Natural behaviours of echolocators, with an emphasis on Vespertilionid bats

by

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Abstract

Here I report on two experimental studies and one observational analysis addressing the flexibility and ecological limitations of sensorimotor integration in echolocating mammals, as they relate to sensory ecology and foraging behaviour. In chapter two, I use acoustic data to comparatively assess echolocation and motor activities of bats and toothed whales to test (*i*) how the presence of a conspecific influences the spectral and temporal content of echolocation signals and (*ii*) whether these species behave similarly when foraging with a conspecific. The results suggest that in the presence of conspecifics (*i*) both bats and toothed whales exhibit the Lombard effect, and (*ii*) bats additionally employ subsets of both a Jamming Avoidance Response and a clutter response. Behaviourally, only porpoises appear to modify their beam direction. In chapter three, I use acoustic, photo, and video data to compare the developmental trajectories of (*i*) flight attempts, (*ii*) wing morphology, and (*iii*) vocalizations of big brown bat pups in the context of landing behavior, specifically with respect to landing buzz production. The results (*i*) clarified previous studies exploring pup vocal and flight ontogeny and (*ii*) identified developmental relationships between wing morphology (RWL) and flight milestones. In chapter four, I comparatively assess acoustic recordings of bat activity before and after the introduction of white-nose syndrome (WNS) to South Eastern Ontario to explore the effects of WNS on (*i*) species abundance and (*ii*) habitat use. I also explore (*iii*) the possibility of niche release and realized niche expansion due

to reduced interspecific competition resulting from species-specific WNS susceptibility. The results confirm that (*i)* endangered species are detected less often foraging in open field habitats since the introduction of WNS, and that (*ii*) relatively unaffected species have increased their presence (but not active foraging) in clutter/edge and open field habitats. The results also indicate that (*iii*) over water habitats showed no difference between pre- and post-WNS bat activity, suggesting that niche expansion for relatively unaffected species may be limited to some habitats and not others.

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Chapter 1

Synopsis

Introduction

Foraging behaviour incorporates the strategies and decisions used to acquire energetic resources (Boogert et al. 2010). Animals must consider where these resources are, how to obtain them, and the most effective way to consume them to ensure their own survival. Foraging behaviours can be highly diverse; differences exist within the sensory systems used, participants involved, and morphologies required (Stephens et al. 2008; Le Roux et al. 2009). Pack hunting in wolves, for example, is vastly different from the web building of spiders or the electroreception of the platypus. As with all traits, foraging behaviour can be comprehensively examined through multiple perspectives. Historically, these perspectives have been characterized by four main questions: (*i*) what is the purpose of the behavior? (*ii*) how does it develop over an individual's lifetime? (*iii*) how did it come to evolve over a species' lineage? and (*iv*) how does it mechanistically function? (Tinbergen 1963). These four approaches, first proposed by Tinbergen, have become more scientifically intricate as our understanding of the natural world and available technology have advanced. The underlying rationale behind these "four questions", however, remains valid and relevant to the study of foraging behavior and sensory ecology.

The tactics and processes involved in foraging are based on the information acquired from an individual's surrounding environment. That is, knowledge regarding the types of available resources, their locations, and appropriate means to obtain them allows for effective and efficient foraging. This environmental information, as well as the mechanisms through which it is acquired and the utility of the information itself, provide insight into the sensory ecology of different organisms (Dusenbery 2001). All organisms

necessarily interact with their environments, and these environments provide unique experiences and perceptions for each organism associated with them. The information received from those environments, the manner in which that information is processed, and the subsequent reactions to that information can, like foraging behaviour, be exceptionally diverse. The perception of electrical stimuli by the platypus, to return to this three species example, also differs greatly from that of mechanical vibrations used by some web-spinning spiders, or the olfactory information used by wolves (Masters and Markl 1981; Scheich et al. 1986; Schmidt and Mech 1997). After processing, this information can be used to inform and support different behaviours, in each of these instances the identification, localization, and interception of prey.

Different organisms, then, rely on different information, behaviours, and associated sensory systems to forage successfully. In some bats and whales, echolocation exemplifies a sensory system that has evolved to support foraging behaviours under conditions where limited visual information is available. Echolocation involves the acquisition and processing of auditory information and involves a variety of adjustments in acoustic behaviour. Based on incoming environmental information (i.e. echoes), some echolocators change the design and pattern of their acoustic signals to optimize foraging efficiency. Echolocation, or biological sonar, is thus an active sensory process used to generate an auditory field of view that is updated at roughly the rate of echolocation signal emission rate (Madsen and Surlykke 2013). The energy emitted during acoustic signal production is transformed by the environment with respect to amplitude and time delay, as well as spectral and temporal properties. Echolocation can

be simply defined as the comparison between the original signal and the returning echo, with respect to changes in time, energy, and frequency content.

What can undisputedly be called echolocation has been recorded from most bats, all toothed whales, some swiftlets, and Oilbirds (Au 1993; Fullard et al. 1993; Holland et al. 2004). All animal echolocators use echolocation for the same basic function of navigation and orientation. That is, to identify where they are in reference to other objects and to move purposefully through space (Brinkløv et al. 2013). An additional function of echolocation, used only by some laryngeal echolocating bats (i.e. non-pteropodid bats) and toothed whales, is that of prey detection, tracking, and interception. In short, only laryngeal echolocating bats (approximately 1100 species) and toothed whales (approximately 80 species) are able to use echolocation signals to hunt (Madsen and Surlykke 2013). Many species of bats and toothed whales hunt for actively moving prey that are typically several times smaller than themselves, and often do so in cluttered, three-dimensional environments. These foraging abilities suggest that bats and toothed whales have exceptional acoustic signal control, often surpassing the sonar of human-made systems (Madsen and Surlykke 2013).

Echolocation for foraging

Echolocation for prey pursuit is similar to echolocation for navigation and orientation. The use of echolocation for both activities involves the production of acoustic signals and the interpretation of temporal, spectral, and amplitude differences between the emitted and returned signals to extract environmental information (Suthers 1988). When foraging, laryngeal echolocating bat echolocation signals are highly flexible and often

can be adjusted to suit environmental conditions (Obrist 1995). To a lesser extent, toothed whales are also able to adjust spectral and temporal components of their echolocation signals to make hunting more efficient (Surlykke and Moss 2000; Tyack 2015; Wisniewska et al. 2015). This increased efficiency is especially beneficial when hunting with conspecifics as well as in cluttered environments, as an individual's relevant echolocation signals and echoes may become buried within the signals and echoes of other echolocators and obstacles (Moss et al. 2014). Sometimes referred to as the "cocktail party nightmare", previous research has explored how some bats might contend with these high levels of acoustic clutter.

In laryngeal echolocating bats, the observed influence of conspecifics on echolocation behaviour has generated two putative mechanisms and one involuntary reflex used by bats to deal with conspecifics: the jamming avoidance response (JAR), the clutter response, and the Lombard effect. Jamming avoidance responses are well known in weakly electric fish and involve two fish each adjusting their emitted signals such that their signals are less likely to overlap with one another (Rose and Heiligenberg 1985). Jamming avoidance response in bats appears to follow roughly the same principle: bats shift their call frequency and / or emission rate, plausibly to avoid spectral and / or temporal overlap with nearby bats (Ulanovsky et al. 2004; Amichai et al. 2015; Corcoan and Moss 2017). Clutter responses have also been widely studied in bats, as many bat species occupy heavily cluttered ecological niches (i.e. dense forests). A clutter response to a conspecific is relatively straightforward: bats interact with the conspecific as though they are simply a flying object in their immediate environment (Obrist 1995; Fawcett et al. 2015; Fawcett and Ratcliffe 2015). With

respect to toothed whales, jamming avoidance response has not been specifically studied however jamming avoidance response-like acoustic responses in the presence of noise have been reported (Tyack and Janik 2013). Additionally, toothed whales also seem to alter their echolocation signals when in a cluttered environment, suggesting possible clutter responses in their echolocation signals as well (Jensen et al. 2013). The Lombard effect, on the other hand, is an involuntary increase in signal amplitude, and associated spectral and temporal properties, resulting from ecologically relevant background noise (Zollinger and Brumm 2011; Hotchkin and Parks 2013).

Laryngeal echolocating bats and toothed whales forage under very different environmental conditions, regardless of clutter or conspecifics. While toothed whales tend to hunt in dark and / or turbid waters, bats have a wide range of terrestrial environments available to them (Evans and Awbrey 1988; Au 1993; Obrist 1995; Vaughan et al. 1997; Grindal and Brigham 1999). Successful foraging within a specific habitat is thought to be driven by morphology and echolocation, allowing spatial resource partitioning between sympatric bat species with similar diets (Norberg and Rayner 1987; Neuweiler 1990; Seimers and Schnitzler 2004). Bats with low wing loading (low mass per unit area of the wing) are, for example, more manoeuvrable and efficient at foraging in and around cluttered environments and tend to use short duration echolocation calls of high peak frequency (frequency component with the highest energy) and broad bandwidth. Such signals minimize call-echo overlap, while providing detailed information on the size and shape of nearby objects. Conversely, bats that emit high intensity, long duration echolocation signals, ideal for long distance detection,

forage more efficiently in open habitats and have long, narrow wings and relatively high wing loading, allowing fast, agile flight (Aldridge and Rautenbach 1987).

All predominantly animal-eating bats are laryngeal echolocators. Many insectivorous bats forage for similar prey within similar habitats and interspecific competition is thought to shape interspecific interactions (Arlettaz et al. 1999; Razgour et al. 2011). This competition helps to promote the distinction between the fundamental niches (potential environment) and realized niches (occupied environment) that describe particular insect-eating bat species. As a result of other bat species being present in a given community, species that could theoretically use multiple habitats for foraging may find themselves restricted to a subset of these habitats by the presence of other species. Reductions in dominant species populations, however, may decrease interspecific competition and allow for niche expansion (Ford et al. 2011; Jachowski et al. 2014).

Since 2006, some, but not all, insectivorous bats in much of Canada and the U.S.A. have been experiencing large population declines due to an infectious disease called white-nose syndrome (WNS). Now spread to over thirty states and seven provinces, white-nose syndrome has reduced some bat populations by over 90% in certain areas (Frick et al. 2010; Moore et al. 2018). White-nose syndrome is caused by the fungus *Pseudogymnoascus destructans* (*Pd*), which causes skin lesions, dehydration, and disrupted hibernation patterns in the most affected species (Mayberry et al. 2017; McGuire et al. 2017). While many bat species test positive for the presence of *Pseudogymnoascus destructans*, not all experience high levels of associated mortality (Bernard et al. 2015). These differences in severity are thought to be related to

the hibernation patterns of different species. Since *Pseudogymnoascus destructans* is a psychrophilic (cold-loving) fungus, it is most dangerous for bats that hibernate in cold areas (Langwig et al. 2015). Migratory bats, which leave the colder northern North American climates during the fall and spend winter in warmer locations, exhibit no detrimental effects of *Pseudogymnoascus destructans* infection (Bernard et al. 2015). Other bats, such as the big brown bat, *Eptesicus fuscus*, exhibit different patterns of thermoregulation during hibernation which seems to reduce the severity of white-nose syndrome (Moore et al. 2018).

Feeding and landing buzzes

During the final phase of foraging (i.e. attempted interception of prey) both laryngeal echolocating bats and toothed whales produce a terminal buzz (Griffin et al. 1960; Madsen and Surlykke 2013). Echolocating birds do not buzz under any circumstances (Brinkløv et al. 2017). This terminal feeding buzz is a ubiquitous feature of echolocation for prey pursuit. For bats, the “buzz” is characterised by an increase in signal production rate (up to ~220 calls per second; Ratcliffe et al. 2013) and often a corresponding decrease in the peak frequency of the signals themselves (Griffin 1958; Surlykke and Moss 2000). This increase in call rate and decrease in signal frequency is thought to provide bats a near-constant update of information as well as produce a broader sound beam (Ratcliffe et al. 2013). In toothed whales, the buzz proceeds similarly, but at much higher rates (>300 signals/s versus >90 signals/s), which are accounted for by the greater speed of sound in water versus air, while the beam broadening is accomplished not through lowering the frequency of the signals, but instead by reducing the effective

aperture size of the melon (DeRuiter et al. 2009; Wisniewska et al. 2015). Both of these aspects of the buzz theoretically help to reduce the chances that the prey will escape. The ability of bats and toothed whales to emit such high signal rates suggests that these species have convergently evolved unique and sophisticated sound-producing structures to allow for the exploitation of poorly lit foraging niches. These feats of acoustic signal production and analysis, coupled with fascinating locomotory behaviours, make bats and toothed whales ideal study species for exploring both the extremes and limits of echolocation behaviour.

Another type of buzz exclusive to laryngeally echolocating bats is the landing buzz. Bats produce landing buzzes when approaching a landing surface. Acoustically, landing and feeding buzzes are similar in that both buzzes involve an increase in call rate. Landing buzzes, however, display lower call rates (i.e. fewer calls per unit of time) and are often missing the second buzz component, a further increase in call rate occasionally coupled with a lowering of frequency observed in many feeding buzzes (Melcón et al. 2007). These differences between the two buzz conditions are thought to reflect the relative difference in difficulty between a three-dimensional aerial interception (feeding buzz) and a two-dimensional landing (landing buzz; Melcón et al. 2007; Hulgard and Ratcliffe 2016).

From a developmental perspective, it is currently unclear when the ability to produce buzzes develops in juvenile bat pups. To date, references to bat pup landing buzz development have been limited to brief mentions in the context of bat pup flight development (Buchler 1980; Brown et al. 1983). In these reports, landing buzzes are described as consistently present as soon as pups were able to fly but were not

described in relation to earlier developmental flight abilities. Bat pups progress through developmental steps when learning how to fly, from “flop” in which they make no effort to move their wings or propel themselves forward, through to “fly”, in which true (i.e. powered and controlled) flight is achieved (Powers et al. 1991). Since the integration of flight and echolocation is vital to the survival of aerial insectivorous bats, it seems likely that the ability to produce sophisticated echolocation signals like a landing buzz would involve gradual change and development over time, likely in parallel with developing flight ability.

Sensorimotor Integration

Much of the physics, physiology, and even genetics underlying acoustic signal production and echo-receiving apparatus have been explored in echolocating bats and toothed whales (Griffin 1958; Au 1993; Shen et al. 2012; Madsen and Surlykke 2013; Ratcliffe et al. 2013; Wisniewska et al. 2015). When considering echolocation for prey pursuit and tracking, however, the necessary evolution of acoustic signal production with corresponding locomotion becomes apparent. The dynamic process of coupling the sensory system (e.g. echolocation) with the motor system (e.g. flight or swimming) is referred to as sensorimotor integration (Wood and Evans 1980; Moss and Sinha 2003; Luo et al. 2017).

Sensorimotor integration with respect to bat and toothed whale echolocation involves using echo information to adjust motor (flight/swim) actions (e.g., speed, direction) while also using these motor outputs to modify current or future acoustic signal production (e.g., pulse rate, beam breadth, signal frequency; Kuc 1994; Valentine

and Moss 1998; Nelson and MacIver 2006). Echolocator sensorimotor integration is essential for effective foraging and survival, as hunting moving prey demands a precise interaction between obtaining prey information and taking actions towards prey interception. Bats and toothed whales have independently evolved to fine-tune both signal emission and motor responses during foraging, suggesting that sensorimotor integration, as well as sensory and motor systems independently, must exhibit some degree of flexibility. However, the extent and limitations of sensorimotor integration remain fertile ground for investigation.

Thesis Outline

By exploring the natural behaviours of mammalian echolocators from multiple ecological perspectives, I will illustrate how echolocation signals and their corresponding locomotory actions can be used to describe evolutionary, developmental, and ecological differences between organisms, individuals, and species. My primary focus will be vespertilionid bats. Furthermore, I have identified conditions under which the sensory and motor systems of echolocators should exhibit high flexibility, and others under which these animals may appear constrained by how they are built. Specifically, this thesis explores how the integrative sensorimotor behaviour of echolocators is expressed (*i*) during conditions of acoustic clutter from conspecifics, (*ii*) throughout early flight and vocal development, and (*iii*) within a species-specific population decline. This thesis is thus the first to explore the acoustic and motor effects of conspecifics on toothed whale echolocation during hunting activities, the first to specifically and comprehensively describe the development of landing buzzes in bat pups, and one of

the first to identify how a widespread disease influences the active foraging environments of un- or less-affected bat species.

Chapter Two

Chapter two describes the influence of conspecifics on acoustic and locomotory behaviours in laryngeal echolocating bats and toothed whales. This chapter also explores the similarities and differences of conspecific influences between these two mammalian groups, which have independently evolved the ability to echolocate. As study species, I used the laryngeal echolocating bat, *Myotis daubentonii* (Vespertilionidae) and the harbor porpoise, *Phocoena phocoena* (Phocoenidae). I hypothesized that the reactions of bats and toothed whales to the presence of a conspecific would be similar, as their use of echolocation is similar despite the obvious differences in locomotion (Jakobsen and Surlykke 2010; Madsen and Surlykke 2013; Wisniewska et al. 2015). For both species, I expected that the presence of a conspecific would elicit one of three probable reactions: (*i*) the conspecific would effectively be treated as a moving, similarly vocalizing object to which individuals would employ a jamming avoidance response (JAR), (*ii*) the conspecific would instead be dealt with simply as a moving or static obstacle, that is, be treated as any other form of clutter (i.e. eliciting a clutter response), or (*iii*) the conspecific would be treated as a form of inflexible acoustic noise, eliciting the Lombard effect.

If bats and toothed whales in my study employed a jamming avoidance response in the presence of a conspecific, I predicted that paired organisms will emit echolocation signals greater spectral and temporal disparity than those emitted when hunting solo

(Ulanovsky et al. 2004; Amichai et al. 2015). If bats and toothed whales instead employed a clutter response, I predicted that these signals would have shorter durations, lower sound levels/intensities, and broader bandwidths (Fawcett and Ratcliffe 2015). If bats and toothed whales exhibit the involuntary Lombard effect, I predicted paired signals would have higher sound intensities, higher frequencies, and longer durations. An additional hypothesis is that bats and toothed whales engage in subsets of both jamming avoidance response and clutter response behaviours simultaneously, potentially in addition to experiencing the Lombard effect. Since a jamming avoidance response helps to reduce interference and confusion from conspecifics, while clutter responses help to reduce interference from echoes from surrounding objects, using aspects of both may produce the most coherent and informative acoustic scene of the surrounding environment. Behaviourally, I also hypothesized that bats and toothed whales would shift their attention (direction of their echolocation beam) when in the presence of a conspecific. That is, I predicted that the average beam direction for solo hunters would be closer to zero degrees (zero degrees representing the direction of the target), and that when hunting in pairs, the average beam direction would be further from zero degrees, suggesting that deviations in beam direction away from the target occur more often. Additionally, I predicted that the average beam directions for paired and solo hunters would be different.

In this chapter, I show that both bats and toothed whales exhibit the Lombard effect, and that bats additionally employ subsets of both jamming avoidance response and clutter responses when in the presence of conspecifics. Porpoises seem to show no acoustic differences between hunting conditions beyond the reflexive Lombard effect

changes. Furthermore, I illustrate that toothed whales hunting both alone and in pairs always emit echolocation beams with an average direction of zero degrees (i.e. towards the target). These average beam directions, however, differ between conditions, such that toothed whales hunting in pairs deviate their beam direction further from the target (i.e. further from zero degrees), as I predicted. Bats hunting both alone and in pairs also typically emit echolocation beams with an average direction of zero degrees, with single trial exceptions in both conditions. Bats hunting in pairs, however, show fewer instances of echolocation beam deviation from the target direction than when hunting solo. Unexpectedly, the average beam directions for bats hunting alone and in pairs are, overall, not different (i.e. both equivalently equal to zero degrees).

Chapter Three

The third chapter in this thesis focuses on the juvenile development of the landing buzz in the big brown bat, *Eptesicus fuscus*. Like *Myotis daubentonii*, *E. fuscus* belongs to the family Vespertilionidae: the most species rich family of bats. This project explores when, and in association with which stages of vocal, morphological, and physiological development, the landing buzz in bat pups occurs. This ontogenetic approach helps to provide some insight into how sensorimotor integration develops over time in bats. Specifically, I investigated how advanced vocal control changes in correlation with volancy, body size, and wing morphology. This chapter also examines sensorimotor integration timing – do advanced echolocation behaviours develop first, and then flight? Or do pups first fly before they produce a buzz?

In bats, juvenile flight and vocalization generally rely on physical growth as the various muscles involved must have grown sufficiently to allow for flight and for vocal control (Powers et al. 1991; Papadimitriou et al. 1996). As such, those animals who reach those morphological milestones faster theoretically have developed their musculature faster as well (Powers et al. 1991). I hypothesized that differences in growth rate would reflect differences in flight and vocal developmental rates. I predicted that pups who grew faster (i.e. reached larger mass and forearm lengths faster) would be able to (*i*) achieve true flight faster and (*ii*) produce landing buzzes, as well as the precursors to landing buzzes (multi-signal sonar strobe groups), at an earlier age. I also hypothesized that flight and landing buzz production would be temporally linked and predicted that the ability to produce a landing buzz should precede the ability to fly. Finally, I hypothesized that relative wing loading (RWL – the mass of the bat per unit of wing area; related to lift, flight speed and maneuverability) would be strongly associated with flight ability and therefore potentially also with buzz production. That is, I predicted that bats who achieve adult-like relative wing loadings earlier in development would both fly and produce landing buzzes earlier.

In this chapter, I demonstrate that bats are able to produce adult-like orientation echolocation calls, sonar strobe groups, and landing buzzes before they are able to use controlled, powered flight. These results suggest that for bats the sensory system is most important for informing in-flight decisions, and develops concurrently with, but ahead of, the flight system. I found no clear relationship between pup physiological growth and the ability to fly or produce landing buzzes. However, a strong positive correlation was found between relative wing loading scores and flight ability, such that

pups that reach adult-like relative wing loading scores more quickly also transition to true flight faster. No such relationship was observed between relative wing loading score and buzz production.

Chapter Four

The final experimental chapter in this thesis explores the influence of a natural ecological population disturbance caused by white-nose syndrome (WNS). The reasons behind the differential susceptibility to white-nose syndrome and the overall impacts on affected species populations have been widely explored since white-nose syndrome introduction (Bernard et al. 2015; Moore et al. 2018). Few studies, however, have explored the secondary impacts of these population reductions on those remaining species (for notable exceptions see Ford et al. 2011 and Jachowski et al. 2014). The purpose of the study I describe in this chapter was to identify how white-nose syndrome has influenced the populations of different bat species in South Eastern Ontario pre- and post- white-nose syndrome introduction, specifically with respect to foraging activity (i.e. presence of feeding buzzes) and foraging/flight locations (i.e. where these species were found, now and then, and whether or not they are actively hunting there). Using acoustic recordings, this project uses species-specific echolocation signals to infer bat location and activity in three habitats: over fields, in clutter/edge areas, and over water. I hypothesized that the massive population decrease of the previously dominant species (the little brown bat, *Myotis lucifigus*) would allow for a shift in the use of the cluttered and over water habitats due to a decrease in interspecific competition. Specifically, I predicted that big brown bats, *Eptesicus fuscus*, as the only relatively unaffected

sedentary species, would show the greatest increase in flight and foraging over water and within clutter. Furthermore, I predicted that migratory species (also unaffected by white-nose syndrome) might also broaden their foraging niches, but perhaps not to the same extent as big brown bats, due in part to their wing morphology.

In this chapter, I show that some endangered bat species exhibit the expected decreases in foraging (i.e. feeding buzzes) and presence (i.e. bat passes), but only over open fields. No differences between pre- and post- white-nose syndrome presence were observed for any endangered species in clutter/edge habitats or over water. I also show that no species (endangered or relatively unaffected) in any habitat exhibited an increase in foraging activity (buzzes), although big brown bats do have an increased presence at clutter/edge sites (passes). Finally, I provide evidence that migratory species increase their presence at both open field and clutter/edge sites, but not their hunting activity. In South Eastern Ontario, the niche of nocturnal insects over water, once occupied by little brown bats, appears now to be essentially vacant.

Chapter Five

In Chapter five, I briefly summarize all data chapters presented in this thesis. I also provide suggestions for future research.

References

Aldridge, H. D. J. N., & Rautenbach, I. L. (1987). Morphology, echolocation and resource partitioning in insectivorous bats. *Journal of Animal Ecology*, *56*, 763-778.

Amichai, E., Blumrosen, G., & Yovel, Y. (2015). Calling louder and longer: how bats use biosonar under severe acoustic interference from other bats. *Proceedings of the Royal Society of London B: Biological Sciences*, *282*, 2064.

Arlettaz, R. (1999). Habitat selection as a major resource partitioning mechanism between the two sympatric sibling bat species *Myotis myotis* and *Myotis blythii*. *Journal of Animal Ecology*, *68*, 460-471.

Au, W. W. L. (1993). *The Sonar of Dolphins*. New York, NY: Springer-Verlag New York, Inc.

Bernard, R. F., Foster, J. T., Willcox, E. V., Parise, K. L., & McCracken, G. F. (2015). Molecular detection of the causative agent of white -nose syndrome on Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) and two species of migratory bats in the southeastern USA. *Journal of Wildlife Diseases*, *51*, 519-522.

Boogert, N. J., Bui, C., Howarth, K., Giraldeau, L. A., & Lefebvre, L. (2010). Does foraging behaviour affect female mate preferences and pair formation in captive zebra finches? *Public Library of Science One*, *5*, 14340.

Brinkløv, S., Fenton, M. B., & Ratcliffe, J. M. (2013). Echolocation in Oilbirds and swiftlets. *Frontiers in Physiology*, *4*, 00123.

Brown, P. E., Brown, T. W., & Grinnell, A. D. (1983). Echolocation, development, and vocal communication in the lesser bulldog bat, *Noctilio albiventris*. *Behavioral Ecology and Sociobiology*, *13*, 287-298.

Buchler, E. R. (1980). The development of flight, foraging, and echolocation in the little brown bat (Myotis lucifugus). *Behavioral Ecology and Sociobiology*, *6*, 211-218.

Corcoran, A. J., & Moss, C. F. (2017). Sensing in a noisy world: lessons from auditory specialists, echolocating bats. *Journal of Experimental Biology*, *220*, 4554-4566.

DeRuiter, S. L., Bahr, A., Blanchet, M. A., Hansen, S. F., Kristensen, J. H., Madsen, P. T., Tyack, P.L., & Wahlberg, M. (2009). Acoustic behaviour of echolocating porpoises during prey capture. *Journal of Experimental Biology*, *212*, 3100-3107.

Dusenbery, D. B. (2001). Physical constraints in sensory ecology. In Barth F.G., Schmid A. (Eds) *Ecology of Sensing* (pp. 1-17). Berlin, Heidelberg: Springer.

Evans, W. E., & Awbrey, F. T. (1988). Natural history aspects of marine mammal echolocation: feeding strategies and habitat. In: Nachtigall P.E., Moore P.W.B. (Eds) *Animal sonar. NATO ASI science (Series A: life sciences), volume 156* (pp. 521-534). Boston, MA: Springer.

Fawcett, K., Jacobs, D. S., Surlykke, A., & Ratcliffe, J. M. (2015). Echolocation in the bat, Rhinolophus capensis: the influence of clutter, conspecifics and prey on call design and intensity. *Biology Open*, *4*, 693-701.

Fawcett, K., & Ratcliffe, J. M. (2015). Clutter and conspecifics: a comparison of their influence on echolocation and flight behaviour in Daubenton's bat, *Myotis daubentonii*. *Journal of Comparative Physiology A*, *201*, 295-304.

Ford, W. M., Britzke, E. R., Dobony, C. A., Rodrigue, J. L., & Johnson, J. B. (2011). Patterns of acoustical activity of bats prior to and following white-nose syndrome occurrence. *Journal of Fish and Wildlife Management*, *2*, 125-134.

Frick, W. F., Pollock, J. F., Hicks, A. C., Langwig, K. E., Reynolds, D. S., Turner, G. G., Butchkoski, C.M., & Kunz, T. H. (2010). An emerging disease causes regional population collapse of a common North American bat species. *Science*, *329*, 679-682.

Fullard, J. H., Barclay, R. M., & Thomas, D. W. (1993). Echolocation in free-flying Atiu swiftlets (*Aerodramus sawtelli*). *Biotropica*, *25,* 334-339.

Griffin, D. R. (1958). *Listening in the Dark*. New Haven: Yale University Press.

Griffin, D. R., Webster, F. A., Michael, C. R. (1960). The echolocation of flying insects by bats. *Animal Behaviour, 8*, 141–154.

Grindal, S. D., & Brigham, R. M. (1999). Impacts of forest harvesting on habitat use by foraging insectivorous bats at different spatial scales. *Ecoscience*, *6*, 25-34.

Holland, R. A., Waters, D. A., & Rayner, J. M. (2004). Echolocation signal structure in the Megachiropteran bat Rousettus aegyptiacus Geoffroy 1810. *Journal of Experimental Biology*, *207*, 4361-4369.

Hotchkin, C., & Parks, S. (2013). The Lombard effect and other noise-induced vocal modifications: insight from mammalian communication systems. *Biological Reviews*, *88*, 809-824.

Hulgard, K., & Ratcliffe, J. M. (2016). Sonar sound groups and increased terminal buzz duration reflect task complexity in hunting bats. *Scientific Reports*, *6*, 21500.

Jachowski, D. S., Dobony, C. A., Coleman, L. S., Ford,- W. M., Britzke, E. R., & Rodrigue, J. L. (2014). Disease and community structure: white nose syndrome alters spatial and temporal niche partitioning in sympatric bat species. *Diversity and Distributions*, *20*, 1002-1015.

Jakobsen, L., & Surlykke, A. (2010). Vespertilionid bats control the width of their biosonar sound beam dynamically during prey pursuit. *Proceedings of the National Academy of Sciences*, *107*, 13930-13935.

Jensen, F. H., Rocco, A., Mansur, R. M., Smith, B. D., Janik, V. M., & Madsen, P. T. (2013). Clicking in shallow rivers: short-range echolocation of Irrawaddy and Ganges river dolphins in a shallow, acoustically complex habitat. *Public Library of Science One*, *8*, 0059284.

Kuc, R. (1994). Sensorimotor model of bat echolocation and prey capture. *Journal of the Acoustical Society of America*, *96*, 1965-1978.

Langwig, K. E., Frick, W. F., Reynolds, R., Parise, K. L., Drees, K. P., Hoyt, J. R., Cheng, T.L., Kunz, T.H., Foster, J.T., & Kilpatrick, A. M. (2015). Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. *Proceedings of the Royal Society of London B: Biological Sciences*, *282*, 2335.

Le Roux, A., Cherry, M. I., Gygax, L., & Manser, M. B. (2009). Vigilance behaviour and fitness consequences: comparing a solitary foraging and an obligate group-foraging mammal. *Behavioral Ecology and Sociobiology*, *63*, 1097-1107.

Luo, J., Kothari, N. B., & Moss, C. F. (2017). Sensorimotor integration on a rapid time scale. *Proceedings of the National Academy of Sciences*, *114*, 6605-6610.

Madsen, P. T., & Surlykke, A. (2013). Functional convergence in bat and toothed whale biosonars. *Physiology*, *28*, 276-283.

Masters, W. M., & Markl, H. (1981). Vibration signal transmission in spider orb webs. *Science*, *213*, 363-365.

Mayberry, H. W., McGuire, L. P., & Willis, C. K. (2018). Body temperatures of hibernating little brown bats reveal pronounced behavioural activity during deep torpor and suggest a fever response during white-nose syndrome. *Journal of Comparative Physiology B*, *188*, 333-343.

McGuire, L. P., Mayberry, H. W., & Willis, C. K. (2017). White- nose syndrome increases torpid metabolic rate and evaporative water loss in hibernating bats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *313,* R680-R686.

Melcón, M. L., Denzinger, A., & Schnitzler, H. U. (2007). Aerial hawking and landing: approach behaviour in Natterer's bats, Myotis nattereri (Kuhl 1818). *Journal of Experimental Biology*, *210*, 4457-4464.

Moore, M. S., Field, K. A., Behr, M. J., Turner, G. G., Furze, M. E., Stern, D. W., Allegra, P.R., Bouboulis, S.A., Musante, C.D., Vodzak, M.E., & Biron, M. E., Meierhofer, M.B., Frick, W.F., Foster, J.T., Howell, D., Kath, J.A., Kurta, A., Nordquist, G., Johnson, J.S., Lilley, T.M., Barrett, B.W., & Reeder, D.M. (2018). Energy conserving thermoregulatory patterns and lower disease severity in a bat resistant to the impacts of white-nose syndrome. *Journal of Comparative Physiology B*, *188*, 163-176.

Moss, C. F., Cechetto, C., Warnecke, M., Chiu, C., Xian, W., & Falk, B. (2014). Echolocating bats face a cocktail party nightmare when they fly together in cluttered environments. *Journal of the Acoustical Society of America*, *135*, 2150-2151.

Moss, C. F., & Sinha, S. R. (2003). Neurobiology of echolocation in bats. *Current Opinion in Neurobiology*, *13*, 751-758.

Nelson, M. E., & MacIver, M. A. (2006). Sensory acquisition in active sensing systems. *Journal of Comparative Physiology A*, *192*, 573-586.

Neuweiler, G. (1990). Auditory adaptations for prey capture in echolocating bats. *Physiological Reviews*, *70*, 615-641.

Norberg, U. M., & Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *316*, 335-427.

Obrist, M. K. (1995). Flexible bat echolocation: the influence of individual, habitat and conspecifics on sonar signal design. *Behavioral Ecology and Sociobiology*, *36*, 207-219.

Papadimitriou, H. M., Swartz, S. M., & Kunz, T. H. (1996). Ontogenetic- and anatomic variation in mineralization of the wing skeleton of the Mexican free tailed bat, *Tadarida brasiliensis*. *Journal of Zoology*, *240*, 411-426.

Powers, L. V., Kandarian, S. C., & Kunz, T. H. (1991). Ontogeny of flight in the little brown bat, Myotis lucifugus: behavior, morphology, and muscle histochemistry. *Journal of Comparative Physiology A*, *168*, 675-685.

Ratcliffe JM, Elemans CPH, Jakobsen L, Surlykke A. (2013). How the bat got its buzz. *Biology Letters, 9*, 20121031.

Razgour, O., Korine, C., & Saltz, D. (2011). Does interspecific competition drive patterns of habitat use in desert bat communities?. *Oecologia*, *167*, 493-502.

Rose, G., & Heiligenberg, W. (1985). Temporal hyperacuity in the electric sense of fish. *Nature*, *318*, 178-180.

Scheich, H., Langner, G., Tidemann, C., Coles, R. B., & Guppy, A. (1986). Electroreception and electrolocation in platypus. *Nature*, *319*, 401-402.

Schmidt, P. A., & Mech, L. D. (1997). Wolf pack size and food acquisition. *American Naturalist*, *150*, 513-517.

Shen, Y. Y., Liang, L., Li, G. S., Murphy, R. W., & Zhang, Y. P. (2012). Parallel evolution of auditory genes for echolocation in bats and toothed whales. *Public Library of Science Genetics*, *8*, 1002788.

Siemers, B. M., & Schnitzler, H. U. (2004). Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature*, *429*(6992), 657-661.

Stephens, D. W., Brown, J. S., & Ydenberg, R. C. (Eds.) (2008). *Foraging: behavior and ecology*. Chicago, IL: University of Chicago Press.

Surlykke, A., & Moss, C. F. (2000). Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory. *Journal of the Acoustical Society of America*, *108*, 2419-2429.

Suthers, R. A. (1988). The production of echolocation signals by bats and birds. In: Nachtigall P.E., Moore P.W.B. (Eds) *Animal sonar. NATO ASI science (Series A: life sciences), volume 156* (pp. 23-45). Boston, MA: Springer.

Tinbergen, N. (1963) On aims and methods of ethology. *Zeitschrift für Tierpsychologie,* 20, 410–433

Tyack, P. (2015). Echolocation: Clicking for supper. *eLife*, *4*, 07690.

