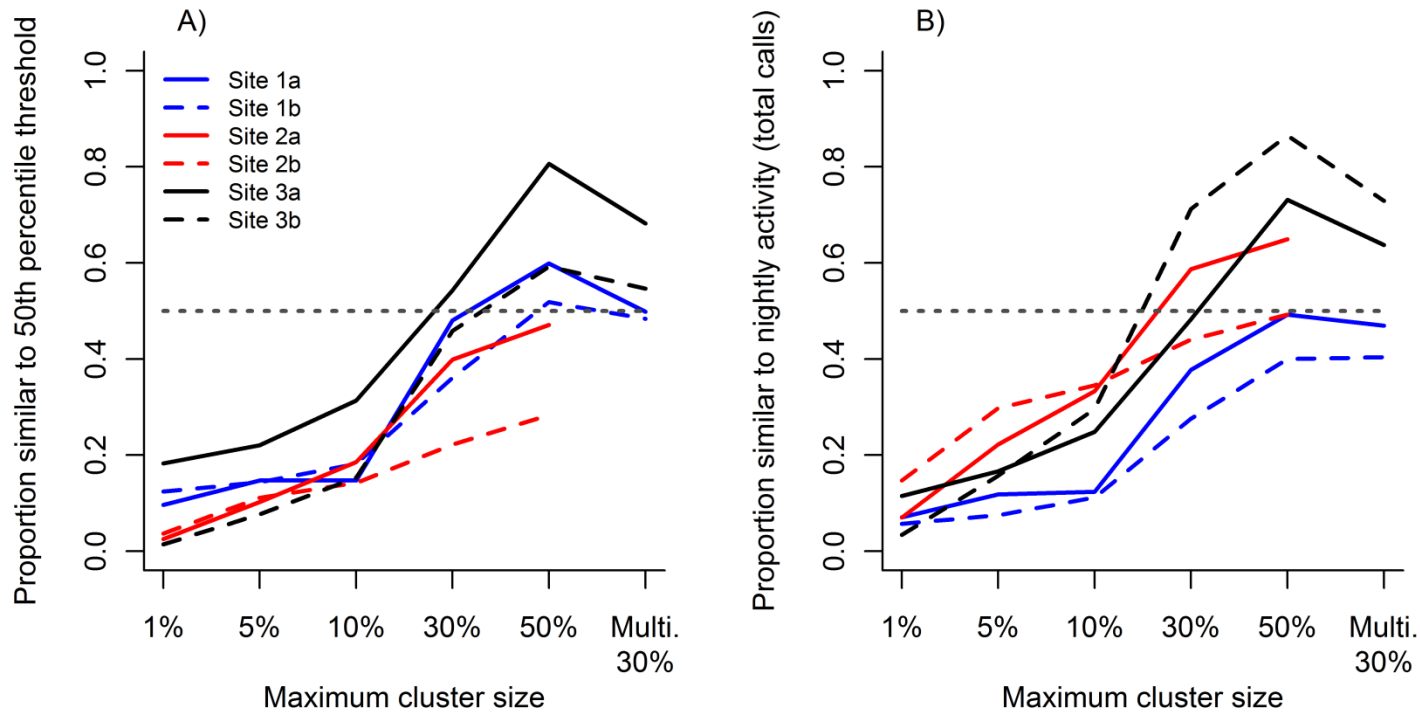
Figure 4.2. Proportion of overall bat activity in SaTScan high cluster time frames at various maximum cluster sizes (MCS) similar to A) total minutes over the 50th percentile threshold, and B) total nightly activity for three sites in Ontario, Canada during spring (a) and late summer (b) in 2008 with calls for all species combined.
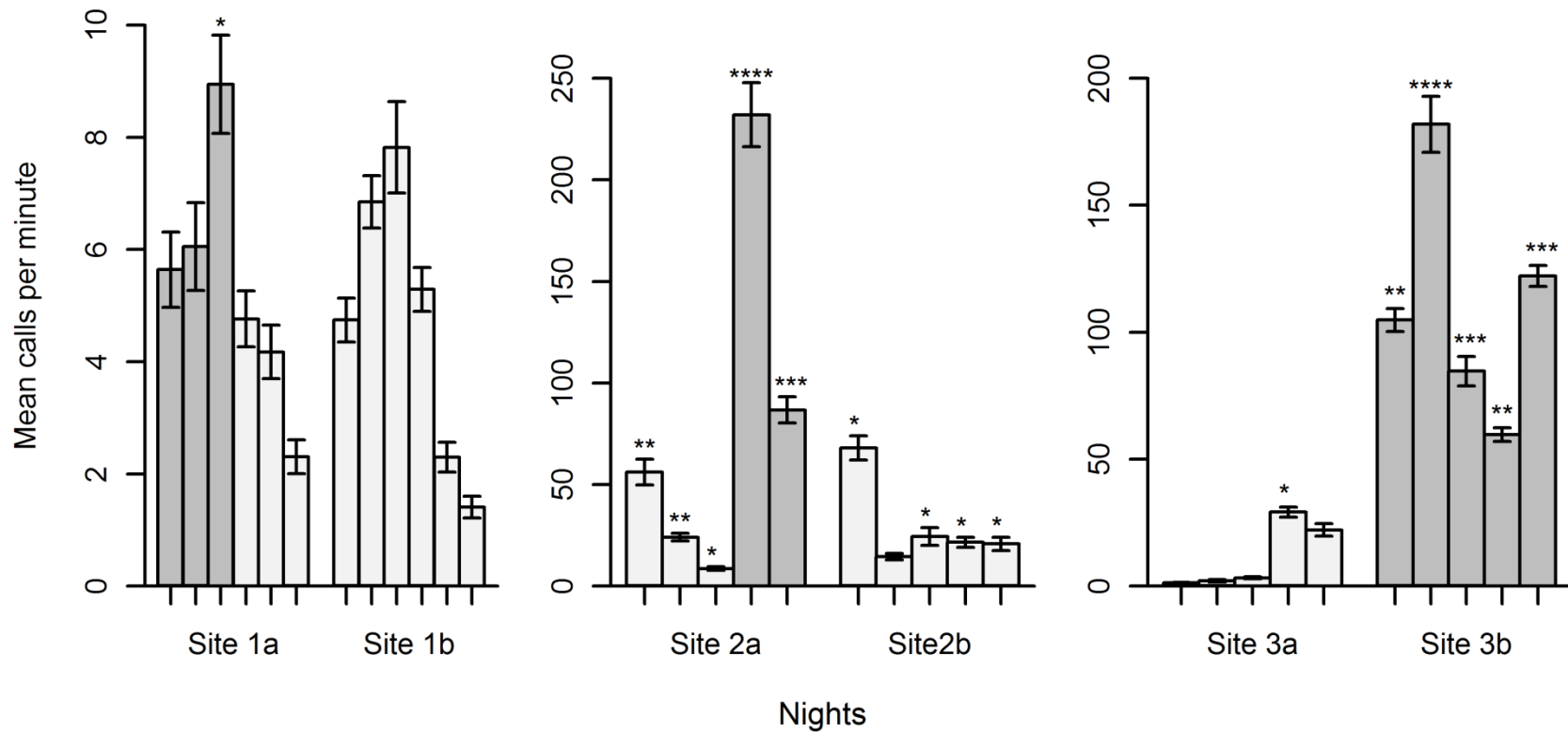
Figure 4.3. Comparison of nights of peak bat activity (mean calls per minute ±SD) identified by SaTScan spatial-temporal analysis (grey bars) and with at least 60 minutes of activity over the five percentile thresholds (*50th percentile, **70th percentile, ***90th percentile, ****95th percentile), for all species combined at three sites in Ontario, Canada during spring (a) and late summer (b) in 2008. Note different y-axes.

4.3.2   Species-specific activity

We found no indication of temporal partitioning among species that was consistent among all sites and seasons (Fig.4.4). *Lasiurus cinereus* always had the earliest peaks of activity in the night at sites where it was present. All species had overlapping peaks of activity at Sites 2 and 3a. The majority of peak activity occurred within the first five hours of the night for all species at all sites, except *L. borealis* at Site 3b (Fig. 4.4). Peaks of activity at Site 1a were very inconsistent, with different timing of peaks among nights for most species (Fig. 4.4G,S,Y). *Lasiurus borealis* activity patterns differed between Site 1a and 1b with timing of peak activity being more consistent at Site 1b (Fig.4.4H). The majority of *M. lucifugus* peaks of activity were two to four hours after sunset and was most consistent at Site 3b, being the only case when all nights were included at all MCS $(50 - 1\%)$.
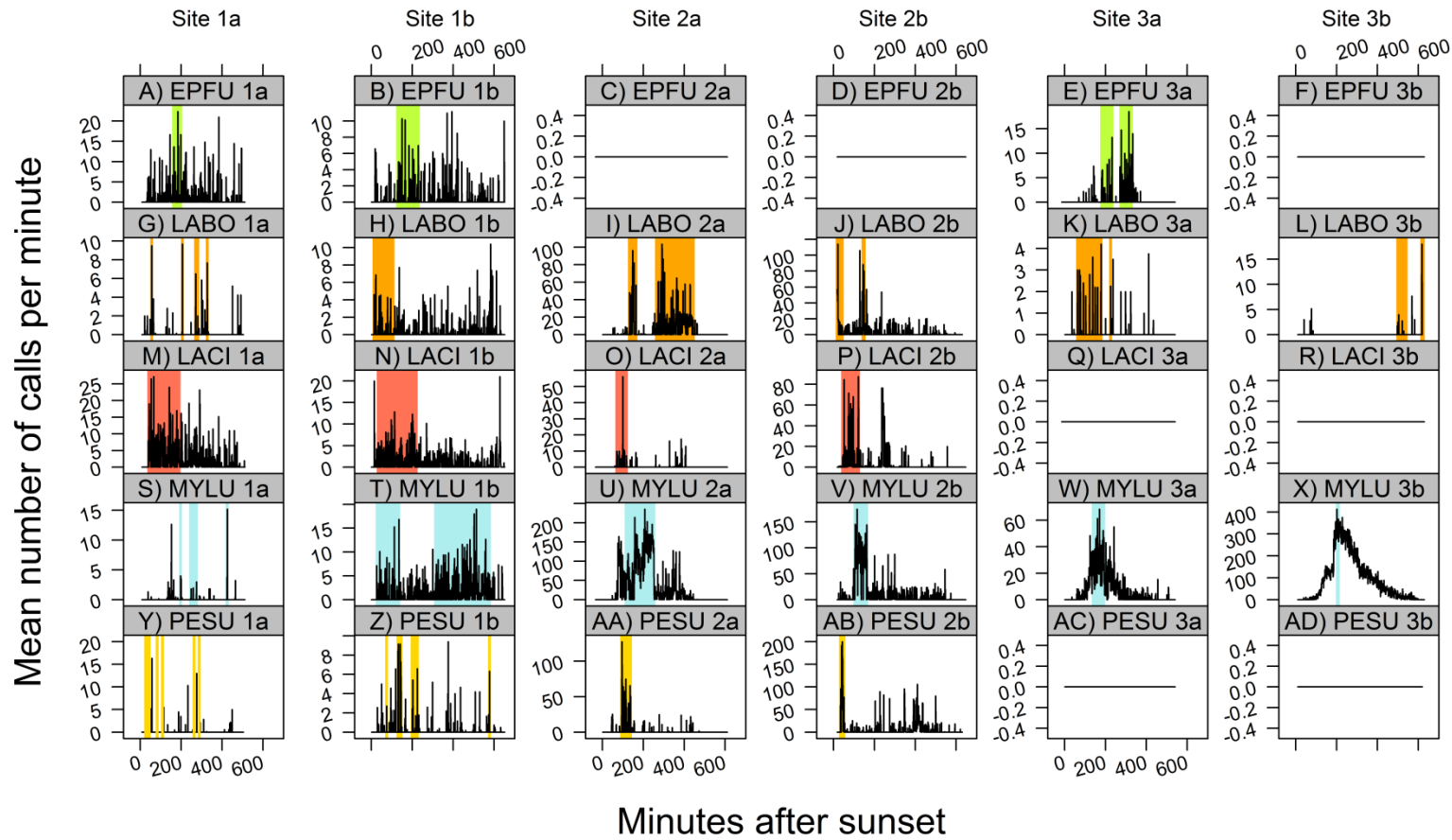
Figure 4.4. Mean species-specific bat activity at three sites in Ontario, Canada during spring (a) and late summer (b) for 2008. Shaded time frames are peaks identified by multivariate SaTScan. Note different y-axes. EPFU – *Eptesicus fuscus*, LABO – *Lasiurus borealis*, LACI – *L. cinereus*, MYLU – *Myotis lucifugus*, PESU – *Perimyotis subflavus*.

4.4 Discussion

SaTScan precisely identified peaks of activity, but cannot identify every individual minute of high activity within a night. There are two main advantages of SaTScan. First, multivariate SaTScan identifies peaks that occur among all datasets. Identifying common peaks within each site/season can be extremely valuable for recognizing important time periods for bat activity, whether to focus sampling efforts or comparing species-specific temporal patterns. Second, the true strength of SaTScan is the ability to simultaneously account for both spatial and temporal patterns and it works with data at any spatial and temporal scales. We analyzed our data by minute because it allowed SaTScan to define more specific peak time periods, but any time scale is possible. Our analysis was at a fairly large spatial scale with coarse resolution, but this method has the potential to be more useful at a much smaller spatial scale without concerns of autocorrelation during analysis.

SaTScan and percentile thresholds both identify peak activity objectively, but do so in different ways making it difficult to compare results. SatScan identifies a single cluster of peak activity, while percentile thresholds identify any time frame with activity above a defined threshold. Using multiple percentile thresholds provides a measure of the magnitude of activity at a site and can be useful for identifying important sites (Adams *et al.* submitted), while SaTScan is best at identifying peak time periods, especially among multiple nights. We recommend using the two methods in combination to have a more complete picture of activity levels and activity patterns at a site. Percentile thresholds will be better for identifying important sites, while SaTScan will identify peaks in activity

among nights. For example, Site 3b is the most important to bats because the majority of nights had overall activity that exceeded the 90th percentile threshold (Fig. 4.3), while it also stood apart from the rest of the sites with peak of activity, identified by SaTScan, three to four hours after sunset. In contrast, Sites 1, 2, and 3a had few to no nights with activity above the 90th percentile and peak activity starting one to two hours after sunset.

We identified peaks in activity with SaTScan that were comparable to findings in other studies. For example, peaks in foraging activity were consistent with foraging patterns reported by temperate species at other sites (Kunz 1973), however, at our sites in Ontario *M. lucifugus* did not have peaks of activity immediately after sunset as observed in Nova Scotia (Broders *et al.* 2003) potentially due to timing of insect prey (Rautenbach *et al.* 1996) or proximity of roosting habitat. The consistency of peaks among nights with SaTScan can potentially indicate bats' use of a site, for example peak activity was most consistent during migration (Site 1b) and swarming (Site 3b) and more variable among nights during foraging (Sites 1a and 2).