Tyack, P. L., & Janik, V. M. (2013). Effects of noise on acoustic signal production in marine mammals. In Brumm H. (Eds) *Animal communication and noise. Animal signals and communication, volume 2* (pp. 251-271). Berlin, Heidelberg: Springer.

Ulanovsky, N., Fenton, M. B., Tsoar, A., & Korine, C. (2004). Dynamics of jamming avoidance in echolocating bats. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *271*, 1467-1475.

Valentine, D. E., & Moss, C. F. (1998). Sensorimotor integration in bat sonar. In: Kunz T.H., Racey P.A. (Eds) *Bat biology and conservation* (pp. 220-230). Washington, DC: Smithsonian Institution University Press.

Vaughan, N., Jones, G., & Harris, S. (1997). Habitat use by bats (Chiroptera) assessed by means of a broad-band acoustic method. *Journal of Applied Ecology*, 716-730.

Wisniewska, D. M., Ratcliffe, J. M., Beedholm, K., Christensen, C. B., Johnson, M., Koblitz, J. C., Wahlberg, M., & Madsen, P. T. (2015). Range-dependent flexibility in the acoustic field of view of echolocating porpoises (*Phocoena phocoena*). *eLife*, *4*, 05651.

Wood, F. G., & Evans, W. E. (1980). Adaptiveness and ecology of echolocation in toothed whales. In Busnel R.G., Fish J.F. (Eds) *Animal sonar. NATO advanced study institute series (Series A: life sciences), volume 28* (pp. 381-425). Boston, MA: Springer.

Zollinger, S. A., & Brumm, H. (2011). The evolution of the Lombard effect: 100 years of psychoacoustic research. *Behaviour*, *148*, 1173-1198.

Chapter 2

Little evidence of a jamming avoidance response in either toothed whales or laryngeal echolocating bats when hunting in pairs

Little evidence of a jamming avoidance response in either toothed whales or laryngeal echolocating bats when hunting in pairs

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Abstract

Laryngeally echolocating bats and toothed whales are the only echolocators known to use biological sonar for prey pursuit / tracking in addition to navigation and orientation. When hunting, both bats and toothed whales must often contend with echolocating conspecifics hunting in proximity. Multiple hypotheses have been proposed to explain how echolocators handle the potentially overwhelming amount of acoustic information during group hunting: jamming avoidance responses (JAR; echolocators actively adjust the spectral and / or temporal components of their signals to reduce overlap with those of other individuals), clutter response (echolocators treat conspecifics as moving obstacles), and the Lombard effect (increases in signal amplitude in response to noise). To explore the similarities and differences between bats and toothed whales in echolocation behaviour when hunting in the presence of conspecifics, I measured acoustic (spectral and temporal parameters) and behavioural (direction of sonar emissions) changes made by Daubenton's bats, *Myotis daubentonii*, and harbour porpoises, *Phocoena phocoena*, hunting alone and in pairs. I found that both bats and porpoises produce acoustic changes indicative of the Lombard effect, however bats showed additional changes that may represent JAR and / or a clutter response. Behaviourally, bats show minimal shifts in sonar beam direction under different hunting conditions, while porpoises consistently exhibit noticeable differences in click direction between solo and paired trials, suggesting they more explicitly attend to conspecifics. Overall, these results suggest that bats and porpoises engage in different acoustic and behavioural adjustments when hunting with conspecifics. While these differences

may reflect unique physiologies, evolutionary histories, and foraging environments, I caution against assigning acoustic signal changes to a specific response type.

Introduction

Echolocation, or biological sonar, is a relatively rare sensory process that has evolved to support activity under conditions with limited potential visual information. All echolocators, from bats to swiftlets, oilbirds to toothed whales, use echolocation to orient themselves within the environment and to navigate through that environment. Of these natural echolocators, only laryngeal echolocating bats (hereafter, bats) and toothed whales use echolocation to detect and pursue prey (Madsen et al. 2005; Clausen et al. 2010; Liu et al. 2010a; Liu et al. 2010b; Madsen and Surlykke 2013). Similar to echolocation for navigation and orientation, echolocation for prey pursuit involves the production of acoustic signals and the interpretation of temporal, spectral, and amplitude differences between the emitted and returned signals to extract environmental information (Suthers 1988). When foraging, many bats' echolocation signals are highly flexible and can be adjusted to suit environmental conditions (Obrist 1995). To a lesser extent, toothed whales also adjust spectral and temporal components of their echolocation signals to make hunting more efficient (Surlykke and Moss 2000; Wisniewska et al. 2015).

The hunting behaviours of bats and toothed whales show similar patterns with respect to biosonar signal production. Prey tracking, pursuit, and interception via echolocation follow the same general progression though the search phase, approach phase, and finally the terminal buzz phase (Surlykke and Moss 2000; Verfuβ et al. 2008; Ratcliffe et al. 2013). Each phase has stereotypical acoustic trademarks that help to define the transition between consecutive phases, such as an increase in signal emission rate and, in some bats, a corresponding shift to lower

frequencies observed in the terminal buzz phase (Griffin et al. 1960; Moss and Surlykke 2001). The ability of bats and toothed whales to produce these terminal buzzes, with call rates of over 200 calls or 300 clicks per second, reflects the sonar sophistication used to offset the complexity of foraging / hunting behaviours (DeRuiter et al. 2009; Ratcliffe et al. 2013).

Most, perhaps all, animals that hear have to contend with the problem of acoustic clutter, or background noise. Humans often experience the “cocktail party effect” when experiencing acoustic clutter: our brain’s ability to attend to our own conversation even in the midst of a noisy background environment (McDermott 2009). The ability to focus one’s auditory attention, or to filter out less salient information, may be a necessary aspect of any animal which relies on acoustic signals for survival (Fullard et al. 2008), and specifically for those using echolocation (Ulanovsky and Moss 2008). As bats and toothed whales often forage in proximity to other actively echolocating conspecifics, the amount of acoustic clutter they experience, as well as the task of identifying their own echoes amidst the emitted and returning signals from other individuals, could, theoretically, be overwhelming.

The influence of conspecifics on echolocation behaviour, specifically in aerially foraging bats, has been widely debated. The initial evidence did not suggest any form of active spectral shifts in signal production, however subsequent research suggested the existence of a jamming avoidance response (JAR). It was widely reported that bats engage in jamming avoidance responses when flying with conspecifics (shifting their echolocation call frequencies in attempt to avoid spectral interference / overlap; Ulanovsky et al. 2004, Bates et al. 2008). Conversely, other

researchers suggest that the design of calls emitted amongst other echolocators does not reflect a jamming avoidance response (Ratcliffe et al. 2004, Fawcett et al. 2015). Instead, bats may simply be treating conspecifics as physical clutter. With respect to toothed whales, a jamming avoidance response has not been specifically studied, however some toothed whales apparently shift signal frequencies away from background noise and / or cease signaling until noise has lessened (Tyack and Janik 2013). Additionally, toothed whales have been observed to decrease the source level and increase the emission rate of their echolocation signals when in a cluttered environment, suggesting a similar clutter response to bats (Jensen et al. 2013).

Another explanation for acoustic changes observed when multiple echolocators are in proximity is the Lombard effect: an involuntary increase in signal amplitude observed under conditions of loud, ecologically relevant noise (Hage et al. 2013; Bunkley et al. 2015). Increases in signal frequency and duration are also thought to represent this effect (Au et al. 1985; Parks et al. 2010). Together, these shifts to a higher vocalization effort under noisy conditions should reduce the likelihood of signal interpretation errors through higher signal energy / signal-to-noise ratios (SNR; Hotchkin and Parks 2013). Amichai et al. (2015) recently showed that the vesper bat, *Pipistrellus kuhlii*, increases echolocation call duration and intensity in the presence of conspecifics, while beluga whales (*Delphinapterus leucas*) and killer whales (*Orcinus orca*) apparently increase echolocation click intensity in the presence of increased noise (Hotchkin and Parks 2013).

I assumed that attacking prey is a demanding task with respect to auditory attention, and thus might be seriously impacted by interference from other

echolocators. To explore these potential influences, I designed similar experiments to compare the echolocation and attention behaviour of both echolocating whales and bats. I elected to explore the hunting behaviours of both bats and toothed whales to (*i*) determine if, and if so where, the behaviour of these two animals differed from one another and (*ii*) assess whether there are any changes that occur when echolocators are hunting alone versus when hunting in pairs. I hypothesized that the reactions of bats and toothed whales to the presence of a conspecific would be similar, as their use of echolocation is similar despite the obvious differences in locomotion (Jakobsen and Surlykke 2010; Madsen and Surlykke 2013; Wisniewska et al. 2015).

For both species, I expected that the presence of a conspecific would elicit one of three probable acoustic reactions: (*i*) the conspecific would effectively be treated as a moving, similarly vocalizing object (jamming avoidance response), (*ii*) the conspecific would be treated as a moving obstacle (clutter response), or (*iii*) the conspecific would be treated similarly to an acoustically noisy environment (Lombard effect). I predicted that under a jamming avoidance response, paired signals would be spectrally and / or temporally separate from one another with respect to peak frequency and / or call emission rate (Ulanovsky et al. 2004; Chiu et al. 2009; Amichai et al. 2015). If bats and toothed whales employed a clutter response, I predicted that paired signals would have shorter durations, lower sound levels, and higher bandwidths (Schnitzler and Kalko 2001; Broders and Forbes 2004; Fawcett and Ratcliffe 2015; Warnecke et al. 2015). If bats and toothed whales responded to conspecifics via the reflexive Lombard effect, I predicted that paired signals would

have longer durations, higher source levels, and higher signal frequency (Parks et al. 2010; Zollinger and Brumm 2011; Hage et al. 2013).

Behaviourally, I hypothesized that bats and toothed whales would shift their attention (direction of echolocation beam) in the presence of a conspecific. I predicted that the average beam direction for solo hunters would be closer to zero degrees (representing the direction of the target), and when hunting in pairs the average beam direction would be further from zero degrees, suggesting that deviations in beam direction away from the target occur more often. Additionally, I predicted that the average beam direction for paired and solo hunters would be different from one another. This study is the first, to my knowledge, to compare bats and toothed whales with respect to the influence of conspecifics during hunting from both an acoustic and behavioural perspective.

Methods and Materials

Bats; flight room and recording set-up

I made bat echolocation call recordings at the University of Southern Denmark (SDU, Odense, Denmark), in an indoor flight room (L 7m x W 4.8m x H 2.5m). The flight room was constructed from aluminum poles covered in cotton batting and foam to attenuate echoes. The walls and ceiling consisted of thin mesh nylon. The floor was carpeted with a square pool (L2.5 x W2.5m x D0.2m) situated in the middle of the room; water depth was 10 cm (see Figure 1).

I obtained acoustic data from bats as they hunted for and attacked prey. To this end, acoustic signals (exclusively echolocation calls) from five adult male

Daubenton's bats (*Myotis daubentonii*) flying in the flight room were recorded. Because I wanted to identify and compare the influence of conspecifics on foraging behaviour, bats were released into the flight room either alone or in pairs. Bats were fed mealworms (*Tenebrio molitor*) during the experimental trials and provided with *ad libitum* access to water while in captivity. Bats were released at capture site upon completion of the experiment.

Recordings were made using a 12-microphone array (1/4" 40 BF G.R.A.S., Sound and Vibration, Denmark, grids off) located above the pool in a 3-dimensional rectangular shape, microphones were spaced 235 cm apart horizontally and 55 cm apart vertically (Figure 1). Before and after each day's trials, each microphone was calibrated using a 1 kHz pure tone at 114 dB SPL (type 42AM G.R.A.S. Sound Calibrator). Avisoft amplifiers were used to amplify the signals from microphones (amplified by 30 dB, high-pass filtered at 13.5 kHz). Signals in each recording were sampled at 500 kHz per channel using a Avisoft USGH 1216 A/D converter (16-bit) and were saved as a multi-microphone .wav file of 5 seconds long (1 s pre-trigger and 4 s hold time) onto a ThinkPad X201 laptop computer (Lenovo, Morrisville, USA).

Bat trials: solo and pairs

For each trial, 2-4 mealworms were placed on the water in the centre of the pool, as Daubenton's bats are trawling bats that hunt for prey located on or slightly above the surface of water (Boonman et al. 1998). Echolocation calls were recorded while the bats were identifying, approaching, and retrieving the mealworms. Three-

dimensional localization of each emitted echolocation call (x, y and z coordinates) was determined using the call's time of arrival differences at a minimum of 4 microphones. Recorded signals were analyzed using MATLAB (MathWorks) and a custom script (written by L. Jakobsen). For spectral and temporal analysis of individual signals, the multi-microphone recordings were split into their individual channels (single microphones) and analyzed using BatSound (v.4, Pettersson Elektronik, Uppsala, Sweden).

The following parameters were measured or determined for each echolocation call: source level (dB re 20 µPa pp), beam aim (0-360 degrees off-target (prey position), based on the acoustic axis of the emitted call), maximum receiver (which microphone received the strongest signal, indicative of the general direction the bat was calling), call duration (ms, measured from start to end of call from the oscillogram), pulse interval (ms, measured from start the focal call to the start of the next call from the oscillogram), peak frequency (kHz, measured from the power spectrum (FFT)), minimum frequency of the fundamental (kHz, -10 dB below peak frequency measured from the power spectrum (FFT)), maximum frequency of the fundamental (kHz, -10 dB above peak frequency measured from the power spectrum (FFT)), and -10 dB bandwidth of the fundamental (kHz, measured as difference between -10 dB above and below peak frequency).

For all recordings (solo and paired), the length of the search phase as well as the post-detection approach and terminal buzz phases were measured, with respect to length of time (in milliseconds) and the number of calls produced. The approach phase was defined as when the call period measured below 50 ms, while the buzz

phase was defined to have started when the call period measured below 11 ms (Surlykke and Moss 2000; Ratcliffe et al. 2013). The search phase contained all calls with PIs ≥ 50 ms.

Porpoises; pool area and recording set-up

I made the porpoise recordings in the Kerteminde harbour at Fjord & Bœlt in Kerteminde, Denmark. The enclosure was outdoors with netting used to create an 8 m x 12 m pool in the harbour at sea level (see Figure 2a). Three adult porpoises were used in these experiments, two females and one male; all had been at the facility for several years. As with the bat trials, I was interested in obtaining acoustic data for the porpoises as they hunted and attacked prey. To this end, acoustic signals from the three harbour porpoises (*Phocoena phocoena*) swimming in the pool were recorded along with corresponding video footage. Since I wanted to identify any influence of conspecifics, porpoises swam alone and in pairs (to allow for comparison between conditions and also between species (bats and porpoises)).

The pool in which the recordings took place was equipped with 16 calibrated Reson TC4014 hydrophones arranged in two horizontal linear arrays (8 hydrophones per array) and spaced 60 cm apart (see Figure 2b). The hydrophones were 75 cm and 125 cm below the surface of the water. All trials were recorded with a video camera (Profiline CTV7040, Abus, Germany). Prey (2-4 freshly thawed dead fish) were dropped into the pool in front of the hydrophones (ESM1). After the prey were introduced, the water above was splashed to attract attention and each porpoise was then released by the handler. For the solo trials, a single porpoise was released

alone and for the paired trials, two porpoises were released at the same time approximately 2-4 m apart (horizontally) at the same short end of the pool (ESM1). Only trials in which the porpoise(s) swam and produced clicks directly towards the hydrophone array were analysed.

Echolocation clicks from the solo and paired porpoises were collected from the moment of release until prey capture, for a maximum of 5 seconds. Signals were amplified and filtered using a custom-made conditioning box and simultaneously converted (analog to digital) with 16-bit resolution at 500 kHz per channel (National Instruments PXI-6123). These echolocation clicks were analyzed with MATLAB (MathWorks) and custom scripts (written by M. Wahlberg). Two-dimensional location (x, y coordinates) was determined for each emitted click using hydrophone arrival time differences. Animal depth was estimated from the videos.

Porpoise trials: solo and pairs

For each recorded click, the following parameters were measured: source level (dB re 1 µPa pp), beam heading (0-360 degrees off-target position, using the acoustic axis of the emitted click), maximum receiver (i.e. which hydrophone picked up the strongest signal), peak frequency (kHz), click duration (µs), -10 dB bandwidth (kHz, measured at 10 dB from peak frequency on the FFT/power spectrum), and click interval (ms, measured from the start of focal click to the start of the next click on the oscillogram). Porpoise recordings consisted only of approach and buzz phases, since the target had already been identified before release (due to splashing the water to draw attention to the target). The approach phase was defined as when the

click interval measured below 50 ms, and the buzz phase was defined as occurring when the click interval was < 13 ms (Verfuβ et al. 2008; Wisniewska et al. 2015). For all recordings (solo and paired), the length of approach and terminal buzz phases were measured, with respect to length of time (in seconds) and the number of clicks produced. These data, however, were not used in the analysis because the assignment of clicks to individual porpoises around the transition between approach and buzz phases was difficult due to the stereotyped nature of porpoise clicks (i.e. clicks exhibit little individual variation). After excluding any clicks without confident individual assignment, I decided not to present data on phase duration and click count, as I believe these might give a false impression of acoustic behaviour between paired *versus* solo hunting conditions.

Matching trajectories: bats and toothed whales

For both the bat and porpoise data, I wanted to compare solo and paired acoustic and behavioural data as robustly as possible. Since the physical trajectory of a given individual can dramatically alter the patterns observed in both physical behaviour as well as in signals emitted, I elected to match flight / swim trajectories prior to analysis and to run statistical comparisons between solo and paired files in which the flight/swim trajectories were similar. Trajectories were re-created from the coordinates (x, y, z for bats; x, y for porpoises) determined by the custom MATLAB scripts. Each solo trajectory was visually matched as closely as possible with a paired trajectory from a paired file and analyses were performed on the acoustic

signals and behavioural data contained within those two files. This process was repeated for each of the solo flight (N = 7) and solo swim (N = 5) trials.

Statistical analyses

Solo v. Paired: Acoustic analysis

Signals from all trials were separated into distinct foraging phases defined by pulse intervals (PIs) prior to analysis. Signals were separated into three phases for bats and two phases for porpoises; search phase (pulse interval > 50 ms; bat data only), approach phase (50 ms ≤ pulse interval ≥ 11 ms for bat data; 50 ≤ pulse interval ≥ 13 for porpoise data), and terminal buzz phase (pulse interval < 11 ms for bats, pulse interval < 13 for porpoises). For all data sets, Wilcoxon Signed-Rank tests were conducted to determine if differences in the following parameters existed between hunting condition (i.e. solo *versus* paired): average phase duration (bats only), number of signals (bats only), pulse interval (both groups), signal duration (both groups), peak frequency (both groups), minimum signal frequency (bats only), maximum signal frequency (bats only), and fundamental -10 dB bandwidth (both groups, see Tables 1 and 2). For bats, source levels (signal intensities) were converted into pascals for solo *versus* paired comparisons and re-converted into dB SPL for presentation. For porpoise data, such conversions were not feasible due to computational limits. Porpoise source levels, then, were compared using median dB values. Source levels were compared using Wilcoxon Signed-Rank tests (z-scores presented in Tables 1 and 2).

Solo v. Paired: Behavioural analysis

When contending with conspecifics during a hunting event, individuals may need to divide their attention between the target they are attempting to intercept and the conspecific, potentially as a form of competition (i.e. to ensure first access to the target) or as a means of avoiding collisions. To determine if echolocators divided their attention between the target and the conspecific during paired motor activities, I analyzed and compared the aim / direction values of the bats and porpoises with the matched flight / swim paths in the paired trials (N = 7 for bats; N = 5 for porpoises) with the aim values for the trajectory-matched solo bat and porpoise (N = 7, N = 5, respectively). Within groups, I also collapsed and compared aim values for all paired bats and all paired porpoises with all solo bats and all solo porpoises of matched trajectories to get a sense of overall echolocator beam direction differences. Aim values are the calculated measurement (in degrees) of the direction that a signals beam was emitted at the moment it was captured by a given microphone and were determined by the custom MATLAB scripts (Jakobsen). Briefly, each signal in a trial had a designated microphone / hydrophone at which most of the energy in the vocalization was directed. Using the source levels of signals at all surrounding microphones, the MATLAB script calculated the direction the signal was emitted (i.e. the direction the bat's mouth was facing, or the direction the porpoise's head was facing).

I only analyzed aim values for vocalizations emitted during the attack sequence (i.e. from the beginning of the approach phase up until the end of the buzz phase) for each individual. For each trial, I set the average aim direction during the

buzz phase to zero, as this was assumed to represent each individual "looking" towards the target. I then re-calculated the remaining aim values to represent deviations from zero (i.e. from the target) throughout the attack sequence. Using a circular statistics analysis program (Oriana, Kovach Computing Services, Anglesey, Wales), I calculated the following information for each paired and solo echolocator with matched flight / swim trajectories in each trial, as well as for all paired and all solo echolocators, combined: mean aim direction (μ), standard deviation. I used Watson's U^2 value to test for von Mises distribution, and V-test's to test for deviations from a set mean. V-test values indicated whether the mean aim value was different from the set direction (zero degrees in this case, see Table 3). Watson's U^2 values indicated if the distribution of aim values conformed to a von Mises distribution (i.e. circular normal distribution / continuous probability distribution on a circle). I also compared mean aim values between a paired and solo echolocator with matching trajectories, using a two-sample *t*-test to determine if mean aim directions for each individual under the separate hunting conditions differed from one another. Since there was no evidence in any trials of a von Mises distribution of aim values ($N = 0$), I compared paired and solo means using a non-parametric Mardia-Watson-Wheeler test (Table 3).

Results

Bat acoustic results

Bat acoustic results are summarized in Table 1. Representative buzzes for both solo and paired bat conditions are depicted in Figure 3a and b. In the search phase, I

found differences in fundamental bandwidth. Paired bats produced calls with narrower bandwidths (35.48 ± 6.8 kHz) than solo bats (50.63 ± 11.4 kHz; Wilcoxon Signed-Rank test: z = 2.3, P = 0.022). Paired bats in the approach phase emitted fewer calls (9.57 ± 3.0 calls) than solo bats (15.29 ± 3.2 calls; Wilcoxon Signed-Rank test: z = 2.69, P = 0.007). Finally, paired bats in the buzz phase produced shorter buzzes (84.58 ± 14.9 ms) than those flying alone (117.74 ± 17.0 ms; Wilcoxon Signed-Rank test: z = 2.68, P = 0.007). Paired bats in the buzz phase also emitted fewer calls (12.43 ± 2.0 calls), had longer pulse intervals (7.05 ± 0.6 ms), and higher minimum fundamental frequencies (30.17 ± 4.2 kHz) when compared to solo measurements (19.29 ± 2.5 calls, 6.41 ± 0.2 ms, and 26.88 ± 1.73 kHz, respectively; Wilcoxon Signed-Rank tests: z = 3.09, P = 0.002, z = -2.68, P = 0.007, and z = -3.07, P = 0.002, respectively). Some trends failed to reach significance, including: in all phases, paired bats produced calls with lower maximum fundamental frequencies and narrower bandwidths. In the search and buzz phases, but not the approach phase, paired bats produced calls that tended to have higher source levels and call durations (Table 1).

Bat behavioural results

Overall, both solo and paired bats produced echolocation signals with a mean direction of zero degrees (i.e. in the assumed direction of the target; 1.6˚ vs 346.6˚, respectively). V-test results for combined paired and combined solo trials suggest that these mean directions are not different from zero degrees. Indeed, the majority of individual trials for solo bats (N = 6) and paired bats (N = 6) also show mean aim

directions as not different from zero degrees (see Figure 3c and d for representative circular arrow graphs of a single trial paired (c) and solo (d) bat). When comparing solo and paired mean aim directions to each other (as opposed to comparing mean aim directions to zero degrees), 3 of the 7 trials had significant Mardia-Watson-Wheeler results suggesting that the mean aim directions were different from one another, while the remaining 4 trials show no difference between solo and paired directions. However, when all paired and all solo trials are collapsed, no difference in mean aim directions was detected. In 6 of 7 trials, the mean direction for solo bats was closer to zero than the mean direction for paired bats, however as indicated above, the majority of these differences were not significant. Bat behavioural results are summarized in Table 2. An illustration of two bats flying together, indicating their simultaneous x-positions and call directions, is provided in Figure 3e, while an illustration of a bat flying alone is depicted in Figure 3f.

Porpoise acoustic results

Since my sample sizes for porpoises was small (N = 3 porpoises, N = 5 matched trials) applying appropriate statistical tests was difficult. As such, I chose to describe the results here, and include the statistical analyses in Table 3. As was the case for the bats, each trial compared a solo porpoise (one of the three porpoises swimming alone) with a paired porpoise (one of the three porpoises, swimming with a second of those three porpoises, along a swim trajectory best matching that of the solo porpoise). Representative buzzes for both solo and paired porpoise conditions are found in Figures 4a and b.

While no significant differences were observed in the approach phase, paired porpoises emitted clicks with higher source levels (more intense signals; 146.52 ± 4.9 dB) than solo porpoises (138.3 ± 4.1 dB) during the buzz phase (Wilcoxon Signed-Rank test: z = -2.09, P = 0.037). With respect to peak frequency and -10 dB bandwidth, no consistent trends were observed in either phase. Click duration and click interval were consistently longer for paired porpoises (approach phase: 94.8 ± 2.2 µs, 22.5 ± 2.8 ms respectively; buzz phase: 100.8 ± 12.4 µs, 3.19 ± 1.2 ms respectively) than for porpoises hunting alone (approach phase: 86.8 ± 6.9 µs; 20.96 ± 5.0 ms respectively; buzz phase: 99.8 ± 16.5 µs, 2.54 ± 0.7 ms respectively).

Porpoise behavioural results

Similar to the bat results, paired and solo porpoises (both in individual trials as well as when combined over all trials) had mean aim directions not different from zero degrees (N = 6 comparisons; 355.2˚ vs 347.4˚ for combined trials, respectively). After comparing solo and paired mean directions to each other, however, my results suggest paired porpoises and solo porpoises differed in the majority of trials, as well as between overall paired and solo data. Refer to Figure 4c and d for representative circular arrow graphs of a paired (c) and solo (d) porpoise from a single trial. In only one porpoise trial were there equivalent mean aim directions for both conditions. In 4 of 5 trials, the mean direction for the solo porpoise was closer to zero than the mean direction for the paired porpoise (see Table 4). An illustration of two porpoises swimming together, indicating their simultaneous x-positions and click directions, is

provided in Figure 4e, while a similar illustration of a porpoise swimming alone is depicted in Figure 4f.

Discussion

My results suggest that the presence of conspecifics when hunting influences bats and porpoises both similarly and dissimilarly with respect to acoustic and behavioural adjustments. Both bats and porpoises hunting with conspecifics show evidence of the Lombard effect (Tables 1 and 3). Bats also appear to show some potential overlap between clutter response and jamming avoidance response but at the same time, no consistent change in call direction under different hunting conditions (Table 2; Figures 3c and d). Shorter buzz durations are consistent with a clutter response, emitting fewer calls with longer pulse intervals would be more beneficial to avoid echolocation signal jamming, and narrower bandwidths with higher minimum frequencies reflect aspects of the Lombard effect (Table 1). These results suggest that the acoustic flexibility observed in aerial hawking bats may allow for the use of multiple vocal and behavioural responses to focus the attention during instances of high acoustic clutter. Porpoises, on the other hand, show few acoustic responses to conspecifics but consistent evidence of signal direction changes when hunting in pairs (Tables 3 and 4; Figures 4c and d) and thus may depend more on behavioural adjustments to contend with conspecifics than spectral / temporal shifts in echolocation signal production.

The three general theories (Lombard effect, jamming avoidance response, and clutter response) each reflect a different interpretation of what conspecifics

represent as well as different goals of acoustic adjustments. Under JAR, conspecifics represent flexible acoustic signals masking the signals and echoes of the focal animal. The acoustic shifts that characterize a jamming avoidance response aim to separate one's vocal signal from the vocal signals of others. In a clutter response, conspecifics can better be thought of as silent, physical obstacles and changes in vocalizations reflect call changes made as bats approach objects and may potentially also reflect an attempt to distinguish between echolocation signals / echoes and the less relevant echoes returning from those obstacles. Finally, the Lombard effect suggests that conspecifics are dealt with the same way as noise that masks the relevant frequencies of echolocation signals and returning echoes. The vocal changes observed during the Lombard effect make one's own signals more salient and thus detectable. The specific acoustic changes thought to represent these hypotheses, specifically jamming avoidance response and clutter, can be contradictory and / or similar between responses. Below I explore each hypothesis with respect to the findings to identify why assigning spectral and / or temporal shifts to a single response type (i.e. jamming avoidance response, clutter, Lombard) may be misleading.

Jamming Avoidance Response

The key feature of a jamming avoidance response is the separation of signals from multiple sources, either with respect to spectral components, temporal components, or both. A jamming avoidance response between echolocators should be reflected by an active change in signal emission such that the vocalizations being produced

become more different from one another (Ulanovsky et al. 2004). Increases in signal frequency have been identified as representative of jamming avoidance as well as clutter and Lombard effect responses. Indeed, I observed an increase in minimum signal frequency in my bat data, although only during the buzz phase (Table 1), which may reflect a potential jamming avoidance response as higher frequencies promote increased signal directionality and, in turn, an increased signal-to-noise ratio (SNR) due to fewer extraneous returning echoes (Jones et al. 2018). Additional purported spectral evidence of a jamming avoidance response is increased bandwidth (Amichai et al. 2015; Hase et al. 2018). Increasing signal bandwidth would permit greater range of available frequencies for spectral shifts, thus allowing the most energy-rich frequency to be shifted more easily (Ulanovsky et al. 2004). However, I observed a decrease in fundamental bandwidth across all bat flight phases, although significant declines were observed only in the search phase and no such differences occurred for porpoise comparisons (see Tables 1 and 3). This decrease in bandwidth could instead reflect an aspect of the Lombard effect (see below).

Other jamming avoidance response evidence includes adjustments in the temporal aspects of vocalizations. Some studies suggest that increasing the number of signals reflects a jamming avoidance response due to simply increasing the number of available signals for echo return (Amichai et al. 2015), however I suspect this response would be more indicative of the Lombard effect (i.e. an attempt to increase signal detectability, as opposed to separating signal overlap). Conversely, other studies suggest that decreasing the number of signals during interference is

more effective in reducing the likelihood of jamming, as there would be fewer vocalizations and echoes with which to overlap (Chiu et al. 2009; Jarvis et al. 2013; Adams et al. 2017). Because click counts were excluded from analysis, I can only speak to the changes in number of signals emitted for bat trials. The decrease in call numbers I observed, then, could theoretically represent a form of jamming avoidance response (see Table 1). The observed increase in pulse intervals (PIs; Table 1) during paired hunting for both bats and toothed whales may also lend support to a jamming avoidance response; signals emitted further apart from one another would reduce the likelihood that those signals would experience temporal overlap. Previous research suggests that extraneous acoustic signals only disrupt bat echolocation when those signals fall within a critical time window (Miller 1991; Ratcliffe and Fullard 2005; Corcoran et al. 2011). Greater pulse intervals, then, may allow conspecifics to better avoid signal overlap within these windows.

Clutter Response

While some characteristics of a putative jamming avoidance response are in dispute, the key aspects of a clutter response are relatively consistent. Most if not all work regarding clutter responses in laryngeally echolocating bats describe such responses as pronounced decreases in signal duration and amplitude in conjunction with increased signal bandwidth and higher peak frequencies (Arlettaz et al. 2001; Schnitzler and Kalko 2001; Broders and Forbes 2004; Sümer et al. 2009). Shorter call durations with lower source levels are thought to reduce pulse-echo overlap, allowing echolocators to distinguish between the relevant and irrelevant echoes

returning from obstacles (Fawcett and Ratcliffe 2015). Increased bandwidths and higher peak frequencies should (*i*) allow for the separation of echoes from multiple sources, (*ii*) enhance spatial resolution, and (*iii*) make signals more directional and informative to allow for safe passage through vegetation (Moss et al. 2006; Boonman and Ostwald 2007). Of these responses, I only found an increase in minimum fundamental signal frequency, theoretically supporting separation between target and clutter echoes (Sümer et al. 2009; Table 1). Increased frequency, however, is also indicative of a jamming avoidance response and the Lombard effect, again consistent with the supposition that response assignment based on spectral and temporal adjustments may be misleading.

Temporally, an increase in signal emission rate would generally indicate a clutter response, as increasing the amount of information returning to the echolocator would reduce the likelihood of collisions and echoic ambiguities (Kalko and Schnitzler 1993; Fawcett and Ratcliffe 2015; Adams et al. 2017). As indicated above, however, I observed a decrease in signal emission by bats and an increase in pulse interval (i.e. greater signal separation) in both bats and porpoises (see Tables 1 and 3). These observed rates and number of signals emitted, therefore, do not support a clutter response. Finally, studies report that within cluttered environments, shorter buzzes are employed to reduce the amount of time in which an echolocator experiences the potentially overwhelming information returned from such a high number of target and clutter echoes (Moss et al. 2006; Hulgard and Ratcliffe 2016). My results, then, may support a clutter response by bats with respect to average buzz duration or, instead, may reflect bats trading off improved accuracy for faster

acquisition times in the face of competition from conspecifics hunting for the same food reward (Table 1).

Lombard Effect

Both jamming avoidance responses and clutter responses suggest intentional changes in signal production. The Lombard effect, on the other hand, happens in humans without conscious thought (Zollinger and Brumm 2011). The Lombard effect has been reported for many different animals, including bats and toothed whales (Tressler and Smotherman 2009; Hotchkin and Parks 2013; Tyack and Janik 2013). The involuntary nature of the effect suggests that shifts to higher signal intensities is not to avoid overlapping with other signals (i.e. jamming avoidance response) or to avoid the distractions of extraneous echoes (i.e. clutter response), but exclusively to increase the saliency of one's own signals when experiencing noise with ecologically relevant frequencies. The primary defining characteristic of the Lombard effect is an increase in source level, with the ultimate goal of increased signal-to-noise ratios and effective communication (Hage et al. 2013; Luo et al. 2017). While I observed no changes in bat source levels, I did observe a significant increase in signal amplitude during the buzz phase in paired versus solo porpoises (Table 3).

Changes in signal frequency, duration, and bandwidth have also been attributed to the Lombard effect (Au et al. 1985; Parks et al. 2010; Bunkley et al. 2015). Higher signal frequencies shift energy away from lower, more easily masked frequency components, reducing interference from background noise. As indicated above, I observed shifts to higher minimum signal frequencies under paired bat

hunting conditions, a response seemingly indicative of all three response types (see Table 1). Longer signal duration increases the amount of energy present, thus effectively increasing signal-to-noise ratios (Chiu et al. 2009; Bunkley et al. 2015; Jones et al. 2018). These signals and echoes, then, become more redundant and easily detectable among similarly pitched noise. While I observed no changes in signal duration between solo and paired conditions in any phase of my bat data trials, I did detect a trend across approach and buzz phases for paired porpoises to emit longer clicks, similar to that described by Tyack and Janik (2013; Table 3). Finally, narrower bandwidths also increase signal-to-noise ratios, and may thus represent outcomes of the Lombard effect, by concentrating signal energy into a narrower band of frequencies and thus activating a smaller number of frequency-tuned neurons but increasing the given activity of a given frequency-tuned neuron within that bandwidth (Bunkley et al. 2015; Jones et al. 2018). My results suggest that bats narrow their bandwidths while hunting in pairs, but that porpoises do not (see Tables 1 and 3).

Directed Attention

With respect to behaviour, I focused on the direction of a signal (and theoretically the direction of that individual's attention) during the attack phase. Under any hunting conditions (solo or otherwise), echolocators will benefit from maintaining directed attention towards their target, as directionality will promote the highest signal-to-noise ratio of echoes returning from the target and maximize the likelihood of capture (Ghose and Moss 2003). When multiple targets are present, however, it may benefit

echolocators to divide their attention amongst these objects of interest. That is, echolocators would benefit from the knowledge of multiple prey items to increase foraging efficiency, as well as from the knowledge of obstacle position to avoid collisions (Fujioka et al. 2014). When an additional target takes the form of a conspecific actively hunting for the same resource, dividing attention between prey items and the nearby conspecific may also give echolocators a competitive advantage.