A limitation is that SaTScan will not identify peaks of activity when activity is consistently high or low throughout a night, but using percentile thresholds in combination with SaTScan would identify when this is the case. SaTScan is also not able to detect multiple temporal peaks, if there are bimodal peaks only one will be identified. Foraging sites are most commonly observed with bimodal activity, with a peak at dusk when bats forage on crepuscular insects and another peak at dawn when returning to their day roost (Kunz 1974, Rydell 1993, Kunz *et al.* 1995, Hayes 1997). It is possible to recognize bimodal patterns of activity with SaTScan by running multiple analyses, each time excluding the peak time period and searching for a secondary peak. For example, it

was possible to identify a secondary peak of *L. borealis* activity just before dawn at Site 1b after excluding the primary peak at dusk (Fig. 4.4H). We did not detect secondary peaks of activity for all species combined at Sites 1 or 3, rather just extensions of the primary peak time period.

SaTScan is a valuable tool to quickly identify peaks with an objective, replicable, and statistically sound method that can be applied at various temporal and spatial scales. As bat detector technologies improve, allowing all night recordings over long periods of time at many locations, it is more difficult to analyze the vast amounts of data. SaTScan identifies when and where bats are most active, which has applications for basic and applied research, such as comparing peaks in activity among habitat types, commercial developments (e.g., wind energy), or years (e.g., meta-analysis of annual fluctuations pre- and post- white-nose syndrome).

4.5   Acknowledgements

4.6 References

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Chapter 5

5 Value in variation? Stationary acoustic transects to account for spatial variation in bat activity

5.1 Introduction

Variation is a challenge to anyone studying ecology. The ability to identify and account for different sources of variation at a particular location can impact the conclusions drawn from the data. This is true whether asking questions about habitat associations or making recommendations for environmental policy. Frequently, high variation in bat activity has been reported (e.g., Hayes and Adam 1996, Milne *et al.* 2009). It is important to account for variation to maximize the chances of obtaining unbiased measures of bat activity (Hayes 1997, 2000) and to detect specific species (Broders 2003, Skalak *et al.* 2012). Acoustic surveys are common practice for studying bat ecology (Britzke *et al.* 2013) and a key assumption is that activity levels recorded from a single bat detector reflect a broader set of locations and times (Hayes 2000). To capture acoustic activity that accurately describes local activity levels, it is essential to account for variation from detectors used (Adams *et al.* 2012), temporal variability within and among nights (Milne *et al.* 2005, Skalak *et al.* 2012), and spatial variation within sites (Hayes 2000, Fischer *et al.* 2009). Both field work and analysis of acoustic data are labor intensive, resulting in a trade-off between sampling effort and collecting sufficient data so as to accurately describe bat activity at a site. A compromise can be made by measuring and understanding activity variability present at a site (Fischer *et al.* 2009). Where should we draw the line when making the trade-off between effort and accuracy?

Decisions on sampling effort are typically based on limitations in funding and personnel, but at ultimately impacted by the research question being asked. The number of sampling nights necessary to accurately estimate levels of bat activity at a site differs among locations. Hayes (1997) recommended six to eight nights of sampling to obtain accurate estimates of overall activity at sites in Oregon, USA, while Broders (2003) found that 14+ nights of sampling were required for accurate estimates of *Myotis lucifugus* activity at sites in New Brunswick, Canada. Identification of species-specific activity may play a role in these different recommendations of sampling effort (Broders 2003).

The validity of the assumption that extrapolation of recording activity from a single detection point reflects activity for an entire site/habitat has received little attention (Hayes 2000, Britzke *et al.* 2013), considering that a single detector has a limited range of 5 m to 40 m for high frequencies (Adams *et al.* 2012). To account for horizontal spatial variation, multiple detectors can be deployed within a site (Krusic *et al.* 1996b, Gannon *et al.* 2003, Duchamp *et al.* 2006, Fischer *et al.* 2009), or a single detector can be moved to a new location within the site each night (Fischer *et al.* 2009). Vertical spatial variation can be addressed with multiple detectors deployed at various heights (Hayes and Gruver 2000, Reynolds 2006, Fischer *et al.* 2009, Staton and Poulton 2012a). The use of multiple detectors simultaneously can increase the probability of detecting different species of bats at large (Skalak *et al.* 2012) and small (Duchamp *et al.* 2006) spatial scales, but there is little evidence of how multiple detectors within a site impact estimates of activity levels (Fischer *et al.* 2009).

It is also important to accurately detect patterns of activity to increase understanding of bat ecology and behavior. Nightly activity patterns vary by species (Kunz 1973, Broders

et al. 2003, Milne *et al.* 2005, Skalak *et al.* 2012) and with extrinsic factors, such as temperature (e.g., Hayes 1997) and insect abundance (e.g., Ciechanowski *et al.* 2007). Temporal and spatial partitioning may underlie resource partitioning among species of bats with similar ecology and morphology (Kunz 1973, Nicholls and Racey 2006, Adams and Thibault 2006) and could be another reason for variation in spatio-temporal activity patterns. If patterns of activity differ spatially within a site, then the placement of detectors could impact depictions of activity patterns.

Our goal was to quantify small-scale spatio-temporal variation in bat activity as determined by monitoring echolocation calls and to demonstrate the effect on acoustic sampling design and interpretation. We did this by addressing three objectives: i) testing whether space, including detection height, or time, explained more of the variation in bat activity to prioritize sampling efforts; ii) assessing within-site spatio-temporal variation in activity among species and how detector location impacted depictions of activity at each site; and iii) investigating how many nights and detectors were necessary to have accurate estimates of mean nightly activity and how this varied by species. We expected that estimates of activity would be more accurate with more detectors within a site. We also expected that activity levels and patterns would vary along a linear, stationary acoustic transect and would require multiple sampling points within a site to accurately assess bat activity. We predicted that species would partition resources in space and time and that species-specific peaks of activity would exhibit little or no overlap in time and space.

5.2   Methods

5.2.1   Study areas

We recorded bat echolocation calls at four sites in Ontario, Canada: first, at Long Point

for a total of 12 nights (June, August, September 2008), which is an important site for

bats during migratory periods (Dzal *et al.* 2009, McGuire *et al.* 2012); second, at the

Queen's University Biological Station (QUBS), for 11 nights (May, June, August 2008),

a site where bats forage and roost nearby (Barclay 1982, Arh 2009); third, at an

abandoned mine near Renfrew for 12 nights in 2008 (May, June, August) and 15

consecutive nights in 2010 (August), which is an important swarming site and

hibernaculum (Fenton 1969, 1970) housing up to 30,000 bats during the winter; lastly,

along the shore of a lake 500 m from the abandoned mine at Renfrew for 12 nights (May,

June, August 2008). Throughout the summer we rotated from site to site, recording for

three clear, consecutive nights before moving to the next site.

5.2.2   Acoustic sampling

We recorded continuously from dusk until dawn with batcorders 1.0 (ecoObs,

Nuremberg, Germany). Batcorders have a sampling rate of 500 kHz and 16 bit sampling

resolution and were set at a critical frequency of 14 kHz (i.e., a trigger event prompted

recordings of sound in the frequency range of 14 - 250 kHz). Recordings were activated

at a low detector sensitivity threshold of -36 dB (1.6% of the microphone's maximum

amplitude) to increase the recording range of the detectors, similar to increasing gain on

other detectors. Batcorders have a pre-trigger of 50 ms and we adjusted the post-trigger to

800 ms, which is the interval between successive detected sounds written into the same

file, the higher value maximizes the number of below-threshold calls recorded. We set

quality to 40, an intermediate value where higher values of the quality detection algorithm are less conservative, to allow for recognition of sounds that are less like echolocation calls. Recordings were saved to an HCSD card in .RAW format and then converted to .WAV with a custom conversion program created with MATLAB software (The MathWorks, Natick, Massachusetts) for future analysis.

We placed detectors along a linear transect 40 m apart, corresponding to the minimum sampling distance without overlap (Stahlschmidt and Brühl 2012). All four sites were forested, but ranged in levels of canopy cover throughout the site (Table 5.1). Transect lines within each site were chosen so detector locations ranged in amount of canopy cover. Detectors were oriented upward at a 45° angle, facing the area with the greatest opening. In 2008, we set out three sets of paired detectors with one low detector at 1.5 m above ground level and one high detector at 4 m in an 80 m transect. One high detector malfunctioned for half of the field season and was not included in the majority of the analysis. In 2010, five detectors were 1.5 m high in a 160 m transect. We measured canopy cover with a densitometer at each detector location. Each night we recorded temperatures at every detector with iButton Thermochron temperature data loggers (Maximum Integrated, San Jose, California) and wind speed and relative humidity with a Kestrel 4000 (Nielsen-Kellerman, Boothwyn, Pennsylvania) at a single sampling location in the site.

Table 5.1. Percent canopy cover at each of the detector locations within A) each of the four sites in Ontario, Canada in 2008 and B) Renfrew mine in 2010.

A)	Detector locations		
Sites	A	B	C
Long Point	20%	37%	5%
QUBS	36%	57%	43%
Renfrew lake	38%	30%	68%
Renfrew mine	75%	69%	39%

B)	Detector locations				
Site	A	B	C	D	E
Renfrew mine	30%	75%	67%	39%	70%

We analyzed acoustic data using callViewer (v. 18, Skowronski 2008), a custom echolocation sound analysis program written with MATLAB. We used the automated detection feature of callViewer to identify echolocation calls in acoustic recordings. We then filtered and identified our data to species using quadratic discriminant function analysis (DFA) in R (v. 2.13.1, R Development Core Team 2011, following Adams *et al.* submitted, Appendix A) for seven species in Ontario: *Eptesicus fuscus, Lasionycteris noctivagans, Lasiurus borealis, L. cinereus, Myotis lucifugus, M. septentrionalis,* and *Perimyotis subflavus*. Classification accuracy from cross-validation was high (Table 5.2), but to further improve classification accuracy and because DFA is not capable of assigning calls to an "unknown" category, we applied a post hoc, species-specific filter to remove any data that were above or below specific durations and minimum frequencies for each species (Table 5.2).

Table 5.2. Classification accuracy for species identification of echolocation calls for bats in Ontario, Canada with quadratic discriminate function analysis (DFA) using cross-validation. Post-DFA filter settings removed echolocation calls identified to species, but outside of the species-specific ranges of duration and minimum frequencies (Fmin) to further improve classification accuracy.

Species	DFA classification accuracy	Post-DFA filter	
		Duration	**Fmin**
Eptesicus fuscus	78 %	3 – 12 ms	20 – 30 kHz
Lasionycteris noctivagans	94 %	4 – 12 ms	21 – 30 kHz
Lasiurus borealis	88 %	5 – 17 ms	29 – 43 kHz
L. cinereus	90 %	8 – 30 ms	15 – 29 kHz
Myotis lucifugus	90 %	3 – 8 ms	30 – 43 kHz
M. septentrionalis	82 %	1 – 3.5 ms	25 – 60 kHz
Perimyotis subflavus	93 %	5 – 15 ms	36 – 46 kHz

5.2.3   Statistical methods

We totaled the number of calls per minute after sunset (MAS), hour after sunset (HAS), and per night for each detector per species. We also summed the calls per MAS, HAS, and night for all seven species, post-DFA and filtering, for activity of all species combined. There were five parts to our analysis: (i) Kruskal-Wallis non-parametric test for overall differences in activity among detectors, (ii) generalized linear mixed models (GLMM) to partition variance components among random effects, (iii) percentile thresholds for magnitude of activity, (iv) space-time scan statistic (SaTScan) for identifying peaks of activity, and (v) sub-sampling to test the effect of sampling effort on estimates of activity. To test the overall significant differences in activity levels (mean calls per hour) among detectors within a site, we used Kruskal-Wallis rank sum test and a pair-wise post-hoc test (*kruskalmc* in pgirmess package in R; Giraudoux 2011).

5.2.4   Generalized linear mixed models

To test the relative contribution of spatial and temporal variation with non-normal data including random effects, we used a GLMM fit by the Laplace approximation and a Poisson distribution (*glmer* in lme4 package in R; Bolker *et al.* 2009, Bates *et al.* 2011). Acoustic data were not transformed to normality because transformations of count data can increase bias that is negligible when using an appropriate model, such as the Poisson distribution (O'Hara and Kotze 2010). We analyzed total calls per night (nightly activity) for each species at each site and detector. Random effects were site (2008 only), detector nested in site, and night nested in site. Fixed effects were canopy cover and detector height (2008 only), each detector-level effects. Like many ecological studies with count data, our dataset was overdispersed (Richards 2008), with greater variability than

expected (variance/mean > 1). We used additive overdispersion to account for overdispersion in our model by including an individual-level random effect, adding a random intercept with one level per observation that captures overdispersion (Elston *et al.* 2001, Browne *et al.* 2005). Residual variation was not included because Poisson GLMM only has one parameter, with a known mean-variance relationship, so there is no estimate for residual variation (Sokal and Rohlf 1981), but the individual-level effect is the variation not explained by other random effects. First we compared models with and without fixed effects; the difference in variance between the models provided an estimate of detector-level variance for the fixed effect (Elston *et al.* 2001). We then continued model selection by dropping random effects with non-significant variance estimates and model comparison with likelihood ratio tests and Akaike's Information Criterion (AIC). We calculated the proportion of variation explained by each factor in the full model in order to test if space or time explains the most variation in activity levels.

5.2.5   Percentile thresholds

To detect peaks and patterns in activity among detectors within nights, we used two methods: percentile thresholds (Adams *et al.* submitted) and SaTScan (Adams and Fenton submitted). We calculated percentile thresholds (50th and 90th percentiles) of activity for each species with a larger distribution of activity from 14,898 detector hours, after excluding hours without activity. We created this larger distribution using data from our four Ontario sites in 2008 and 2010, four sites in Saskatchewan, Canada in 2009, and one site near the Hudson River in New York, USA in 2009. When the number of calls per hour is greater than the 50th percentile threshold, we consider activity to be "high" since it is above the majority of the hourly activity for a species from the larger distribution.

Activity over the 90th percentile threshold was considered "very high" activity. We then

identified the number of hours above each threshold for each species on every night at all

detectors and sites in Ontario.