These behavioural results suggest that bats, in general, did not divide their attention between their prey target and their conspecific during paired hunting. Solo and paired bats both emitted calls that, on average, did not deviate from the target during the attack phase (6 of 7 solo bats, 6 of 7 paired bats). Furthermore, in most trials, the direction of solo bat calls was not different from the direction of paired bat calls (Table 2, Figure 3). In 2 of the 3 trials in which solo and paired directions were not equivalent, the solo bats aimed their signals closer to the target than the paired bat. The ability of bats to engage in competitive foraging without noticeable deviations in attention away from the target suggests bats do not consider conspecifics to be a cause for concern. Bats do, however, make some acoustic adjustments when flying with conspecifics. These results may instead indicate that bats are aware of their conspecifics but choose to maintain directional attention on the target. Perhaps bats listen to one another to track each other's position in shared space (see below).

All porpoises (solo and paired) appear to maintain a focused attention on their targets but do so differently between hunting conditions. Under both conditions, all

porpoise trials analyzed showed click directions towards the target (Table 4, Figure 4). When comparing the directions between solo and paired (as opposed to between attention direction and the direction of the target), I found that in 5 of 6 comparisons, including the combined average of all trials, the direction of the paired porpoise clicks is significantly different from that of the solo porpoises and that, in general, the paired porpoises emitted their clicks at an average direction that was further away from the target than solo porpoises, suggesting that when hunting in pairs porpoises do track one another using echolocation.

The differences observed between the bat and porpoise behavioural / attention data may reflect a trade-off between acoustic signal adjustments and attentional adjustments: bats make several acoustic changes in the presence of conspecifics, whereas porpoises significantly alter only the intensity of their signals, a change that would not promote the identification or localization of their conspecific. Bats appear to forgo behavioural changes due to their use of spectral / temporal adjustments, while porpoises instead engage in attention splitting between targets and their environment. In other words, if echolocators are able to contend with conspecifics through acoustic changes (i.e. bats), they may not also need to shift attention towards them, but if echolocator vocalizations are so precise and conserved that adjusting them is not realistic (i.e. porpoises), they might need to resort to attention deviations to avoid collisions or as a means of securing a competitive advantage. However, this is only conjecture and further research is required to determine how echolocators attend to each other.

Concluding Remarks

In describing my results as they support or refute various acoustic responses to conspecifics, it is clear that the literature-based descriptions and definitions of jamming avoidance response, clutter response, and the Lombard effect overlap which may lead to confusion. Additional complications in response assignment arise from the understanding that different bat species, engage in acoustic responses to obstacles, noise, and conspecifics in different ways, depending on the types of signals typically produced (Jones et al. 2018). Indeed, I observed individual differences along the entire spectrum of acoustic adjustments. Making specific predictions regarding which response is being employed based on observed acoustic changes is complex. Exploring each response with respect to the ideal outcomes of spectral and / or temporal adjustments, as well as the unique representation of conspecifics, allows for a more informative explanation of signal changes. Under these conditions, and in conjunction with my behavioural results, I argue that these acoustic measurements best support the Lombard effect as opposed to any intentional signal shifts.

A jamming avoidance response necessarily indicates that signal adjustments are being made to minimize signal overlap, and I observed only minor temporal changes from paired bat data that could theoretically support this notion. Clutter responses require echolocators to actively attend to their conspecifics in order to avoid collisions. My behavioural results, however, provide no evidence that bats acknowledge the position of conspecifics. Porpoises do appear to shift their attention away from the target under paired conditions, likely towards the other porpoise. Most

of my results support the goal of increasing vocalization / echo signal-to-noise ratio and therefore individual signal detectability. In other words, my results suggest that conspecifics likely represent acoustic background noise as opposed to static obstacles or flexible signals and thus echolocators experience the Lombard effect. Since echolocation evolved in both bats and toothed whales tens of millions of years ago, it seems reasonable both contend with conspecifics via cross correlation of signals to echoes as opposed to active signal changes.

An additional, but not mutually exclusive, explanation for some of the more unexpected bat results (specifically narrower bandwidths) could be simple competition. A trade-off exists between narrowband and broadband frequency modulated (FM) signals: typically, narrowband signals are better for target detection and classification, especially of moving objects, but broadband signals are better for target localization (Schnitzler and Kalko 2001). Furthermore, frequency modulated signals with narrower bandwidths increase an echolocators ability to detect small objects at greater distances (Boonman and Schnitzler 2004; Jones and Holderied 2007). Although narrowband signals would cause a decrease in ranging accuracy, these signals would enhance their ability to identify an object at greater distances, thus perhaps allowing echolocators hunting in pairs to (*i*) detect prey faster than their conspecifics and also (*ii*) be consistently aware of the general location of their conspecific, either to avoid collisions or to ensure a competitive advantage with respect to prey. Assuming that paired hunting conditions involve conspecifics attempting to acquire a limited resource, my results suggest that the spectral

changes with respect to bat bandwidth may reflect the bats attempting to detect and intercept a target more quickly and efficiently than nearby conspecifics.

In summary, I have provided the first comparison of bat and toothed whale foraging behaviour under the condition of conspecific influence and identified several areas that require further research. I have reported differences in both behavioural and signal flexibility between these two animals. I conducted trials with relatively small sample sizes, especially for the toothed whales. Additional trials with different individuals would likely increase the relevance of these results, as well as provide more information regarding how echolocators respond to group hunting conditions. That said, the differences I report here between the two species groups, vespertilionid bats and toothed whales, may reflect real differences in evolutionary foraging history / behaviours between these animals.

References

Adams, A. M., Davis, K., & Smotherman, M. (2017). Suppression of emission rates improves sonar performance by flying bats. *Scientific Reports*, *7*, 41641.

Amichai, E., Blumrosen, G., & Yovel, Y. (2015). Calling louder and longer: how bats use biosonar under severe acoustic interference from other bats. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20152064.

Arlettaz, R., Jones, G., & Racey, P. A. (2001). Effect of acoustic clutter on prey detection by bats. *Nature*, *414*, 742-745.

Au, W. W., Carder, D. A., Penner, R. H., & Scronce, B. L. (1985). Demonstration of adaptation in beluga whale echolocation signals. *The Journal of the Acoustical Society of America*, *77*, 726-730.

Bates, M. E., Stamper, S. A., & Simmons, J. A. (2008). Jamming avoidance response of big brown bats in target detection. *Journal of Experimental Biology*, *211*, 106-113.

Boonman, A. M., Boonman, M., Bretschneider, F., & van de Grind, W. A. (1998). Prey detection in trawling insectivorous bats: duckweed affects hunting behaviour in Daubenton's bat, *Myotis daubentonii*. *Behavioral Ecology and Sociobiology*, *44*, 99-107.

Boonman, A., & Ostwald, J. (2007). A modeling approach to explain pulse design in bats. *Biological Cybernetics*, *97*, 159-172.

Boonman, A., & Schnitzler, H. U. (2005). Frequency modulation patterns in the echolocation signals of two vespertilionid bats. *Journal of Comparative Physiology A*, *191*, 13-21.

Broders, H. G., & Forbes, G. J. (2004). Interspecific and intersexual variation in roost-site selection of northern long eared and little brown bats in the Greater Fundy National Park ecosystem. *The Journal of Wildlife Management*, *68*, 602-610.

Bunkley, J. P., McClure, C. J., Kleist, N. J., Francis, C. D., & Barber, J. R. (2015). Anthropogenic noise alters bat activity levels and echolocation calls. *Global Ecology and Conservation*, *3*, 62-71.

Chiu, C., Xian, W., & Moss, C. F. (2009). Adaptive echolocation behavior in bats for the analysis of auditory scenes. *Journal of Experimental Biology*, *212*, 1392-1404.

Clausen, K. T., Wahlberg, M., Beedholm, K., Deruiter, S., & Madsen, P. T. (2011). Click communication in harbour porpoises *Phocoena phocoena*. *Bioacoustics*, *20*, 1-28.

Corcoran, A. J., Barber, J. R., Hristov, N. I., & Conner, W. E. (2011). How do tiger moths jam bat sonar?. *Journal of Experimental Biology*, *214*, 2416-2425.

Corcoran, A. J., & Conner, W. E. (2014). Bats jamming bats: food competition through sonar interference. *Science*, *346*, 745-747.

DeRuiter, S. L., Bahr, A., Blanchet, M. A., Hansen, S. F., Kristensen, J. H., Madsen, P. T., & Wahlberg, M. (2009). Acoustic behaviour of echolocating porpoises during prey capture. *Journal of Experimental Biology*, *212*, 3100-3107.

Fawcett, K., Jacobs, D. S., Surlykke, A., & Ratcliffe, J. M. (2015). Echolocation in the bat, *Rhinolophus capensis*: the influence of clutter, conspecifics and prey on call design and intensity. *Biology Open*, *4*, 693-701.

Fawcett, K., & Ratcliffe, J. M. (2015). Clutter and conspecifics: a comparison of their influence on echolocation and flight behaviour in Daubenton's bat, *Myotis daubentonii*. *Journal of Comparative Physiology A*, *201*, 295-304.

Fujioka, E., Aihara, I., Watanabe, S., Sumiya, M., Hiryu, S., Simmons, J. A., Riquimarous, H., Watanabe, Y. (2014). Rapid shifts of sonar attention by *Pipistrellus abramus* during natural hunting for multiple prey. *The Journal of the Acoustical Society of America*, *136*, 3389-3400.

Fullard, J. H., Ratcliffe, J. M., & Jacobs, D. S. (2008). Ignoring the irrelevant: auditory tolerance of audible but innocuous sounds in the bat-detecting ears of moths. *Naturwissenschaften*, *95*, 241.

Ghose, K., & Moss, C. F. (2003). The sonar beam pattern of a flying bat as it tracks tethered insects. *The Journal of the Acoustical Society of America*, *114*, 1120-1131.

Gillam, E. H., Ulanovsky, N., & McCracken, G. F. (2006). Rapid jamming avoidance in biosonar. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 651-660.

Griffin, D. R., Webster, F. A., & Michael, C. R. (1960). The echolocation of flying insects by bats. *Animal Behaviour*, *8*, 141-154.

Hase, K., Kadoya, Y., Maitani, Y., Miyamoto, T., Kobayasi, K. I., & Hiryu, S. (2018). Bats enhance their call identities to solve the cocktail party problem. *Communications Biology*, *1*, 39.

Hage, S. R., Jiang, T., Berquist, S. W., Feng, J., & Metzner, W. (2013). Ambient noise induces independent shifts in call frequency and amplitude within the Lombard effect in echolocating bats. *Proceedings of the National Academy of Sciences*, *110*, 4063-4068.

Hotchkin, C., & Parks, S. (2013). The Lombard effect and other noise-induced vocal modifications: insight from mammalian communication systems. *Biological Reviews*, *88*, 809-824.

Hulgard, K., & Ratcliffe, J. M. (2016). Sonar sound groups and increased terminal buzz duration reflect task complexity in hunting bats. *Scientific Reports*, *6*, 21500.

Jakobsen, L., & Surlykke, A. (2010). Vespertilionid bats control the width of their biosonar sound beam dynamically during prey pursuit. *Proceedings of the National Academy of Sciences*, *107*, 13930-13935.

Jarvis, J., Jackson, W., & Smotherman, M. (2013). Groups of bats improve sonar efficiency through mutual suppression of pulse emissions. *Frontiers in Physiology*, *4*, 140.

Jensen, F. H., Rocco, A., Mansur, R. M., Smith, B. D., Janik, V. M., & Madsen, P. T. (2013). Clicking in shallow rivers: short-range echolocation of Irrawaddy and Ganges river dolphins in a shallow, acoustically complex habitat. *Public Library of Science One*, *8*, e59284.

Jones, G., & Holderied, M. W. (2007). Bat echolocation calls: adaptation and convergent evolution. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 905-912.

Jones, T. K., Wohlgemuth, M. J., & Conner, W. E. (2018). Active acoustic interference elicits echolocation changes in heterospecific bats. *Journal of Experimental Biology*, *221*, jeb176511.

Kalko, E. K., & Schnitzler, H. U. (1993). Plasticity in echolocation signals of European pipistrelle bats in search flight: implications for habitat use and prey detection. *Behavioral Ecology and Sociobiology*, *33*, 415-428.

Liu, Y., Cotton, J. A., Shen, B., Han, X., Rossiter, S. J., & Zhang, S. (2010). Convergent sequence evolution between echolocating bats and dolphins. *Current Biology*, *20*, R53-R54.

Liu, Y., Liu, Z., Shi, P., & Zhang, J. (2010). The hearing gene Prestin unites echolocating bats and whales. *Current Biology*, *20*, R55-R56.

Luo, J., Lingner, A., Firzlaff, U., & Wiegrebe, L. (2017). The Lombard effect emerges early in young bats: Implications for the development of audio-vocal integration. *Journal of Experimental Biology*, *220*, 1032-1037.

Madsen, P. T., Johnson, M., De Soto, N. A., Zimmer, W. M. X., & Tyack, P. (2005). Biosonar performance of foraging beaked whales (*Mesoplodon densirostris*). *Journal of Experimental Biology*, *208*, 181-194.

Madsen, P. T., & Surlykke, A. (2013). Functional convergence in bat and toothed whale biosonars. *Physiology*, *28*, 276-283.

Mao, B., Aytekin, M., Wilkinson, G. S., & Moss, C. F. (2016). Big brown bats (*Eptesicus fuscus*) reveal diverse strategies for sonar target tracking in clutter. *The Journal of the Acoustical Society of America*, *140*, 1839-1849.

McDermott, J. H. (2009). The cocktail party problem. *Current Biology*, *19*, R1024-R1027.

Miller, L. A. (1991). Arctiid moth clicks can degrade the accuracy of range difference discrimination in echolocating big brown bats, Eptesicus fuscus. *Journal of Comparative Physiology A*, *168*, 571-579.

Moss, C. F., & Surlykke, A. (2001). Auditory scene analysis by echolocation in bats. *The Journal of the Acoustical Society of America*, *110*, 2207-2226.

Moss, C. F., Bohn, K., Gilkenson, H., & Surlykke, A. (2006). Active listening for spatial orientation in a complex auditory scene. *Public Library of Sscience Biology*, *4*, e79.

Moss, C. F., Chiu, C., & Surlykke, A. (2011). Adaptive vocal behavior drives perception by echolocation in bats. *Current Opinion in Neurobiology*, *21*, 645-652.

Obrist, M. K. (1995). Flexible bat echolocation: the influence of individual, habitat and conspecifics on sonar signal design. *Behavioral Ecology and Sociobiology*, *36*, 207-219.

Parks, S. E., Johnson, M., Nowacek, D., & Tyack, P. L. (2010). Individual right whales call louder in increased environmental noise. *Biology Letters*, *7*, 33-35.

Ratcliffe, J. M., Hofstede, H. M. T., Avila -Flores, R., Fenton, M. B., McCracken, G. F., Biscardi, S., Blasko, J., Gillam, E., Orprecio, J., & Spanjer, G. (2004). Conspecifics influence call design in the Brazilian free-tailed bat, *Tadarida brasiliensis*. *Canadian Journal of Zoology*, *82*, 966-971.

Ratcliffe, J. M., & Fullard, J. H. (2005). The adaptive function of tiger moth clicks against echolocating bats: an experimental and synthetic approach. *Journal of Experimental Biology*, *208*, 4689-4698.

Ratcliffe, J. M., Elemans, C. P., Jakobsen, L., & Surlykke, A. (2013). How the bat got its buzz. *Biology Letters*, *9*, 20121031.

Schnitzler, H. U., & Kalko, E. K. (2001). Echolocation by Insect-Eating Bats: I define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group. *Bioscience*, *51*, 557-569.

Sümer, S., Denzinger, A., & Schnitzler, H. U. (2009). Spatial unmasking in the echolocating Big Brown Bat, *Eptesicus fuscus*. *Journal of Comparative Physiology A*, *195*, 463-472.

Surlykke, A., & Moss, C. F. (2000). Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory. *The Journal of the Acoustical Society of America*, *108*, 2419-2429.

Suthers, R. A. (1988). The production of echolocation signals by bats and birds. In: Nachtigall P.E., Moore P.W.B. (Eds.) *Animal Sonar* (volume 156, pp. 23-45). Boston, MA: Springer.

Tressler, J., & Smotherman, M. S. (2009). Context -dependent effects of noise on echolocation pulse characteristics in free-tailed bats. *Journal of Comparative Physiology A*, *195*, 923-934.

Tyack, P. L., & Janik, V. M. (2013). Effects of noise on acoustic signal production in marine mammals. In: Brumm H. (Eds.) *Animal communication and noise* (volume 2, pp. 251-271). Berlin, Heidelberg: Springer.

Ulanovsky, N., Fenton, M. B., Tsoar, A., & Korine, C. (2004). Dynamics of jamming avoidance in echolocating bats. *Proceedings of the Royal Society of London B: Biological Sciences*, *271*, 1467-1475.

Ulanovsky, N., & Moss, C. F. (2008). What the bat's voice tells the bat's brain. *Proceedings of the National Academy of Sciences*, *105*, 8491-8498.

Verfuß, U. K., Miller, L. A., Pilz, P. K., & Schnitzler, H. U. (2009). Echolocation by two foraging harbour porpoises (*Phocoena phocoena*). *Journal of Experimental Biology*, *212*, 823-834.

Warnecke, M., Chiu, C., Engelberg, J., & Moss, C. F. (2015). Active listening in a bat cocktail party: adaptive echolocation and flight behaviors of big brown bats, *Eptesicus fuscus*, foraging in a cluttered acoustic environment. *Brain, Behavior and Evolution*, *86*, 6-16.

Wisniewska, D. M., Ratcliffe, J. M., Beedholm, K., Christensen, C. B., Johnson, M., Koblitz, J. C., Wahlberg, M., & Madsen, P. T. (2015). Range-dependent flexibility in the acoustic field of view of echolocating porpoises (*Phocoena phocoena*). *Elife*, *4*, e05651.

Zollinger, S. A., & Brumm, H. (2011). The evolution of the Lombard effect: 100 years of psychoacoustic research. *Behaviour*, *148*, 1173-1198.

Figure Legends

Figure 1. Flight room set-up for bat trials. Dimensions of the room (length, width, height) and floor pool (length, width) are indicated with black and white arrows, respectively. Microphones (*N* = 12) are indicated by white, dashed circles.

Figure 2. (A) Outdoor enclosure for porpoise acoustic recordings. Dimensions of pool (length, width) indicated with white arrows. Location of prey placement indicated by white circle and location of porpoise release indicated by white "X". **(B)** Three-dimensional view of the underwater recording area. Hydrophones (*N* = 16) are depicted by black circles.

Figure 3. Summary of paired and solo bat acoustic and behavioural attention data. **(A)** Terminal feeding buzz of Bat 1 with Bat 2 orientation calls indicated by an asterisk (*) at the top of the oscillogram (top) and along the bottom of the spectrogram (bottom). (**B)** Terminal feeding buzz of bat flying alone. Calls are shown in an oscillogram (top) and spectrogram (bottom). **(C)** Circular arrow graph indicating the direction of calls up until the end of the buzz for Bat 1 in the paired trial. 0 / 360˚ represents the direction of the target, and the length of each arrow corresponds to the frequency at which calls were emitted in that direction (i.e. longer arrows indicate the bat called in that direction relatively more often). The mean call direction (*N* = 83 calls, μ = 351.7˚) and standard error (SE = 21.1) are indicated by the thicker black line extending beyond the outer circle. **(D)** Circular arrow graph indicating the

direction of attention of calls up until the end of the buzz for a bat in a solo trial. The mean call direction (*N* = 40 calls, μ = 350.2˚) and standard error (SE = 15.0) are indicated. Position and beam direction of calls emitted by bats flying together (simultaneous x-coordinates of Bat 1 and Bat 2 in panels **A** and **C**) are shown in panel **(E)** while those for the solo bat (from panels **B** and **D**) are shown in panel **(F)**. Note that arrows indicate the direction of emitted beam, in reference to a hypothetical circle (shown in light grey around the flight paths) in which the target is located at 0 / 360˚ (direction (not specific location) indicated by "**X**").

Figure 4. Summary of paired and solo porpoise acoustic and behavioural attention data. **(A)** Terminal feeding buzz of Porpoise 1 with Porpoise 2 orientation clicks indicated by an asterisk (*) at the top of the oscillogram (top) and along the bottom of the spectrogram (bottom). (**B)** Terminal feeding buzz of porpoise swimming alone. Clicks are shown in an oscillogram (top) and spectrogram (bottom). **(C)** Circular arrow graph indicating the direction of clicks up until the end of the buzz for Porpoise 1 in the paired trial. 0 / 360˚ represents the direction of the target, and the length of each arrow corresponds to the frequency at which clicks were emitted in that direction (i.e. longer arrows suggest the porpoise clicked in that direction relatively more often). The mean click direction (*N* = 96 clicks, μ = 336.2˚) and standard error (SE = 1.8) are indicated by the thicker black line extending beyond the outer circle. **(D)** Circular arrow graph indicating the direction of attention of clicks up until the end of the buzz for the porpoise in the solo trial. The mean click direction (*N* = 48 clicks, μ = 15.0˚) and standard error (SE = 2.6) are indicated. Position and beam direction

of clicks emitted by porpoises swimming together (simultaneous x-coordinates of Porpoise 1 and Porpoise 2 in panels **A** and **C**) are shown in panel **(E)** while those for the solo porpoise (from panels **B** and **D**) are shown in panel **(F)**. Note that arrows indicate the direction of emitted beam, in reference to a hypothetical circle (shown in light grey around the swim paths) in which the target is located at 0 / 360˚ (direction (not specific location) indicated by "**X**").

Table Headings

Table 1. Summary of echolocation behaviours in each phase for bat trials. Shown are the mean (± SD) spectral and temporal call parameters, number of calls, and call intensity for solo and paired bats in the search, approach, and terminal buzz phases across 7 trials. Results of Wilcoxon Signed-Rank tests between solo and paired hunting conditions are reported for each measure along with *P*-values.

Table 2. Summary of call directions for solo and paired bat trials. Shown are the mean (ρ 1 Standard deviation, SD) directions of bat calls emitted for 7 trials (7 solo trials and 7 paired trials), as well as for the average of all trials together. Results of V-tests for solo and paired call directions (whether the mean is different from zero degrees) are reported for each trial along with *P*-values. When $P < 0.05$, mean call direction was not different from zero degrees. Results of Mardia-Watson-Wheeler tests for assessing the similarity between average solo and average paired call directions are reported for each trial along with *P*-values. When $P < 0.05$, mean call directions of solo and paired trials are different from one another.

Table 3. Summary of echolocation behaviours in each phase for porpoise trials. Shown are the mean (± SD) spectral and temporal click parameters, number of clicks, and click intensity for solo and paired porpoises in the search, approach, and terminal buzz phases across 5 trials. Results of Wilcoxon Signed-Rank tests between solo and paired hunting conditions are reported for each measure along with *P*-values.

Table 4. Summary of click directions for solo and paired porpoise trials. Shown are the mean (± SD) directions of porpoise clicks emitted for 5 trials (5 solo trials and 5 paired trials), as well as for the average of all trials together. Results of V-tests for solo and paired click directions (whether the mean is different from zero degrees) are reported for each trial along with *P*-values. When P < 0.05, mean click direction was not different from zero degrees. Results of Mardia-Watson-Wheeler tests for assessing the similarity between average solo and average paired click directions are reported for each trial along with *P*-values. When *P* < 0.05, mean click directions of solo and paired trials are different from one another.

Figure 1.

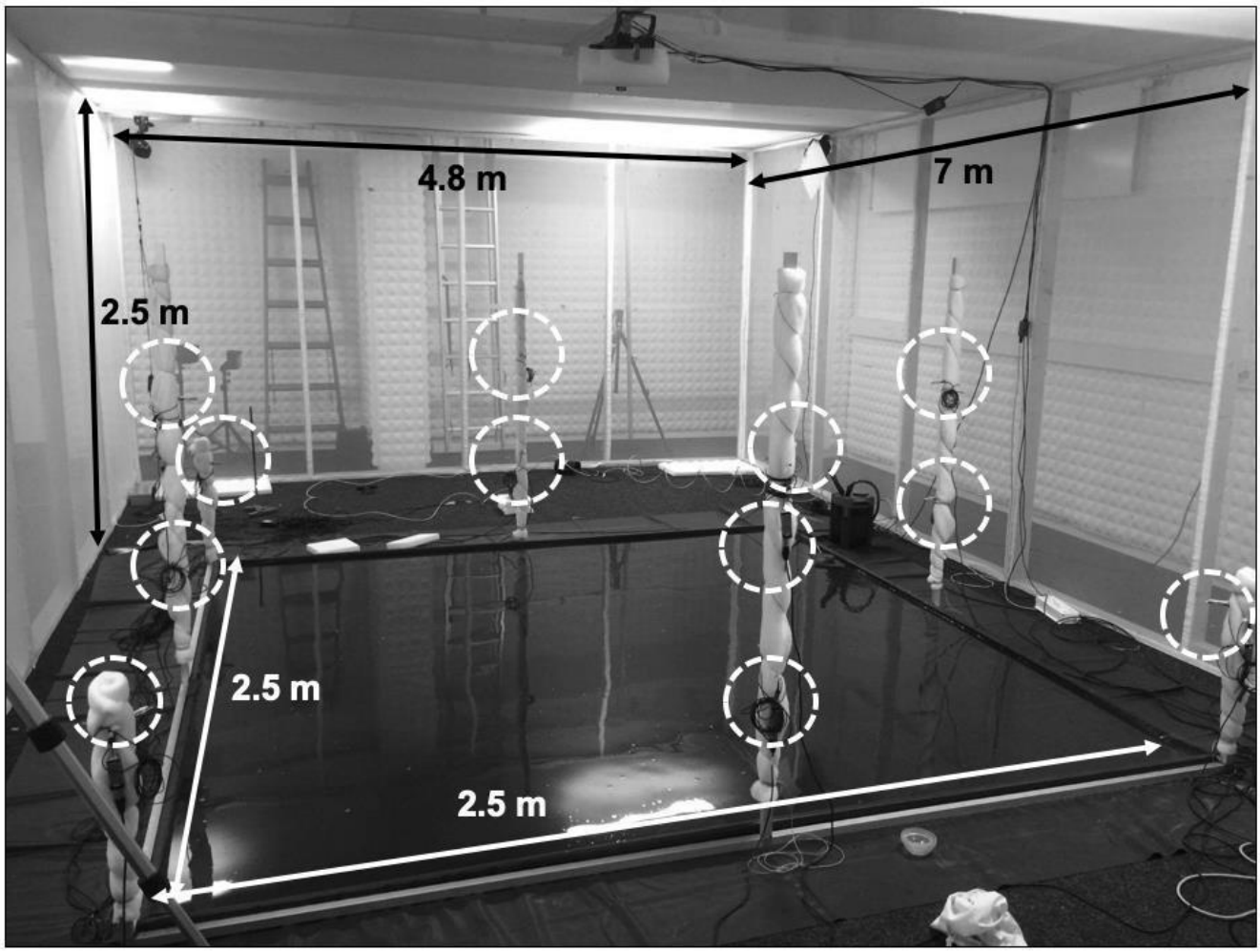


Figure 2.

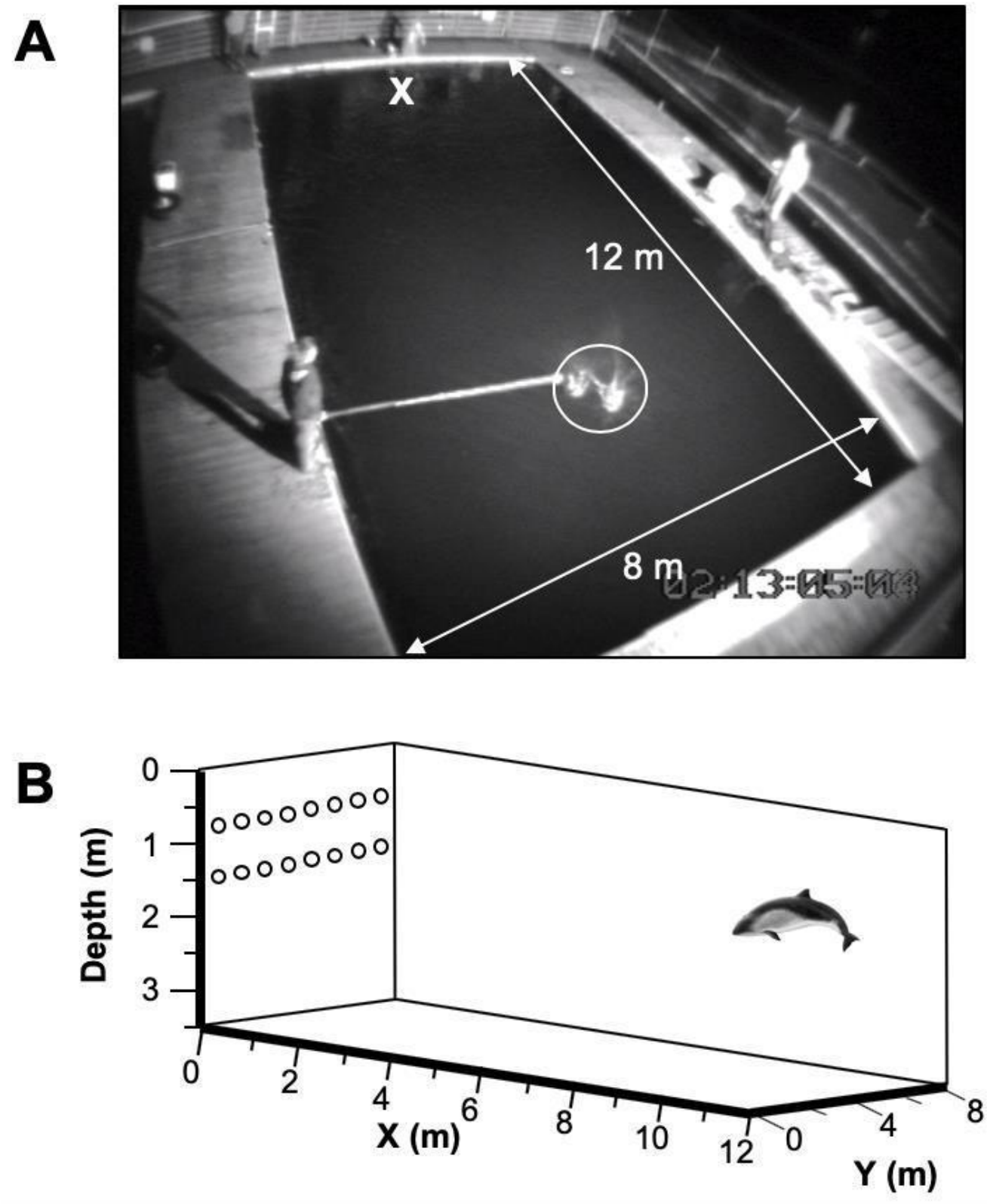


Figure 3.

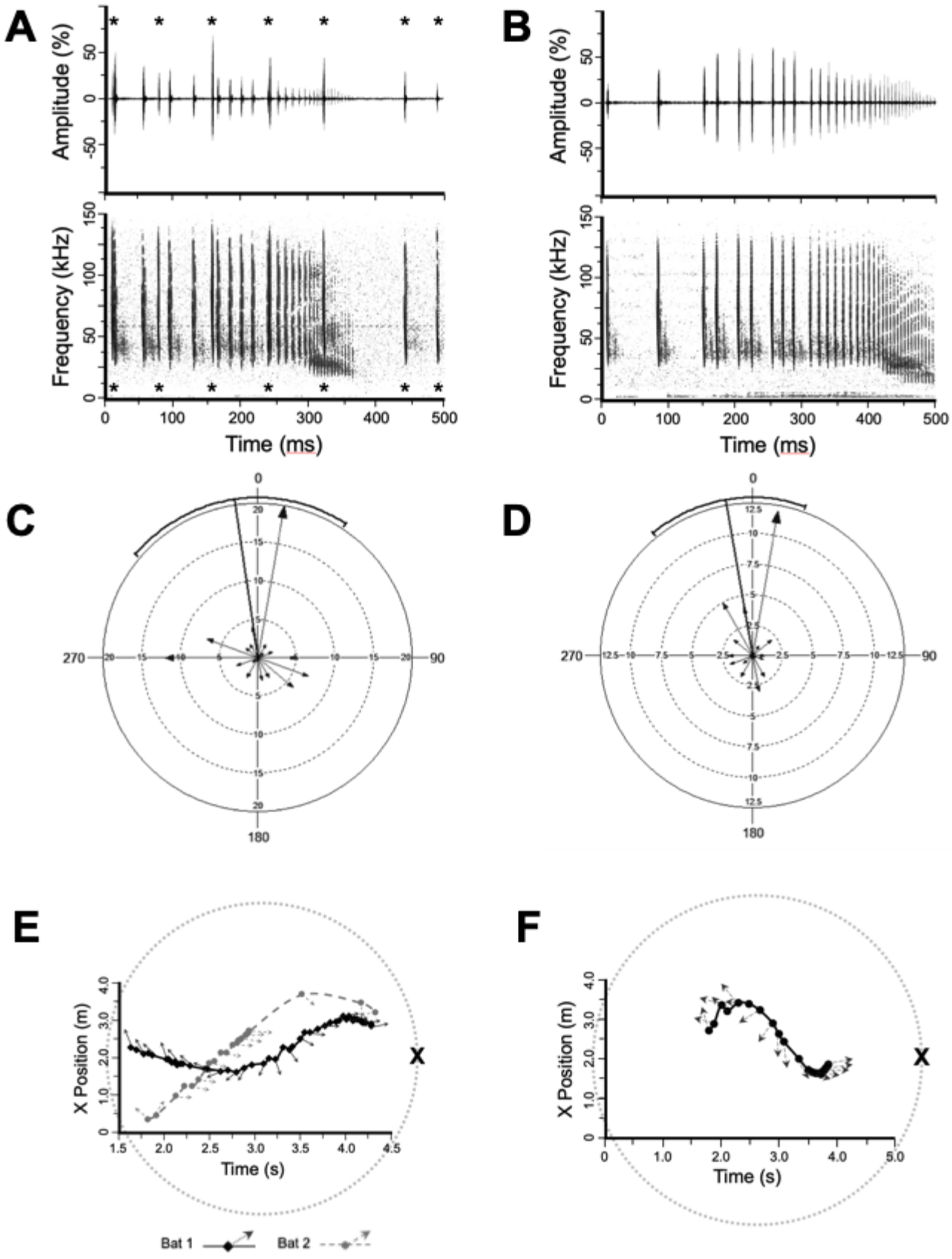
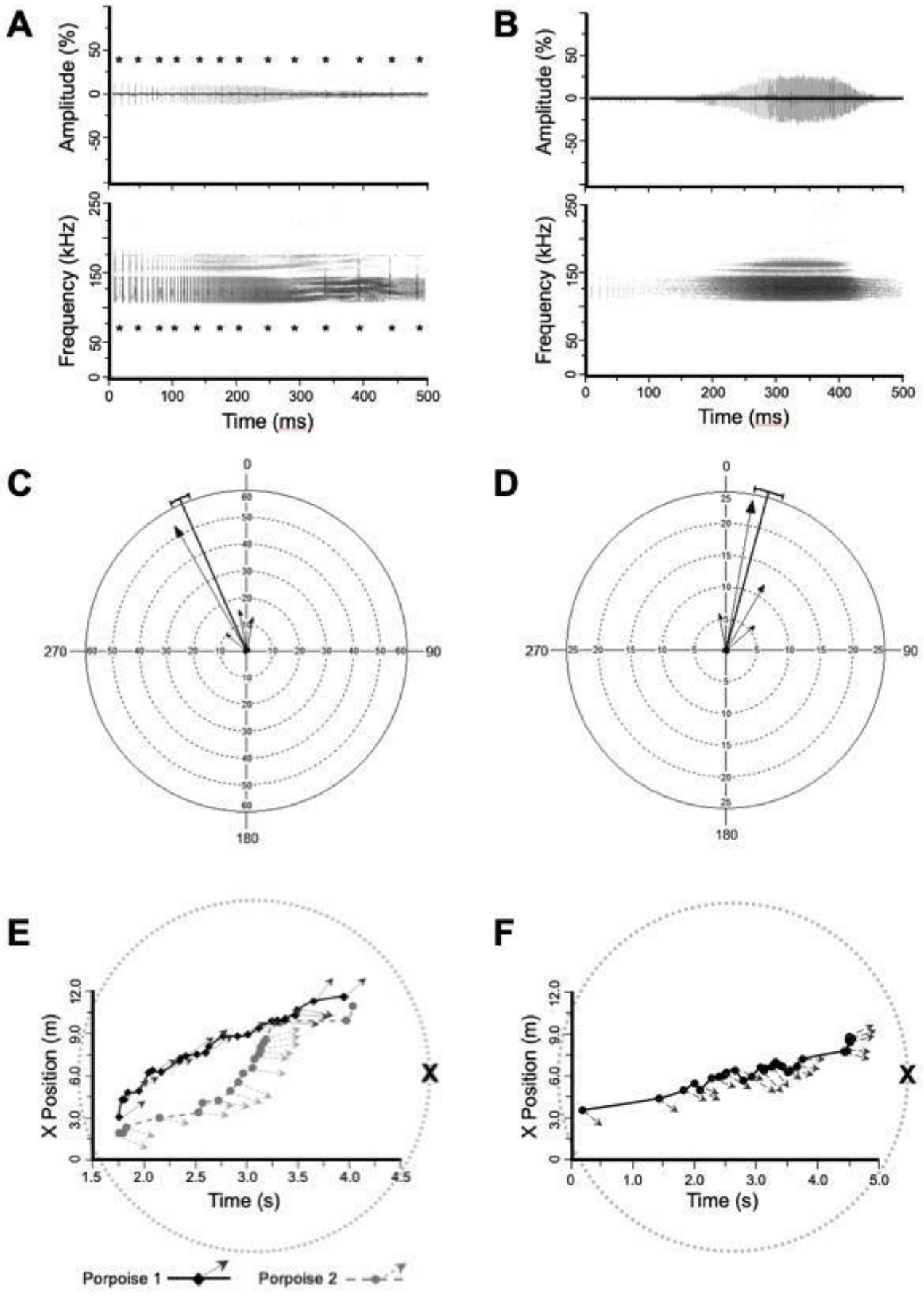

A
B
Amplitude (%)
Frequency (kHz)
Time (ms)
C
D
0
90
180
270
E
F
X Position (m)
Time (s)
X
Bat 1
Bat 2

Figure 4.