5.2.6   SaTScan

We identified peaks in activity with SaTScan software package (v. 9.1.1, Kulldorff *et al.*

2005, following Adams and Fenton submitted), which can identify clusters of activity in

space and time that are significantly different from the background expectation. We

specified a one-tailed analysis to look for clusters with unexpectedly high numbers of

calls with the space-time permutation model (Kulldorff *et al.* 2005). We used

retrospective analysis, specified spatial and temporal maximum cluster sizes (MCS) at

50%, and used 999 Monte Carlo replications. We input case files consisting of the

number of calls per minute, MAS, and detector location within the site. The coordinates

file included the Cartesian coordinates (x, y, z) of each detector location. The analysis

output included the "most likely cluster" and "secondary clusters" which described the

locations (detectors) and time frame(s) when activity was significantly higher than the

background expectation with the observed and expected number of cases and p-value.

We analyzed activity for each species individually. We used multivariate SaTScan

(Kulldorff *et al.* 2007) with case files for each night at a site as separate datasets. For

2008, we ran multivariate SaTScan twice for each site, for the first six nights (spring) and

the last six nights (summer). Since multivariate SaTScan is limited to 12 datasets, we ran

the 15 consecutive nights from 2010 in two batches, the first 12 nights and again with

nights four to 15. We then compared locations and timing of peak activity that occurred

on the majority of nights among all species with Gantt charts.

5.2.7 Sub-sampling for estimates of activity

To determine the effect of number of nights and amount of space sampled on estimates of

activity levels, we randomly sampled subsets of nights to compare the mean activity level

of the subsets to a grand mean of the total sample (following Hayes 1997). We sampled

two to ten night subsets 100 times for each species and all species combined for each

detector individually. We created a loop to run *sample* without replacement 100 times in

R for each subset of nights. We calculated the mean nightly activity for each subset and

compared it to the grand mean of the full dataset of all 15 nights at each detector in 2010.

We determined the proportion of subsamples within 10-50% of the mean of the grand

mean. We then expanded the analysis for the full model to include mean activity of five

detectors over all nights (2008 = 12 nights, 2010 = 15 nights). We sampled one to five

detector subsets for each two to ten night subset 100 times and determined the proportion

of subsamples within 30% of the grand mean. We based recommendations on how many

nights had at least 80% of the subsamples within 30% of the grand mean (following

Hayes 1997).

5.3 Results

5.3.1 Differences among detectors

Every species was present at all sites, but species-specific activity levels varied

dramatically among and within sites. Overall activity, for all species combined, was

significantly different among detectors within each site, except Renfrew mine in 2008

(Long Point $H_4 = 182.49$, $p < 0.001$; QUBS $H_4 = 45.46$, $p < 0.001$; Renfrew lake $H_4 =$

96.66, $p < 0.001$; Renfrew mine 2010 $H_4 = 45.08$, $p < 0.001$). The majority of species-

specific activity was significantly different among detectors within each site, except *L.*

noctivagans, and *M. septentrionalis* at QUBS and Renfrew lake (Table B1). Overall,

estimates of activity levels at a site differed substantially depending on detector

placement (Fig. 5.1, B1).
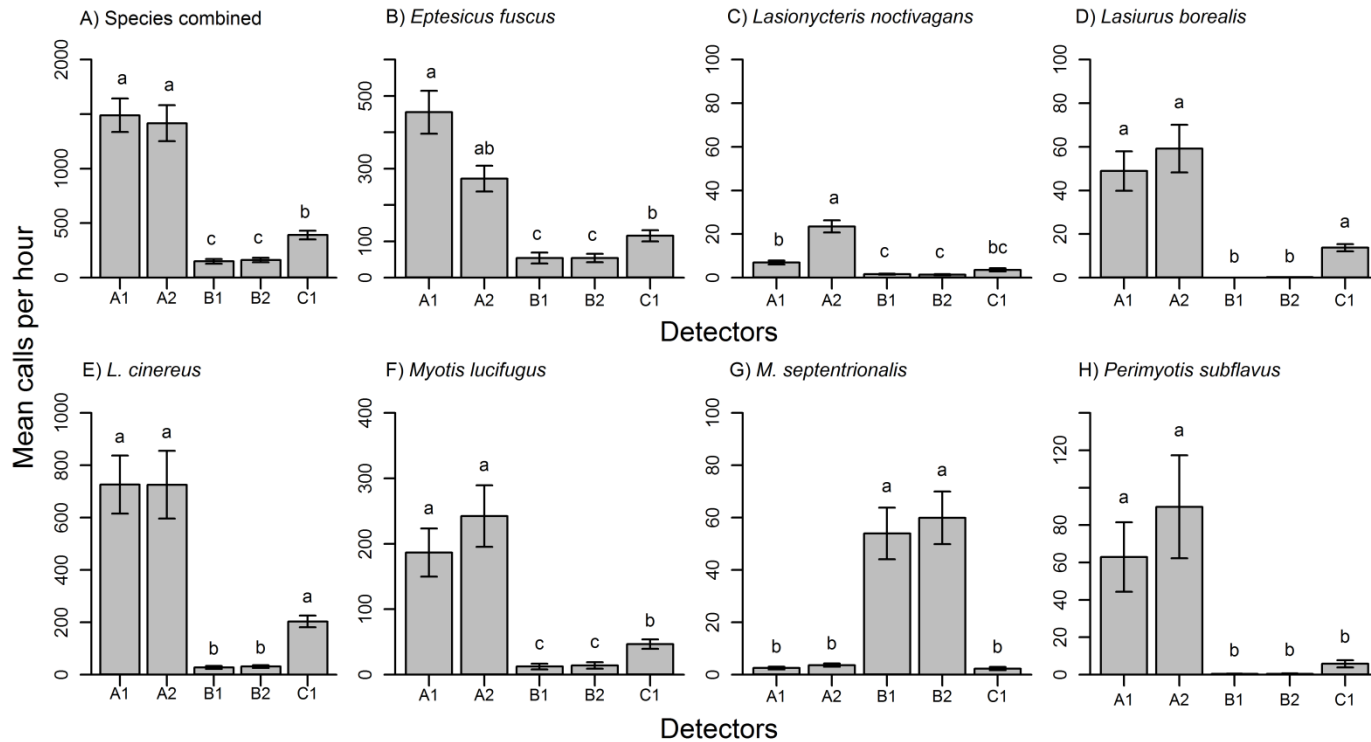
Figure 5.1. Mean calls per hour ± SD for each species of bat at five detectors at Long Point, Ontario (n = 12 nights). Note different y-axes. Detectors of the same letter were paired at the same location and first detectors in the transect (A1/A2) had intermediate openness at the site (20% canopy cover), B1/B2 were the most enclosed (37% canopy cover), and C1 was the most open (5% canopy cover). Detector number denotes height: 1 was low (1.5 m above ground) and 2 was high (4 m). Means, within each species, with the same lowercase letter are not significantly different (p > 0.05) according to a Kruskal-Wallis multiple comparison post hoc test.

5.3.2 Generalized linear mixed models: variance partitioning of random effects

The proportion of variance explained by each random effect differed among species in 2008 (Fig. 5.2). Detector position and night-to-night differences significantly explained of activity levels for every species ($p < 0.05$), but site was not significant for all species (Fig. 5.2, Table B2). Spatial components had the greatest association with variation in activity levels for *L. noctivagans*, *L. borealis*, and *M. lucifugus*. Detector height was only significant for explaining variation in *L. noctivagans* activity ($p = 0.0251$, Table B2) with activity levels higher at higher detectors. Canopy cover explained a significant amount of variation for all species ($p < 0.05$, Table B2) with activity increasing as canopy cover decreased, but *M. septentrionalis* was more active with increased canopy cover. Canopy cover had the greatest impact on *L. borealis,* with 27% of the variation attributable to detector location due to the effects of canopy cover.

During 2010, the majority of within-site variation was associated with the individual-level effect, except for *M. septentrionalis* where the majority of variation was explained by differences among detector locations. The two species with the least activity at the site in 2010, *E. fuscus* and *L. noctivagans,* had the most variation associated with the individual-level effect. Between detector location and night-to-night differences, spatial effects contributed more to variation in activity for *L. borealis, L. cinereus,* and *M. septentrionalis*. Spatial and temporal effects equally contributed to variation in activity for *M. lucifugus* and *P. subflavus*. Canopy cover was a significant factor for *L. borealis* and *L. cinereus*, explaining 37% and 21% of the detector-level effects, with activity increasing as canopy cover decreased.
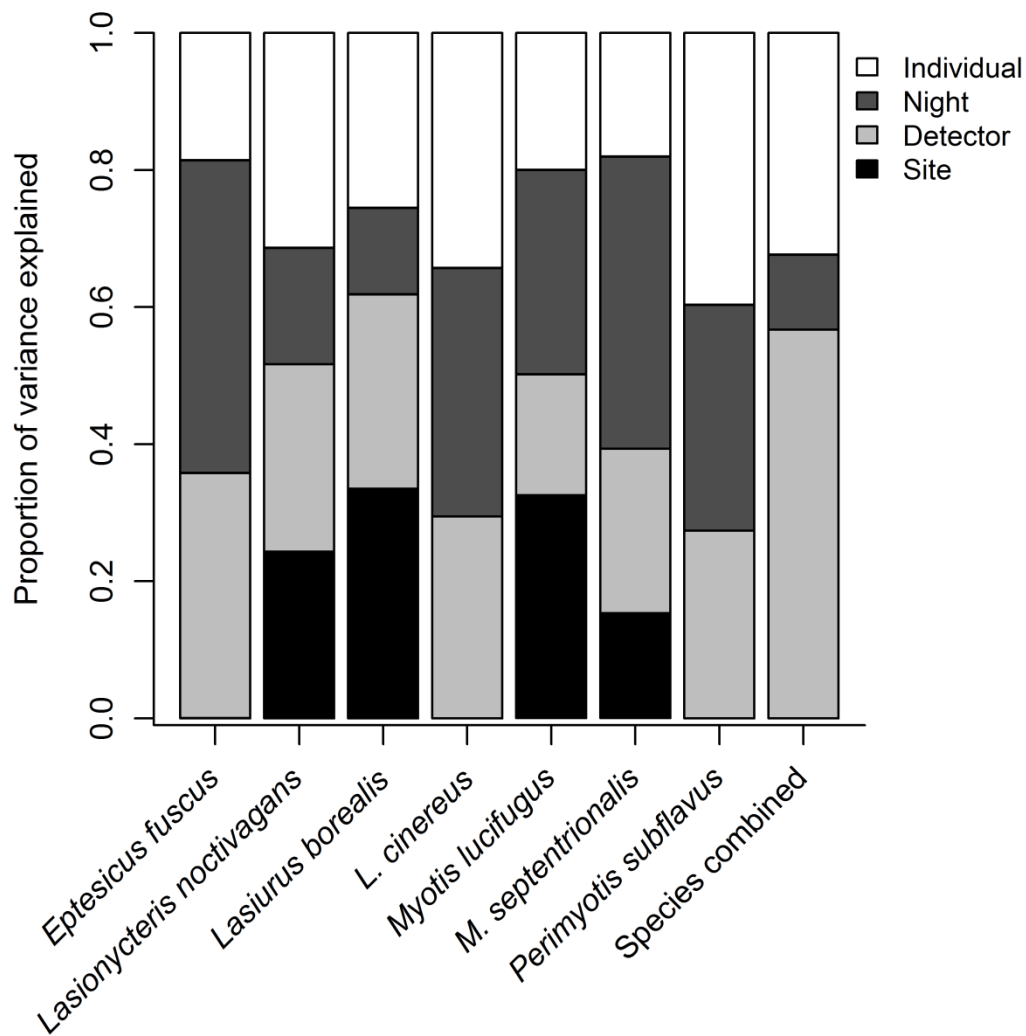
Figure 5.2. Proportion of variance explained by spatial (detector, site) and temporal (night) random effects for seven species of bats at four sites in Ontario, Canada. Within-site variation (detector location) is an important component in explaining variation in activity levels of all species combined. The importance of within-site, among-site, and night-to-night effects differs by species.

5.3.3 Percentile thresholds: magnitude of activity

The number of nights that each species had high activity, above the 50th and 90th percentile thresholds (Table 5.3), differed among all detectors at all sites (Table 5.4,B3). The "picture" of activity differed depending on detector placement. For example, at Renfrew mine in 2010 both *Myotis* species had high activity on the majority of the nights at all detectors. However, if only detector C were present, it would not be evident that *M. lucifugus* and *L. borealis* had very high activity at the site (Table 5.4). If not all detectors were present at the site in 2010 then the magnitude of activity for each species would not be evident. It was not necessary to have detectors at both heights in 2008 because the number of nights with high activity between paired detectors (1.5 and 4 m) was always the same or within one to two nights, except at QUBS (*L. noctivagans, L. cinereus,* and *M. septentrionalis*) and Long Point (*L. noctivagans*) where there was higher activity on more nights at one of the high detectors (Table B3).

Table 5.3. Number of bat echolocation calls per hour at the 50th and 90th percentile thresholds for each species and all species combined for a distribution of activity at eight sites in Canada and New York. Activity above the 50th percentile (median) is considered high and activity above the 90th percentile threshold is very high.

Species	Number of calls per hour at percentile thresholds	
	50th	90th
Species combined	218	2793
Eptesicus fuscus	35	308
Lasionycteris noctivagans	6	32
Lasiurus borealis	20	166
L. cinereus	31	327
Myotis lucifugus	87	1426
M. septentrionalis	42	960
Perimyotis subflavus	21	140

Table 5.4. Number of nights when at least one hour of bat activity was above the 50th and 90th percentile thresholds at each detector (A-E) over 15 nights at an abandoned mine near Renfrew, Ontario in 2010.