A
Amplitude (%)
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Amplitude (%)
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Time (ms)
C
D
E
X Position (m)
Time (s)
X
F
X Position (m)
Time (s)
X
Porpoise 1
Porpoise 2

Table 1.

Echolocation Behaviour	Search Phase: Solo mean (SD)	Search Phase: Paired mean (SD)	Search Phase: Solo v. Paired z-score	Search Phase: Solo v. Paired *P* (α)	Approach Phase: Solo mean (SD)	Approach Phase: Paired mean (SD)	Approach Phase: Solo v. Paired z-score	Approach Phase: Solo v. Paired *P* (α)	Terminal Buzz Phase: Solo mean (SD)	Terminal Buzz Phase: Paired mean (SD)	Terminal Buzz Phase: Solo v. Paired z-score	Terminal Buzz Phase: Solo v. Paired *P* (α)
Phase Duration (ms)	459.50 (29.8)	383.70 (151)	1.66	0.097 (0.05)	398.36 (129.8)	290.54 (149.3)	1.41	0.16 (0.05)	117.7 (17.0)	84.58 (14.9)	2.68	**0.007 (0.05)**
# of calls	6.43 (1.3)	5.00 (2.2)	1.5	0.135 (0.05)	15.29 (3.2)	9.57 (3.0)	2.69	**0.007 (0.05)**	19.29 (2.5)	12.43 (2.0)	3.09	**0.002 (0.05)**
Pulse Interval (ms)	84.23 (12.9)	99.44 (33.1)	-0.89	0.371 (0.05)	26.54 (4.2)	30.84 (9.2)	-0.64	0.523 (0.05)	6.41 (0.2)	7.04 (0.6)	-2.68	**0.007 (0.05)**
Call Duration (ms)	2.40 (0.3)	2.56 (0.3)	-0.38	0.702 (0.05)	2.31 (0.2)	2.09 (0.3)	1.53	0.125 (0.05)	0.75 (0.1)	0.93 (0.3)	-1.53	0.125 (0.05)
Peak Frequency (kHz)	53.81 (4.1)	55.79 (2.9)	-0.38	0.702 (0.05)	52.57 (3.3)	53.18 (2.8)	-0.38	0.702 (0.05)	38.01 (3.4)	38.84 (4.3)	-0.51	0.609 (0.05)
Min. Fund. Freq. (kHz)	41.24 (2.4)	42.11 (3.0)	-0.89	0.371 (0.05)	40.95 (2.5)	40.09 (2.0)	0.77	0.443 (0.05)	26.88 (1.7)	30.17 (4.2)	-3.07	**0.002 (0.05)**
Max. Fund. Freq. (kHz)	91.88 (12.2)	81.42 (11.8)	1.53	0.125 (0.05)	98.37 (7.9)	92.40 (14.4)	0.51	0.609 (0.05)	99.34 (11.7)	89.18 (10.6)	1.41	0.16 (0.05)
Fund. Bandwidth (kHz)	50.63 (11.44)	35.48 (6.8)	2.3	**0.022 (0.05)**	57.42 (6.9)	52.27 (15.0)	0	1 (0.05)	72.18 (11.1)	57.55 (14.3)	1.66	0.097 (0.05)
Call Intensity (dB)	107.06 (100.3, 110.8)	109.23 (107.7, 110.6)	-0.64	0.522 (0.05)	110.33 (108.1, 112.1)	109.64 (106.8, 111.8)	-0.13	0.897 (0.05)	99.9 (91.0, 104.2)	100.82 (95.1, 104.3)	-0.38	0.704 (0.05)

Table 2.

Bat Trials	Average Solo Aim			Average Paired Aim			Average Solo Aim v. Average Paired Aim	
	mean (SD)	V test statistic	P (α)	mean (SD)	V test statistic	P (α)	W value	P (α)
1	4.20 (99.2)	3.95	**< 0.001 (0.05) mean = 0°**	347.32 (79.5)	5.44	**< 0.001 (0.05) mean = 0°**	3.21	0.201 (0.05) solo mean = paired mean
2	2.91 (91.4)	3.86	**< 0.001 (0.05) mean = 0°**	21.36 (173.7)	1.99	**0.023 (0.05) mean = 0°**	1.8	0.406 (0.05) solo mean = paired mean
3	3.53 (41.8)	5.16	**< 0.001 (0.05) mean = 0°**	266.72 (141.7)	-0.15	0.56 (0.05) mean ≠ 0°	20.13	**< 0.001 (0.05) solo mean ≠ paired mean**
4	2.17 (157.7)	2.65	**0.004 (0.05) mean = 0°**	353.16 (97.1)	3.22	**< 0.001 (0.05) mean = 0°**	1.4	0.498 (0.05) solo mean = paired mean
5	350.16 (94.6)	3.6	**< 0.001 (0.05) mean = 0°**	351.74 (192.2)	2.66	**0.004 (0.05) mean = 0°**	3.55	0.169 (0.05) solo mean = paired mean
6	342.34 (291.1)	1.25	0.106 (0.05) mean ≠ 0°	8.61 (34.1)	5.22	**< 0.001 (0.05) mean = 0°**	7.29	**0.026 (0.05) solo mean ≠ paired mean**
7	9.45 (75.5)	4.83	**< 0.001 (0.05) mean = 0°**	336.21 (50.3)	4.67	**< 0.001 (0.05) mean = 0°**	17.77	**< 0.001 (0.05) solo mean ≠ paired mean**
All trials combined	1.61 (103.3)	9.31	**< 0.001 (0.05) mean = 0°**	346.64 (118.5)	8.19	**< 0.001 (0.05) mean = 0°**	3.84	0.147 (0.05) solo mean = paired mean

Table 3.

Echolocation Behaviour	Approach Phase: Solo mean (SD)	Approach Phase: Paired mean (SD)	Approach Phase: Solo v. Paired z-score	Approach Phase: Solo v. Paired *P* (α)	Terminal Buzz Phase: Solo mean (SD)	Terminal Buzz Phase: Paired mean (SD)	Terminal Buzz Phase: Solo v. Paired z-score	Terminal Buzz Phase: Solo v. Paired *P* (α)
Pulse Interval (ms)	20.96 (5.0)	22.5 (2.8)	-0.6	0.285 (0.05)	2.54 (0.7)	3.19 (1.2)	-0.53	0.599 (0.05)
Click duration (μs)	86.8 (6.9)	94.8 (2.2)	-1.79	0.073 (0.05)	99.8 (16.5)	100.8 (12.4)	-0.53	0.599 (0.05)
Peak Frequency (kHz)	126.12 (1.6)	129.0 (6.2)	-0.52	0.600 (0.05)	125.28 (1.9)	124.14 (2.2)	0.63	0.526 (0.05)
Fund. Bandwidth (kHz)	26.14 (2.5)	27.96 (7.0)	-0.1	0.917 (0.05)	29.96 (5.6)	26.9 (4.1)	1.27	0.205 (0.05)
Click Intensity (dB)	154.9 (3.5)	152.6 (3.8)	0.63	0.530 (0.05)	138.3 (4.1)	146.52 (4.9)	-2.09	**0.037 (0.05)**

Table 4.

Porpoise Trials	Average Solo Aim			Average Paired Aim			Average Solo Aim v. Average Paired Aim	
	mean (SD)	V test statistic	P (α)	mean (SD)	V test statistic	P (α)	W value	P (α)
1	14.98 (17.7)	9.02	**< 0.001 (0.05) mean = 0°**	336.17 (17.4)	12.1	**< 0.001 (0.05) mean = 0°**	60.67	**< 0.001 (0.05) solo mean ≠ paired mean**
2	343.02 (20.8)	11.94	**< 0.001 (0.05) mean = 0°**	356.02 (8.6)	6.39	**< 0.001 (0.05) mean = 0°**	11.79	**0.003 (0.05) solo mean ≠ paired mean**
3	358.47 (9.8)	15.33	**< 0.001 (0.05) mean = 0°**	353.26 (11.0)	18.8	**< 0.001 (0.05) mean = 0°**	40.92	**< 0.001 (0.05) solo mean ≠ paired mean**
4	348.86 (11.5)	8.49	**< 0.001 (0.05) mean = 0°**	339.46 (28.8)	7.99	**< 0.001 (0.05) mean = 0°**	18.76	**< 0.001 (0.05) solo mean ≠ paired mean**
5	355.08 (7.8)	13.96	**< 0.001 (0.05) mean = 0°**	352.74 (15.5)	8.34	**< 0.001 (0.05) mean = 0°**	0.52	0.772 (0.05) solo mean = paired mean
All trials combined	355.24 (16.6)	26.92	**< 0.001 (0.05) mean = 0°**	347.37 (18.2)	25.27	**< 0.001 (0.05) mean = 0°**	26.59	**< 0.001 (0.05) solo mean ≠ paired mean**

Chapter Three

Sonar strobe groups and buzzes are produced before powered flight is achieved in juvenile big brown bats, *Eptesicus fuscus*

Sonar strobe groups and buzzes are produced before powered flight is achieved in juvenile big brown bats, *Eptesicus fuscus*

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Abstract

Laryngeally echolocating bats produce a rapid succession of echolocation calls just before landing. These landing buzzes exhibit an increase in call rate and decreases in call peak frequency and duration relative to pre-buzz calls and resemble the terminal buzz phase calls of an aerial hawking bat's echolocation attack sequence. Sonar strobe groups (SSGs) are clustered sequences of non-buzz calls whose pulse intervals (PIs) are fairly regular and shorter than the PIs both before and after the cluster, but longer than the PIs of buzz calls. Like buzzes, SSGs are thought to indicate increased auditory attention. I recorded the echolocation calls emitted by juvenile big brown bats (*Eptesicus fuscus*) over postnatal development from birth to 32 days old, when full flight has normally been achieved, and tested the following hypotheses: (*i*) buzz production precedes the onset of controlled, powered flight; (*ii*) the emission of SSGs precedes buzzes and coincides with the onset of fluttering behaviour and, (*iii*) the onset of flight is attained first by young bats with adult-like wing loadings. I found that *E. fuscus* pups emitted landing buzzes before they achieved powered flight and produced SSGs several days before emitting landing buzzes. Both observations indicate the onset of adult-like echolocation behaviour occurs prior to adult-like flight behaviour. Pups that achieved flight first were typically those that also first achieved low, adult-like wing loadings. My results demonstrate that echolocation and flight develop in parallel but maybe temporally offset, such that sensory system precedes locomotory system.

Introduction

Bats are uniquely characterized as the only mammals capable of powered flight. While a minority of today's bat species do not echolocate, the common ancestor of all bats was likely both a laryngeal echolocator and an adept flier (Veselka et al. 2010; Thiagavel et al. 2018). As such, the first bat and the majority of chiropteran species alive today reflect an ancient transition (> 65 mya) from a non-volant, vision-reliant, nocturnal mammal to one almost exclusively reliant on flight using echolocation to orient and find food (Griffin, 1958; Maor et al. 2017; Thiagavel et al. 2018). The ontogeny of laryngeally-produced echolocation calls has been studied in a number of bat species (e.g., Brown et al. 1983; Balcombe 1990; Jones et al. 1991; de Fanis and Jones 1995; Kunz and Robson 1995; Moss et al. 1997; Zhang et al. 2005; Mayberry and Faure 2015; Mehdizadeh et al. 2018), providing a comprehensive understanding of when specific acoustic features appear in the vocal repertoire of pups. Some features include the ubiquitous nature of isolation calls: relatively long duration, low frequency, multi-harmonic vocalizations made in the first few days of life which facilitate infant-mother communication (Balcombe 1990; Gelfand and McCracken 1986; Bohn et al. 2007; Mayberry and Faure 2015). Adult-like echolocation call designs and the emission of rapid buzzes are present in some bat species at one month of age, around the time flight is first achieved (Jones et al. 1991; Moss et al. 1997; de Fanis and Jones 1995).

Powered flight, laryngeal echolocation, and the ability to produce an acoustic buzz were three key innovations that allowed the first bats to exploit the then unrealized foraging niche of nocturnal flying insects (Griffin 1958; Ratcliffe et al. 2013). Documenting flight and vocalization ontogenies is crucial for understanding the

concurrent development of this integrated sensorimotor system. The ability of individual bats to achieve powered flight is associated with body size and wing morphology. Wing loading (WL = m•g/S [N/m2]) describes the relationship of body mass (m [kg]) multiplied by the net acceleration due to Earth's standard gravity (g [m/s2]) divided by the wing area (S [m2]) (Findley et al. 1971; Norberg and Rayner 1987; Norberg and Fenton 1988; Adams 1996; Stern et al. 1997). All else equal, animals with lower wing loadings produce greater lift that allow slower, more manoeuvrable flight than those with higher wing loadings (Norberg and Rayner 1987). Wing aspect ratio (henceforth referred to as aspect ratio; AR = B^2/S) is the ratio of wing span (B) to wing area (S); bats with long, narrow wings have higher aspect ratios than bats with short, wide wings. During development, juvenile bats have higher wing loadings and lower aspect ratios than adults because their wing area is lower. Wing area also initially increases more rapidly than wing span, but both measures reach near adult values around the time of a pup's first sustained flight around 24 days after birth (O'Farrell and Studier 1973; Powers at al. 1991; de Fanis and Jones 1995; Papadimitriou et al. 1996; McLean and Speakman 2000). It is not entirely clear how wing development relates to echolocation development and flight transitions.

Echolocation works by comparing temporal and spectral differences between outgoing calls and reflected echoes to infer information about the environment (Neuweiler 1990; Moss and Surlykke 2010). Echolocation is an active and dynamic sensing process where bats not only alter the design of individual call emissions (e.g. peak frequency, bandwidth, duration, beam width) but also their temporal patterning to maximize information acquisition for goal-directed behaviour and perception (Moss and

Surlykke 2001; Moss et al. 2006). Bats decrease the pulse interval (PI) between successive vocalizations with decreasing distance to a target during hunting and landing manoeuvres. For example, in free flight the big brown bat (*Eptesicus fuscus*) emits search phase calls up to 20 ms in duration with pulse intervals ranging from 20 to >100 ms, but during the terminal buzz phase they decrease call durations and pulse intervals to < 1 ms and < 13 ms, respectively (Surlykke and Moss 2000; Moss and Surlykke 2001). Most laryngeally echolocating bats also emit a rapid burst of short duration calls with pulse intervals < 13 ms (i.e. at rates over 76 calls/s) when landing on surfaces—the so-called landing buzz (Melcón et al. 2007; Ratcliffe et al. 2013). Landing buzzes closely resemble the initial component of buzzes emitted by bats during aerial hawking attack sequences (Griffin et al. 1960; Tian and Schnitzler 1997; Melcón et al. 2007) and those buzzes emitted prior to drinking (Greif and Siemers 2010). It is believed that landing buzzes help bats to accurately and efficiently land on surfaces (Melcón et al. 2007). Among mammals, the ability to emit calls at the rate observed during landing, feeding and drinking buzzes reflects a speed of vocal-motor control unique to laryngeally echolocating bats (Elemans et al. 2011).

Additional evidence that bats actively control the temporal patterning of their calls is seen by sonar strobing. A sonar strobe / sound group (SSG) is a sequence of clustered calls with short pulse intervals bracketed by calls with longer pulse intervals. When a sonar strobe / sound group contains ≥ 3 calls, the pulse intervals within the group are remarkably stable (Moss et al. 2006). Bats emit sonar strobe / sound groups during the search and approach but not terminal phases of echolocation, and when faced with challenging tasks, or those that require increased attention. For example,

bats commuting from a roost do not emit sonar strobe / sound groups, whereas bats foraging in the field and the lab do (Kothari et al. 2014). Use of relatively constant pulse intervals within the sonar strobe / sound group is thought to reflect increased auditory attention to sharpen the bat's neural representation of spatial information (Petrites et al. 2009; Hulgard and Ratcliffe 2016). Similarly, both their frequency of occurrence and number of calls emitted per sonar strobe / sound group increases with task complexity (Moss et al. 2006; Hulgard and Ratcliffe 2016).

The co-development of vocal and motor abilities has been observed in many different species, including humans (Abney et al. 2014). Although the timing of flight and vocal development have, independently, been well studied in juvenile bats, their concurrent maturity with respect to landing buzz emissions, the onset of sonar strobe / sound group production, and flight milestone transitions have not been systematically explored. The big brown bat (*E. fuscus*) is arguably the world's most thoroughly studied bat species. To my knowledge, the developmental trajectory of buzz call production has yet to be systematically explored in any bat, including *E. fuscus* (but see Moss et al. 1997 for observations on buzz production in juvenile little brown bats, *Myotis lucifugus*). Previous authors mentioning landing buzzes in *E. fuscus* pups reported that they occurred at the offset of every flight (Buchler 1980; Brown et al. 1983); however, those studies looked exclusively at landing buzzes emitted close to a pup's first flights (i.e. 3-4 weeks after birth). Here, I report on the development of laryngeal echolocation—both call design and the temporal pattern of emission—and the acquisition of controlled, powered flight in the same bat pups over the first 32 days of post-natal life. I sought to determine at what ages landing buzzes and sonar strobe / sound groups first appear

and become indistinguishable from those emitted by adults. I also sought to reveal developmental correlations between flight ability, wing morphology, and the production of landing buzzes.

Specifically, I predicted that the development of landing buzzes and adult-like echolocation calls would precede powered flight. I also predicted that the emission of sonar strobe / sound groups would precede landing buzzes, with sonar strobe / sound groups first observed around the time that bats transition between flopping flight *versus* fluttering to the ground. I reasoned that echolocation abilities would develop more quickly than flight abilities, hence the timing of sonar strobe / sound group production would correspond to the onset of wing beating behaviour in pups. I also tested two additional and non-mutually exclusive hypotheses by predicting that bats which moved quickly through the various flight phase transitions would develop echolocation abilities and reach adult-like (i.e. lower) wing loadings faster compared to conspecifics who transitioned through the same flight phases more slowly.

Methods and Materials

Subjects and trial conditions

This study was conducted at McMaster University, Hamilton, Ontario, Canada. All procedures met the guidelines for the care / use of wild animals in research as per the Canadian Council on Animal Care and were approved by the Animal Research Ethics Board of McMaster University. Data were collected from 8 male big brown bat pups (*Eptesicus fuscus,* Palisot de Beauvois 1796) born in captivity to wild-caught mothers in 2017 (March to May). The day of birth was defined as post-natal day zero (PND 0).

Pups were recorded either every day or every second day between post-natal day 1 and post-natal day 32. I stopped recording after post-natal day 32 because at this age *E. fuscus* have reached adult size and are exclusively producing adult-like vocalizations (Kurta and Baker 1990; Moss et al. 1997; Mayberry and Faure 2015). Pups not discovered on their date of birth and / or deemed too vulnerable to risk maternal separation were first recorded on post-natal day 2.

To ensure I had at least one matched set of adequate acoustic and video files per animal per recording day, several trials were conducted each day and a single trial with the best matched audio and video recordings was used for analysis. Multiple trials were necessary because some bats were uncooperative about making flight attempts or they would fly and land in an area not suitably monitored by microphones and cameras. When not tested, pups and mothers were housed together in stainless steel wire (1/4" mesh) holding cages (28 × 22 × 18 cm; l × w × h) in a temperature and humidity-controlled room and were provided food (mealworms, *Tenebrio molitor*) and water *ad libitum* (Skrinyer et al. 2017).

Acoustic data collection

Acoustic recordings were collected in a flight room (4.9 × 3.3 × 3.3 m) whose ceiling, walls and floor were lined with sound attenuating foam (Sonex® Classic; Pinta Acoustic, U.S.A.). Recordings took place in darkness and two different microphone set-ups were used. At first, the set-up consisted of 6, CM16 microphones (Avisoft Bioacoustics, Glienicke, Germany; frequency sensitivity 2 to 200 kHz, frequency response approximately flat (± 3 dB) from 25 to 140 kHz). Midway through, the set-up was

changed to consist of 9, CM16 microphones. In both configurations, the microphones were connected to an Avisoft USGH 1216 A/D converter (16-bit). Thus, bat calls were first sampled at 375 kHz per channel, after the microphone configuration changes signals were sampled at 250 kHz per channel to accommodate the 3 additional microphones.

The quality of the audio and video data collected in both set-ups was indistinguishable and therefore data from both configurations was used in my analysis. Microphones were placed throughout the flight room and aimed towards the airspace where bats were expected to fall / fly (Figure 1). Acoustic files were 11 s in duration (1 s pre-trigger, 10 s hold time) to record all relevant information, as pups would often take a few seconds to initiate a flight attempt. Signals were saved to a ThinkPad X240 laptop computer (Lenovo, Morrisville, NC, U.S.A.).

Bats were hand released 60 cm above a table covered with foam. I did this to safely break the fall of young pups still unable to fly. This translates to a release point 130 cm above the floor and in line with the highest recording microphone (Figure 1a, 1c). The drop site was positioned 30 cm from the wall. Bats departed from the researcher's hand on their own initiative. Young pups simply fell from the researcher's hand onto the foam-lined table, while older pups often took flight immediately. For each trial, I noted the category of flight exhibited by the pup (see *Flight data collection* for flight classification key). Upon landing and cessation of movement, the bat's horizontal distance from the drop site was quantified with a measuring tape. I also set up reference points with two parallel "hot pack lines" (i.e. two hand warmers per line; Hot Hands, Kobayashi, Dalton, GA, U.S.A.) to measure landing distances on images recorded by 1

or 2 thermal cameras (T480, FLIR, Wilsonville, OR, U.S.A.), depending on camera availability. Hot pack lines ran parallel to the drop site, with the heat packs positioned 1 m and 2 m from the wall closest to the release site (Figure 1c).

Flight data and analysis

After every trial I made notes on a pup's flight attempts and movements. Flight abilities at different ages were classified into one of 4 categories using criteria established by Powers et al. (1991): (*i*) Flopping flight: pups fell straight down upon leaving the researcher's hand and exhibited no wing movements or horizontal displacement; (*ii*) Fluttering flight: pups fell straight down upon leaving the researcher's hand and exhibited stretching or flapping wing movements but without achieving horizontal displacement; (*iii*) Flapping flight: pups exhibited wing flapping and achieved horizontal displacement upon leaving the researchers hand but with no control over their descent; and (*iv*) Powered flight: pups exhibited sustained (true) powered flight upon leaving the researcher's hand with horizontal displacement and clear evidence of path control (e.g. turning).

Acoustic analysis

Trials in which the animal did not leave the researcher's hand or when it flew to / landed in areas of the recording room not adequately monitored were not analyzed. Of the remaining files, acoustic analysis was conducted on those trials where bats landed in areas covered by the microphone set-up. Because multiple microphones were used in every trial, calls were recorded on multiple channels at different points along the pup's

flight trajectory. I typically analyzed signals recorded closest to the landing site and / or with the highest signal-to-noise ratio. Clipped signals were not analyzed.

Once signals to be analyzed were identified for each trial, I assigned calls emitted by pups to one of three phases: (*i*) pre-flight, defined as the 500 ms period before the pup left the researcher's hand, (*ii*) in-flight, defined as the time between when the pup left the researcher's hand and when it first contacted the landing surface, and (*iii*) post-flight, defined as the 500 ms period after the pup first contacted the landing surface. I then used oscillogram, power spectrum, and spectrogram displays (BatSound software v.4.2, Pettersson Electronik AB, Uppsala, Sweden) to manually measure temporal and spectral parameters of pup calls. From the oscillograms, I measure signal duration (ms; defined as time between signal onset and offset) and pulse interval (ms; defined as time from the onset of one call to the onset of the next call). From the spectrogram, I measured maximum fundamental frequency (kHz; defined as the highest frequency of the fundamental), minimum frequency (kHz; defined as lowest frequency of the fundamental), and fundamental bandwidth (kHz; defined as highest frequency minus lowest frequency in fundamental FM signal). From the power spectrum [automatic fast Fourier transform function (FFT), size 1024, Hann window] of each call, I measured peak frequency (kHz; defined as frequency of maximum energy). I also estimated the number of harmonics in each call using information combined from spectrogram and power spectrum displays. Sound analysis settings and displays were kept constant for all analyses to allow for comparisons between individuals and developmental milestones.

The presence or absence of landing buzzes and sonar strobe / sound groups was noted, and if present, the landing buzz duration and number of calls per sonar strobe / sound group was recorded. Landing buzzes are sequences of calls characterized by decreasing signal durations and peak frequencies, with pulse intervals < 13 ms (i.e. call rates ≥ 75 Hz). A sonar strobe / sound group is a temporal cluster of calls with short, stable pulse intervals bracketed by calls with longer pulse intervals. I used the Island Criterion and Stability Criterion for the identification and quantification of sonar strobe / sound groups. The Island Criterion identifies the temporal isolation of a sonar strobe / sound group within a continuous stream of biosonar emissions, whereas the Stability Criterion identifies the nearly constant pulse intervals within a sonar strobe / sound group (see Figure 1 in Kothari et al. 2014). When a sonar strobe / sound group consists of ≥ 3 calls, the individual pulse intervals within the group must be stable (i.e. ≤ 5% deviation from mean pulse interval within cluster) and shorter than the pulse intervals flanking the group (i.e. flanking pulse intervals ≥ 1.2 times mean pulse interval within cluster; Moss et al. 2006). Note that the Stability Criterion cannot be used to identify a doublet sonar strobe / sound group.

My analysis primarily focused on calls emitted in-flight because these vocalizations were most relevant to the study. I tested for differences in emitted call parameters for pups at different ages (i.e. between post-natal days 2, 12, 22 and 32) and flight ability phases (i.e. between each pup's transition from the flop to flutter, flutter to flap, and flap to fly phases; see *Flight data and analysis*).

Morphological data collection and other pertinent observations

I recorded morphological, developmental, and behavioural milestones for each pup on every trial: age (post-natal day), forearm length (mm), mass (g), eye status (open/closed), and whether the pup was attached to its mother when removed from the cage (yes/no). These milestones helped to ensure that pup development was healthy, normal, and conformed to growth trajectories reported in the literature (Kurta and Baker 1990; Mayberry and Faure 2015). Forearm length and mass were also used to corroborate date of birth (Mayberry and Faure 2015).

Photos of the left wing of each pup were taken every recording day and analyzed using ImageJ (National Institutes of Health, Bethesda, MD, U.S.A.) to measure the half wingspan (distance from tip of left wing to midline of torso) and half wing area (area of the entire left wing, including the left halves of the body and tail membrane). Measures were taken in triplicate before they were averaged and doubled to estimate the full wingspan (B) and full wing area (S), respectively.

I calculated the aspect ratio and relative wing loading of pups on each recording day. Aspect ratio (AR) is a dimensionless number that describes wing narrowness ($AR = B^2 / S$; = wing span [B] squared divided by wing area [S]). Wing loading (WL) describes lift and flight maneuverability ($WL = m \times g / S$; = mass [m] times acceleration due to Earth's standard gravity [$g = 9.81\ m/s^2$] divided by wing area [S]) (Findley et al. 1971; Norberg and Rayner 1987; Norberg and Fenton 1988; Adams 1996; Stern et al. 1997). Because wing loading increases with body mass in geometrically similar animals, for scaling reasons, larger bats will have higher wing loadings than geometrically similar but smaller bats (Norberg and Fenton 1988). To correct for scaling effects, I used

relative wing loading (RWL = WL / $m^{1/3}$) because this index is independent of body size (Norberg and Fenton 1988). As with my acoustic data, I compared aspect ratios and relative wing loadings of pups across developmental ages (i.e. post-natal day 2, 12, 22, and 32) and behavioural milestones (i.e. flight transition phases).

Rank correlations

To reveal potential developmental relationships that exist between wing morphology, echolocation behaviour, and flight proficiency, I ranked the eight pups with respect to relative wing loading on post-natal day 2, 12, 22, and 32. I then compared the ranks with respect to the age when pups transitioned from flop to flutter, flutter to flap, and flap to fly, and also to the post-natal day ranks when sonar strobe / sound groups and landing buzzes were first observed. When comparing ranks of different variables, I selected variables that temporally corresponded with one another. In other words, I compared the age of milestone achievement with other, subsequent milestones and / or the most reasonable post-natal days around which the milestones occurred. For example, bats transitioned from fluttering to flapping flight around post-natal day 17. I compared these flight rankings to relative wing loading score rankings on post-natal day 12 and 22 because these morphological rankings were most temporally similar to the behavioural rankings. For comparisons in which both variables were measured in post-natal days (i.e. consecutive flight transitions, or flight transitions *versus* age of first ≥ 3 signal sonar strobe / sound group or buzz), I used raw ages to explore linear relationships instead of ranked data.

Captive versus wild-born pups

I compared the forearm length, mass, and relative wing loading of captive-born pups on post-natal day 32 to their mothers (see Mayberry and Faure 2015) and to wild-caught, volant adult and juvenile male *E. fuscus* captured from field sites in southern Ontario. I also compared the age of first flight in my pups to published reports of wild *E. fuscus* (Kurta and Baker 1990).

Statistics

Data are reported as the mean ± standard deviation (SD). My analysis of pup calls focused on temporal and spectral parameters providing the most insight into sonar strobe / sound group and landing buzz development (i.e. minimum pulse interval, maximum and minimum call duration, maximum peak frequency, minimum number of harmonics, maximum fundamental bandwidth, and maximum number of calls per sonar strobe / sound group). As previous literature has described the developmental trajectories of echolocation behaviours and morphometrics, I was able to make reasonable predictions regarding how these variables would change between consecutive time points. As such, I used pre-planned, 1-tailed, paired *t*-tests to compare call parameters measured in-flight and morphological measurements (body mass, wing span, relative wing loading, and aspect ratio) for selected post-natal days (i.e. post-natal day 2 *versus* post-natal day 12, post-natal day 12 *versus* post-natal day 22, and post-natal day 22 *versus* post-natal day 32) and between consecutive flight transitions (i.e. last day of flop *versus* last day of flutter behaviour, and last day of flutter *versus* last day

of flap behaviour). I also used one-tailed, paired *t*-tests to compare the ages at which pups transitioned to new flight behaviours.

For those comparisons in which I had no *a priori* information, I used one-way between subjects analyses of variance (ANOVA). Specifically, I used ANOVAs to compare the ages at which pups first produced both landing buzzes and sonar strobe / sound groups with ≥ 3 calls across the three phases of pup flight. I used 2-sample *t*-tests to compare parameters between pups and adults. I used Spearman's rank and Pearson's correlation coefficients to identify relationships between individual pup development with respect to flight transitions, in-flight ≥ 3 call sonar strobe / sound group production, in-flight landing buzz production, and relative wing loading scores. Pearson's correlation coefficients were used when the variables being compared were both measured in post-natal days, whereas Spearman's rank correlation coefficients were used when variables were of different types (i.e. post-natal day and relative wing loading score). Alpha (α) values were adjusted using Bonferroni corrections (Rice 1989).

Results

I compared in-flight call parameters across sequential pup ages at 10-day intervals (i.e. post-natal day 2 *vs* post-natal day 12, post-natal day 12 *vs* post-natal day 22, and post-natal day 22 *vs* post-natal day 32), and between post-natal day 32 pups and their mothers (Table 1). Single representative pup calls on each analysis day are illustrated as oscillogram, spectrogram, and power spectrum displays in Figure 2. All call parameters of post-natal day 2 pups differed from post-natal day 12 pups. Minimum call

duration, maximum peak frequency, maximum bandwidth of the fundamental, and minimum pulse interval differed between post-natal day 12 and 22 pups. When comparing post-natal day 22 and post-natal day 32 pups, only the maximum bandwidth of the fundamental differed. Post-natal day 2 pups did not emit sonar strobe / sound groups or buzzes. Average in-flight call parameters between post-natal day 32 pups and their mothers were indistinguishable from one another.

Very young *E. fuscus* pups (post-natal days 1 and 2) always exhibited flopping behaviour upon leaving the researcher's hand. On average, pups transitioned from flopping to fluttering by 5.6 ± 2.5 days. Fluttering pups subsequently transitioned to flapping behaviour by 16.4 ± 3.8 days, and pups achieved true powered flight by 23.9 ± 4.3 days. As expected, pups were significantly younger on the last day of flopping behaviour compared to the last day of fluttering behaviour and were also younger on the last day of fluttering behaviour compared to the last day of flapping behaviour (paired *t*-tests: flopping *versus* flutter: $t = 6.45$, $P < 0.001$; fluttering *versus* flapping: $t = 4.23$, $P < 0.002$). All pups transitioned from flapping to fluttering flight and from fluttering to flapping flight; however, only 7 of 8 pups transitioned from flapping to powered flight in the trials.

To document how calls changed across successive flight classifications, I compared the temporal and spectral parameters of pup calls recorded in-flight on the final day when pups transitioned between flight ability categories described above (Table 2). I found differences in the minimum number of harmonics, maximum fundamental bandwidth, maximum peak frequency, minimum pulse interval, and maximum number of calls within a sonar strobe / sound group between calls emitted on

the final day of flopping flight and those emitted on the final day of fluttering flight. In contrast, only the maximum peak frequency differed between calls emitted on the final day of fluttering flight and the final day of flapping flight. Single representative pup calls on each final flight transition day, as well as a representative adult mother call, are depicted in Figure 3.

Very young pups emitted vocalizations with no discernible temporal pattern (i.e. no obvious clusters or groupings). However, over time, pups started emitting bursts of vocalizations clustered into sonar sound groups with an increasing number of calls per group (Table 1; Figure 4). I focused my analysis on the ages when pups began to emit sonar strobe / sound groups with ≥ 3 calls because these sonar strobe / sound groups satisfy both the Island Criterion and Stability Criterion (see Kothari et al. 2014). There were no age differences when pups first began emitting sonar strobe / sound groups with ≥ 3 calls across the three flight phases (one-way between-subjects ANOVA, $F = 3.42$, $P = 0.052$). Specifically, pups first emitted sonar strobe / sound groups with ≥ 3 calls at 11.75 ± 6.84 days during the pre-flight phase, 6.38 ± 2.56 days during the in-flight phase, and 6.38 ± 3.78 days during the post-flight phase.

Buzzes are defined as a sequence of rapidly emitted calls that have decreasing signal durations, peak frequencies, fundamental bandwidths, and pulse intervals < 13 ms (Schnitzler and Kalko 1989; Moss and Surlykke 2001). The average age when pups first began to emit adult-like landing buzzes in-flight was 17.38 ± 4.93 days. By post-natal day 32, landing buzzes emitted by pups were indistinguishable from those emitted by mothers with respect to minimum pulse interval and buzz duration (Table 1; Figure 4). All pups emitted landing buzzes during the in-flight phase; however, some also

emitted pre-flight and post-flight buzzes (i.e. some pups emitted buzzes before leaving the researchers hand and/or after landing). Of 8 pups examined, 4 emitted at least 1 pre-flight buzz at an average age of 20 ± 8.6 days, and 7 emitted at least 1 post-flight buzz at an average age of 19.9 ± 6.9 days. There were no age differences when pups first emitted landing buzzes between the different flight phases (one-way between-subjects ANOVA, F = 0.35, *P* = 0.708). The timing of in-flight buzzes with respect to landing varied with age; younger pups often produced landing buzzes hundreds of milliseconds prior to landing, while older pups tended to produce in-flight buzzes immediately before landing. Pre- and post-flight buzzes were also indistinguishable from adult landing buzzes with respect to minimum pulse interval and buzz duration. Pre-flight buzzes were first produced at an age on (*N* = 2) or after (*N* = 2) the pup had already emitted an in-flight landing buzz. Of 7 pups who emitted post-flight buzzes, 5 produced them after their first in-flight landing buzz, and 2 produced them either on the same day (*N* = 1) or 3 days prior to emitting their first in-flight landing buzz (*N* = 1).

Landing buzz durations varied throughout development and across the different flight categories. Fluttering pups emitted landing buzzes with the longest durations (69.1 ± 48.9 ms) compared to the intermediate buzz durations emitted during flapping (58.25 ± 39.7 ms) and powered flight (54.0 ± 40.0 ms). A single pup emitted the shortest duration landing buzzes in flopping flight (17.5 ± 6.2 ms), but these buzzes contained only 1 or 2 PIs that reached the threshold for defining a landing buzz (i.e. the pulse interval < 13 ms). Although buzzes emitted in the flutter, flap, and powered flight categories were longer in duration compared to the flop category (paired *t*-tests: *t* = 6.87, *P* < 0.001 for flop *versus* flutter; *t* = 8.88, *P* < 0.001 for flop *versus* flap; *t* = 7.58, *P*

< 0.001 for flop *versus* fly), there were no differences in the buzz durations among bats that had achieved the 3 most advanced flight capabilities (paired *t*-tests: t = -1.43, P = 0.158 for flutter *versus* flap; t = -0.84, P = 0.402 for flap *versus* fly; t = -1.94, P = 0.056 for flutter *versus* fly).

Because wing morphology directly affects when a bat first achieves and maintains powered flight, I correlated wingspan, mass, relative wing loading, and aspect ratio with preselected post-natal day ages (i.e. post-natal day 2, 12, 22, and 32; Table 3), and flight transition days (i.e. last day of flopping, fluttering, and flapping flight; Table 4). All morphometric measurements differed between post-natal day 2 and 12 pups, and all but aspect ratio differed between post-natal day 12 and 22 pups. Only forearm length continued to increase between post-natal day 22 and 32 pups; all other measures appeared to plateau (Table 3). Wing aspect ratio remained constant throughout flight transitions (Table 4), and relative wing loading differences were only observed between the final days of flopping and final fluttering flight. Body mass and wingspan continually increased throughout flight development; I found differences for both measurements between the last day of flop versus flutter flight, and the last day of flutter versus flapping flight (Table 4).

I also wanted to determine if the oldest pups (post-natal day 32) were morphologically distinct from their mothers as well as wild-caught juvenile male and wild-caught adult male *E. fuscus* (Table 3; note: wing span, aspect ratio, and relative wing loading were not measured in wild-caught bats). There was no difference in mass or forearm length between post-natal day 32 pups and wild-caught adult or juvenile males (adult data from Mayberry and Faure 2015). Moreover, the forearm length,

aspect ratio, and relative wing loading of post-natal day 32 pups did not differ from their mothers. Post-natal day 32 pups (all of which were male) did, however, have lower masses and shorter wingspans compared to their mothers (Table 3).

Finally, I explored relationships between when pups accomplish locomotory and vocal developmental milestones by comparing the average ages when pups achieve flight transitions and first emit in-flight sonar strobe / sound groups (≥ 3 calls/ sonar strobe / sound group) or landing buzzes. I found that pups transition from flopping to fluttering flight at roughly the same age (5.63 ± 2.45 days) when they first emit in-flight sonar strobe / sound groups with ≥ 3 calls per group (6.38 ± 2.56 days; paired *t*-tests, $t = -0.56$, $P = 0.591$). Furthermore, the age at which pups transition from fluttering to flapping flight (16.5 ± 3.85 days) did not differ from when pups first produced in-flight landing buzzes (17.38 ± 4.93 days; $t = -0.41$, $P = 0.696$). I also wanted to determine if pups that quickly reached flight milestones also reached acoustic milestones more quickly, and if pups that quickly reached an adult-like morphology also achieved flight and echolocation milestones more quickly. For each comparison, I used either the raw post-natal day or the relative rankings of each pup, with higher ranks representing more advanced progression (i.e. earlier accomplishment of SSG or landing buzzer production, flight ability, or adult-like relative wing loading). I found that the age when a pup transitioned from flapping to powered flight was strongly correlated with the relative wing loading score on post-natal day 22 (Spearman's rank correlation, $R = 0.97$, $P < 0.001$). All other correlations were not significant.

Discussion

The developmental trajectories of echolocation call design and flight abilities observed in this study corroborate previous observations collected independently on bat pup vocal development and flight in vespertilionid and other laryngeal echolocating bats (Balcombe 1990; Jones et al. 1991; De Fanis and Jones 1995; Moss et al. 1997; Zhang et al. 2005; Mayberry and Faure 2015). That is, I observed the same changes from isolation call production in very young pups to adult-like echolocation calls over the first 32 days of life (Moss et al. 1997), as well as transitions between historically described flight categories in pups to adult-like powered flight over this same time period (Powers et al. 1991).

By post-natal day 6, big brown bat pups transitioned from flopping flight (i.e. falling with no wing movements) to fluttering flight (i.e. wing movements that enable a softer landing). Although fluttering flight does not result in horizontal displacement, the wing movements serve as a precursor to more advanced flying abilities (Powers et al. 1991). Interestingly, pups also started emitting their first sonar strobe / sound groups with ≥ 3 calls at the same time. After reaching fluttering behaviour, pups effectively reduced the number of harmonic elements in their orientation calls and decreased their minimum call durations to adult levels measured in the same flight room (Tables 1 and 2; Mayberry and Faure 2015). However, the peak frequencies and fundamental bandwidths of non-buzz calls only reached adult values around the time that pups transitioned to flapping flight (Tables 1 and 2). Controlled, powered flight was first observed in bats that were ~24 days old. These results demonstrate that the ability to emit SSGs in *E. fuscus* pups precedes the ability to produce landing buzzes. Moreover,

echolocation call designs and the temporal emission patterns used by adult bats to orient, attend to objects, and land develop before controlled, powered flight. I did not find that pups emitting sonar strobe groups and / or buzzes earlier in development were also those that transitioned through flight categories most quickly.

Because data were obtained from captive-born pups born it is possible that the results may not apply to *E. fuscus* and or other bats raised in the wild. For example, captive bats typically have *ad libitum* access to food, so they may gain mass more rapidly and have more resources to allocate to laryngeal and/or neural development compared to individuals in nature. Alternatively, laboratory-born pups may have fewer opportunities to listen to the vocalizations of flying and / or landing adult bats so they may acquire echolocation skills more slowly (Prat et al. 2015). However, field and lab developmental studies on other bat species do not differ dramatically with respect to the ontogeny of echolocation call design and buzz emission (Scherrer and Wilkinson 1993; Moss et al. 1997). Similarly, *ab libitum* access to nutrition may promote faster flight development, but the confines of captivity could provide fewer opportunities for young pups to practice flying, plus a greater wing loading may slow development. In this study, pups had attained controlled, powered flight by post-natal day ~24 and this closely matches reports from the wild (post-natal day ~25; Kurta and Baker, 1990). Although adult *E. fuscus* kept in long-term captivity can weigh more than age-matched adults from the wild (Mayberry and Faure 2015), I found no difference in mass or relative wing loading between captive-bred male pups at post-natal day 32 compared to adult or juvenile volant males captured nearby (Mayberry and Faure 2015).

Orientation calls

Big brown pups reach adult proportions approximately 30 days after birth (Kurta and Baker 1990; Mayberry and Faure 2015). Over this time, I observed a transition from intermittently produced, long duration, low frequency, multi-harmonic vocalizations (i.e. isolation and/or rudimentary echolocation calls) to more frequently and regularly produced echolocation calls with shorter durations, broader fundamental bandwidths, and fewer harmonics (Figure 2, Table 1). By post-natal day 32, pups were emitting orientation calls indistinguishable from adults (Table 1, Figures 2 and 3). These changes, consistent across trial phases, matched the general trends of previous studies on pup vocal development (Brown et al. 1983; Balcombe 1990; Jones et al. 1991; de Fanis and Jones 1995; Moss et al. 1997; Zhang et al. 2005; Monroy et al. 2011; Mayberry and Faure 2015; Mehdizadeh et al. 2018). Specifically, I observed that maximum and minimum call durations went from being relatively long and highly variable (Tables 1 and 2) on post-natal day 2 through the flop-to-flutter transition (post-natal day ~6) to adult-like call durations before pups (*i*) reached post-natal day 12 (Table 1), and (*ii*) transitioned from fluttering to flapping flight (post-natal day ~17; Table 2). Maximum peak frequency and maximum fundamental bandwidth only reached adult levels after pups began to emit sonar strobe / sound groups with ≥3 calls, around the same time they began to emit landing buzzes, and just before transitioning to powered flight (Table 2).

From a biomechanical perspective, the order of the developmental changes makes sense. Similar to other laryngeally-echolocating bats, the larynx of the big brown bat is hypertrophied, due in large part to its massive cricothyroid musculature (Moss

1988; Metzner and Schuller 2010). Calls are produced as the cricothyroid muscle relaxes and the rate of relaxation influences frequency-modulated sweep rates, while the tension achieved during contraction determines the highest frequency of the fundamental element (Elemans et al. 2011; Ratcliffe et al. 2013). My data suggest that over the first 32 days of life, the cricothyroid musculature obtains the ability to produce calls with adult-like durations before it acquires the ability to produce adult-like peak frequencies and fundamental bandwidths. In other words, the cricothyroid muscle can already relax sufficiently fast enough to produce short duration calls before it can achieve sufficient tension to produce higher peak frequencies and wider fundamental bandwidths. The fact that pups emitted calls with fewer harmonics between post-natal day 6 and post-natal day 12 demonstrates that the filtering properties of the pup's supralaryngeal vocal tract are present early in development. Functionally, the early decrease in call duration should translate to fewer instances of call-echo overlap, and thus improved echolocation in cluttered conditions. The concurrent decrease in number of harmonics and increase in call peak frequency and fundamental bandwidth should maintain and/or improve object resolution and frequency-dependent timing.

Sonar sound groups (SSGs)

Sonar sound groups were first identified in the echolocation call sequences of flying big brown bats (Moss et al. 2006) and are produced most frequently when bats face perceptually challenging tasks (e.g., maneuvering in clutter, capturing moving prey; Moss et al. 2006; Petrites et al. 2009; Kothari et al. 2014; Hulgard and Ratcliffe 2016). I found that pups began emitting sonar strobe / sound groups with ≥3 calls around post-

natal day 6, as they transitioned from flopping to fluttering flight. Previous studies have suggested that SSG production is synchronized with respiration and the wing beat cycle (Petrites et al. 2009; Hulgard and Ratcliffe 2016), and the emergence of sonar strobe / sound groups concurrent with the first wing movements supports this idea. Also, pups emitted the maximum number of calls in sonar strobe / sound groups around the same time they transitioned from fluttering to flapping flight, suggesting a link between wing beats and sonar strobe / sound group production.

In these pups, sonar strobe / sound group production always preceded landing buzzes, leaving open the possibility that sonar strobe / sound groups are a precursor to landing buzz production. With respect to individual trajectories, I found no individual correlation between the production of sonar strobe / sound groups and the production of first landing buzzes. In other words, pups who produced sonar strobe / sound groups earliest in development did not also emit the first landing buzzes, suggesting that the shift from sonar strobe / sound groups to buzz production is unidirectional but not individually conserved. Interestingly, sonar strobe / sound groups emitted on post-natal day 12 contained as many calls per group as on post-natal day 32, a period before pup calls had acquired adult-like peak frequencies and fundamental bandwidths (Table 1). Functionally, increasing the number of calls per sonar strobe / sound group during the switch from wing movements on post-natal day 5.63 to horizontal displacement on post-natal day 16.5 may reflect selection on pups to acquire more information. Indeed, the subsequent increases in call peak frequency and bandwidth would also assist pups in acquiring finer details with echolocation (e.g. object size, texture, velocity).

Buzzes

Adult laryngeally-echolocating bats emit buzzes when they are about to intercept airborne prey and land on or drink from surfaces (Griffin et al. 1960; Melcón et al. 2007; Greif and Siemers 2010). Buzzes are characterized by a decrease in call duration, peak frequency, and pulse interval (Britton and Jones 1999; Surlykke and Moss 2000; Ratcliffe et al. 2013). Because buzz calls are emitted at higher rates, the returning echoes provides bats with faster updates about their surroundings (Petrites et al. 2009; Ratcliffe et al. 2013). In this study, 6 of 8 bats began emitting landing buzzes before taking flight, and 4 bats began to emit landing buzzes prior to transitioning from fluttering to flapping flight. One pup emitted his first landing buzz on the same day it transitioned from flopping to fluttering flight; however, this transition was noticeably later compared to other pups (~4 days after the average transition time). In general, pups did not begin to emit landing buzzes or call sequences with pulse intervals approaching those of landing buzzes until ~11 days after they had begun emitting sonar strobe / sound groups and achieving adult-like echolocation call designs.

Each echolocation call emitted by a laryngeally-echolocating bat is under independent neuromuscular control. Producing calls at landing buzz rates demands that the cricothyroid muscles contract and relax at rates much higher than possible in striated muscles of most vertebrates. In the larynges of vespertilionid bats, the buzz is powered by so-called superfast muscles (Elemans et al. 2011). Given the ubiquity of buzzes in echolocating bats, this data from *E. fuscus* pups suggest that superfast muscles continue to develop postnatally, reaching their fast rates of contraction near post-natal day 17 (i.e. approximately two-thirds through development).

Controlled, powered flight

As in other vespertilionids, achieving controlled powered flight was correlated with pups having reached adult-like morphologies, specifically RWLs (Table 3; Hughes et al. 1995; Elangovan et al. 2007). My post-natal day 32 pups did not differ in mass or forearm length from wild adult or juvenile male *E. fuscus* (Mayberry and Faure 2015), or from their mothers with respect to forearm length, relative wing loading, and orientation call design (Tables 1 and 3). Although this study was laboratory-based, the fact that pups first emitted landing buzzes ~6 days before they began to fly is likely to occur in the wild. Indeed, in nature, juvenile *E. fuscus* achieve controlled, powered flight by post-natal day ~25 (Kurta and Baker 1990), around the same time that my captive-bred male pups achieve the same milestone (post-natal day ~24).

I found no association between the transition to powered flight and any echolocation behaviour milestone, indicating that, in general, pups develop echolocation abilities slightly in advance of flight abilities. Two pups in my study achieved powered flight 1 day prior to the production of their first landing buzz. Because I defined buzzes as having pulse intervals ≤ 13 ms, these individuals may have been emitting calls at near-buzz rates but without technically meeting my definition. Near-buzzes likely provide pups with comparably relevant information. Indeed, pups that flew before emitting buzzes with pulse intervals ≤ 13 ms had been emitting multi-call sonar strobe / sound groups with pulse intervals < 15 and < 18 ms, respectively, on or prior to their first day of flight.

A single pup in my study failed to transition from flapping to powered flight. This pup also had the highest and least adult-like relative wing loading score and greatest

mass on post-natal day 32 compared to other pups (non-flier relative wing loading = 56.94 N/m^2, average relative wing loading = 45.4 $\pm$ 6.7 N/m^2; non-flier mass = 19.7 g, average mass = 17 $\pm$ 2.1 g). These data suggest that this pup may have been unable to achieve powered flight due to high transport costs associated with a higher mass and relative wing loading (see below).

Wing morphology

The change in mass, wingspan, relative wing loading, and aspect ratio in *E. fuscus* closely match developmental trajectories described in previous studies (de Fanis and Jones 1995; Hoying and Kunz 1998; McLean and Speakman 2000; Mayberry and Faure 2015). I found that both mass and wingspan increased during development and plateaued between post-natal day 22 and 32, while relative wing loading decreased early in development and reached adult-like values shortly before a pup's first flight. The decrease in relative wing loading results from differences between body mass and wing area growth; wing area increases faster than mass (Hughes et al. 1995; Stern et al. 1997). A decrease in relative wing loading early in development also reflects the lowering of transport costs as pups begin to achieve sustained flight and has been observed in multiple species (O'Farrell and Studier 1973; Buchler 1980; Powers et al. 1991; Adams 1996; Stern et al. 1997; McLean and Speakman 2000). Transport costs of sustained flight directly relate to wing loading; as relative wing loading decreases, the energy and power required for flight decreases due to proportionately larger wing areas relative to body mass (Powers et al. 1991). Transport costs often decrease further at the time of first flight because of a measurable reduction in mass (Hughes et al. 1995).

Wing aspect ratio shows a slight increase immediately after post-natal day 2 but plateaus quickly (Table 3). The overall development consistency in aspect ratio has been reported (O'Farrell and Studier 1973; de Fanis and Jones 1995; Hughes et al. 1995) and reflects the parallel development of wingspan and wing area. As pup wings grow longer, they also increase in surface area. The change in aspect ratio immediately after birth may represent an initial divergence of wingspan and wing area; initially, pup wing area may increase faster than wingspan, resulting in a temporarily lower aspect ratio.

Concluding remarks

I observed younger pups emitting landing buzzes at non-optimal times. For example, buzzes emitting during the pre-flight and post-flight phases would not be helpful for acquiring updated environmental information immediately prior to landing. Pre-flight buzzes may reflect pups attempting to acquire information prior to movement due to inexperience with motor procedures involved with landing, while post-flight buzzes could represent vocal practice and / or learning. Because observations were restricted to a short period of time each day, pups may have emitted buzzes that were not documented. These results illustrate the trajectory of vocal development (i.e. production of sonar / strobe groups prior to landing buzzes).

There was no clear association between the age when pups first emitted buzzes and their flight ability milestones; pups that buzzed earlier did not necessarily acquire powered flight earlier. This suggests that vocal development and flight ability, while clearly related and dependent on one another, are not tightly linked developmentally in

a step-wise fashion. These results provide further information on the development of flight and echolocation abilities in aerially hawking bats. While it is clear that sensory system development precedes motor development with respect to echolocation signal sophistication and powered flight, further research on the ontogeny of terminal feeding buzzes during aerial hawking prey captures as well as landing buzzes of gleaning bats could provide additional insight into sensorimotor integration in bats.

References

Adams, R.A. (1996). Size-specific resource use in juvenile little brown bats, *Myotis lucifugus* (Chiroptera: Vespertilionidae): Is there an ontogenetic shift? *Canadian Journal of Zoology*, *74*, 1204-1210.

Balcombe, J. P. (1990). Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. *Animal Behavior*, *39*, 960-966.

Bohn, K. M., Wilkinson, G. S., & Moss, C. F. (2007). Discrimination of infant isolation calls by female greater spear-nosed bats, *Phyllostomus hastatus*. *Animal Behaviour*, *73*, 423-432.

Britton, A. R., & Jones, G. (1999). Echolocation behaviour and prey-capture success in foraging bats: laboratory and field experiments on *Myotis daubentonii*. *Journal of Experimental Biology*, *202*, 1793-1801.

Brown, P. E., Brown, T. W., & Grinnell, A. D. (1983). Echolocation, development, and vocal communication in the lesser bulldog bat, *Noctilio albiventris*. *Behavioral Ecology and Sociobiology*, *13*, 287-298.

Buchler, E. R. (1980). The development of flight, foraging, and echolocation in the little brown bat (*Myotis lucifugus*). *Behavioral Ecology and Sociobiology*, *6*, 211-218.

de Fanis, E., & Jones, G. (1995). Post-natal growth, mother-infant interactions and development of vocalizations in the vespertilionid bat *Plecotus auritus*. *Journal of Zoology*, *235*, 85-97.

Elangovan, V., Priya, E. Y. S., Raghuram, H., & Marimuthu, G. (2007). Wing morphology and flight development in the short-nosed fruit bat *Cynopterus sphinx*. *Zoology*, *110*, 189-196.

Elemans, C. P., Mead, A. F., Jakobsen, L., & Ratcliffe, J. M. (2011). Superfast muscles set maximum call rate in echolocating bats. *Science*, *333*, 1885-1888.

Findley, J.S., Studier E.H. & Wilson, D.E. (1971) Morphologic properties of bat wings. *Journal of Mammology*, *53*, 429-444.

Gelfand, D. L., & McCracken, G. F. (1986). Individual variation in the isolation calls of Mexican free-tailed bat pups (*Tadarida brasiliensis mexicana*). *Animal Behaviour*, *34*, 1078-1086.

Greif, S. & Siemers, B. M. (2010). Innate recognition of water bodies in echolocating bats. *Nature Communications*, *1*, 1-6.

Griffin, D. R., Webster, F. A., & Michael, C. R. (1960). The echolocation of flying insects by bats. *Animal Behaviour*, *8*, 141-154.

Griffin, D. R. (1958). *Listening in the dark: the acoustic orientation of bats and men* (pp. 413). New Haven, Connecticut: Yale University Press.

Hoying, K. & Kunz, T. (1998). Variation in size at birth and post-natal growth in the insectivorous bat *Pipistrellus subflavus* (Chiroptera: Vespertilionidae). *Journal of Zoology, 245*, 15-27.

Hughes, P.M., Rayner, J.M.V. & Jones, G. (1995). Ontogeny of 'true' flight and other aspects of growth in the bat *Pipistrellus pipistrellus*. *Journal of Zoology*, *235*, 291-318.

Hulgard, K., & Ratcliffe, J. M. (2016). Sonar sound groups and increased terminal buzz duration reflect task complexity in hunting bats. *Scientific Reports*, *6*, 21500.

Jones, G., Hughes, P. M., & Rayner, J. M. V. (1991). The development of vocalizations in *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during post-natal growth and the maintenance of individual vocal signatures. *Journal of Zoology*, *225*, 71-84.

Kalko, E. K., & Schnitzler, H. U. (1989). The echolocation and hunting behavior of Daubenton's bat, *Myotis daubentoni*. *Behavioral Ecology and Sociobiology*, *24*, 225-238.

Kothari, N. B., Wohlgemuth, M. J., Hulgard, K., Surlykke, A., & Moss, C. F. (2014). Timing matters: sonar call groups facilitate target localization in bats. *Frontiers in Physiology*, *5*, 168.

Kurta, A., & Baker, R. H. (1990). *Eptesicus fuscus*. *Mammalian Species*, 356, 1-10.

Kunz, T.H. & Robson, S.K. (1995). Postnatal growth and development in the Mexican free-tailed Bat (*Tadarida brasiliensis mexicana*): birth size, growth rates, and age estimation. *Journal of Mammalogy*, *76*, 769–783

Maor, R., Dayan, T., Ferguson-Gow, H. & Jones, K. E. (2017). Temporal niche expansion in mammals from a nocturnal ancestor after dinosaur extinction. *Nature Ecology and Evolution*, *1*, 1889–1895.

Mayberry, H. W. & Faure, P. A. (2015). Morphological, olfactory, and vocal development in big brown bats. *Biology Open, 4*, 22-34.

McLean, J.A. & Speakman, J.R. (2000). Morphological changes during postnatal growth and reproduction in the brown long-eared bat *Plecotus auritus*: implications for wing loading and predicted flight performance. *Journal of Natural History*, *34*, 773-791.

Mehdizadeh, R., Eghbali, H., & Sharifi, M. (2018). Postnatal growth and vocalization development in the long-fingered bat, *Myotis capaccinii* (Chiroptera, Vespertilionidae). *Zoological Studies*, *57*, ZS.2018.57-37.

Melcón, M. L., Denzinger, A., & Schnitzler, H. U. (2007). Aerial hawking and landing: approach behaviour in Natterer's bats, *Myotis nattereri* (Kuhl 1818). *Journal of Experimental Biology*, *210*, 4457-4464.

Metzner, W., & Schuller, G. (2010). Vocal control in echolocating bats. In Brudzynski S.M. (Ed) *Handbook of behavioral neuroscience, Vol. 19* (pp. 403-415). Oxford, UK: Elsevier BV.

Monroy, J. A., Carter, M. E., Miller, K. E., & Covey, E. (2011). Development of echolocation and communication vocalizations in the big brown bat, *Eptesicus fuscus*. *Journal of Comparative Physiology A*, *197*, 459-467.

Moss C.F. (1988) Ontogeny of vocal signals in the big brown bat, *Eptesicus fuscus*. In: Nachtigall P.E., Moore P.W.B. (Eds) *Animal Sonar. NATO ASI Science (Series A: Life Sciences), vol 156*. Boston, MA: Springer.

Moss, C. F., & Surlykke, A. (2001). Auditory scene analysis by echolocation in bats. J Acoust Soc Am 110:2207-2226

Moss, C. F., & Surlykke, A. (2010). Probing the natural scene by echolocation in bats. *Frontiers in Behavioral Neuroscience*, *4*, 33

Moss, C. F., Redish, D., Gounden, C., & Kunz, T. H. (1997). Ontogeny of vocal signals in the little brown bat, *Myotis lucifugus*. *Animal Behaviour*, *54*, 131-141.

Moss, C. F., Bohn, K., Gilkenson, H., & Surlykke, A. (2006). Active listening for spatial orientation in a complex auditory scene. *Public Library of Science Biology*, *4*, e79.

Neuweiler, G. (1990). Auditory adaptations for prey capture in echolocating bats. Physiological Reviews 70:615–641

Noberg, U. M., & Fenton, M. B. (1988). Carnivorous bats? *Biological Journal of the Linnean Society*, *33*, 383-394.

Norberg, U. M., & Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *316*, 335-427.

O'Farrell, M.J. & Studier, E.H. (1973). Reproduction, growth, and development in *Myotis thysanodes* and *M. lucifugus* (Chiroptera: Vespertilionidae). *Ecology*, *54*, 18-30.

Papadimitriou, H.M., Swartz, S.M. & Kunz, T.H. (1996). Ontogenetic and anatomic variation in mineralization of the wing skeleton of the Mexican free-tailed bat, *Tadarida brasiliensis*. *Journal of Zoology*, *240*, 411-426.

Powers, L. V., Kandarian, S. C., & Kunz, T. H. (1991). Ontogeny of flight in the little brown bat, *Myotis lucifugus*: behavior, morphology, and muscle histochemistry. *Journal of Comparative Physiology A*, *168*, 675-685.

Ratcliffe, J.M., Elemans, C.P.H., Jakobsen, L., & Surlykke, A. (2013) How the bat got its buzz. *Biology Letters*, 9, 2012103.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution*, *43*, 223-225.

Scherrer, J. A., & Wilkinson, G. S. (1993). Evening bat isolation calls provide evidence for heritable signatures. *Animal Behaviour*, *46*, 847-860.

Skrinyer, A.J., Faure, P.A., Dannemiller, S., Ball, H.C., Delaney, K.H., Orman, R., Stewart, M., & Cooper, L.N. (2017) Care and husbandry of the world's only flying mammals. Laboratory Animal Science Professional June:24-27

Stern, A.A., Kunz, T.H., & Bhatt, S.S. (1997). Seasonal wing loading and the ontogeny of flight in *Phyllostomus hastatus* (Chiroptera: Phyllostomidae). *Journal of Mammalogy*, *78*, 1199–1209

Surlykke, A., & Moss, C. F. (2000). Echolocation behavior of big brown bats, *Eptesicus fuscus*, in the field and the laboratory. *Journal of the Acoustical Society of America*, *108*, 2419-2429.

Thiagavel, J., Santana, S. E., & Ratcliffe, J. M. (2017). Body size predicts echolocation call peak frequency better than gape height in vespertilionid bats. *Scientific Reports*, *7*, s41598-017-00959-2

Tian, B., & Schnitzler, H-U. (1997). Echolocation signals of the greater horseshoe bat *(Rhinolophus ferrumequinum)* in transfer flight and during landing. *Journal of the Acoustical Society of America* 101, 2347-2364.

Veselka, N., McErlain, D. D., Holdsworth, D. W., Eger, J. L., Chhem, R. K., Mason, M. J., Brain, K. L., Faure, P. A., & Fenton, M. B. (2010). A bony connection signals laryngeal echolocation in bats. *Nature*, *463*, 939-942.

Zhang, L., Jones, G., Parsons, S., Liang, B., & Zhang, S. (2005). Development of vocalizations in the flat-headed bats, *Tylonycteris pachypus* and *T. robustula* (Chiroptera: Vespertilionidae). *Acta Chiropterologica*, *7*, 91-99.

Figure Legends

Figure 1. Full room set-up for acoustic and video data collection. **(A)** Diagram of room, with microphones 1 through 9 indicated by *grey numbered circles*. Thermal cameras 1 and 2 indicated by *dark grey numbered rectangles*. Plastic sheeting was affixed over the foam closest to the thermal cameras to dissuade bats from landing behind the recorded area. **(B)** Photograph of partial room; placement of thermal cameras and partial microphone placement indicated. **(C)** Photograph of partial room; parallel "hot pack lines" for thermal camera measurements are depicted, as well as the foam-covered table for safe flop flight landings and remaining microphone placements. Pup flight drop site indicated by a *white asterisk*.

Figure 2. Single representative orientation calls emitted by *E. fuscus* pups on **(A)** PND 2, **(B)** PND 12, **(C)** PND 22, and **(D)** PND 32. Shown for each call is an oscillogram (*top*), spectrogram (*bottom left*) and power spectrum (*bottom right*).

Figure 3. Single representative orientation calls emitted by an *E. fuscus* pup on the last days of **(A)** flop, **(B)** flutter, and **(C)** flap flight behaviour. Shown in panel **(D)** is a typical orientation (non-buzz) call emitted by the pup's mother. Each panel shows an oscillogram (*top*), spectrogram (*bottom left*) and power spectrum (*bottom right*). I note that panels **B** and **D** each show a faint echo immediately following the orientation call.

Figure 4. Sequences of clustered orientation (non-buzz) calls emitted by a **(A)** PND 32 *E. fuscus* pup and an **(B)** adult mother, and sequences of approach and landing buzz calls emitted by a **(C)** PND 32 pup and an **(D)** adult mother. Each column shows an oscillogram (*top*), spectrogram (*middle*), and a plot of PIs over time (*bottom*). For panels **A** and **B**, the *red circles* in the PI plots show calls that satisfy one or both criteria that define SSGs (see Material and Methods). For panels **C** and **D**, the rapid series of calls emitted at the end of both sequences have PIs < 13 ms (*horizontal dashed line*) and thus meet the technical criteria for a true buzz.

Table Headings

Table 1. Echolocation behaviour measurements as a function of age in big brown bat pups. Shown are the mean (± SD) spectral and temporal call parameters and number of calls per sonar sound group (SSG) on consecutive 10-day intervals for 8 pups measured at ages PND 2, 12, 22, and 32, as well as for adult mothers. Results of paired *t*-tests between consecutive age groups (i.e. PND 2 *versus* 12, PND 12 *versus* 22, PND 22 *versus* 32, and PND 32 *versus* adult mothers) are reported for each measure along with P-values and Bonferroni corrected α-values.

Table 2. Echolocation behaviour measurements as a function of flight ability in developing big brown bat pups. Shown are the mean (± SD) spectral and temporal call parameters and number of calls per sonar sound group (SSG) measured on consecutive flight transition days for 8 pups. Flight transitions are the days when a pup last exhibited the less-advanced flight behaviour (i.e. the last day a pup exhibited flopping flight before transitioning to fluttering flight). Results of paired *t*-tests between consecutive flight transitions (i.e. flop / flutter *versus* flutter / flap, and flutter / flap *versus* flap / fly) are reported for each measure along with P-values and Bonferroni corrected α-values.

Table 3. Morphometric measurements as a function of age in big brown bat pups. Shown are the mean (± SD) body mass, forearm length, wingspan, relative wing loading, and aspect ratio on consecutive 10-day intervals for 8 pups measured at ages

PND 2, 12, 22, and 32, as well as for adult mothers (captive females), wild-caught juvenile males, and wild-caught adult males. Results of paired *t*-tests between consecutive age groups (i.e. PND 2 vs 12, PND 12 vs 22, and PND 22 vs 32) as well as between PND 32 pups and adult mothers, wild-caught juvenile males, and wild-caught adult males are reported for each measure along with P-values and Bonferroni corrected α-values. Wingspan, RWL, and AR were not measured for any wild-caught animal.

Table 4. Morphometric measurements as a function of flight ability in big brown bat pups. Shown are the mean (± SD) body mass, forearm length, wingspan, relative wing loading, and aspect ratio measured on consecutive flight transition days for 8 pups. Flight transitions are the days when a pup last exhibited the less-advanced flight behaviour (i.e. the last day a pup exhibited flopping flight before transitioning to fluttering flight). Results of paired *t*-tests between consecutive flight transitions (i.e. flop / flutter *versus* flutter / flap, and flutter / flap *versus* flap / fly) are reported for each measure along with P-values and Bonferroni corrected α-values.

Figure 1.

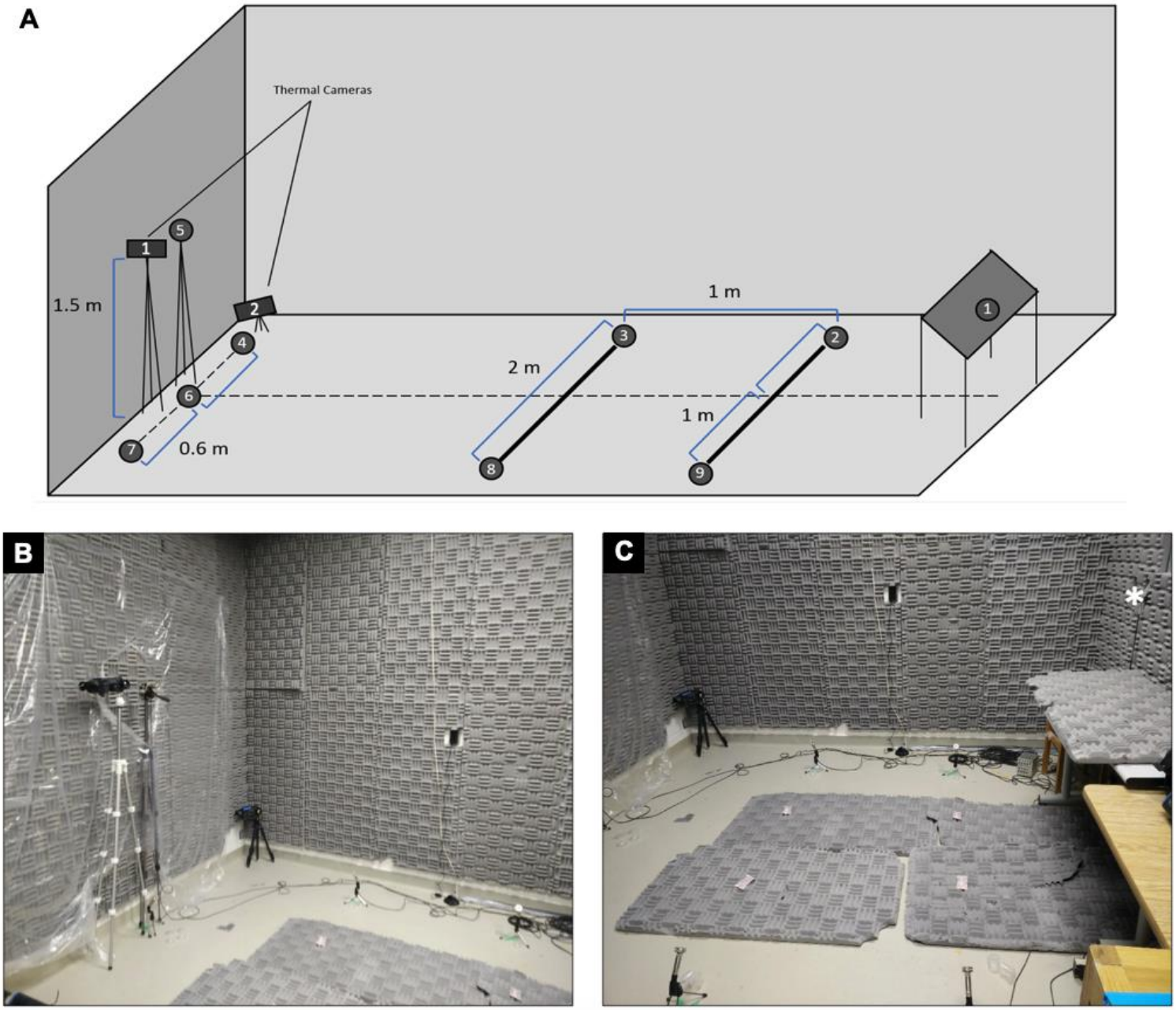

Figure 2.