Species	50th percentile Detectors					90th percentile Detectors				
	A	B	C	D	E	A	B	C	D	E
Species combined	10	15	15	15	13	7	14	5	5	2
Eptesicus fuscus	1	1		2		1				
Lasionycteris noctivagans	4	1		1		1	1		1	
Lasiurus borealis	9	5		15	8		1		13	
L. cinereus	2	1		7	1				1	
Myotis lucifugus	10	15	15	15	14	6	7		7	2
M. septentrionalis	10	15	15	13	15	4	14	10		1
Perimyotis subflavus	3	4		10	3	1	1		1	

5.3.4 SaTScan: peaks of activity in space and time

Peaks of activity, detected by SaTScan, differed among detector locations, even between paired detectors at two heights, at all sites (Fig. 5.3,B1). Although activity patterns varied, we were unable to detect evidence of spatial partitioning among species, every species had overlapping activity on at least one detector at each site. All species had peaks of activity at every detector during at least one season, except *E. fuscus* that never had peaks of activity at detectors B1/B2 Long Point (Fig. 5.3A) or detector C1 at QUBS (Fig. B1.2). There was some degree of temporal partitioning among species within sites, for example *Myotis lucifugus* and *M. septentrionalis* did not have overlapping activity peaks at any detector at Long Point (Fig. 5.3A); neither did *L. noctivagans, L. borealis,* and *P. subflavus* (Fig. 5.3A). At Renfrew 2010 only three species had significant peaks of activity on the majority of nights and timing of peaks overlapped among all three species (Fig. 5.3B). *Lasionycteris noctivagans* and *L. borealis* peaks of activity rarely overlapped temporally in 2008 (Fig. B1).
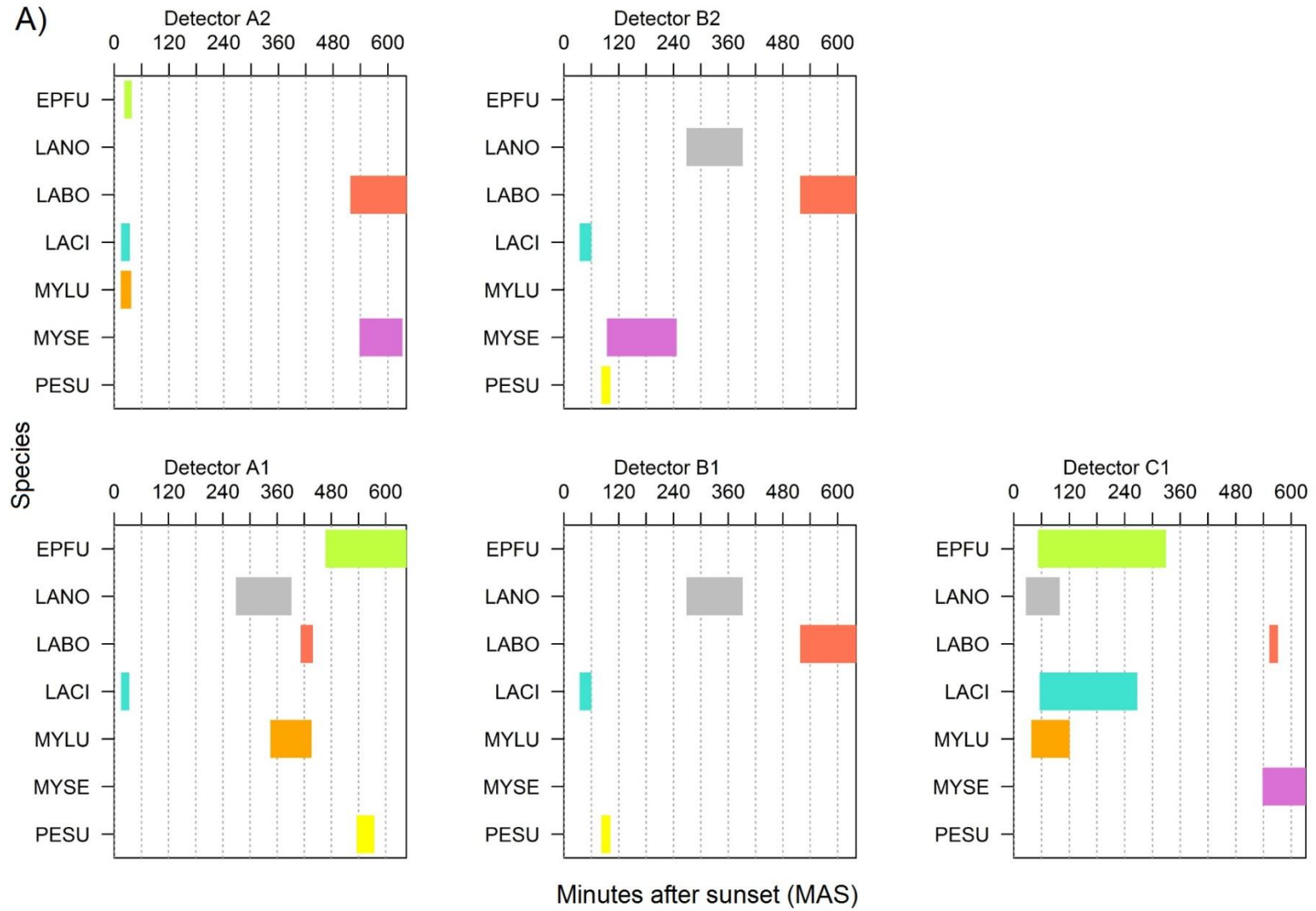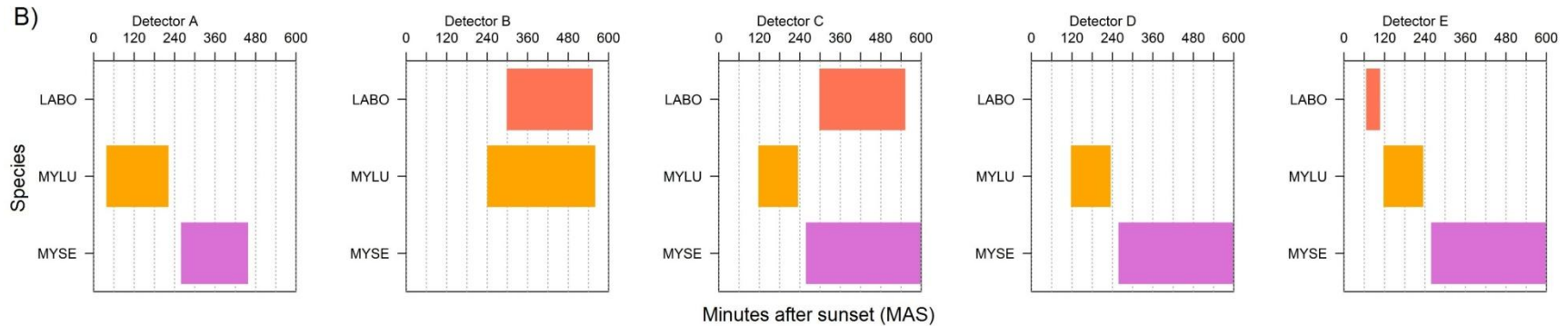
Figure 5.3. Peak periods of bat activity along linear, stationary transects within two sites: A) during late summer (migration) at Long Point, Ontario in 2008 and B) during swarming (August) at an abandoned mine near Renfrew, Ontario in 2010. Species-specific activity patterns vary among detectors. Detectors at Long Point were paired at two heights, low (1.5 m; A1, B1, and C1) and high (4 m; A2 and B2). All detectors at Renfrew were at 1.5 m. There is no evidence of spatial partitioning, but temporal partitioning (no time overlap of peak activity) is evident between several species, such as *L. noctivagans* and *L. borealis.* EPFU – *Eptesicus fuscus*, LANO – *Lasionycteris noctivagans,* LABO – *Lasiurus borealis*, LACI – *L. cinereus*, MYLU – *Myotis lucifugus*, MYSE – *M. septentrionalis,* PESU – *Perimyotis subflavus*.

5.3.5   Sub-sampling for estimates of activity

As the number of nights increased so did the probability of obtaining mean estimates of

activity more similar to the grand mean (Table 5.5,5.6). When sampling with one detector

and species identification was not taken into account, it required three to nine nights to

have at least 80% of the subsamples within 30% of the grand mean in 2010 (Table 5.5).

When we accounted for species, the number of nights increased; the most active species,

M. lucifugus and *M. septentrionalis*, required fewer nights of sampling than any of the

other species at the site (Table 5.5). When the grand mean included multiple detectors

within a site, we required at least four detectors recording for a minimum of four nights to

have accurate estimates of overall activity across a site (Table 5.6A,B). We required

sampling for at least five nights with five detectors for the most active species within a

season (Table 5.6B). Even within a season it was not possible for a single detector

moving locations for fifteen nights to be within 30% of the grand mean. Fewer nights

were required when all nights in the grand mean were during a single season (Table

5.6B), as opposed to during multiple periods in the year (Table 5.6A).

Table 5.5. Mean ± SD number of nights required to have at least 80% of random samples with mean nightly bat activity within 10-50% of the grand mean of the entire dataset (total calls per night for one detector over 15 nights) for six detectors at a mine near Renfrew, Ontario, Canada in 2010. Data were randomly sampled 100 times to include nightly activity for one detector for two to ten night sample periods. Empty cells are when more than ten nights of sampling were required for all detectors.

Species	Percent deviation from grand mean				
	≤10%	≤20%	≤30%	≤40%	≤50%
Species combined		8.4 ± 3.4	5.6 ± 3.4	4.3 ± 1.7	3.0 ± 1.4
Eptesicus fuscus					9.2 ± 1.8
Lasionycteris noctivagans					8.8 ± 4.7
Lasiurus borealis			8.4 ± 4.2	6.8 ± 4.1	5.6 ± 3.8
L. cinereus				10.6 ± 1.7	8.0 ± 2.5
Myotis lucifugus			6.8 ± 3.7	5.8 ± 4.0	4.4 ± 2.9
M. septentrionalis			7.6 ± 3.6	5.6 ± 3.7	4.4 ± 3.8
Perimyotis subflavus			10.5 ± 2.1	8.5 ± 1.5	7.4 ± 1.8

Table 5.6. Number of nights required to have at least 80% of random samples with mean nightly bat activity within 30% of the grand mean of the entire dataset at A) four sites in Ontario, Canada in 2008 (five detectors over 12 nights) and B) one site in 2010 (five detectors over 15 nights). Data were randomly sampled 100 times to include nightly activity for one to five detectors for two to ten night sample periods for each site. Empty cells are when more than ten nights of sampling were required for all sites.

A) Species	1	2	3	4	5
Species combined		10.5 ± 3	10.3 ± 3.5	9.8 ± 4.5	7.3 ± 4.0
Eptesicus fuscus				11.0 ± 2.0	9.5 ± 3.3
Lasionycteris noctivagans			11.3 ± 1.5	9.8 ± 2.6	8.5 ± 4.1
Lasiurus borealis				10.5 ± 3.0	8.8 ± 3.0
L. cinereus			11.2 ± 2.0	8.8 ± 3.8	9.8 ± 4.5
Myotis lucifugus					10.3 ± 3.5
M. septentrionalis			10.3 ± 3.5	8.5 ± 4.1	7.8 ± 5.1
Perimyotis subflavus					11.0 ± 1.7

B) Species	1	2	3	4	5
Species combined				4	3
E. fuscus					
L. noctivagans					
L. borealis					4
L. cinereus					
M. lucifugus				9	5
M. septentrionalis					5
P. subflavus					9

5.4   Discussion

Patterns of bat activity vary within a habitat in response to a variety of biotic (e.g., prey abundance) and abiotic factors (e.g., habitat structure). Our results demonstrate significantly different depictions of bat activity depending on where a detector was placed within a site, even when relatively close together (40 m), including differences in number of calls, magnitude of activity, and temporal patterns of activity. While there was some effect of detector height and canopy cover on activity levels, these factors did not explain the majority of the variation from detector location. Differences in where peaks of activity occurred among detectors indicate that it is important to place multiple detectors throughout a site in order to capture spatial variation in activity.

Our results differed from those in other regions, suggesting that the relative importance of sources of variation can vary considerably regionally. Variation among sites was less important in our study than in Australia (e.g., Fischer *et al.* 2009), accounting for only 13% of variation on average. Within-site heterogeneity was most important at our sites, accounting for two-thirds of variation in activity levels, while it was not as significant in other studies (Krusic *et al.* 1996b, Fischer *et al.* 2009). It is clear that experimental design will impact what we see in snap-shots of bat activity at a site. The goal is to have the most accurate estimate of activity, while the extent of variation determines where effort needs to be focused to increase accuracy. When interested in overall activity levels, it is most important to sample for fewer nights and more locations within a site, but the number of nights sampled must increase when estimating species-specific activity.

Detectors placed at greater heights did not help to account for variation in activity levels, except for one species. It is possible that the small difference in heights (2.5 m) and low

vertical vegetation structure was not biologically meaningful or high enough to reveal a difference. However, detectors with a small difference in height have shown disparity of activity levels (Fischer *et al.* 2009), although these differences could be attributed to slightly different detector locations within the site. There is value in sampling at heights greater than 1.5 m (e.g., Krusic *et al.* 1996, Reynolds 2006), but our study suggests this is only worthwhile if detectors are at heights greater than 4 m. While we saw differences between detector heights in timing of activity peaks (e.g., Fig. 5.3) and the number of nights with activity above various percentile thresholds, we suggest a better use of multiple detectors would be to sample at more points throughout the site to account for horizontal heterogeneity in similar regions.

The only evidence of temporal partitioning was between *L. noctivagans* and *L. borealis,* which corresponds to findings by Kunz (1973) in Iowa, most likely driven by preferences for different insect prey. It is possible that this finding is an artifact of relatively low activity levels of *L. noctivagans* at the majority of our sites, but warrants further investigation into these species' activity patterns. Sampling for the entire night is a requirement for any study interested in activity levels (Hayes 1997, Skalak *et al.* 2012). While many studies detect a peak of activity in the first two hours after sunset (e.g., Kunz 1973, Hayes 1997, Broders *et al.* 2003), only *L. cinereus* regularly had peaks of activity in this period during our study. Where and when peaks in species-specific activity occurred differed enough among detectors to suggest that sampling at a single point or for a portion of the night would give only a partial picture of bat activity at a given site.

Greater sampling effort, in both space and time, will always lead to more accurate estimates of activity levels. When sampling with a single detector, regardless of species,

our findings agreed with those of Hayes (1997), which recommended sampling for no less than six nights. It appears we had greater variation among detectors within a site (Table 5.5) than Hayes (1997) did among sites and years. Once species-specific estimates of activity for a site include multiple sampling points within a site, it is not possible to have accurate estimates of activity with a single detector. Species with relatively high activity levels require fewer nights of sampling; the number of nights sampled at a site would need to be either based on a particular species of interest or on the least active species at the site. Acoustically rare species, with lower detection probabilities, have higher variation in activity levels, more time with no activity, requiring more nights to simply detect these species (Skalak *et al.* 2012). Using one detector and moving it to a different location each night, while an option at a large spatial scale (Skalak *et al.* 2012), is not as desirable at a small scale. We required fewer nights when recording with multiple detectors simultaneously within a site. Blocked sampling designs are more efficient than completely randomized designs for comparisons among sites, requiring fewer nights of sampling (Hayes 1997), which was also the case for sampling within sites.

There is growing concern of inadequate accounts of variation in acoustic studies (Hayes 2000, Sherwin *et al.* 2000, Gannon *et al.* 2003, Skalak *et al.* 2012). Considering our results, the use of multiple detectors within a site should be standard practice for all acoustic surveys (Duchamp *et al.* 2006, Fischer *et al.* 2009, Stahlschmidt and Brühl 2012). Our findings caution extrapolation of results from a single detector to estimate activity of a site. The importance of among- and within-site variation differs among regions and it may be possible to use fewer detectors with less structural complexity at a site (Britzke 2003). If using fewer detectors it would be worthwhile conducting a

preliminary field season to see where important sources of variation are in order to focus sampling efforts more efficiently in future seasons. If experimental designs do not adequately account for within-site variation, activity estimates will be biased.