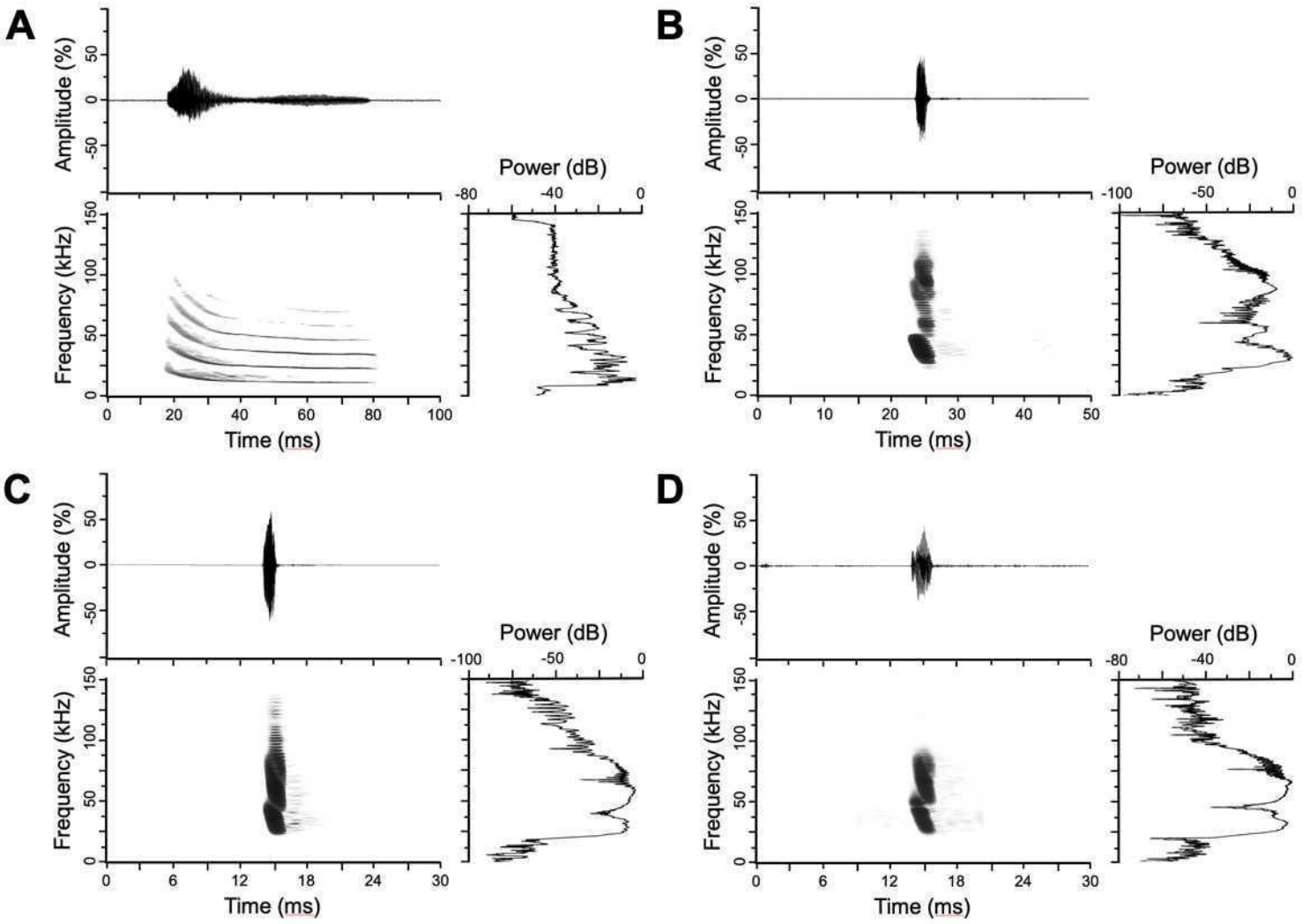

Figure 3.

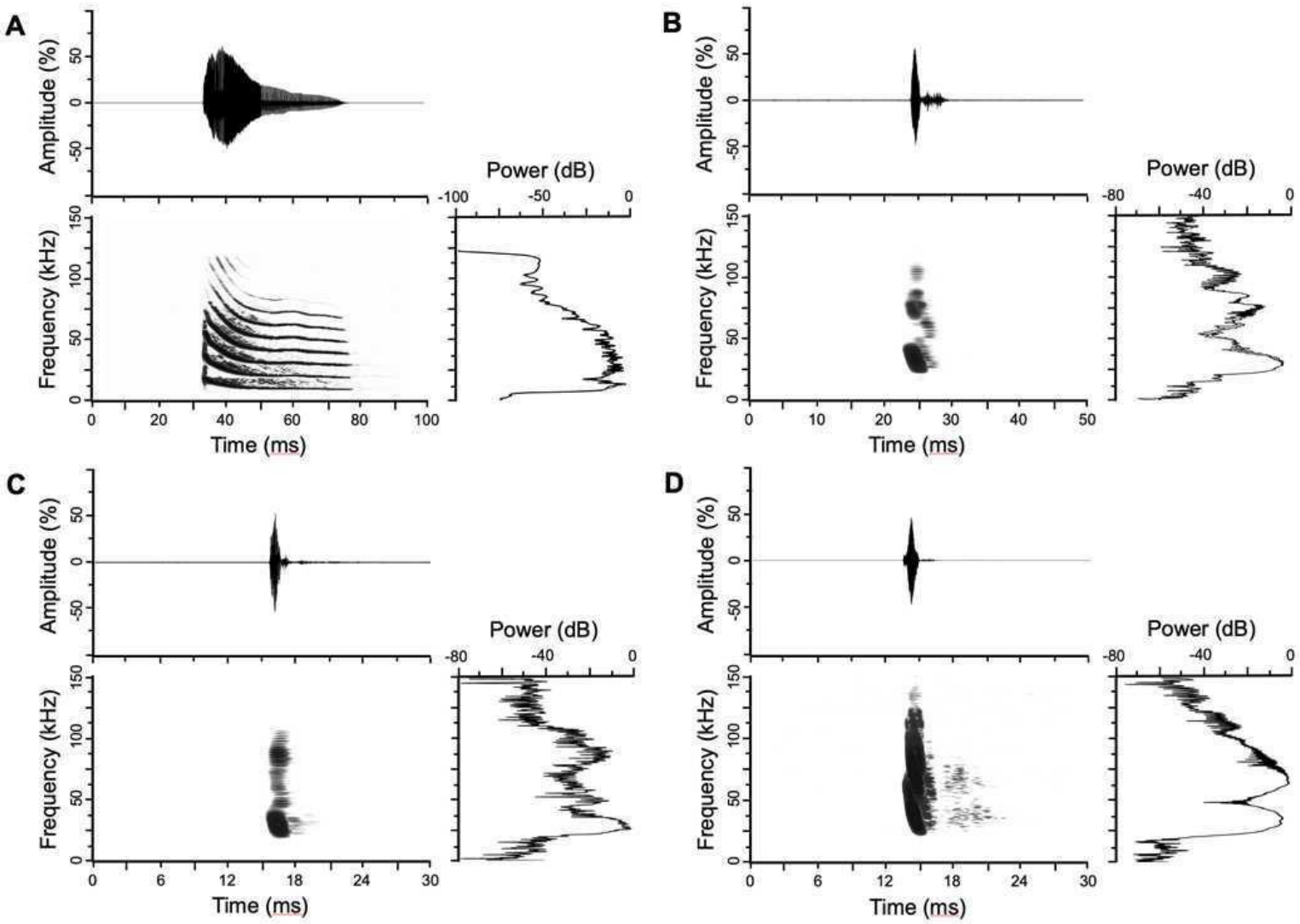
A
Amplitude (%)
50
0
-50
Power (dB)
-100
-50
0
Frequency (kHz)
150
100
50
0
0
20
40
60
80
100
Time (ms)
B
Amplitude (%)
50
0
-50
Power (dB)
-80
-40
0
Frequency (kHz)
150
100
50
0
0
10
20
30
40
50
Time (ms)
C
Amplitude (%)
50
0
-50
Power (dB)
-80
-40
0
Frequency (kHz)
150
100
50
0
0
6
12
18
24
30
Time (ms)
D
Amplitude (%)
50
0
-50
Power (dB)
-80
-40
0
Frequency (kHz)
150
100
50
0
0
6
12
18
24
30
Time (ms)

Figure 4.

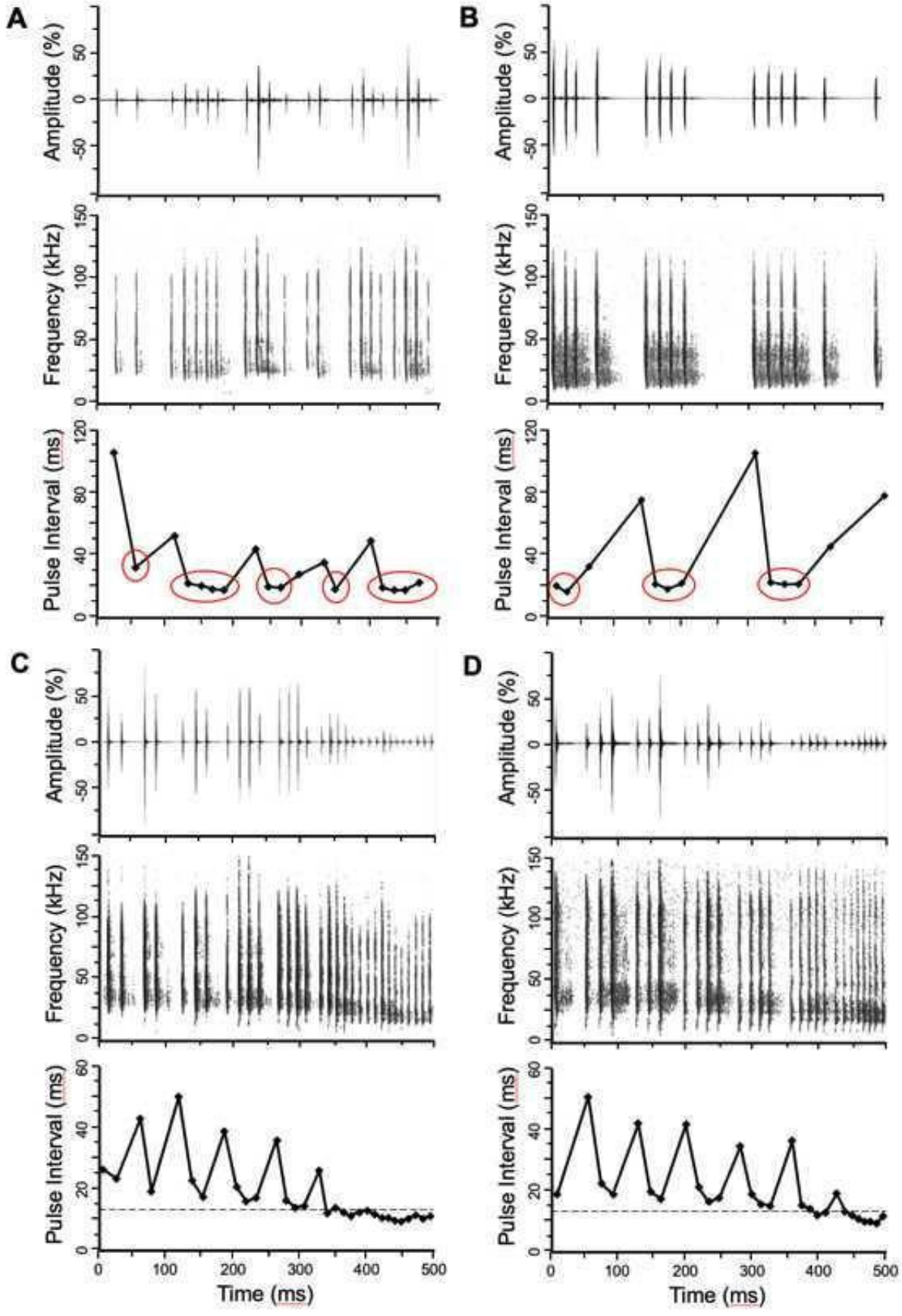

Table 1.

Echolocation behaviour	PND 2 mean (SD)	PND 12 mean (SD)	PND 22 mean (SD)	PND 32 mean (SD)	Adult (Mothers) mean (SD)	PND 2 v. 12 *t*-statistic	PND 2 v. 12 *P* (α)	PND 12 v. 22 *t*-statistic	PND 12 v. 22 *P* (α)	PND 22 v. 32 *t*-statistic	PND 22 v. 32 *P* (α)	PND 32 v. Adult (Mothers) *t*-statistic	PND 32 v. Adult (Mothers) *P* (α)
Max. call duration (ms)	37.7 (17.9)	2.5 (0.9)	2.5 (0.8)	2.8 (0.5)	3.4 (0.7)	*-5.28*	**<0.001** (0.013)	*-0.26*	0.4 (0.013)	*0.82*	0.78 (0.013)	-1.8	0.11 (0.013)
Min. call duration (ms)	19.2 (10.1)	1.1 (0.4)	0.9 (0.3)	0.8 (0.5)	0.8 (0.4)	*-4.73*	**<0.002** (0.013)	*-2.63*	0.02 (0.013)	*-1.03*	0.17 (0.013)	0.04	0.967 (0.013)
Min. # of call harmonics	6.5 (2.1)	2.9 (0.9)	2.9 (0.6)	2.5 (0.8)	2.7 (1)	*-4.22*	**<0.003** (0.013)	*-0.23*	0.41 (0.013)	*-1*	0.18 (0.013)	-0.34	0.745 (0.013)
Max. fund. harm. bandwidth (kHz)	17.2 (5)	30.5 (6.9)	50.6 (7.7)	58.6 (5.7)	56.3 (5.9)	*5.36*	**<0.001** (0.013)	*4.1*	**<0.004** (0.013)	*3.95*	**<0.004** (0.013)	0.72	0.486 (0.013)
Max. peak frequency (kHz)	16.6 (2)	29.5 (3.4)	38.8 (7.2)	38.6 (6.1)	39.9 (6.2)	*7.91*	**<0.001** (0.013)	*2.63*	0.02 (0.013)	*0.9*	0.23 (0.013)	-0.38	0.709 (0.013)
Min. pulse interval (ms)	85.6 (41.6)	17.6 (7.6)	11.2 (2.3)	11.3 (2.5)	9.1 (1.4)	*-4.12*	**<0.004** (0.013)	*-2.5*	0.02 (0.013)	*-0.79*	0.23 (0.013)	2.13	0.057 (0.013)
Max. # of calls in SSG	2 (0)*	7.9 (6.1)	11.5 (4.4)	9.4 (4)	15.7 (6.6)	*NA*	*NA*	*0.53*	0.32 (0.013)	*0.03*	0.49 (0.013)	-2.07	0.077 (0.013)
Average buzz duration (ms)	*NA*	75.1 (59.7)	58.1 (35.9)	46 (32.2)	41.7 (34.1)	*NA*	*NA*	0.74	0.47 (0.013)	0.2	0.4 (0.013)	0.24	0.816 (0.013)

Table 2.

Echolocation behaviour	**Last flop** mean (SD)	**Last flutter** mean (SD)	**Last flap** mean (SD)	**Last flop v. Last flutter** *t*-statistic	*P* (α)	**Last flutter v. Last flap** *t*-statistic	*P* (α)
Max. call duration (ms)	16.7 (18.8)	2.2 (0.3)	2.4 (0.7)	*-1.98*	0.048 (0.025)	*0.53*	0.31 (0.025)
Min. call duration (ms)	4.5 (5.4)	1.1 (0.2)	0.9 (0.2)	*-1.86*	0.056 (0.025)	*-1.3*	0.12 (0.025)
Min. # of call harmonics	4.3 (1.2)	3.1 (0.8)	2.9 (0.7)	*-3.36*	**<0.008** (0.025)	*-0.68*	0.26 (0.025)
Max. fund. harm. bandwidth (kHz)	19.2 (6)	38.9 (10.1)	45.4 (8.8)	*5.95*	**<0.001** (0.025)	*2.42*	0.026 (0.025)
Max. peak frequency (kHz)	22.8 (5)	32 (3.8)	36.4 (4.7)	*4.35*	**<0.003** (0.025)	*4.24*	**<0.003** (0.025)
Min. pulse interval (ms)	36 (16.7)	16.3 (7.9)	11.9 (4.2)	*-3.61*	**<0.006** (0.025)	*-1.43*	0.1 (0.025)
Max. # of calls in SSG	3.5 (1.7)	9 (5.4)	10.6 (5.9)	*2.7*	**0.018** (0.025)	*1.03*	0.17 (0.025)

Table 3.

Morphometric measure	PND 2	PND 12	PND 22	PND 32	Adult (Mothers)	Wild-Caught Juvenile Males	Wild-Caught Adult Males
	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)
Body mass (g)	5.6 (1.1)	11.8 (0.7)	15.1 (0.9)	17 (2.1)	22.7 (4.8)	14.4 (0.4)	16.5 (0.8)
Forearm (mm)	22.7 (1.7)	37.6 (1.2)	43.8 (1.8)	44.8 (1.9)	46.3 (0.3)	45.3 (1.9)	44.0 (0.3)
Wingspan (cm)	13.7 (1.3)	23.6 (1.5)	29.3 (1.3)	31 (1)	33.2 (1.2)	*NA*	*NA*
RWL	98.2 (4.7)	62.4 (7.3)	47.7 (2.8)	45.4 (6.7)	49.0 (3.8)	*NA*	*NA*
AR	6 (0.6)	6.7 (0.3)	6.8 (0.2)	6.7 (0.4)	6.7 (0.6)	*NA*	*NA*

PND 2 v. 12		PND 12 v. 22		PND 22 v. 32		PND 32 v. Adult (Mothers)		PND 32 v. Wild-Caught Juvenile Males		PND 32 v. Wild-Caught Adult Males	
t-statistic	*P* (α)	*t*-statistic	*P* (α)	*t*-statistic	*P* (α)	*t*-statistic	*P* (α)	*t*-statistic	*P* (α)	*t*-statistic	*P* (α)
9.83	**<0.001** (0.017)	*16.55*	**<0.001** (0.017)	*1.68*	0.07 (0.017)	-4.84	**<0.001** (0.008)	3.44	0.010 (0.008)	0.66	0.528 (0.008)
19.77	**<0.001** (0.017)	*9.76*	**<0.001** (0.017)	*6.02*	**<0.001** (0.017)	-2.19	0.065 (0.008)	-0.53	0.608 (0.008)	1.22	0.263 (0.008)
18.54	**<0.001** (0.017)	*11.16*	**<0.001** (0.017)	*2.4*	0.027 (0.017)	-3.64	**0.005** (0.008)	*NA*	*NA*	*NA*	*NA*
-11.23	**<0.001** (0.017)	*-5.53*	**<0.001** (0.017)	*-2.04*	0.044 (0.017)	-1.29	0.22 (0.008)	*NA*	*NA*	*NA*	*NA*
3.15	**<0.01** (0.017)	*0.22*	0.42 (0.017)	*-0.16*	0.92 (0.017)	0.1	0.92 (0.008)	*NA*	*NA*	*NA*	*NA*

Table 4.

Morphometric measure	**Last flop** mean (SD)	**Last flutter** mean (SD)	**Last flap** mean (SD)	**Last flop v. Last flutter** *t*-statistic	*P* (α)	**Last flutter v. Last flap** *t*-statistic	*P* (α)
Body mass (g)	8.3 (1.9)	13.5 (1.7)	16 (0.9)	*8.09*	**<0.001** (0.025)	*4.56*	**<0.002** (0.025)
Wingspan (cm)	17.4 (2.3)	26.5 (2)	30.1 (0.6)	*10*	**<0.001** (0.025)	*4.41*	**<0.003** (0.025)
Rel. wing loading	88.7 (9.6)	52.8 (6.5)	46.4 (0.4)	*-8.78*	**<0.001** (0.025)	*-1.92*	0.052 (0.025)
Aspect ratio	6.7 (0.3)	6.7 (0.4)	6.8 (0.3)	*-0.52*	0.69 (0.025)	*1.28*	0.12 (0.025)

Chapter Four

Foraging niche release in North American bat species relatively unaffected by white-nose syndrome

Foraging niche release in North American bat species relatively unaffected by white-nose syndrome

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Abstract

White-nose syndrome (WNS), a fungal disease which is responsible for killing many bats across North America, has rendered 4 of Ontario's species endangered, while leaving the other 4 species found there relatively unaffected. The causes and extent of the declines have been widely studied. However, the influence of the population declines on the remaining bat species, including possible competition-related niche release, has not. Comparing acoustic data recorded ~10 years apart, I evaluated if, and if so how, the 8 bat species found in southeastern Ontario used different foraging habitats 1 or 2 years before, and 9 years, after WNS was detected. I found that the reduction of the once most common species, the little brown bat, *Myotis lucifugus*, has essentially left the foraging niche of flying insects near lake surfaces locally vacant. I also found expected decreases in now-endangered species foraging activity over open fields (*Myotis leibii*, *M. lucifugus*, *M. septentrionalis*, and *Perimyotis subflavus*). My data indicate these declines may have allowed for greater presence, but not hunting, in open field habitats and clutter/edge environments by the big brown bat, *Eptesicus fuscus*, and three migratory species (*Lasiurus borealis*, *L. cinereus*, and *Lasionycteris noctivagans*). Overall, my results suggest that the WNS-induced population reductions of 4 of 5 resident bat species, including two that were common and abundant, have allowed species relatively unaffected by WNS to expand their foraging niches into habitats to which they are less well-suited but now face reduced competition. Sensory and / or biomechanical constraints, however, may limit these species from exploiting prey in these new habitats.

Introduction

Bat communities are rarely monospecific and local assemblages can include over 100 bat species in equatorial regions of Africa, Asia, and South America (Fenton & Ratcliffe 2010). Even in less species-rich communities, interspecific competition might be expected between species with similar diets (Gordon et al. 2019). However, species with similar diets and foraging strategies often appear to co-exist in the same broad niche with respect to diet (Aldridge and Rautenbach 1987; Jacobs and Barclay 2009). At such locations, interspecific competition may be offset by spatial separation driven by sensory, morphological and behavioural differences (Aldridge and Rautenbach 1987; Fullard 1987; Norberg and Rayner 1987; Fenton 1990; Brigham et al. 1997; Arlettaz 1999, Siemers and Swift 2006; Emrich et al. 2014). For example, differences in wing shape and limits on echolocation call design influence a species' flight behaviour and ability to emit effective and ultimately informative echolocation signals. Wing morphology and signal characteristics are considered to be linked with a species' general ecology and may predict habitats in which species are more likely to be present (Norberg and Rayner 1987).

My study focuses on a relatively small community of bat species found in south-eastern Ontario, Canada. I summarize these bats' characteristics (wing design, typical echolocation calls, diet, migratory status, and preferred foraging habitats) in Table 1. With respect to migratory status, I have classified each of the 8 species as either "sedentary" or "migratory". While I am aware that migration can refer to a variety of seasonal movements, in this paper I am exclusively using the term "migratory" to refer to species who participate in seasonal, two-way movements with relatively large

latitudinal shift from colder to more favourable climatic environments (i.e. from South Eastern Ontario to / beyond the southern United States). I classified those species making seasonal roost shifts (e.g. *M. lucifugus*) and / or those who remain in the same general area throughout the winter months (i.e. without movement to warmer climates) as “sedentary”.

For bats, potential foraging locations in rural Ontario can be broadly segregated into four main habitats: open areas (open fields with little to no tall vegetation (>2m)), cluttered areas (heavily wooded), edge habitats (transitional areas between wooded areas and adjacent open areas), and over water (including lakes, streams and ponds; Furlonger et al. 1987; Emrich et al. 2014). All eight species are insectivorous and sympatric during the summer at the study sites. While some species are markedly distinct from one another in body size and signal design, others share similar morphologies (body size, wing shape, etc.) and acoustic signatures (Fenton and Bell 1981). These bats often forage for similar prey in habitats close to one another (Saunders and Barclay 1992; Bowie et al. 1999), suggesting interspecific competition. Interspecific competition can directly influence ecosystem biodiversity. Extreme reductions in a species population can have dramatic effects on the composition, abundance and niche use of any remaining species (Abul-Fatih and Bazzaz 1979; Power et al. 1996; Kunte 2008). The removal of dominant species through extinction or ecosystem manipulation can increase competitor abundance, reducing subsequent prey numbers and/or overall ecosystem biodiversity (Abul-Fatih and Bazzaz 1979; Smith and Knapp 2003; Langwig et al. 2012; Alves et al. 2014). Furthermore, removing a dominant species can release previously inhabited and competed for foraging areas open to

exploitation by the remaining species, thus causing a shift in habitat use (Jachowski et al. 2014).

White-nose syndrome (WNS) is a fungal disease caused by a European strain of *Pseudogymnoascus destructans* introduced to North America in 2006 (Blehert et al. 2009). First observed in New York State, the fungus has since spread to over thirty states and most Canadian provinces. White-nose syndrome has caused species-specific declines of over 90% in some areas (Moore et al. 2018). White-nose syndrome most severely impacts species that rely on prolonged hibernation during the winter months. Species that migrate from cold areas (e.g. SE Ontario) to warmer climates do not appear to experience population declines after infection (Ford et al. 2011; Langwig et al. 2012; Alves et al. 2014; Jachowski et al. 2014; Bernard et al. 2015). The roosting temperatures experienced by these migratory species during winter are likely too warm for *Pseudogymnoascus destructans* growth, and / or the availability of prey in warmer climates may allow these species to avoid prolonged hibernation. *M. lucifigus*, previously the most abundant bat species in Ontario, is among those most severely impacted by white-nose syndrome (Frick et al. 2010). Another common hibernating species, *E. fuscus*, exhibits significantly lower mortality rates after infection. These differences in white-nose syndrome susceptibility are thought to be due to disparities in hibernation physiology and behaviour (Moore et al. 2018).

The introduction of white-nose syndrome to Ontario has provided an opportunity to explore the effects of the reduction in a naturally dominant species on the remaining species with respect to species abundance, niche use, and changes to foraging habitats. Previous studies exploring species-specific white-nose syndrome population

declines have identified increases in overall activity and a shift in the niche partitioning of less-affected species, suggesting a relaxation of interspecific competition (Dzal et al. 2010; Ford et al. 2011; Jachowski et al. 2014). These studies, however, focused on single habitats (i.e. close to / over water) and did not consider changes in foraging activity (i.e. the presence of feeding buzzes) across multiple environments (open fields, open water, and clutter/edge habitats). Comparing acoustic recordings from several sites in South Eastern Ontario 1 to 2 years before and ~9 years after the appearance of white-nose syndrome, I hypothesize that species-specific population reductions will have influenced the remaining species abundance and foraging activity within and between three distinct habitats (open fields, open water, and clutter/edge habitats). I predicted that a decrease in the overall activity of endangered species would promote higher activity levels in the remaining four species within clutter/edge environments and open water due to reduced interspecific competition. Specifically, I suspected that the big brown bat, *E. fuscus*, would exploit the decline of the little brown bat, *M. lucifugus*, as *E. fuscus* is the only relatively unaffected sedentary species in South Eastern Ontario's bat community.

Methods and Materials

Field Sites / Data Collection

I collected acoustic data at and around the Queen's University Biological Station (QUBS) near Chaffey's Lock, Ontario (44˚34'N, 79˚15'W) during the summers (early June to late September) of 2007, 2008, and 2017. The Queen's University Biological

Station and the surrounding area comprise a variety of terrestrial and wetland ecosystems. This area, as with much of South Eastern Ontario, encompasses mixed deciduous and coniferous forest/shrubs, agricultural land, freshwater lakes, small ponds, swamps, and marsh areas. Of the 18 bat species occurring in Canada, 8 reside in Ontario. All 8 of these species occur in South Eastern Ontario, and specifically at and around the Queen's University Biological Station. Of these 8 species, I classified 5 as sedentary (*E. fuscus, M. lucifigus*, *M. septentrionalis*, *M. leibii*, and *P. subflavus* (previously misclassified as *Pipistrellus subflavus*)) and 3 as migratory (*L. noctivagans, L. cinereus*, and *L. borealis*), as per the migratory status specifications indicated both above and in Table 1. I collapsed data from 2007 and 2008 into a single data set, referred to as 2007/8, for ease of comparison and because both of these summers represented a pre-WNS environment in this area (Dzal et al. 2010). For accurate and robust comparisons among data sets, I selected the nights for data collection in 2017 to closely match the Julian calendar dates of the 2007/8 recording nights (maximum of five days difference, most matched dates were within two days of one another).

I began data collection at 9 pm and recorded continuously until 4 am, or as long as weather permitted. Due to the sensitivity of the recording devices to moisture (as well as the reluctance of bats to forage in rainy conditions (Fenton 1970)), recording nights were cancelled or cut short if rain was present. Any night from one season missing a matched night in the other season was discarded. In total, data from 26 matched nights were recorded (see *Time Matching* and *Weather* below).

Data were collected from six sites: Station Road, Lane Sergeant, Elbow Lake, Boat House, East Field, and New Field (Figure 1). Station Road is a fallow field with

little vegetation over 1 m high (Figure 2a). One side is bordered by forest, another by a more highly vegetated field, and the remaining sides were lined with fences to separate the field from two gravel roads. Lane Sergeant, East Field, and New Field are all open hay fields (harvested in October), each bordered by dense forest along one side (Figure 2b-d). These four sites represented the “open field” sites as well as four “clutter/edge” sites in my analyses. Boat House and Elbow Lake were located along the shores of Lake Opinicon and Elbow Lake, respectively (Figure 2e and f).

Recordings

At terrestrial sites in both seasons, I positioned two Avisoft (CM16, Avisoft Bioacoustics, Glienicke, Germany) condenser ultrasound microphones attached to tripods such that one microphone was aimed out over the “open field” site and the second microphone aimed over the “clutter/edge” site. I placed microphones recording from the space above the open areas at least 5 metres away from the edge of the forested/cluttered areas and aimed them towards the open field / water (and directly away from the forest edge). I placed microphones recording the clutter/edge areas approximately 0.5 metre from the edge of the forest (placing the microphones and tripods further into the forested areas was often impossible due to thick vegetation). The same microphones were used at open water sites and were positioned at, and aimed away from, shore. All microphones were 1.5 m high and aimed upwards approximately 45 degrees from horizontal.

Microphones were connected to a designated Avisoft UltraSoundGate (4 channel (416), 8 channel (816) or 12 channel (1216); Avisoft Bioacoustics, Glienicke, Germany)

device associated with a laptop computer (ThinkPad X240, ThinkPad X220 or ThinkPad T430; Lenovo, Morrisville, U.S.A.). Data were automatically saved to computers as a .wav file and transferred to external hard drives after each recording session. The recording interface only recorded echolocation signals that exceeded a manually designated threshold. Automatic 5 second recordings (2 second pre-trigger, 3 second hold time; 500 kHz sampling rate, 16-bit format) began when any signal containing frequencies between 20 and 250 kHz, and which had an intensity above 1% of the maximum amplitude of the recording system, was detected. Using pre-determined thresholds with automatic detection and recording minimized the likelihood that the recording equipment would be triggered by, and thus record, other sources of noise beyond bat calls (i.e. other animals, vehicles, etc.). Moreover, automatic recordings eliminated the potential concern of missing echolocation signals. That said, all recordings were made with a researcher present and attending to the visual display of detected acoustic activity.

Time Matching

For each recording night (i.e. from 9 p.m. to 4 a.m.), I identified the start and end times of bat activity, determined by the first and last .wav file recorded for that night. Using these times, I calculated the total active recording time for each night. I note that bat activity is often not consistent throughout the night, however species-specific differences are generally conserved (Kunz 1973; Jachowski et al. 2014) and it is possible that the matched nights may not represent accurate comparisons of bat

activity. In an attempt to ensure that activity was comparable between seasons, I trimmed both matched nights to include only files recorded between the latest start time and the earliest end time of both nights. For example, an active recording time span of, say, between 9 p.m. and 3 a.m. in 2007/8 and an active recording between 10 pm and 4 am on the matched 2017 date would result in an analysis of files recorded between 10 pm and 3 am on both nights. Any nights in which no files were recorded during the recording period in either season (N = 2) were eliminated. While I acknowledge that file trimming may exclude some species-specific activity, I believe these trimmed files permit the most robust comparisons between seasons while still providing data on all species activity.

Species Identification

I identified species based on echolocation calls and call sequences using two complementary methods, one semi-automated, one manual. First, I used SonoBat (version 4; North America, North-Northeast Regional Classifier; DNDesign, Arcata, CA, USA), software that automatically filters environmental noise and rejects files with low-quality signals. In SonoBat, species classifications were made from full spectrum analyses of acceptable call sequences and determined by assessing the overall agreement of species identification between individual calls within that sequence. Probabilities of final species decisions were provided for each file. I accepted, after manual verification, species identifications with decision probabilities of 0.9 or greater. Species identifications with probabilities below this threshold were eliminated from analysis, except those that listed only *E. fuscus* and *L. noctivagans* as potential species.

Since *E. fuscus* and *L. noctivagans* echolocation signals have a high degree of spectral and temporal overlap, and manual inspection also failed to differentiate between species, *E. fuscus* / *L. noctivagans* classifications were accepted and subsequently treated as a composite species.

Manual species identifications were also made using a subset of files (roughly 15% of the files captured for each summer) using BatSoundPro (Version 3.31, Pettersson Elektronik AB, Uppsala, Sweden). For individual calls within each file, I measured call duration, peak frequency from BatSound oscillograms and power spectrums (Fourier transforms) and made species identifications based on the average / range data present in Table 1. Additional information, such as call shape and pulse interval, were considered when classification was ambiguous. These manual classifications were then compared with SonoBat's automated classifications. These comparisons indicated that while the species identifications were nearly identical, SonoBat was, overall, more conservative than trained personnel. Moreover, SonoBat often distinguished between the three myotid species as well as between big brown bats (*E. fuscus*) and silver haired bats (*L. noctivagans*), a challenge because of similarities among call parameters (Table 1). As both classifications were broadly similar, and SonoBat provided a more conservative approach with additional specificity, I felt confident in using SonoBat's accepted species identifications for analyses. Because SonoBat assumes each call sequence is from one animal, all files were visually assessed for the presence of multiple individuals / species. For any files in which multiple species were present (see Figure 3a, b), SonoBat's automated

identification was considered accurate only for that species with the highest call energy. Any additional species, therefore, were classified manually using call design parameters based on the literature (Table 1).