5.5 Acknowledgements

5.6 References

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6   Conclusion

The main goal of this dissertation was to investigate sources of variation in acoustic

monitoring of bat activity and how this impacts acoustic survey design. I addressed this

goal in three ways: i) variation from bat detectors, ii) methods for objective identification

of peaks of activity, and iii) the use of stationary, linear transects to address within-site

spatial and temporal variation. While several studies have examined variation in acoustic

monitoring of bats and made recommendations for future surveys (Hayes 1997, 2000,

Fischer *et al.* 2009, Skalak *et al.* 2012), more are needed to understand sources of

variation and improve methods to develop standardized sampling protocols. Combining

the results of this research, I have provided insight into how sources of variation and

methods of data analysis can impact interpretation of acoustic data of bat activity.

Detailed discussions of specific experimental results are presented in the pertinent

chapters. Here, I summarize the cumulative findings of my dissertation and make

recommendations for future acoustic bat surveys. Finally, I conclude with a description of

challenges and gaps in our knowledge challenges to our greater understanding of bat

activity, and suggestions for future research directions.

6.1   Contribution to acoustic studies of bats

An integral component of conducting effective acoustic surveys is accounting for sources

of variation, an essential step to collecting unbiased data (Hayes 2000), while using

objective methods to analyze that data. This information is necessary for effective

sampling design, data analysis, and interpretation of results. I have contributed to this

field in three ways:

6.1.1   Variation from detectors

Many factors can increase variation in an acoustic dataset, one of which is the detector

used (Adams *et al.* 2012/Chapter 2). I found that detector performance varied among

systems, differing in sensitivity and directionality, and is an important source of variation

in acoustic monitoring methods that must be accounted for when creating sampling

protocols and comparing datasets. The value of these results is not simply as a

comparison of different detectors, but as a demonstration of how different technologies

can give different results; an issue that needs to be considered when designing and

evaluating any studies using these technologies.

6.1.2   Objective methods for detecting peaks in bat activity

A limitation of acoustic monitoring is the relative nature of data interpretation.

Previously, conclusions have been based on subjective assessments about the relative

importance of sites or species-specific activity patterns. I proposed two methods for

objectively identifying peaks in bat activity at various scales: percentile thresholds

(Chapter 3) and SaTScan (Chapter 4). Using percentile thresholds to assess acoustic data

permits an unbiased measure of the importance of a site and is a replicable method of

describing within-night activity patterns. The strength of this method is evaluating

activity levels at several thresholds based on a larger distribution of activity among sites.

SaTScan is a valuable tool for quickly identifying peaks with an objective, replicable, and

statistically-sound method that can be applied at various temporal and spatial scales.

Using these two methods in combination permits a thorough investigation of activity

levels and patterns at a site, from the magnitude of species-specific activity to comparison of timing of peaks among species or sites.

6.1.3 Variation within sites

Bat activity can vary temporally (e.g, Hayes 1997, Milne *et al.* 2005), but within-site spatial variation has been too often overlooked (Britzke 2003, Fischer *et al.* 2009). I found that within site factors are very important for understanding variation in bat activity, being as or more important than differences among sites (Chapter 5). The high degree of variation within sites can affect sampling design, including necessary sampling effort, and requires the use of multiple detectors recording simultaneously within a site. Detector placement within a site dramatically impacts the depictions of activity, in turn impacting estimates of levels and patterns of activity. An *a priori* understanding of the survey effort necessary should ensure statistically powerful sampling designs, clearer data interpretation, and more successful management and conservation actions.

6.2 Recommendations for future acoustic surveys

To use acoustic monitoring to address ecological questions, it is important to know how sources of variation affect data collection and thus the data itself. While there is no simple formula for what constitutes an ideal survey effort, it is clear that additional effort will result in more precise estimates of activity. Accuracy increases with the number of nights sampled and detectors deployed. It is important to first clearly define the research question and decide on the best study design to test the predictions. If the aim of a study is to determine overall activity levels at a site then a site in Ontario would require

sampling for at least four nights with four detectors within a season, but would require an increased sampling effort when evaluating species-specific activity. It is difficult to extrapolate from my results because the degree of habitat heterogeneity differs among sites. I recommend using preliminary studies to determine the number of detectors and nights necessary to obtain an accurate estimate of activity before establishing a long-term monitoring program. I echo the recommendations of other authors (Hayes 1997, Skalak *et al.* 2012) that monitoring should be done continuously through the night. Ideally, sampling should occur for as long as possible; this is relatively easy with passive methods, but long-term datasets can be inhibiting in terms of analysis.

It is important to use a single brand of detector for a monitoring program and to report detector settings in publications to ensure comparable results among locations and years (Adams *et al.* 2012/Chapter 2). Detectors should be calibrated to reduce variation among detectors of the same brand and among sampling periods (Larson and Hayes 2000). Passive detection systems with an automatic trigger are best for developing standardized sampling protocols because they remove biased sampling methods and require little effort for deployment (Stahlschmidt and Brühl 2012). Choice of bat detector will depend on the research question being asked and potentially be influenced by budgetary constraints. Study location and focal species will determine which detectors are appropriate based on their frequency response. Wildlife Acoustics' SongMeter SM2BAT has two different models that differ in sampling rate and the lower sampling rate model would not be adequate to record all species present in the Neotropics. Full-spectrum detectors are a better choice for the majority of research questions since they are more sensitive, with greater detection ranges (Adams *et al.* 2012/Chapter 2), and collect more information than

frequency division systems, leading to more accurate species identification (Fenton 2000). If asking questions about echolocation behavior then a more sensitive and calibrated microphone will be important. Research questions about activity levels at a particular site will require decisions on a trade-off between the more expensive detector (i.e., Avisoft, Batlogger) that detects calls in a larger volume of airspace at a given location or a less expensive option (i.e., SongMeter). Also involved in the decision is the importance of simultaneously monitoring multiple locations within a site. Sampling area heterogeneity and access to multiple detectors will impact this decision.

Successful application of acoustic monitoring to detect within-site variation requires the use of multiple detectors simultaneously (Chapter 5). Understanding structural heterogeneity at a site can determine the number of detectors necessary to capture vertical and horizontal variation in bat activity. A reasonable survey effort will depend on the objectives of a particular study. While my recommendations are for surveys sampling patterns and levels of activity, they are relatively in line with surveys for species richness. Recording continuously for the entire night together with increased sampling effort for more nights at more locations will increase chances of detecting rare species (Skalak *et al.* 2012).

Finally, it is necessary to use objective analytic methods for acoustic data because of the already inherent relative nature of the data. Use of programs, such as SaTScan, makes analysis consistent and replicable. It is necessary to measure activity levels relative to a large distribution, which is closer to the ground truth of what is present in nature. The next step is to create a public repository of acoustic datasets to evaluate activity of a species in the context of its entire range, allowing standardization of terms such as "high

activity." Standardization makes it possible to review methods used for environmental assessments and creation of protocols for unified monitoring programs among regions.

6.3   Future research directions

It is clear from my results (Chapter 5) and those of Fischer *et al.* (2009) that activity from a single location does not reflect all locations within a site. My specific findings about within-site variation are unlikely to be directly applicable to other regions because of varying habitat heterogeneity. Vertical spatial partitioning is evident in many habitats (Hecker and Brigham 1999, Kalcounis *et al.* 1999, Hayes and Gruver 2000), but it has not been established what acoustic sampling effort is necessary to detect these patterns. Sampling vertical distributions is limited by the logistics of raising detectors to greater heights; most successful is opportunistic placement on manmade structures, such as towers and poles (Kalcounis *et al.* 1999, Hamilton 2012) and wind turbines (Reynolds 2006), or attaching them to trees (Staton and Poulton 2012b). Further research into patterns of vertical and horizontal spatial variation among habitats is necessary, especially with respect to how habitat structure and differences in insect distributions play roles in determining bat activity. Ideally, future research will provide insights into how transferable findings regarding sampling effort are to other regions with the goal of establishing what measures are necessary to determine sampling efforts in new regions without extensive preliminary study.

Most bat surveys in temperate areas primarily use acoustic methods because of the detectability of the echolocation calls of most insectivorous species, allowing development of standardized protocols based on acoustic monitoring in these regions.

However, acoustic monitoring is not a "silver-bullet" for sampling all bat communities; capture methods are required to sample whispering bats with low intensity echolocation calls and those that do not echolocate at all (Griffin 1958, Fenton 2003). Creating standardized sampling protocols for regions with greater species diversity, such as the tropics, will require an understanding of factors influencing capture success (Kalko and Handley 2001) and how recommendations for acoustic sampling effort would differ.

A major limitation to conservation and management efforts is knowledge of where important sites are for bats. Research into the detectability of special sites, such as roosts, hibernacula, swarming sites, and migration stopover sites, would be extremely valuable. Understanding horizontal spatial variation will play a role, with how patterns of activity drop off as bats move away from these special sites and the proximity to a special site required to detect levels of activity high enough to be notable.

Walking and driving transects are methods used increasingly for standardized bat surveys. The UK's National Bat Monitoring Program uses volunteers with detectors walking 1 km transects to sample bats and has been successful at detecting population trends over time (Walsh *et al.* 2001). At least 17 states (Herzog and Britzke 2009) and one province use driving transects to sample bat activity levels post-white-nose syndrome (WNS, Britzke and Herzog). Efforts to collect long-term datasets in a standardized and comparable fashion are laudable, but there is little scientific literature to support the use of this method. Russ *et al.* (2003) describe the use of a driving transect and discuss its validity, but make no effort to compare the method, and this is likely what most driving surveys protocols are based on. Stahlschmidt and Brühl (2012) have been the only researchers to compare moving transects to stationary detectors, finding that walking transects fail to

represent the heterogeneous bat activity patterns and stationary detectors have the greatest potential for standardized surveys. There is an urgent need for research into the feasibility of moving transect surveys.

While much research is focused on how to survey at wind energy developments to determine which sites will be high-risk for bats (Reynolds 2006, Arnett *et al.* 2011, Korner-Nievergelt *et al.* 2011), there is still little information available to guide policy and permit critical evaluation of wind energy development proposals and environmental assessment reports. Percentile thresholds (Chapter 3) are the first proposed method for objectively comparing the importance of a site to a species of bat, but we need continued development and research into how environmental recommendations, with the potential to impact survival of numerous bats species, are determined.

6.4 Concluding remarks

Acoustic monitoring studies have a number of inherent limitations and assumptions, but the use of bat detectors can be a very powerful tool for insights into the ecology and behavior of bats. Design of any study includes trade-offs between research objectives and logistics. As acoustic monitoring is increasingly used for large-scale management and conservation efforts in response to growing threats to bat populations, the need for research to support standardized protocols is also rising. The results of my dissertation have provided an increased understanding of how variation plays a role in sampling bat activity. I hope that the information presented here will provide a platform for continued research into objective methods and standardized protocols of studying bats in order to streamline bat conservation efforts of this ecologically important group of mammals.

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Appendices

Appendix A: Discriminant function analysis for species identification

Passive acoustic monitoring makes it easy to collect large amounts of data; the challenge

is analyzing all of it. Identification of echolocation calls to species is a daunting task

when done manually, but automated methods make processing many recordings quick

and efficient. Automated methods are more consistent and predictable, and can be more

accurate than manual analysis (Jennings *et al.* 2008). A common technique for automated

identification of bat calls is discriminant function analysis (DFA), it is not only a classic

statistical technique, available in all statistical software packages, but is also very

effective for classifying echolocation calls to species. DFA has outperformed other classic

statistical methods (cluster analysis) and nonconventional methods (classification and

regression trees, and neural networks) with higher classification accuracy of identifying

calls to species (Preatoni *et al.* 2005).

I developed a DFA for the seven species of bats in Ontario, Canada to analyze my data

(Chapters 3, 4, 5). The reference dataset of search-phase calls from recordings of free-

flying bats recorded outside of known roosts or at foraging sites where species and

individual bats were identified unambiguously (assembled by Lauren Hooton, MSc). No

reference recordings came from hand-released individuals. The reference dataset included

calls for seven species of bats in Ontario (Table A1). An eighth species (*Myotis leibii*) is

found in Ontario, but was not included in the DFA due to the relative rarity of the species,

lack of verified recording for the reference dataset, and difficulty differentiating calls from the much more common *M. lucifugus.* The training dataset for the DFA included one, randomly selected call per individual in order to avoid pseudoreplication (Mundry and Sommer 2007). I had unequal sample sizes among species (Table A1), but this is not a problem for DFA. Because of unequal covariance I used quadratic DFA (Vaughan *et al.* 1997b, Parsons and Jones 2000).

The DFA included 11 predictor variables (call parameters), minimum frequency (Fmin), maximum frequency (Fmax), duration, frequency of most energy (FME), 10th percentile of energy (F10), 60th percentile of energy (F60), 90th percentile of energy (F90), median frequency slope (dFmedian), median energy slope (dEmedian), median frequency smoothness (sFmedian), and median energy smoothness (sEmedian). All call parameters were automatically extracted by the automated detection feature in callViewer (v. 18; Skowronski 2008). CallViewer is a custom echolocation sound analysis program written with MATLAB software (The MathWorks, Natick, Massachusetts). The automated detection parameters were the default settings, except minimum link length was set at 10, minimum energy was set at 14 dB, echo filter threshold was set at 10 dB, and lower frequency cutoff was set to 14 kHz. I chose call parameters to maximize classification accuracy with backwards, stepwise selection, starting with all 21 variables extracted by callViewer and removing them one at a time. The DFA should have fewer predictor variables than the smallest sample size; following the 1/3 rule I reduced down to 11 variables. Classification accuracy with leave-one-out cross-validation was high (Table A1), even between *Eptesicus fuscus* and *Lasionycteris noctivagans*, which cannot be discriminated with manual analysis (Betts 1998).

Table A1. Species-specific details for discriminant function analysis (DFA) to identify unclassified bat echolocation calls to species. The sample size per species was the number of echolocation calls per species included in the reference database (training data) for DFA. Each call was from a different individual. Classification accuracy of the DFA with leave-one-out cross-validation is the percent correct identifications of the training data.

Species	Sample size	Classification accuracy (%)
Eptesicus fuscus	50	78
Lasionycteris noctivagans	50	94
Lasiurus borealis	58	88
L. cinereus	52	90
Myotis lucifugus	50	90
M. septentrionalis	33	94
Perimyotis subflavus	42	93

Before identification of unidentified recordings with DFA, I filtered all acoustic data to eliminate noise and weak or fragmented calls, only including detections with duration 0.99 – 30 ms and Fmin 15 - 60 kHz. The filter parameters were selected based on conservative estimates of the echolocation call structure of the species of bats present in Ontario. The DFA compared our unidentified data to the training dataset and identified each call to species. To further improve classification accuracy, and because DFA does not assign calls to an "unknown" category, I applied a post-hoc, species-specific filter to remove any data that were above or below typical durations and minimum frequencies for each species (Table A2).

DFA and filters were performed in R (v. 2.13.1, R Development Core Team 2011).

Table A2. Post-discriminant function analysis filter to increase classification accuracy of automated identification of bat echolocation calls. The filter removed identified calls that were outside the species-specific ranges of call duration and minimum frequency.

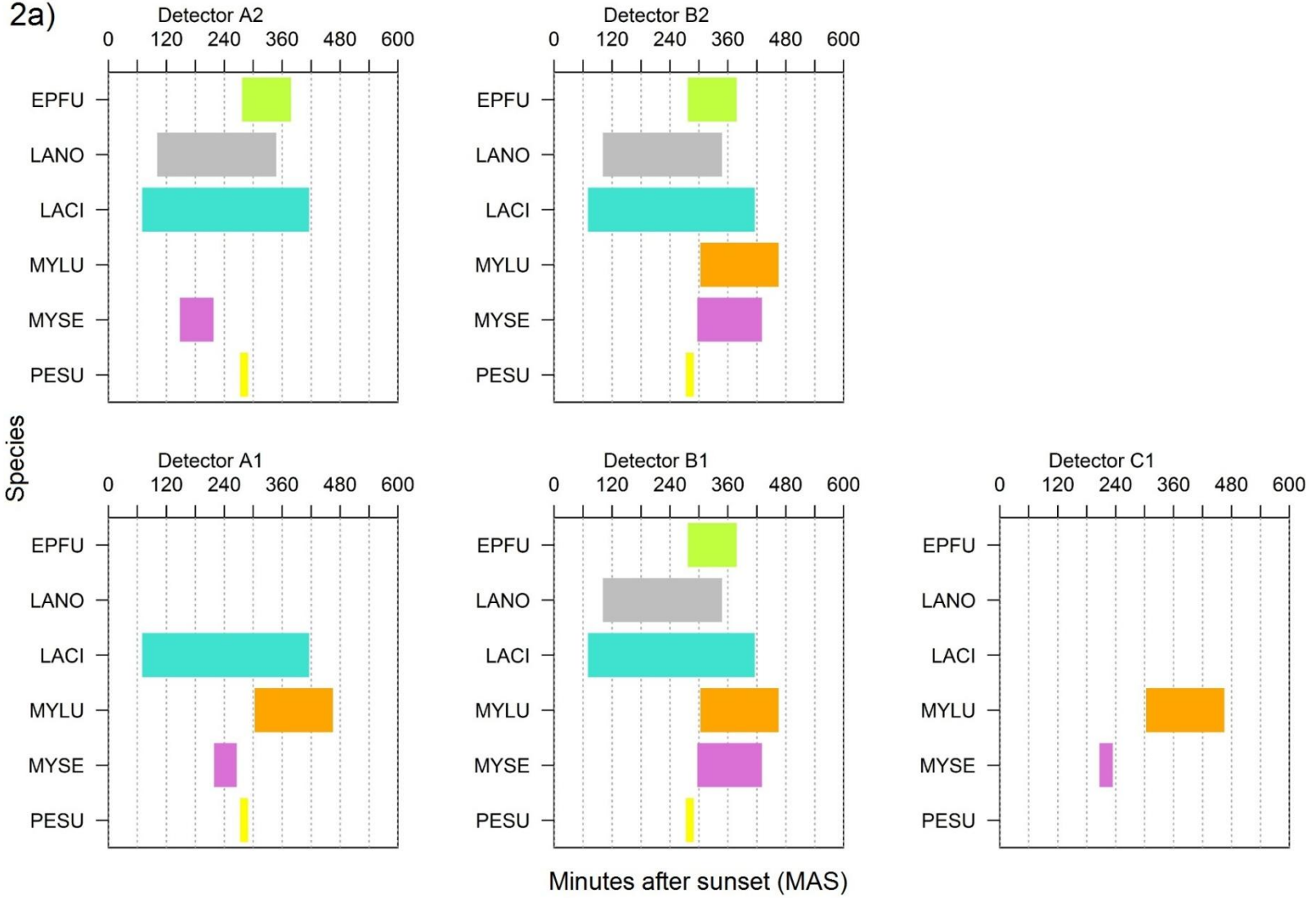| Species	Duration (ms)	Minimum frequency (kHz)
Eptesicus fuscus	3 – 12	20 – 30
Lasionycteris noctivagans	4 - 12	21 - 30
Lasiurus borealis	5 - 17	29 – 43
L. cinereus	8 - 30	15 – 29
Myotis lucifugus	3 - 8	30 – 43
M. septentrionalis	1 – 3.3	25 - 60
Perimyotis subflavus	5 - 15	36 - 46

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Appendix B: Chapter 5 supplementary material

1)

2a)

2b)

Detector A2

Detector B2

Detector A1

Detector B1

Detector C1

Minutes after sunset (MAS)

Species

3a)

3b)

Minutes after sunset (MAS)
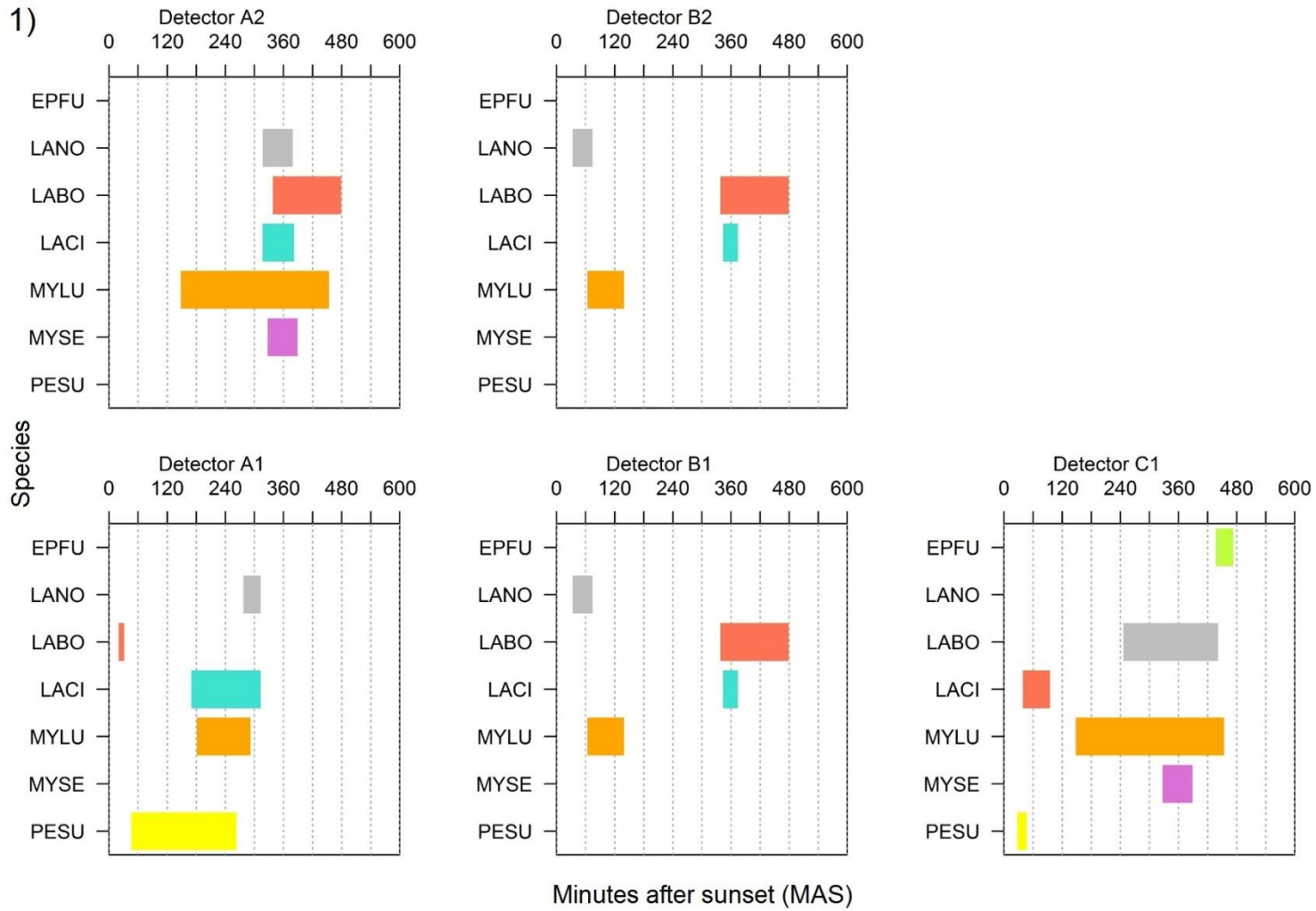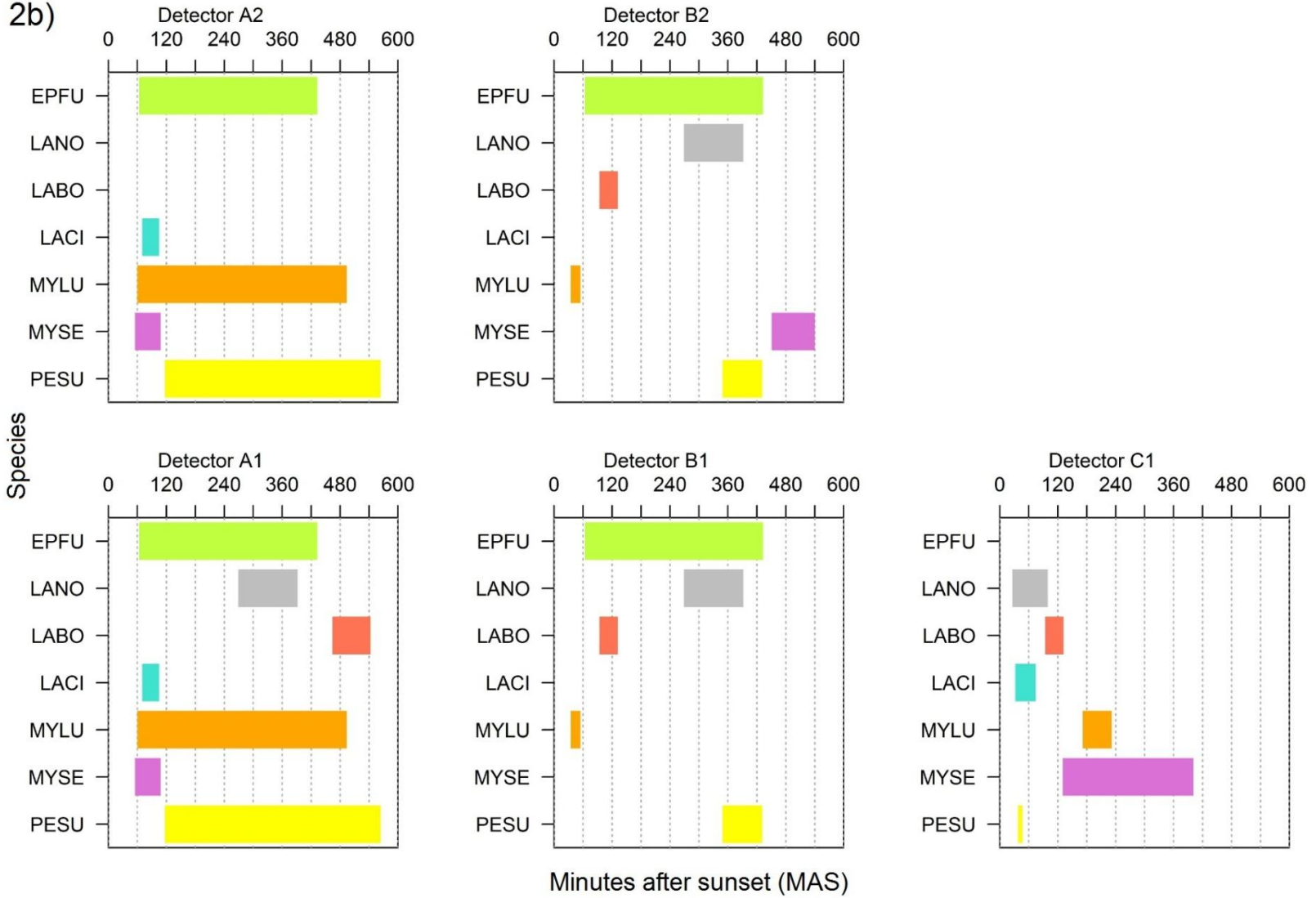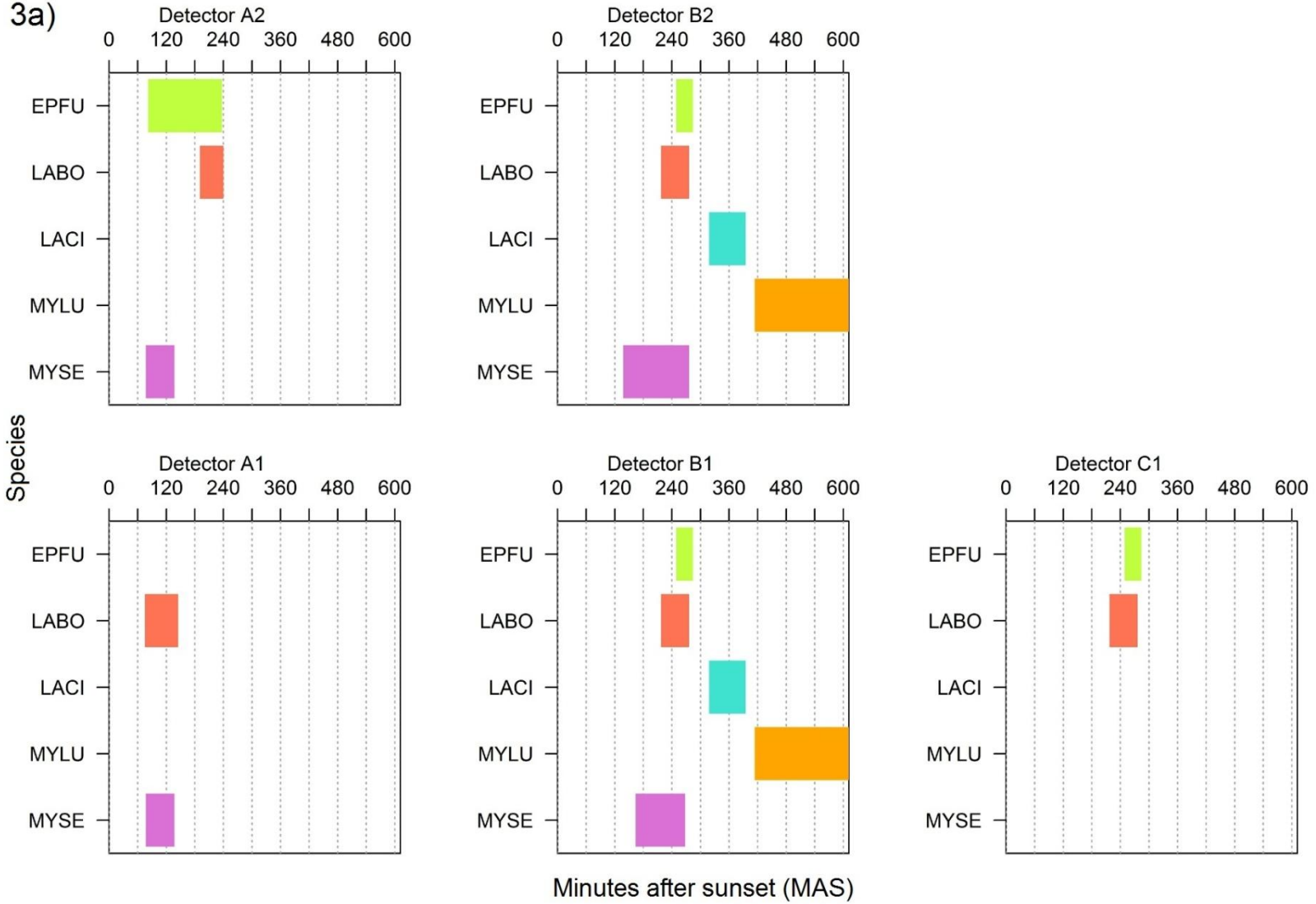
4a)