Species Activity

For each file, I report the estimated number of individual bats present, the number of distinct bat passes observed (i.e. unbroken echolocation sequences apparently from a single bat, Figure 4a, b), and the number of feeding buzzes recorded (Figure 5a, b). I summed all individual, pass, and buzz data for each bat species present from files recorded within the matched start and end times for each night.

Buzzes were identified by a noticeable increase in call rate (to greater or equal to 90 calls/s) and a decrease in signal frequency (Griffin et al. 1960; Figure 5a and b). Buzzes are difficult to distinguish among Ontario bat species and therefore species identification was based on immediately preceding call sequences. I assumed buzzes represent feeding (hunting) attempts. Passes, representing bat presence, were defined as a set of sequential calls ($N \geq 5$) with similar pulse intervals (Dzal et al. 2010; Figure 4a, b). Each pass was assumed to have been emitted by a single individual unless the start times of each sequence were within one minute of one another; those passes recorded within one minute of one another and identified as the same species were conservatively assumed to be produced by the same individual. I acknowledge that individual call assignment with bats is difficult and suggest that the passes and buzzes data are likely more accurate, informative, and comparable to other acoustic monitoring

research than are the individual estimates (Fenton 1970; Kalcounis et al. 1999; Dzal et al. 2009; Ford et al. 2011; Jachowski et al. 2014).

Weather

Cold temperatures and precipitation negatively influence bat activity. Rain and cold increase the cost of energetic activities, limit echolocation signal and flight efficiency, and may be associated with reduced insect densities (Taylor 1963; Catto et al. 1995; Vaughan et al. 1997). Using historical weather data (Queens University Biology Station Weather Station Data, Elgin, ON, Canada; Weather Underground http://www.wunderground.com, The Weather Company, IBM, Brookhaven GA, U.S.A.), I determined the average temperature and total precipitation for each active recording night in 2007/8 and in 2017. Comparing between 2007/8 and 2017, I eliminated from analysis any nights (N = 8) for which (*i*) the average temperature difference between the years was greater than 5 degrees Celsius, and/or (*ii*) the total precipitation difference between the years was greater than 5 mm. There were no differences in average nightly temperature or total nightly precipitation between 200/8 and 2017 (paired t-tests: t = 0.261, $P > 0.1$; t = 0.125, $P > 0.1$ respectively).

Statistical Analysis

After identifying overall differences in the number of individuals, passes and buzzes between 2007/8 and 2017 recording seasons, I analyzed pass and buzz data for each species individually. I compared both average counts and proportions between seasons for both passes and buzzes of each species. I used Wilcoxon signed rank tests to identify general and relative species activity. To explore species-specific temporal

changes, I calculated the cumulative sums of passes and buzzes across each season (i.e. 2007/8 and 2017). These data were log transformed and compared using two-tailed tests for differences between two population regression coefficients (Zar 1999). The slopes of the linear regressions represent overall rates of activity.

Results

All pass and buzz data comparisons between 2007/8 and 2017 for each species within each site-habitat condition are summarized in Tables 2 and 3. Results of the statistical analyses are presented with and without Bonferroni correction. I collected a roughly equivalent number of files in 2007/8 and 2017 (1063 and 1317 respectively) with no difference in average number of files recorded each night (20.44 ± 5.86 and 25.32 ± 6.01 respectively; paired *t*-test: $t = -0.70$, $P > 0.1$). Count, proportion, and cumulative sum data for all habitats in both seasons are included in the supplementary data section.

Open field, sedentary species

M. lucifugus activity (passes and buzzes) declined from 2007/8 to 2017 in open field habitats, as expected. I also found a decrease in *M. septentrionalis* (also endangered) passes between 2007/8 to 2017. While no difference in average or proportional data was detected for the 2 remaining endangered species (*M. leibii* and *P. subflavus*), all endangered sedentary species had higher regression coefficients (steeper slopes) in 2007/8 than in 2017. As I was interested in species-specific changes between pre- and post- white-nose syndrome introduction, I avoided any pooling of endangered species. This trend was conserved for the cumulative sums both pass and buzz data. No

differences were observed for the final sedentary species, *E. fuscus*, between the pre- and post- white-nose syndrome seasons in open habitats (Table 2). See Figure 6 for single call examples of each sedentary species in open habitats.

Open field, migratory species

Considering *L. borealis*, *L. cinereus*, and *L. noctivagans* together, migratory bat abundance (i.e. average number and / or proportion of passes), overall, increased from 2007/8 to 2017 in open field habitats. No differences in foraging (buzzes) between the decade-apart seasons were detected for any migratory species. My cumulative data for migratory species were highly variable (see Table 2). No comparative buzz data was available for *E. fuscus* / *Lasionycteris noctivagans.* Examples of each migratory species call recorded in open habitats are shown in Figure 7.

Edge and forest, sedentary species

Surprisingly, I found no difference in *M. lucifugus* activity with respect to presence (passes), foraging (buzzes), or rate of pass/buzz increase (cumulative sum slopes) between 2007/8 and 2017 in clutter/edge habitats. Furthermore, I found none of the expected declines in any endangered species (*M. lucifugus*, *M. septentrionalis, M. leibii,* and *P. subflavus*) for the average number or proportion of passes between the two seasons. The probable explanation for these findings is that edge/clutter activity for all species prior to 2017 was relatively rare. Regression coefficients of the cumulative sums of passes were higher in 2007/8 for all other endangered species. *E. fuscus* showed no difference in rate of pass increase (cumulative sum slope) between seasons, however I did observe an increase in the average number of passes from

2007/8 to 2017. All sedentary species with buzz data in both years (except *M. lucifugus*) had higher regression coefficients in 2017 for cumulative buzz data. I found no differences in buzz data (average number or proportions of buzzes) for any sedentary species within the edge/clutter habitats (see Table 3). See Figure 8 for single call examples of each species.

Edge and forest, migratory species

I observed an increase in the average number and proportion of passes from 2007/8 to 2017 for all migratory species (Table 3). Furthermore, all migratory species had higher rates of both pass and buzz increase (cumulative slope sums) in 2017 than in 2007/8. However, I found no differences in buzz data (average number or proportions of buzzes) for any migratory species within the edge/clutter habitats. Single call examples are depicted in Figure 9. In general, calls (from both sedentary and migratory species) emitted in clutter/edge habitats had relatively higher peak frequencies and longer durations than those in open habitats (average open peak frequency = 39.2 kHz, average clutter/edge peak frequency = 44.3 kHz, paired *t*-test, $t = -4.92$, $P = 0.002$; average open call duration = 7.43 ms, average clutter/edge call duration = 5.48 ms, paired *t*-test, $t = 4.75$, $P = 0.002$).

Over water, all species

No differences between 2007/8 and 2017 data (number/proportion of passes and buzzes, regression coefficients of all cumulative sums) were detected for any species over water. Several species (4 of 8) did not have any foraging data (buzzes) in either pre- or post- white-nose syndrome seasons and therefore comparisons were not

possible. This absence of statistical difference may be due to (*i*) few instances of activity over water even in 2007/8 and (*ii*) bats formerly active over water (*M. lucifugus*, *P. subflavus*) having decreased due to white-nose syndrome. Further, sample sizes for over water comparisons were small (*N* = 5 nights) due to few pre- white-nose syndrome over water recording nights and weather/time matching data removal.

Discussion

Comparing species-specific data from pre- and post- white-nose syndrome introduction in South Eastern Ontario, I observed a profound change in bat activity over ten years. Those species most heavily affected by white-nose syndrome have experienced severe declines in activity, while those less affected may be expanding their realized niche. Activity by relatively unaffected migratory species has increased (i.e. average number and proportion of passes) in open habitats as well as previously unexploited clutter/edge environments (Tables 2 and 3). Endangered species (especially *M. lucifugus*) have not reduced use of clutter/edge habitats, however they have decreased their presence and hunting activity (i.e. average number and proportion of buzzes) dramatically over open fields. Likely due to small sample sizes, no statistical difference in over water activity was detected for any species (residential, migratory, or endangered). However, I note that while in 2007/8 bats were sometimes recorded hunting over water, no feeding buzzes were recorded there in 2017. Bat passes were recorded over water in both seasons, perhaps from bats in transit or in search of a drink. *E. fuscus*, the only relatively unaffected residential species, did not exploit the reduction in *M. lucifugus* activity as I predicted. *E. fuscus* exhibited an increase only in

the average number of clutter/edge habitat passes, with no similar changes observed in open areas, nor with respect to feeding buzzes (i.e. hunting behaviour).

My results confirm many previously identified destructive effects of white-nose syndrome (Dzal et al. 2010; Ford et al. 2011; Langwig et al. 2012; Jachowski et al. 2014). In Ontario, *M. leibii*, *M. lucifigus*, *M. septentrionalis*, and *P. subflavus* have been listed as endangered as a result of white-nose syndrome as well as habitat destruction due to urbanization (Government of Ontario 2018). As such, the significant overall reductions in *M. lucifigus* and *M. septentrionalis* activity in 2017 were not unexpected. Meanwhile, it is encouraging to report that *P. subflavus* and *M. leibii* abundance and activity have not significantly declined at the study site, at least proportionately.

Bearing in mind the life history of insectivorous bats (long lived, low fecundity), the reported declines in previously dominant species (*M. lucifigus*) should have decreased the total bat activity (species combined) observed in 2017. In other words, since *M. lucifigus* activity was higher than all other species pre- white-nose syndrome, decreases in *M. lucifigus* populations should have translated into fewer automatically triggered recordings. Instead, I collected roughly the equivalent number of files in the 2007/8 and 2017 seasons. These results suggest that I may have inadvertently oversampled during the post- white-nose syndrome season. While this oversampling (perhaps due to unidentified differences in recording systems) is regrettable, the results obtained still provide an accurate representation of the current bat population levels in Ontario. It is also possible, though unlikely, that the while total number of bats found in this local community has declined, individual activity levels for some species has increased at the recording sites. Alternatively, this data may reflect a greater number of

multi-individual recordings, suggesting that the total number of bats have not changed but individual bats are more active in the recording areas. Without season- / habitat-specific quantitative prey information or mist-net capture data, I cannot differentiate between these explanations, however I do acknowledge the limitations of using activity recordings to reflect overall abundance.

Despite controlling for temperature, precipitation, and active recording time, it is possible that nights with atypical activity levels could also have skewed my results. However, comparing averaged count data with proportionality data as well as assessing the slope differences between years confirms that my data appears unaffected by unusually high or low activity levels. In general, shifts in average number of passes or buzzes between years was conserved with respect to proportionality. For example, an increase in the average number of *L. borealis* clutter/edge passes was consistent with a higher proportion of *L. borealis* in the same habitat. Further, most species whose activity declined in 2017 activity also exhibited lower rates of cumulative sum increases of passes and / or buzzes. Less steep slopes in 2017 suggest that (*i*) the number of passes counted for that species were lower in 2017 and (*ii*) they remained lower throughout the entire 2017 season.

My results suggest that some relatively unaffected species have expanded their realized niche and now are more common in different habitats. *L. borealis* and *L. cinereus*, for example, are proportionately more common in open and clutter/edge habitats, while *L. noctivagans* and *E. fuscus* exhibited an increase in clutter/edge activity. No relatively unaffected species, however, show any evidence of hunting (as

indicated by feeding buzzes) more frequently in any habitat in 2017 compared to 2007/8. I expected and found that relatively unaffected species would hunt more in clutter/edge habitats given their increased presence in these areas. I did not, however, detect any change in the average number / proportion of feeding buzzes, suggesting that the ability of white-nose syndrome un- or less-affected species to actively hunt within previously un- or under-used habitats remains limited. This distinction between presence (as determined by pass data) and feeding (as determined by buzz data) in clutter/edge habitats may reflect the difference between the realized niches (occupied environment) and fundamental niches (potential environment) of relatively unaffected species.

Foraging in dense forest or edge environments requires relatively slow, manoeuvrable flight and short, broadband echolocation calls to successfully detect and avoid obstacles and extract echoic prey information from an acoustically noisy background (Fenton 1990). Bats hunting in clutter/edge habitats typically have lower wing loading scores and produce higher frequency broadband echolocation signals at relatively low intensities (Aldridge and Rautenbach 1987; Norberg and Rayner 1987). The production of high frequency signals is directly related to mass and larynx size. Physically lighter bats with smaller larynges produce acoustic signals with higher frequencies (Thiagavel et al. 2017). Furthermore, wing shape and area being equal, bats with lower masses will also have lower wing loading scores. Species relatively unaffected by white-nose syndrome tend to be heavier than those more affected and emit search calls with lower average peak frequencies. They also tend to have wing designs better suited for open space than clutter (i.e. relatively high wing loading and

aspect ratios). As such, the production of high frequency signals and the necessary maneuverability for hunting fast moving insects within clutter may be unattainable (refer to Table 1 for species-specific mass and frequency information).

Relatively unaffected species may also refrain from hunting in clutter/edge habitats despite their increased presence there due to lower prey availability. Insects predominantly present in these environments may be most suitable for smaller bats (i.e. myotids) but too small for larger bats to bother with. That is, small prey may be (*i*) energetically insufficient for the diet of larger bats and / or (*ii*) too small for detection due to longer echolocation signal wavelengths (from lower frequency calls; Hickey et al. 1996; Ford et al. 2011). Further research into the diversity of insects in clutter/edge and open habitats in South Eastern Ontario is needed to clarify this issue.

Surprisingly, I observed that *E. fuscus* do not hunt more often in clutter/edge habitats post- white-nose syndrome. *E fuscus* readily forages in many habitats when prey is available, thereby earning its description as a generalist species (Brigham 1991; Agosta 2002). I expected that with a reduction in *M. lucifugus* population, *E. fuscus* activity (specifically hunting behavior) would expand into previously less-occupied habitats, including clutter/edge habitats and over open water. Although I did observe an increase in the average number of *E. fuscus* passes in clutter/edge habitats in 2017, I did not observe any increase in activity (i.e. number of passes) over water, nor in foraging attempts (i.e. number of feeding buzzes) in any habitat. Unlike the migratory species, *E. fuscus* has suffered population declines due to white-nose syndrome (Jachowski et al. 2014; Moore et al. 2018). These declines are less severe than those experienced by *M. lucifugus* and other endangered species, thus the designation of

“relatively unaffected”. This difference in white-nose syndrome susceptibility is thought to be the result of different patterns of thermoregulation during hibernation (Moore et al. 2018). The lack of (*i*) increased hunting (buzzes) in any habitat and (*ii*) increased activity (passes) over water may simply reflect the fact that some *E. fuscus* have experienced negative effects of white-nose syndrome. This big brown bat population may remain relatively stable instead of permitting the increase in activity and foraging I predicted. A more in-depth study into the population changes of *E. fuscus* across South Eastern Ontario is required to resolve this issue.

My results with respect to feeding buzzes may also indicate an unintended sampling issue. Throughout my data set, buzzes are consistently recorded less often than passes. In 2007/8, the approximate ratio of recorded buzzes to passes was 1:6. In 2017, however, this ratio shifted to 1:20 buzzes to passes. As such, the lack of feeding behavior observed for the relatively unaffected species may reflect an under-sampling of capture attempts (successful or otherwise) in 2017 as opposed to a restriction to niche expansion. Regardless of sampling concerns, I am confident that the activity exhibited by all bats in South Eastern Ontario during these Julian days should reflect active foraging, including capture attempts. Bats mate immediately prior to hibernation and / or migration (Cryan et al. 2012). To prepare for hibernation, sedentary bats enter a state of hyperphagia to increase fat stores (Speakman and Rowland 1999; McGuire et al. 2009b). As such, residential bat activity during the time period I sampled should reflect these bats searching for and consuming prey (and little else). Migrating bats recorded in South Eastern Ontario between June and September are likely exhibiting both foraging and migratory behaviours (McGuire et al. 2009a; McGuire et al. 2013).

Flying in clutter/edge habitats, however, should impede the migratory process. Maneuvering through obstacles takes more time and energy than flying in open areas. It seems likely, then, that migratory species are now using clutter/edge habitats in search of prey.

Bats face population declines from a number of sources, including climate change, wind energy installations, and habitat destruction (Baerwald et al. 2008; Sherwin et al. 2013; O'Shea et al. 2016). Additionally, white-nose syndrome continues to threaten the conservation of insectivorous bats across North America despite innovative attempts at disease mitigation (Boyles and Willis 2010; Cheng et al. 2017; McGuire et al. 2019). While the direct effects of white-nose syndrome have been widely studied, the indirect consequences have been less thoroughly explored. For example, even after the successful survival of white-nose syndrome exposure, previously infected bats may experience reduced survival and reproductive success (Davy et al. 2017). Other incidental effects include those on populations of relatively unaffected species. These results provide clear evidence of habitat changes resulting from species-specific population declines and suggest that relatively unaffected species have been able to extend their presence, but not hunting, into new habitats. Physiological limitations, on both flight and echolocation behaviour, may constrain the ecological flexibility of relatively unaffected bats. Further research is required to explore new venues for supporting North American bat biodiversity and conservation.

References

Abul-Fatih, H. A., & Bazzaz, F. A. (1979). The biology of *Ambrosia trifida* L. I. Influence of species removal on the organization of the plant community. *New Phytologist*, *83*, 813-816.

Agosta, S. J. (2002). Habitat use, diet and roost selection by the big brown bat (*Eptesicus fuscus*) in North America: a case for conserving an abundant species. *Mammal Review*, *32*, 179-198.

Aldridge, H. D. J. N., & Rautenbach, I. L. (1987). Morphology, echolocation and resource partitioning in insectivorous bats. *Journal of Animal Ecology*, *56*, 763-778.

Alves, D. M., Terribile, L. C., & Brito, D. (2014). The potential impact of white-nose syndrome on the conservation status of North American bats. *Public Library of Science One*, *9*, 0107395.

Arlettaz, R. (1999). Habitat selection as a major resource partitioning mechanism between the two sympatric sibling bat species *Myotis myotis* and *Myotis blythii*. *Journal of Animal Ecology*, *68*, 460-471.

Baerwald, E. F., D'Amours, G. H., Klug, B. J., & Barclay, R. M. (2008). Barotrauma is a significant cause of bat fatalities at wind turbines. *Current Biology*, *18*, R695-R696.

Balcombe, J. P., & Fenton, M. B. (1988). Eavesdropping by bats: the influence of echolocation call design and foraging strategy. *Ethology*, *79*, 158-166.

Best, T. L., & Jennings, J. B. (1997). *Myotis leibii. Mammalian Species*, *547*, 1-6.

Bernard, R. F., Foster, J. T., Willcox, E. V., Parise, K. L., & McCracken, G. F. (2015). Molecular detection of the causative agent of white -nose syndrome on Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) and two species of migratory bats in the southeastern USA. *Journal of Wildlife Diseases*, *51*, 519-522.

Blehert, D. S., Hicks, A. C., Behr, M., Meteyer, C. U., Berlowski-Zier, B. M., Buckles, E. L., Coleman, J.T.H., Darling, S.R., Gargas, A., Niver, R., Okoniewski, J. C., Rudd, R.J., & Stone, W.B. (2009). Bat white-nose syndrome: an emerging fungal pathogen? *Science*, *323*, 227-227.

Bowie, R. C., Jacobs, D. S., & Taylor, P. J. (1999). Resource use by two morphologically similar insectivorous bats (*Nycteris thebaica* and *Hipposideros caffer*). *South African Journal of Zoology*, *34*, 27-33.

Boyles, J. G., & Willis, C. K. (2010). Could localized- warm areas inside cold caves reduce mortality of hibernating bats affected by white nose syndrome? *Frontiers in Ecology and the Environment*, *8*, 92-98.

Brigham, R. M. (1991). Flexibility in foraging and roosting behaviour by the big brown bat (*Eptesicus fuscus*). *Canadian Journal of Zoology*, *69*, 117-121.

Brigham, R. M., Grindal, S. D., Firman, M. C., & Morissette, J. L. (1997). The influence of structural clutter on activity patterns of insectivorous bats. *Canadian Journal of Zoology*, *75*, 131-136.

Brigham, R. M., & Cebek, J. E. (1989). Intraspecific variation in the echolocation calls of two species of insectivorous bats. *Journal of Mammalogy*, *70*, 426-428.

Caceres, M. C., & Barclay, R. M. (2000). *Myotis septentrionalis*. *Mammalian Species*, *48*, 1-4.

Catto, C. M. C., Racey, P. A., & Stephenson, P. J. (1995). Activity patterns of the serotine bat (*Eptesicus serotinus*) at a roost in southern England. *Journal of Zoology*, *235*, 635-644.

Cheng, T. L., Mayberry, H., McGuire, L. P., Hoyt, J. R., Langwig, K. E., Nguyen, H., Parise, K.L., Foster, J.T., Willis, C.K.R., Kilpatrick A.M., & Frick,-W. F. (2017). Efficacy of a probiotic bacterium to treat bats affected by the disease white nose syndrome. *Journal of Applied Ecology*, *54*, 701-708.

Cryan, P. M., Jameson, J. W., Baerwald, E. F., Willis, C. K., Barclay, R. M., Snider, E. A., & Crichton, E. G. (2012). Evidence of late-summer mating readiness and early sexual maturation in migratory tree-roosting bats found dead at wind turbines. *Public Library of Science One*, *7*, 0047586.

Davy, C. M., Mastromonaco, G. F., Riley, J. L., Baxter-Gilbert, J. H.,- Mayberry, H., & Willis, C. K. (2017). Conservation- implications of physiological carry over effects in bats recovering from white nose syndrome. *Conservation Biology*, *31*, 615-624.

Dzal, Y., Hooton, L. A., Clare, E. L., & Fenton, M. B. (2009). Bat activity and genetic diversity at Long Point, Ontario, an important bird stopover site. *Acta Chiropterologica*, *11*, 307-315.

Dzal, Y., McGuire, L. P., Veselka, N., & Fenton, M. B. (2010). Going, going, gone: the impact of white-nose syndrome on the summer activity of the little brown bat (*Myotis lucifugus*). *Biology Letters*, *7*, 392-394.

Emrich, M. A., Clare, E. L., Symondson, W. O., Koenig, S. E., & Fenton, M. B. (2014). Resource partitioning by insectivorous bats in Jamaica. *Molecular Ecology*, *23*, 3648-3656.

Farney, J., & Fleharty, E. D. (1969). Aspect ratio, loading, wing span, and membrane areas of bats. *Journal of Mammalogy*, *50*, 362-367.

Fenton, M. B. (1970). A technique for monitoring bat activity with results obtained from different environments in southern Ontario. *Canadian Journal of Zoology*, *48*, 847-851.

Fenton, M. B. (1990). The foraging behaviour and ecology of animal-eating bats. *Canadian Journal of Zoology*, *68*, 411-422.

Fenton, M. B., & Bell, G. P. (1981). Recognition of species of insectivorous bats by their echolocation calls. *Journal of Mammalogy*, *62*, 233-243.

Fenton, M. B., & Ratcliffe, J. M. (2010). Bats. *Current Biology*, *20*, R1060-R1062.

Ford, W. M., Britzke, E. R., Dobony, C. A., Rodrigue, J. L., & Johnson, J. B. (2011). Patterns of acoustical activity of bats prior to and following white-nose syndrome occurrence. *Journal of Fish and Wildlife Management*, *2*, 125-134.

Frick, W. F., Pollock, J. F., Hicks, A. C., Langwig, K. E., Reynolds, D. S., Turner, G. G., Butchkoski, C.M., & Kunz, T. H. (2010). An emerging disease causes regional population collapse of a common North American bat species. *Science*, *329*, 679-682.

Fullard, J. H. (1987). Sensory ecology and neuroethology of moths and bats: interactions in a global perspective. In: Fenton M.B., Racey P.A., Rayner J.M.V. (Eds) *Recent advances in the study of bats* (pp. 244-272). Cambridge: Cambridge University Press.

Fujita, M. S., & Kunz, T. H. (1984). *Pipistrellus subflavus*. *Mammalian Species*, *228*, 1-6.

Furlonger, C. L., Dewar, H. J., & Fenton, M. B. (1987). Habitat use by foraging insectivorous bats. *Canadian Journal of Zoology*, *65*, 284-288.

Gordon, R., Ivens, S., Ammerman, L. K., Fenton, M. B., Littlefair, J. E., Ratcliffe, J. M., & Clare, E. L. (2019). Molecular diet analysis finds an insectivorous desert bat community dominated by resource sharing despite diverse echolocation and foraging strategies. *Ecology and Evolution*, *9,* 3117-3129.

Government of Ontario (2018). Ontario Regulation 230/08: Species at risk in Ontario list. Retrieved from: <www.ontario.ca/laws/regulation/R18404>

Griffin, D. R., Webster, F. A., & Michael, C. R. (1960). The echolocation of flying insects by bats. *Animal Behaviour*, *8*, 141-154.

Hickey, M. B. C., Acharya, L., & Pennington, S. (1996). Resource partitioning by two species of vespertilionid bats (*Lasiurus cinereus* and *Lasiurus borealis*) feeding around street lights. *Journal of Mammalogy*, *77*, 325-334.

Jachowski, D. S., Dobony, C. A., Coleman, L. S., Ford, W. M., Britzke, E. R., & Rodrigue, J. L. (2014). Disease and community structure: white nose syndrome alters spatial and temporal niche partitioning in sympatric bat species. *Diversity and Distributions*, *20*, 1002-1015.

Jacobs, D. S., & Barclay, R. M. (2009). Niche differentiation in two sympatric sibling bat species, *Scotophilus dinganii* and *Scotophilus mhlanganii*. *Journal of Mammalogy*, *90*, 879-887.

Kalcounis, M. C., Hobson, K. A., Brigham, R. M., & Hecker, K. R. (1999). Bat activity in the boreal forest: importance of stand type and vertical strata. *Journal of Mammalogy*, *80*, 673-682.

Kunte, K. (2008). Competition and species diversity: removal of dominant species increases diversity in Costa Rican butterfly communities. *Oikos*, *117*, 69-76.

Kunz, T. H. (1973). Resource utilization: temporal and spatial components of bat activity in central Iowa. *Journal of Mammalogy*, *54*, 14-32.

Kunz, T. H. (1982). *Lasionycteris noctivagans*. *Mammalian Species*, *172*, 1-5.

Kurta, A., & Baker, R. H. (1990). *Eptesicus fuscus*. *Mammalian Species*, *356*, 1-10.

Langwig, K. E., Frick, W. -F., Bried, J. T., Hicks, A. C., Kunz, T. H., & Marm Kilpatrick, A. (2012). Sociality, density dependence and microclimates determine- the persistence of populations suffering from a novel fungal disease, white nose syndrome. *Ecology Letters*, *15*, 1050-1057.

McGuire, L. P., Fenton, M. B., & Guglielmo, C. G. (2009a). Effect of age on energy storage during prehibernation swarming in little brown bats (*Myotis lucifugus*). *Canadian Journal of Zoology*, *87*, 515-519.

McGuire, L. P., & Guglielmo, C. G. (2009b). What can birds tell us about the migration physiology of bats?. *Journal of Mammalogy*, *90*, 1290-1297.

McGuire, L. P., Guglielmo, C-. G., Mackenzie, S. A., & Taylor,- P. D. (2012). Migratory stopover in the long distance migrant silver haired bat, *Lasionycteris noctivagans*. *Journal of Animal Ecology*, *81*, 377-385.

McGuire, L. P., Fenton, M. B., & Guglielmo, C. G. (2013). Phenotypic flexibility in migrating bats: seasonal variation in body composition, organ sizes and fatty acid profiles. *Journal of Experimental Biology*, *216*, 800-808.

McGuire, L. P., Mayberry, H. W., Fletcher, Q. E., & Willis, C. K. (2019). An experimental test of energy and electrolyte supplementation as a mitigation strategy for white-nose syndrome. *Conservation Physiology*, *7*, coz006.

Meteyer, C. U., Valent, M., Kashmer, J., Buckles, E. L., Lorch, J. M., Blehert, D. S., Lollar, A., Berndt, D., Wheeler, E., White, C.L., & Ballmann, A. E. (2011). Recovery of little brown bats (*Myotis lucifugus*) from natural infection with *Geomyces destructans*, white-nose syndrome. *Journal of Wildlife Diseases*, *47*, 618-626.

Miller, L. A., & Treat, A. E. (1993). Field recordings of echolocation and social signals from the gleaning bat *Myotis septentrionalis*. *Bioacoustics*, *5*, 67-87.

Mills, S. C., Adams, A. M., & Phoenix, R. D. (2013). Bat species diversity in the boreal forest of northeastern Ontario, Canada. *Northeastern Naturalist*, *20*, 309-325.

Moore, M. S., Field, K. A., Behr, M. J., Turner, G. G., Furze, M. E., Stern, D. W., Allegra, P.R., Bouboulis, S.A., Musante, C.D., Vodzak, M.E., Biron, M. E., Meierhofer, M.B., Frick, W.F., Foster, J.T., Howell, D., Kath, J.A., Kurta, A., Nordquist, G., Johnson, J.S., Lilley, T.M., Barrett, B.W., & Reeder, D.M. (2018). Energy conserving thermoregulatory patterns and lower disease severity in a bat resistant to the impacts of white-nose syndrome. *Journal of Comparative Physiology B*, *188*, 163-176.

Murray, K. L., Britzke, E. R., & Robbins, L. W. (2001). Variation in search-phase calls of bats. *Journal of Mammalogy*, *82*, 728-737.

Norberg, U. M., & Rayner, J. M. (1987). Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, *316*, 335-427.

O'shea, T. J., Cryan, P. M., Hayman, D. T., Plowright, R. K., & Streicker, D. G. (2016). Multiple mortality events in bats: a global review. *Mammal Review*, *46*, 175-190.

Power, M. E., Tilman, D., Estes, J. A., Menge, B. A., Bond, W. J., Mills, L. S., Daily, G., Castilla, J.C., Lubchenco, J., & Paine, R. T. (1996). Challenges in the quest for keystones: identifying keystone species is difficult—but essential to understanding how loss of species will affect ecosystems. *BioScience*, *46*, 609-620.

Powers, L. V., Kandarian, S. C., & Kunz, T. H. (1991). Ontogeny of flight in the little brown bat, *Myotis lucifugus*: behavior, morphology, and muscle histochemistry. *Journal of Comparative Physiology A*, *168*, 675-685.

Phillips, G. L. (1966). Ecology of the big brown bat (Chiroptera: Vespertilionidae) in northeastern Kansas. *American Midland Naturalist*, *75*, 168-198.

Ratcliffe, J. M., & Dawson, J. W. (2003). Behavioural flexibility: the little brown bat, *Myotis lucifugus*, and the northern long-eared bat, *M. septentrionalis*, both glean and hawk prey. *Animal Behaviour*, *66*, 847-856.

Salcedo, H. D. L. C., Fenton, M., Hickey, M., & Blake, R. (1995). Energetic consequences of flight speeds of foraging red and hoary bats (*Lasiurus borealis* and *Lasiurus cinereus*; Chiroptera: Vespertilionidae). *Journal of Experimental Biology*, *198*, 2245-2251.

Saunders, M. B., & Barclay, R. M. (1992). Ecomorphology of insectivorous bats: a test of predictions using two morphologically similar species. *Ecology*, *73*, 1335-1345.

Sherwin, H. A., Montgomery, W. I., & Lundy, M. G. (2013). The impact and implications of climate change for bats. *Mammal Review*, *43*, 171-182.

Shump, K. A., & Shump, A. U. (1982a). *Lasiurus borealis*. *Mammalian species*, *183*, 1-6.

Shump, K. A., & Shump, A. U. (1982b). *Lasius cinereus*. *Mammalian species*, *185*, 1-5.

Siemers, B. M., & Swift, S. M. (2006). Differences in sensory ecology contribute to resource partitioning in the bats *Myotis bechsteinii* and *Myotis nattereri* (Chiroptera: Vespertilionidae). *Behavioral Ecology and Sociobiology*, *59*, 373-380.

Smith, M. D., & Knapp, A. K. (2003). Dominant species maintain ecosystem function with non random species loss. *Ecology Letters*, *6*, 509-517.

Speakman, J. R., & Rowland, A. (1999). Preparing for inactivity: how insectivorous bats deposit a fat store for hibernation. *Proceedings of the Nutrition Society*, *58*, 123-131.

Taylor, L. R. (1963). Analysis of the effect of temperature on insects in flight. *The Journal of Animal Ecology*, 99-117.

Thiagavel, J., Santana, S. E., & Ratcliffe, J. M. (2017). Body size predicts echolocation call peak frequency better than gape height in vespertilionid bats. *Scientific Reports*, *7*, s41598-017-00959-2

Vaughan, N., Jones, G., & Harris, S. (1997). Habitat use by bats (Chiroptera) assessed by means of a broad-band acoustic method. *Journal of Applied Ecology*, *34*, 716-730.

Weller, T. J. (2008). Using occupancy estimation to assess the effectiveness of a regional multiple-species conservation plan: bats in the Pacific Northwest. *Biological Conservation*, *141*, 2279-2289.

Woodsworth, G. C. (1981). Spatial partitioning by two species of sympatric bats, *Myotis californicus* and *Myotis leibii* (Unpublished master's thesis). Carleton University, Ottawa, Canada.

Zarr, J.H. (1999). *Biostatistical analysis* (3rd ed.). Upper Saddle River, New Jersey: Prentice Hall.

Figure Legends

Figure 1. (A) Map of SE Ontario, with recording sites located within indicated area. **(B)** Aerial view of area indicated in Figure 1A, showing all 6 recording sites (Elbow Lake (A), New Field (B), Lane Sergeant (C), East Field (D), Station Road (E), and Boat House (F)). Latitude and longitude of the Queen's University Biology Station (QUBS) are indicated. QUBS is located at / around Boat House site (F).

Figure 2. Sites (*N* = 4; **(A)** Station Road, **(B)** Lane Sergeant, **(C)** East Field, and **(D)** New Field) representing "open field" and "clutter/edge" habitats. Microphone placements indicated by white "X". Direction of microphone (*N* = 2 per site) coverage also indicated. Sites (*N* = 2; **(E)** Boat House, and **(F)** Elbow Lake) representing "open water" habitats. Microphone placements indicated by white "X". Direction of microphone (*N* = 1 per site) coverage also indicated.

Figure 3. Examples of open **(A)** and clutter/edge **(B)** recordings in which multiple species were present. The species with the highest call energy was classified automatically by SonoBat and any additional species present were classified manually using call design parameters described in **Table 1**. For each sequence I show an oscillogram (top) and a spectrogram (bottom).

Figure 4. Examples of individual bat passes in both open **(A)** and clutter/edge **(B)** habitats. Passes were defined as unbroken echolocation sequences (*N* τ 5 calls) with

similar pulse intervals that appeared to originate from a single bat. For each sequence I show an oscillogram (top) and a spectrogram (bottom).

Figure 5. Examples of terminal feeding buzzes in open **(A)** and clutter/edge **(B)** habitats. Buzzes were defined by a noticeable (*i*) increase in call rate (to τ 90 calls/s; pulse intervals below 11 ms) and (*ii*) decrease in peak frequency. For each sequence I show an oscillogram (top) and a spectrogram (bottom).

Figure 6. Single call examples for each sedentary species (**(A)** *E.fuscus*, **(B)** *M. leibii*, **(C)** *M. lucifugus*, **(D)** *M. septentrionalis*, and **(E)** *P. subflavus*) in an open habitat. For each call I show an oscillogram (top), spectrogram (bottom left) and power spectrum (bottom right).

Figure 7. Single call examples for each migratory species (**(A)** *L. borealis*, **(B)** *L. cinereus*, and **(C)** *L. noctivagans* in an open habitat. For each call I show an oscillogram (top), spectrogram (bottom left) and power spectrum (bottom right).

Figure 8. Single call examples for each sedentary species (**(A)** *E.fuscus*, **(B)** *M. leibii*, **(C)** *M. lucifugus*, **(D)** *M. septentrionalis*, and **(E)** *P. subflavus*) in a clutter/edge habitat. For each call I show an oscillogram (top), spectrogram (bottom left) and power spectrum (bottom right).

Figure 9. Single call examples for each migratory species: **(A)** *L. borealis*, **(B)** *L. cinereus*, and **(C)** *L. noctivagans* in a clutter/edge habitat. For each call I show an oscillogram (top), spectrogram (bottom left) and power spectrum (bottom right).