Figure B1. Peak periods of bat activity along linear, stationary transects within four sites in Ontario, Canada in 2008:1) spring at Long Point, 2a) spring at QUBS, 2b) late summer at QUBS, 3a) spring at Renfrew lake, 3b) late summer at Renfrew lake, 4a) spring at Renfrew mine, and 4b) late summer (swarming) at Renfrew mine. Detectors were paired at two heights, low (1.5 m; A1, B1, and C1) and high (4 m; A2 and B2).

Table B1. Comparison of mean hourly bat activity among detectors for each species within sites in Ontario, Canada in 2008: A) Long Point, B) QUBS, C) Renfrew lake, D) Renfrew mine, and E) Renfrew mine in 2010 (df = 4). Significant Kruskal-Wallis tests indicate that activity was significantly different among detectors within a site.

A) Long Point

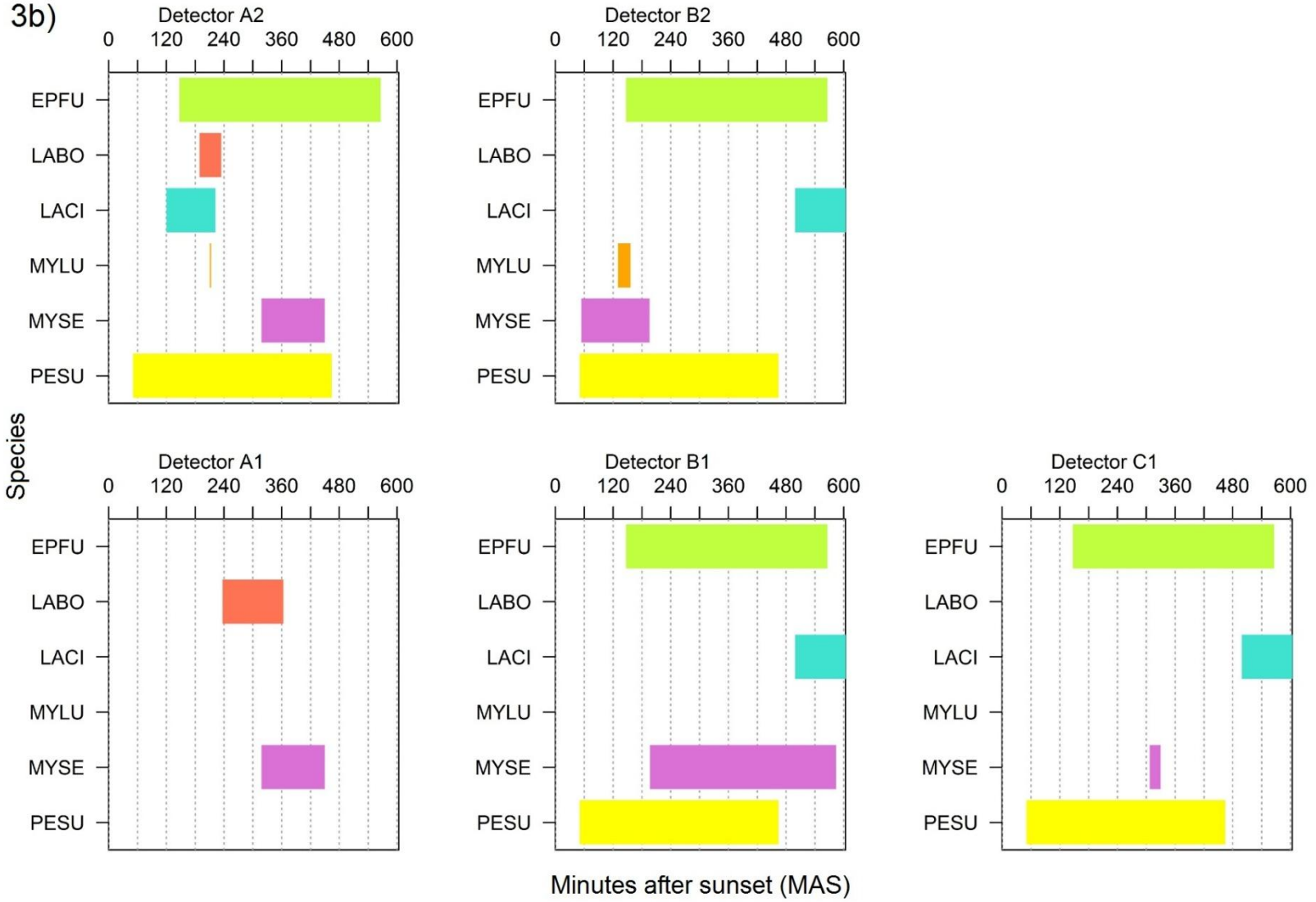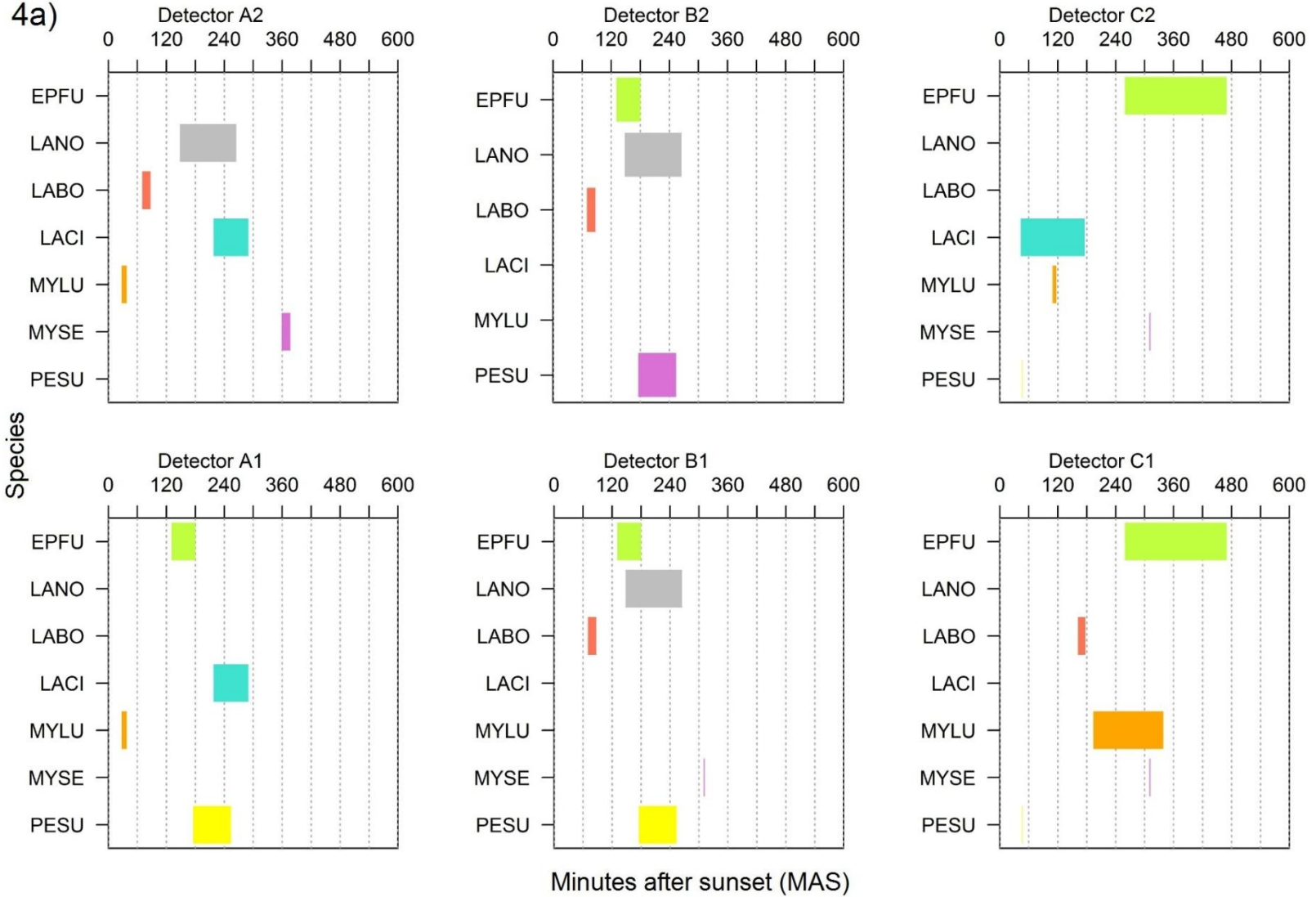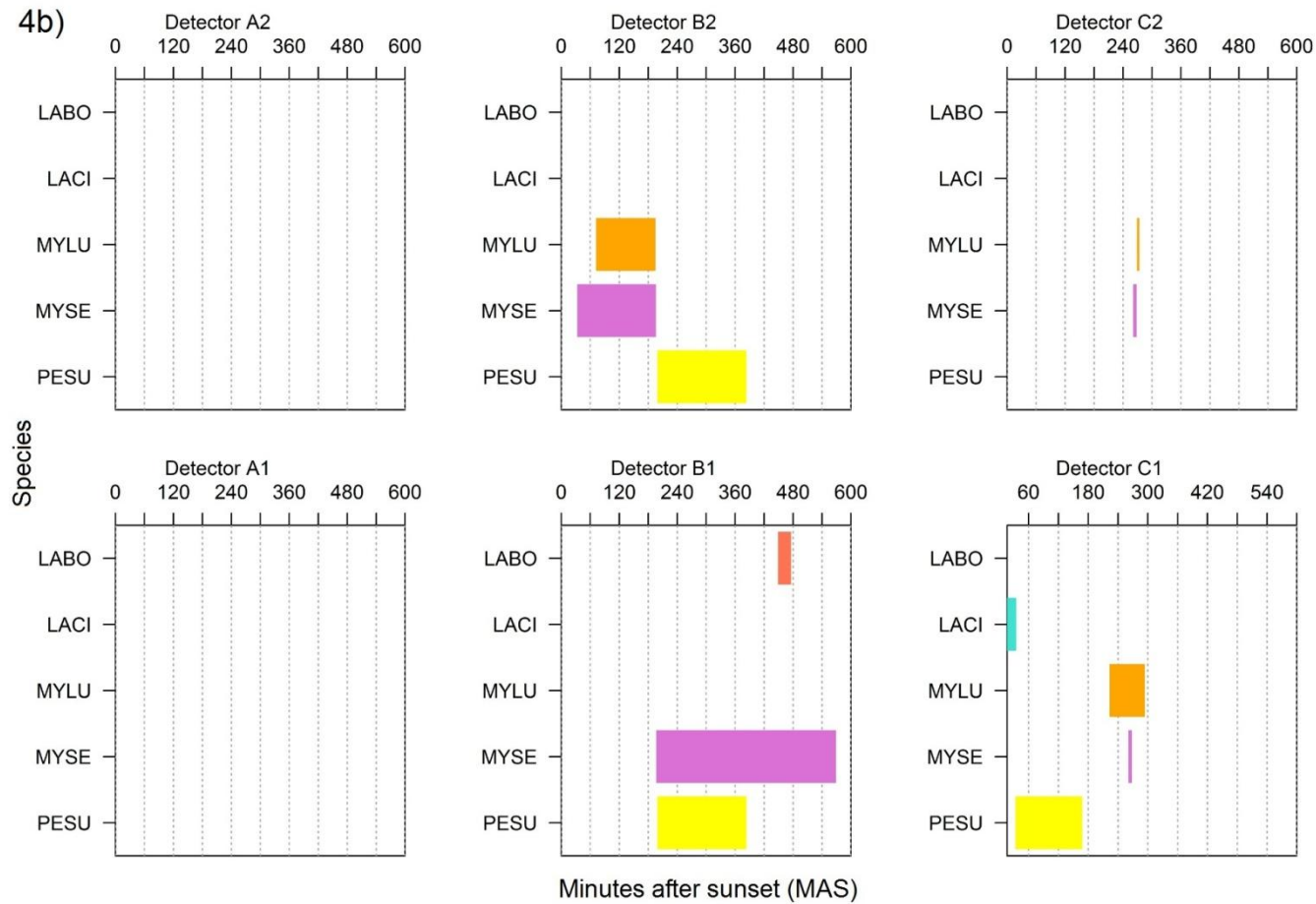| Species	H statistic	p-value
E. fuscus	130.94	< 0.001
L. noctivagans	104.21	< 0.001
L. borealis	252.66	< 0.001
L. cinereus	182.01	< 0.001
M. lucifugus	133.94	< 0.001
M. septentrionalis	146.94	< 0.001
P. subflavus	202.91	< 0.001

B) QUBS

Species	H statistic	p-value
E. fuscus	58.45	< 0.001
L. noctivagans	8.11	0.09
L. borealis	64.53	< 0.001
L. cinereus	36.80	< 0.001
M. lucifugus	34.41	< 0.001
M. septentrionalis	3.34	0.50
P. subflavus	42.23	< 0.001

C) Renfrew lake

Species	H statistic	p-value
E. fuscus	48.40	< 0.001
L. noctivagans	7.51	0.11
L. borealis	172.92	< 0.001
L. cinereus	19.55	0.001
M. lucifugus	118.41	< 0.001
M. septentrionalis	3.98	0.41
P. subflavus	54.54	< 0.001

D) Renfrew mine

Species	H statistic	p-value
E. fuscus	29.77	< 0.001
L. noctivagans	37.11	< 0.001
L. borealis	80.88	< 0.001
L. cinereus	33.76	< 0.001
M. lucifugus	18.34	0.001
M. septentrionalis	34.56	< 0.001
P. subflavus	11.64	0.02

E) Renfrew mine 2010

Species	H statistic	p-value
E. fuscus	14.93	0.005
L. noctivagans	24.16	< 0.001
L. borealis	239.68	< 0.001
L. cinereus	50.69	< 0.001
M. lucifugus	47.14	< 0.001
M. septentrionalis	157.46	< 0.001
P. subflavus	40.95	< 0.001

Table B2. Final models of species-specific hourly bat activity (activity) among detectors and four sites in Ontario, Canada in 2008. Generalized linear mixed models (GLMM) included random and fixed effects. Random effects were detector location nested in site (detector), night nested in site (night), and sites (site). Fixed effects were percent canopy cover (canopy) and detector height (height), both at the detector-level. Models were selected by likelihood ratio tests and AIC. Parameter estimates, standard error, and p-values are included for significant fixed effects in the models.

Species	GLMM model	Fixed effect explanatory variable	Parameter Estimate	Standard error	p-value
Species combined	activity ~ (1|detector) + (1|night)	Intercept	8.183	0.379	< 0.001
E. fuscus	activity ~ canopy + (1|detector) + (1|night)	Intercept	7.291	0.831	< 0.001
		canopy	-7.534	1.728	< 0.001
L. noctivagans	activity ~ canopy + height + (1|detector) + (1|night) + (1|site)	Intercept	2.152	1.128	0.056
		canopy	-6.950	2.047	< 0.001
		height	1.385	0.591	0.019
L. borealis	activity ~ canopy + (1|detector) + (1|night) + (1|site)	Intercept	7.880	1.312	< 0.001
		canopy	-11.628	2.276	< 0.001
L. cinereus	activity ~ canopy + (1|detector) + (1|night)	Intercept	7.915	0.620	< 0.001
		canopy	-8.441	1.299	< 0.001
M. lucifugus	activity ~ canopy + (1|detector) + (1|night) + (1|site)	Intercept	7.662	1.13	< 0.001
		canopy	-4.921	1.764	0.005
M. septentrionalis	activity ~ canopy + (1|detector) + (1|night) + (1|site)	Intercept	0.819	2.449	0.014
		canopy	6.417	1.490	< 0.001
P. subflavus	activity ~ canopy + (1|detector) + (1|night)	Intercept	4.503	1.051	< 0.001
		canopy	-7.703	2.248	< 0.001

Table B3. Number of nights when bat activity was over the 50th and 90th percentile thresholds at each detector at four sites in Ontario, Canada in 2010: A) Long Point, B) QUBS, C) Renfrew lake, D) Renfrew mine. Detectors were paired at two heights, low (1.5 m; A1, B1, C1) and high (4 m; A2, B2).

A) Long Point	50th percentile Detectors					90th percentile Detectors				
Species	A1	A2	B1	B2	C1	A1	A2	B1	B2	C1
Species combined	12	12	11	9	12	7	7			1
E. fuscus	12	12	10	11	12	11	10	2	3	6
L. noctivagans	8	9	5	4	5	2	2			
L. borealis	12	12	8	10	12	11	11	1	1	10
L. cinereus	10	11			10	3	3			
M. lucifugus	12	12	1	1	7	4	6			1
M. septentrionalis	12	12	6	7	7	2	10			3
P. subflavus	1		10	12						

B) QUBS	50th percentile Detectors					90th percentile Detectors				
Species	A1	A2	B1	B2	C1	A1	A2	B1	B2	C1
Species combined	3	7	4	4	8		2			
E. fuscus	1	1	2	3	9		1			4
L. noctivagans	5	7	4	5	3		2			
L. borealis	5	9		1	10					5
L. cinereus	6	8			5	3	5			1
M. lucifugus	2	7	1	3	6					1
M. septentrionalis		2		1						
P. subflavus	3	6	6	7	1					

C) Renfrew lake	50th percentile Detectors					90th percentile Detectors				
Species	A1	A2	B1	B2	C1	A1	A2	B1	B2	C1
Species combined	11	11	8	8		2	3			
E. fuscus	11	11	6	7		7	5	1	1	
L. noctivagans	11	11	5	7		3	3			
L. borealis	8	9	7	8	2		1			
L. cinereus	11	11		3		4	3			
M. lucifugus	7	8				3	2			
M. septentrionalis	4	6	3	4	1					
P. subflavus	7	7	9	9	5					

D) Renfrew mine	50th percentile Detectors					90th percentile Detectors				
Species	**A1**	**A2**	**B1**	**B2**	**C1**	**A1**	**A2**	**B1**	**B2**	**C1**
Species combined	4	6	10	11	10	1	2	6	5	6
E. fuscus	3	4	3	1	7		3			
L. noctivagans	4	6	9	10	10		1	5	4	6
L. borealis	1	3	1	1	7		1			1
L. cinereus		2		1	11		1			7
M. lucifugus		2	1		5		2			
M. septentrionalis		1		1						
P. subflavus	5	6	11	12	9	4	3	6	6	

Appendix C: Permission to reproduce published material

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Appendix D: Animal use protocol approvals

Western

April 2, 2008

This is the Original Approval for this protocol
A Full Protocol submission will be required in 2012

Dear Dr. Fenton:

Your Animal Use Protocol form entitled:
Behavioural Ecology of Bats
Funding Agency NSERC - Grant #R3516A03

has been approved by the University Council on Animal Care. This approval is valid from **April 2, 2008 to April 30, 2009.** The protocol number for this project is **#2008-003-04 and replaces #2004-027-03** .

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 1 YR.

Species	Strain	Other Detail	Pain Level	Animal # Total for 1 Year
Other, add to detail	Bats	various species M/F	C	~ 1500

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

1. Please ensure that the approximate number of bats used under this protocol is submitted to the AUS office by December each year for the annual CCAC report.

c.c. Approved Protocol - B. Fenton, L. McGuire, J. Weber, D. Cheshuk
Approval Letter   - L. McGuire, J. Weber, D. Cheshuk

The **University** *of* **Western Ontario**
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, ● London, Ontario ● CANADA – N6A 5C1
PH· 519-661-2111 ext 86770 ● FI 519-661-2028 ● www uwo ca / animal

Western

Dear Dr. **Fenton**

Your Animal Use Protocol form entitled:

Behavioural Ecology of Bats

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from **05.01.09** to **04.30.10**

The protocol number for this project remains as **2008-003**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.
The holder of this *Animal Use Protocol* is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c.   L McGuire, J Wasylenko

The University *of* Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, ● London, Ontario ● CANADA – N6A 5C1
PH: 519-661-2111 ext. 86770 ● FL 519-661-2028 ● www.uwo.ca / animal

Western

Dear Dr. **Fenton**

Your Animal Use Protocol form entitled:

Behavioural ecology of bats

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from **05.01.2010** *to* **05.01.2011**

The protocol number for this project remains as **2008-003**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this *Animal Use Protocol* is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c.   J. Wasylenko

Appendix E: Research permits

Ministry of **Natural Resources** Planning Unit Manager Southern Region P.O. Box 7000, 300 Water Street Peterborough, Ontario K9J 8M5	**Ministère des** **Richesses naturelles** Telephone: (705) 755-5164 Facsimile: (705) 755-3292

▷ Ontario

May 9, 2008

Mr. Brock Fenton
University of Western Ontario
Department of Biology

Dear Mr. Fenton:

Subject: **Wildlife Scientific Collector's Authorization**
Authorization Number: 1045694

Enclosed please find Wildlife Scientific Collector's Authorization form number 1045694. This authorization is valid only for the person(s), species, numbers, areas and calendar year indicated.

Please review, sign and return the WSC Authorization and attachments to the following address:

Manager
Southern Region Planning Unit
Ministry of Natural Resources

Once returned, the authorization will be given final approval and the original mailed back to you. Please note that you are required to carry a copy of the authorization at all times while collecting specimens.

A written report, as outlined in the Authorization Conditions, covering the operation of the preceding year must be submitted within 30 days of the termination date to the Manager of the Southern Region Planning Unit.

The report shall contain a statement outlining the objectives of the operations, the methods used, the number and species of wildlife caught and their fate as well as a map indicating where the collections took place. The submission of a satisfactory report is a prerequisite to any subsequent Wildlife Scientific Collector's Authorization renewals.

If you have any questions please do not hesitate to contact Michael Gatt, Senior Regional Wildlife Biologist, at

Yours truly,

Monique Rolf von den Baumen-Clark
Manager
Southern Region Planning Unit

Attachments

Ministry of Natural Resources	Ministère des Richesses naturelles
Southern Region	Région du Sud
P.O Box 7000 300 Water Street Peterborough, ON K9J 8M5 Tel: 705-755-3243 Fax: 705-755-3292	P.O. Box 7000 300, rue Water Peterborough (Ontario) K9J 8M5 Tél.: 705-755-3243 Téléc.: 705-755-3292

Ontario

April 17, 2009

Brock Fenton
University of Western Ontario
Department of Biology

Dear Mr. Fenton

Subject: Wildlife Scientific Collector's Authorization
**   Authorization Number: 1050823**

Enclosed please find Wildlife Scientific Collector's (WSC) Authorization form number 1050823. This authorization is valid only for the person(s), species, numbers, areas and calendar year indicated.

Please review, sign and return the WSC Authorization and attachments to the following address:

Manager
Southern Region Planning Unit
Ministry of Natural Resources

Once returned, the authorization will be given final approval and the original mailed back to you. Please note that you are required to carry a copy of the authorization at all times while collecting specimens.

A written report, as outlined in the Authorization Conditions, covering the operation of the preceding year must be submitted within 30 days of the termination date to the Manager of the Southern Region Planning Unit.

The report shall contain a statement outlining the objectives of the operations, the methods used, the number and species of wildlife caught and their fate as well as a map indicating where the collections took place. The submission of a satisfactory report is a prerequisite to any subsequent Wildlife Scientific Collector's Authorization renewals.

If you have any questions please do not hesitate to contact Michael Gatt, Senior Regional Wildlife Biologist,

Yours truly,

Monique Rolf von den Baumen-Clark
Manager
Southern Region Planning Unit

Attachments

Ministry of Natural
Resources

Ministère des Richesses
naturelles

Southern Region

Région du Sud

P.O Box 7000
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Peterborough, ON
K9J 8M5
Tel: 705-755-3243
Fax: 705-755-3292

P.O. Box 7000
300, rue Water
Peterborough (Ontario)
K9J 8M5
Tél.: 705-755-3243
Téléc.: 705-755-3292

Ontario

April 30, 2010

Brock Fenton
University of Western Ontario
Dept. of Biology & Geological Sciences Bldg.
London, ON N6A 5B7

Dear Mr. Fenton

Subject: **Wildlife Scientific Collector's Authorization**
Authorization Number: 1055825

Enclosed please find Wildlife Scientific Collector's (WSC) Authorization form number 1055825. This authorization is valid only for the person(s), species, numbers, areas and calendar year indicated.

Please review, sign and return the WSC Authorization and attachments to the following address:

Manager
Southern Region Planning Unit
Ministry of Natural Resources

Once returned, the authorization will be given final approval and the original mailed back to you. Please note that you are required to carry a copy of the authorization at all times while collecting specimens.

A written report, as outlined in the Authorization Conditions, covering the operation of the preceding year must be submitted within 30 days of the termination date to the Manager of the Southern Region Planning Unit.

The report shall contain a statement outlining the objectives of the operations, the methods used, the number and species of wildlife caught and their fate as well as a map indicating where the collections took place. The submission of a satisfactory report is a prerequisite to any subsequent Wildlife Scientific Collector's Authorization renewals.

If you have any questions please do not hesitate to contact Michael Gatt, Senior Regional Wildlife Biologist,

Yours truly,

Bob McColm
Manager
Regional Operations

Attachments

Curriculum Vitae

Name:	Amanda Adams
Post-secondary Education and Degrees:	University of Western Ontario London, Ontario, Canada 2007-2013 Ph.D. University of California, San Diego La Jolla, California, USA 2004-2006 M.Sc. University of California, San Diego La Jolla, California, USA 2001-2005 B.Sc.
Selected Honors and Awards:	Centre for Environment & Sustainability Excellence Award 2010-2011, 2011-2012 Bat Conservation International Student Scholarship 2010 Graduate Teaching Assistant Union Community Service Award 2009-2010
Related Work Experience:	Teaching Assistant University of Western Ontario 2007-2013 Course Instructor Continuing Education, University of Western Ontario 2012 Course Development Distance Studies, University of Western Ontario 2010 Research Assistant University of Michigan 2007 Intern/Lab Technician Nautilus Environmental, LLC 2004-2006

Publications:

Published

Adams, A. M., M. K. Jantzen, R. M. Hamilton, and M. B. Fenton. 2012. A comparison of bat detectors: performance with synthetic playback and free-flying bats. Methods in Ecology and Evolution 3: 992–998.

Clare, E. L., **A. M. Adams***, A. Maya-Simoes, J. Eger, P. D. N. Hebert, and M. B. Fenton. 2013. Diversification and reproductive isolation: cryptic species in the only New World high-duty cycle bat, *Pteronotus parnellii*. BMC Evolutionary Biology, 13: doi:10.1186/1471-2148-13-26.**

Mills, S.C., **A.M. Adams**, R.D. Phoenix. 2013. Bat Species Diversity in the Boreal Forest of Northeastern Ontario, Canada. Northeastern Naturalist, 20(2): 309–324.

* Co-first author
** rated as highly accessed by Bio-Medical-Central

Accepted

Clare, E.L., H.R. Goerlitz, V.A. Drapeau, M.W. Holderied, **A.M. Adams**, J. Nagel, E.R. Dumont, P.D.N. Hebert, M.B. Fenton. Trophic niche flexibility in *Glossophaga soricina*: how a nectar seeker sneaks an insect snack. Functional Ecology: accepted.

Submitted

Adams, A. M., L. P. McGuire, and M. B. Fenton. How high is high? Using percentile thresholds to identify peak bat activity. Acta Chiropterologica: in review.

Adams, A. M. and M. B. Fenton. Identifying peaks in bat activity: a new application of the space-time scan statistic. Journal of Applied Ecology: in review.