Table Legends

Table 1. Summary of SE Ontario bat species characteristics. Data were taken from the literature indicated in the far right column, and data for each species on ten characteristics are provided. For all measurements (average mass, average forearm length (FAL), wing loading (WL), aspect ratio (AR), search call duration, and peak frequency), averages are indicated with ranges in parentheses. If no averages were found, only ranges were provided. Preferred habitats are defined as: OF (open field), OW (over water), CL (within dense clutter), and ED (within edge habitats). An asterisk indicates which habitat, if any, that species is considered a specialist in. Migratory status is classified as either “migratory” or “sedentary”, as per my definitions. “Migratory” refers to any species that participates in seasonal, two-way movements with relatively large latitudinal shifts from colder climates to warmer (more favourable) climates. “Sedentary” refers to any species who either (*i*) remain in the same general area over winter, or (*ii*) participate in movements with relatively shorter latitudinal shifts. At-risk status is defined as LC (least concern), T (threatened), or E (endangered), as listed by the Government of Ontario as of 2018.

Table 2. Summary of pass and buzz data comparisons between 2007/8 and 2017 for open habitats. For each species, the results of either the Wilcoxon signed rank tests (for passes, proportion of passes, buzzes, and proportion of buzzes data) or two-tailed tests for differences between two population regression coefficients (regression coefficients

of cumulative passes and buzzes data) are presented. For p values less than 0.05, the direction of the difference is noted (i.e. which years had the higher and lower average / proportion / regression coefficient). When no data was available for a given species in one or both years, these results were displayed as “N/A”.

Table 3. Summary of pass and buzz data comparisons between 2007/8 and 2017 for clutter/edge habitats. For each species, the results of either the Wilcoxon signed rank tests (for passes, proportion of passes, buzzes, and proportion of buzzes data) or two-tailed tests for differences between two population regression coefficients (regression coefficients of cumulative passes and buzzes data) are presented. For p values less than 0.05, the direction of the difference is noted (i.e. which years had the higher and lower average / proportion / regression coefficient). When no data was available for a given species in one or both years, these results were displayed as “N/A”.

Supplementary Table Legends

Supplementary Table 1. Summary of the 2007/8 (above) and 2017 (below) total count, average proportion, and log-transformed cumulative sum slope data for each of the five sedentary species. Summaries for pass and buzz data in each of the three habitats (open field, clutter/edge, and over water) are provided.

Supplementary Table 2. Summary of the 2007/8 (above) and 2017 (below) total count, average proportion, and log-transformed cumulative sum slope data for each of the three migratory species. Summaries for pass and buzz data in each of the three habitats (open field, clutter/edge, and over water) are provided.

Figure 1.

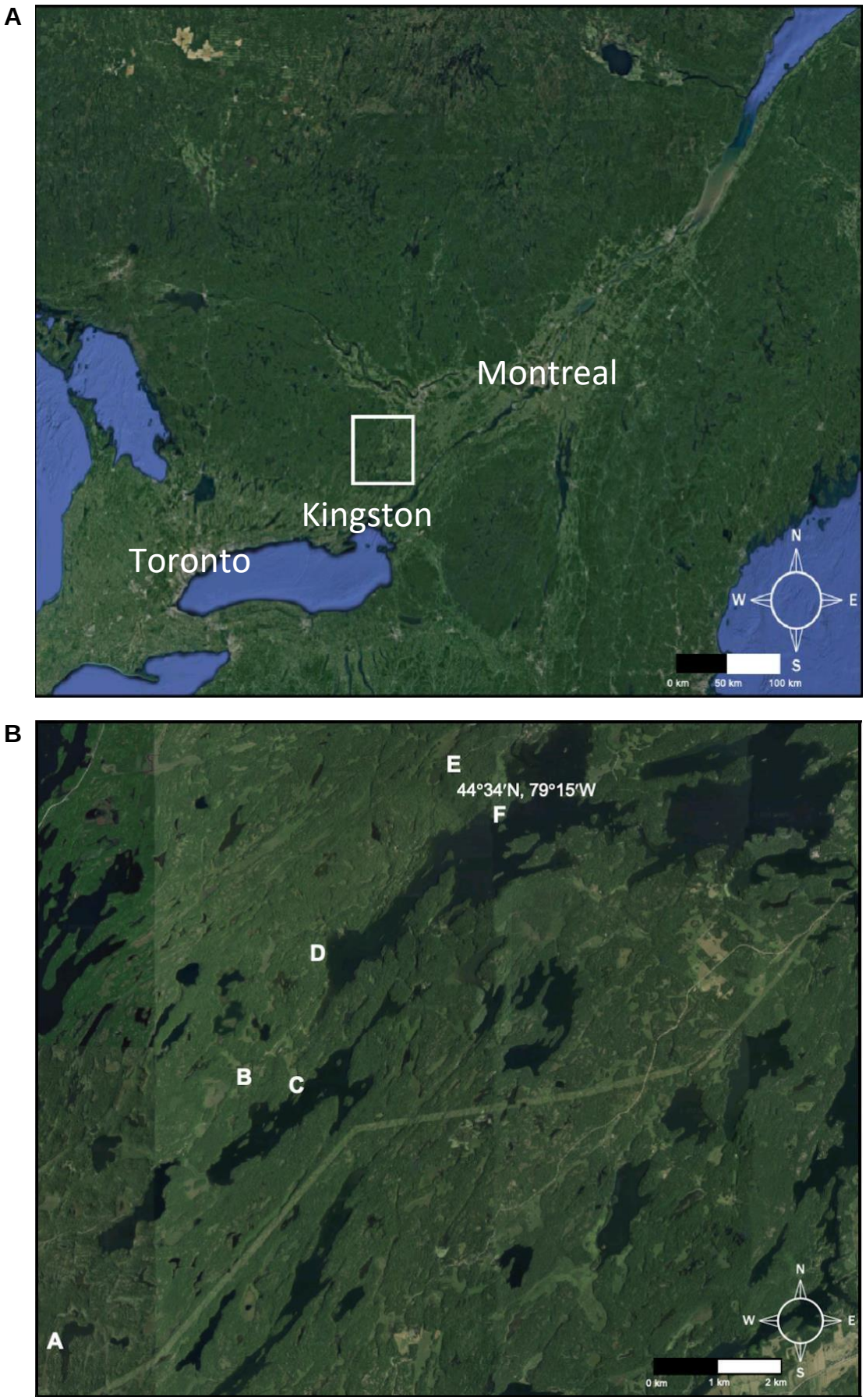

A
Montreal
Kingston
Toronto
N
W
E
S
0 km
50 km
100 km
B
E
44°34′N, 79°15′W
F
D
B
C
A
N
W
E
S
0 km
1 km
2 km

Figure 2.

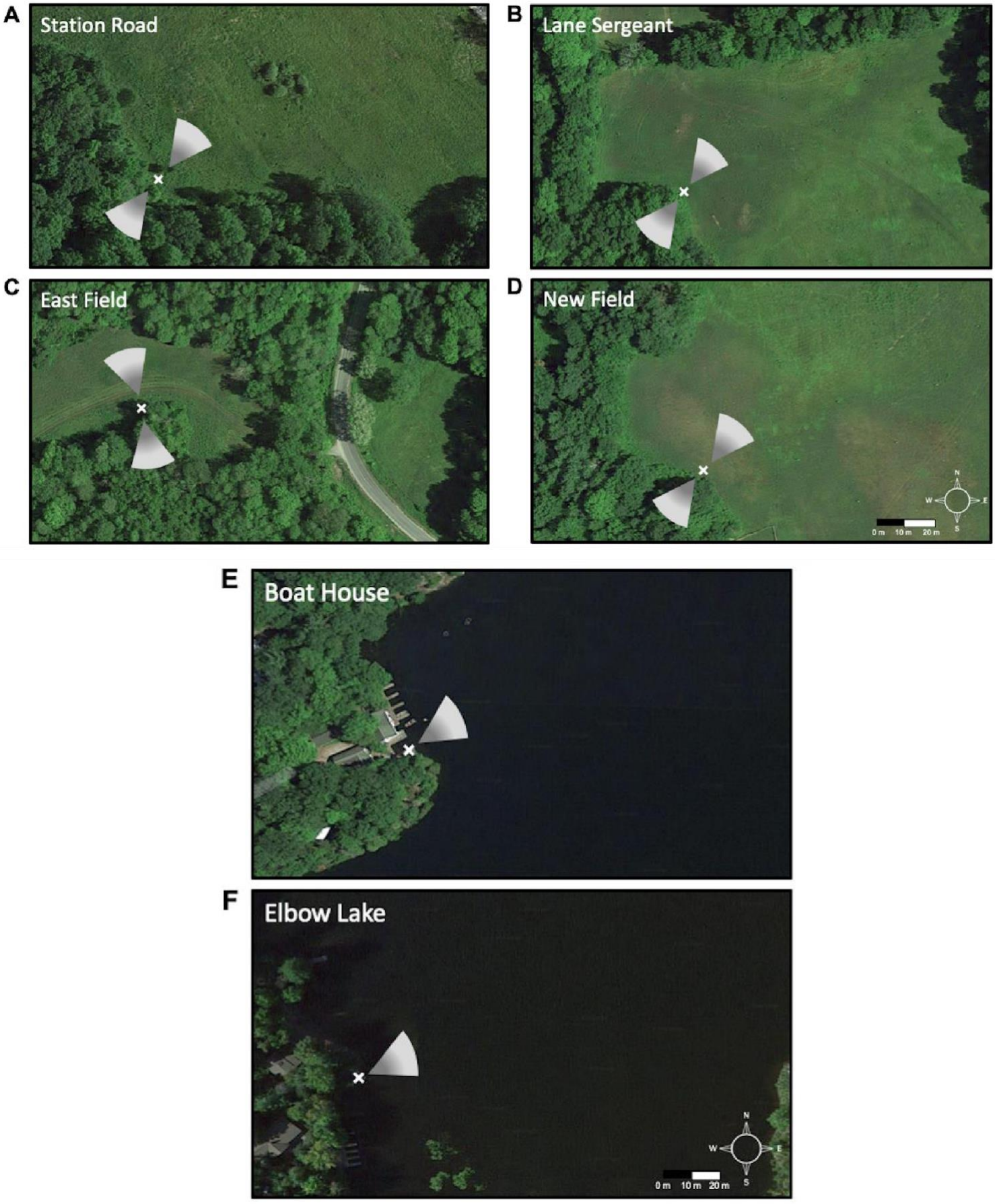

A
Station Road
B
Lane Sergeant
C
East Field
D
New Field
N
W
E
S
0 m 10 m 20 m
E
Boat House
F
Elbow Lake
N
W
E
S
0 m 10 m 20 m

Figure 3.

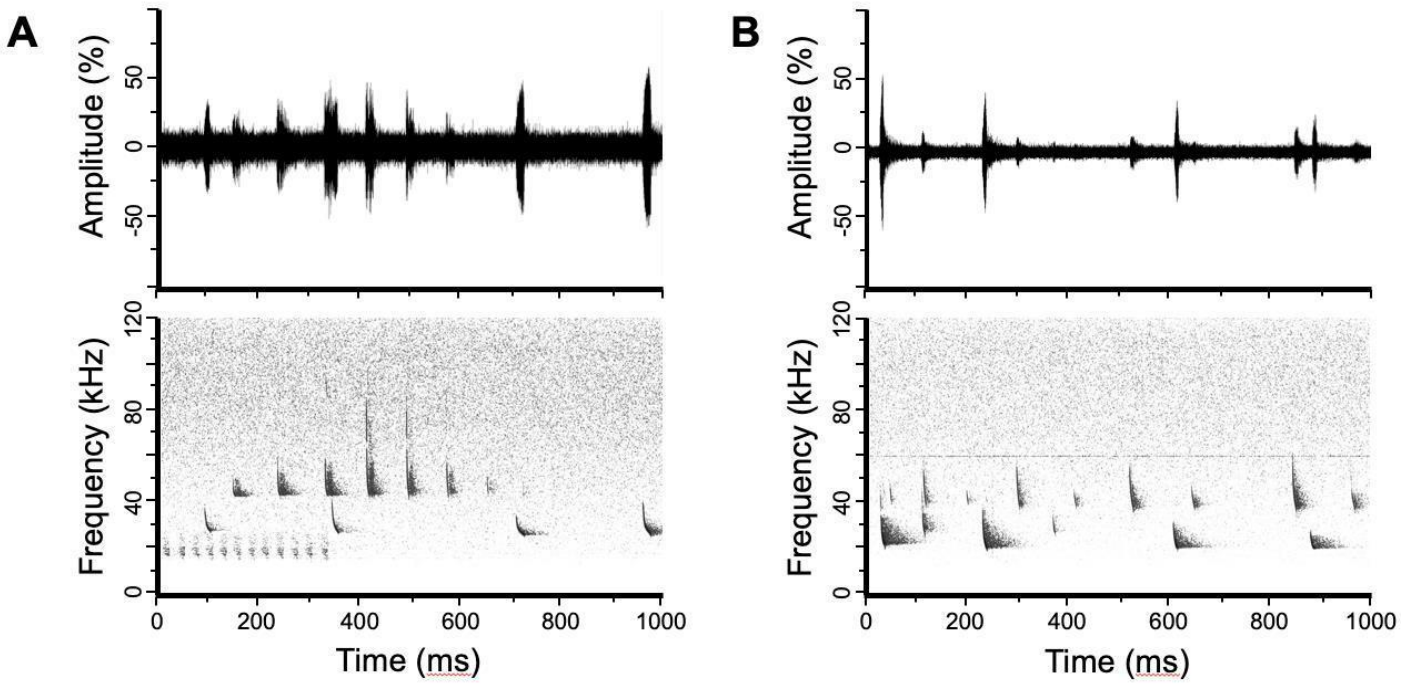

A
Amplitude (%)
50
0
-50
Frequency (kHz)
120
80
40
0
0
200
400
600
800
1000
Time (ms)
B
Amplitude (%)
50
0
-50
Frequency (kHz)
120
80
40
0
0
200
400
600
800
1000
Time (ms)

Figure 4.

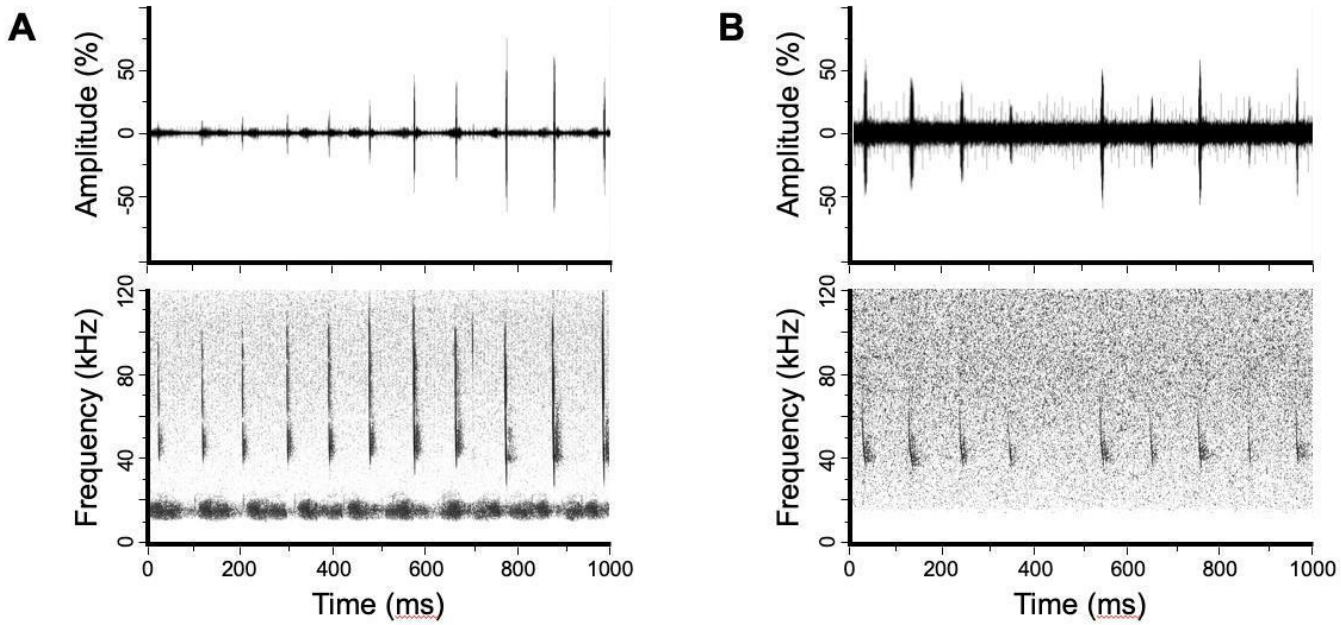

A
Amplitude (%)
50
0
-50
Frequency (kHz)
120
80
40
0
0
200
400
600
800
1000
Time (ms)
B
Amplitude (%)
50
0
-50
Frequency (kHz)
120
80
40
0
0
200
400
600
800
1000
Time (ms)

Figure 5.

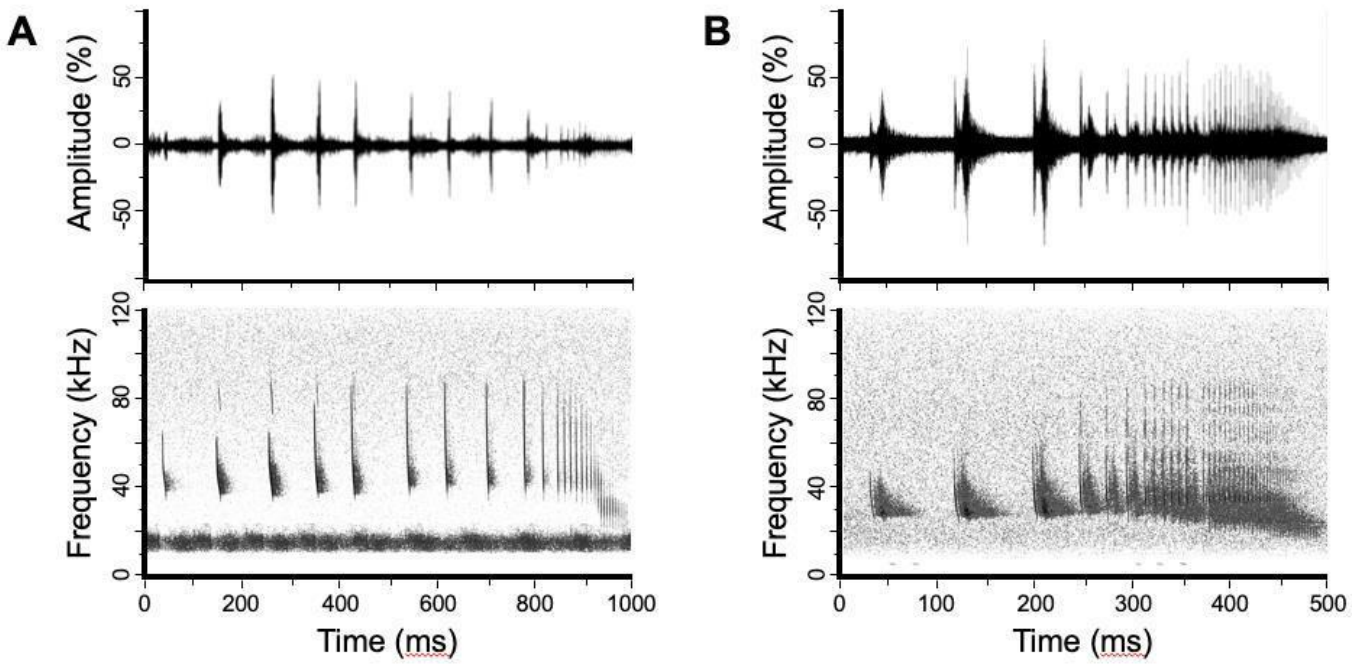
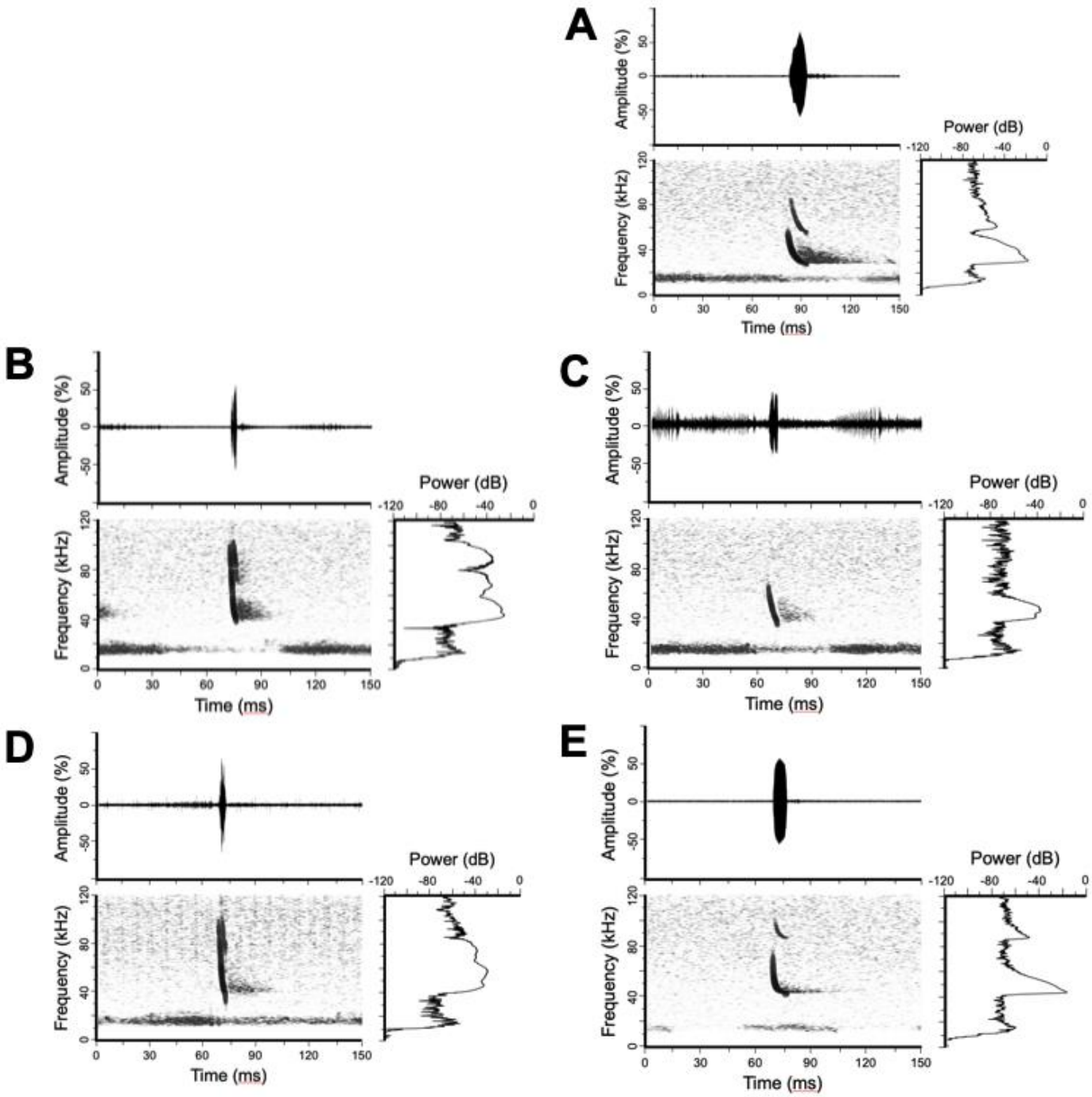

A
Amplitude (%)
50
0
-50
Frequency (kHz)
120
80
40
0
0
200
400
600
800
1000
Time (ms)
B
Amplitude (%)
50
0
-50
Frequency (kHz)
120
80
40
0
0
100
200
300
400
500
Time (ms)

Figure 6.



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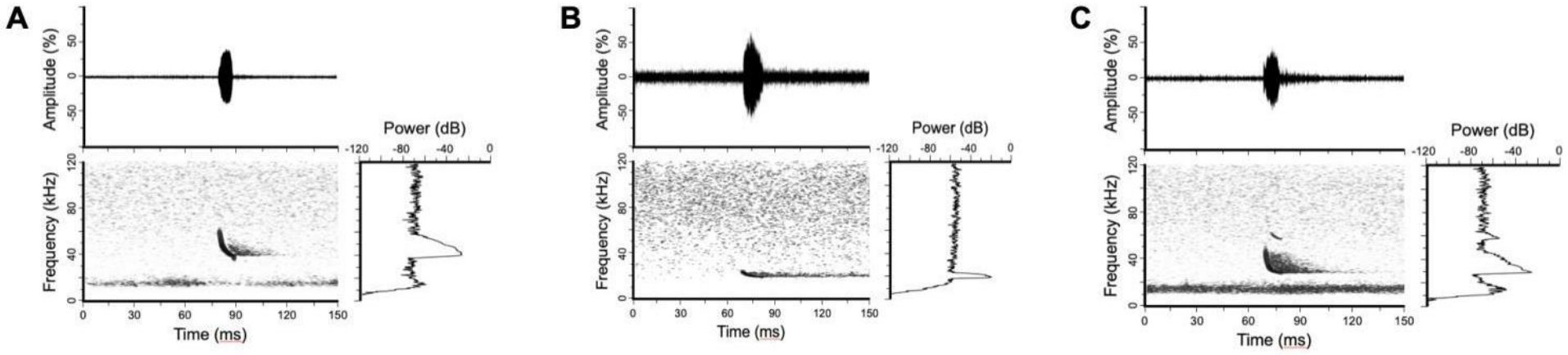

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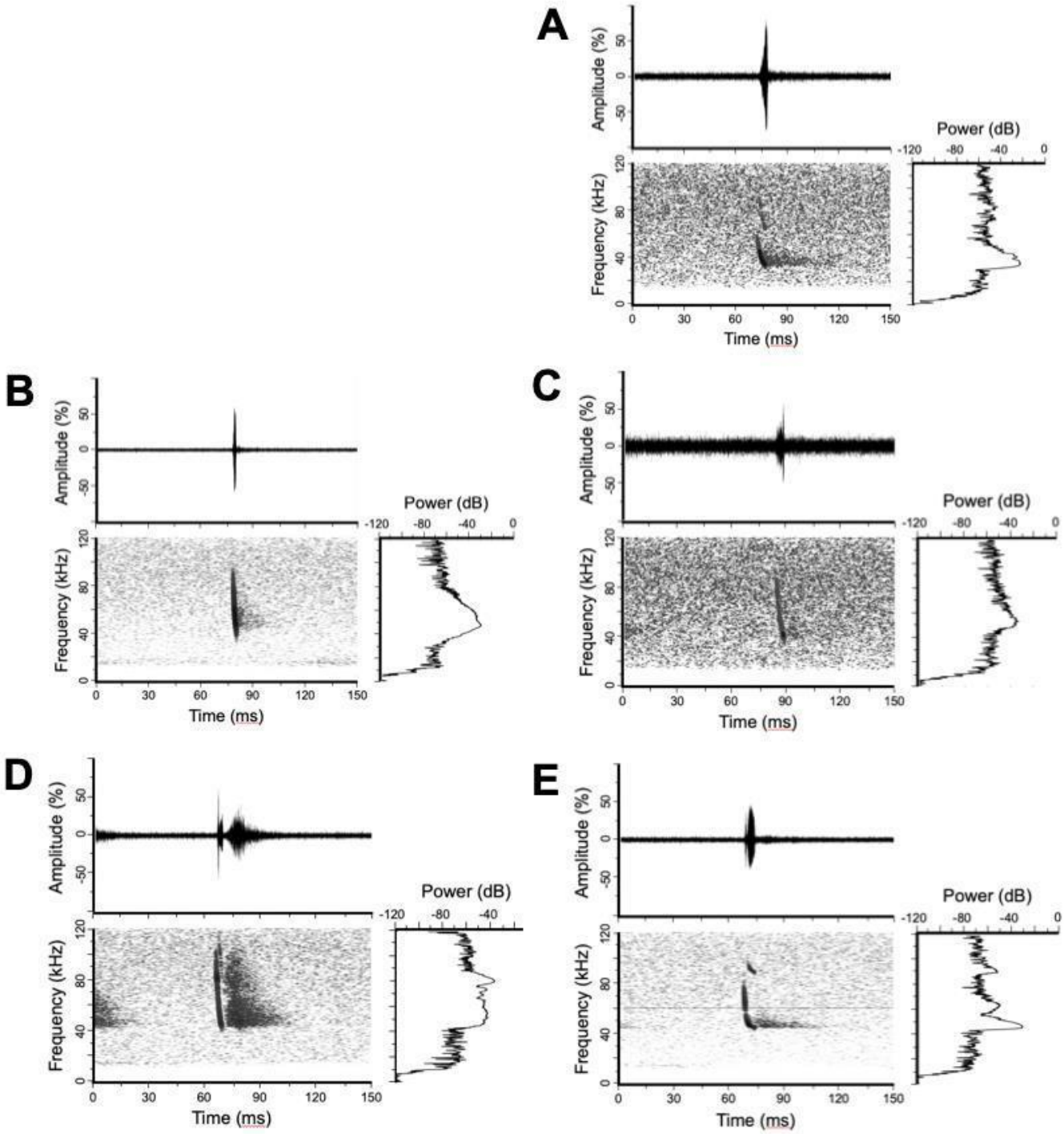

Figure 9.

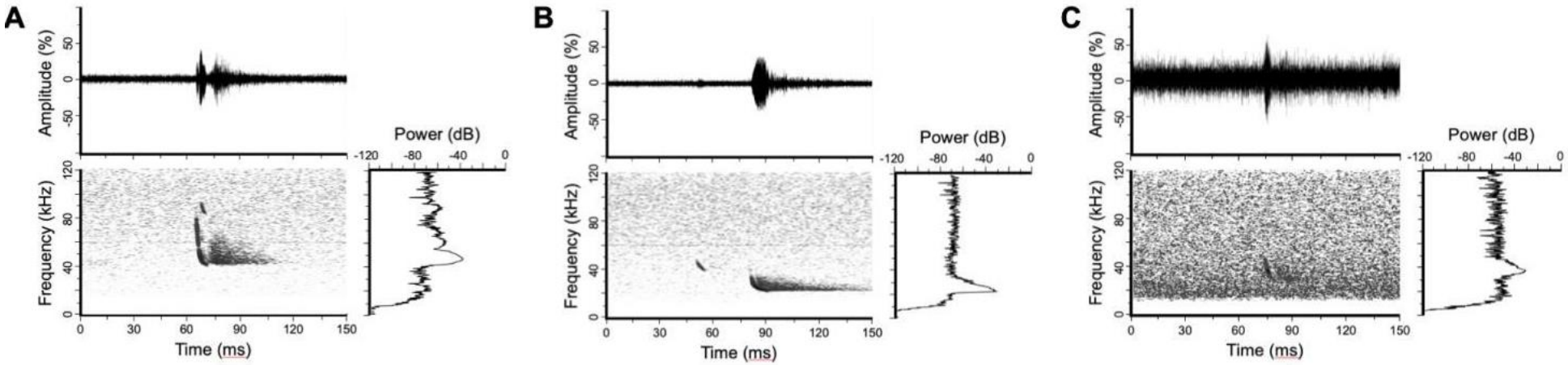
A
B
C
Amplitude (%)
50
0
-50
Power (dB)
-120
-80
-40
0
Frequency (kHz)
120
80
40
0
0
30
60
90
120
150
Time (ms)

Table 1.

Species	Mass (g)	FAL (mm)	WL	AR	Call duration (ms)	Peak freq. (kHz)	Diet	Habitat	Migratory Status	SARO Status	Sources
E. fuscus	11-23	39-54	9.4 (8.2-10.6)	7.06 (6.12-8.19)	7-10	28 (28-33)	**Coleoptera**, Hemiptera, Diptera, Hymenoptera, Homoptera, Lepidoptera	OF, OW, CL, ED	Sedentary	LC	Phillips 1966; Mills 2013; Kurta and Baker 1990; Agosta 2002; Furlonger et al 1987; Fenton and Bell 1981; Balcombe and Fenton 1988; Brigham and Cebek 1989; Norberg and Rayner 1987; Farney and Fleharty 1969
L. borealis	13 (8-16)	40.6 (39-41)	10.6 (8.4-11.4)	6.7 (6-7.55)	> 5 (5-10)	29-43	Homoptera, Coleoptera, Hymenoptera, Diptera, Lepidoptera	OF, ED	Migratory	LC	Shump and Shump 1982a; Salcedo et al 1995; Mills 2013; Furlonger et al 1987; Balcombe and Fenton 1988; Brigham and Cebek 1989; Norberg and Rayner 1987; Farney and Fleharty 1969
L. cinereus	31 (25-35)	52.6 (50.2-54.2)	15.6 (12.4-16.5)	8.25 (7-9.2)	> 5 (5-15)	16-28	**Lepidoptera**. Coleoptera, Diptera, Orthoptera, Blattodea, Odonata, Hymenoptera	OF, ED	Migratory	LC	Shump and Shump 1982b; Salcedo et al 1995; Mills 2013; Furlonger et al 1987; Fenton and Bell 1981; Balcombe and Fenton 1988; Norberg and Rayner 1987; Farney and Fleharty 1969
L. noctivagans	8-11	41 (40-43)	7.9 (6-10.3)	7.3 (6.5-7.9)	> 5 (5-9)	28-30	**Lepidoptera**, Hemiptera, Coleoptera, Diptera, Trichoptera	OF, ED	Migratory	LC	Kunz 1982; Mills 2013; Dzal et al 2009; McGuire et al 2012; Norberg and Rayner 1987; Farney and Fleharty 1969
M. lebeii	4 (3.2-6.5)	32	6.5-6.7	6.1-7.04	5 (2-5)	44	Trichoptera, Coleoptera, Lepidoptera, Diptera	OF, ED	Sedentary	E	Best and Jennings 1997; Woodsworth 1981; Fenton and Bell 1981; Murray et al 2001; Norberg and Rayner 1987; Farney and Fleharty 1969
M. lucifugus	7 (6-11)	33-41	7.5 (5.4-9.6)	6 (5.78-7.19)	< 5 (2-4)	45 (44-55)	Trichoptera, Diptera, Isoptera, Coleoptera, Ephemeroptera, Lepidoptera, Homoptera	OF, OW, CL, ED	Sedentary	E	Fenton and Barclay 1980; Meteyer et al 2011; Powers et al 1991; Kalcounis and Brigham 1995; Ratcliffe and Dawson 2003; Dzal et al 2009; Mills 2013; Fenton and Bell 1981; Norberg and Rayner 1987; Farney and Fleharty 1969
M. septentrionalis	5-8	36 (34-38)	5.1 (4.8-6.8)	6.2 (5.8-6.5)	<4 (1-3)	59	**Lepidoptera**, Coleoptera, Neuroptera, Diptera, Hemiptera, Homoptera, Hymenoptera	OW, CL*, ED	Sedentary	E	Caceres and Barclay 2000; Ratcliffe and Dawson 2003; Miller and Treat 1993; Mills 2013; Fenton and Bell 1981
P. subflavus	4.6-8	31.4-34	6.2 (4.9-8.1)	6.9 (6.2-7.6)	6.9 (6.2-7.6)	43-46	Coleoptera, Homoptera, Diptera, Hymenoptera, Lepidoptera	OF, OW*, ED	Sedentary	E	Fujita and Kunz 1984; Mills 2013; Norberg and Rayner 1987; Farney and Fleharty 1969

Table 2.

Species	Passes	Prop. passes	Reg. coeff. passes	Buzzes	Prop. buzzes	Reg. coeff. buzzes
E. fuscus	*P*= 0.47	*P*= 0.15	*P*= 0.22	*P*= 0.41	*P*= 0.08	*P*= 0.18
L. borealis	*P*= 0.16	*P* = 0.04 2017>2007/8	*P* < 0.001 2017<2007/8	*P*= 0.69	*P*= 0.44	*P*= 0.08
L. cinereus	*P* = 0.002 2017>2007/8	*P* = 0.001 2017>2007/8	*P* < 0.005 2017>2007/8	*P*= 1	*P*= 1	*P*= 0.09
L. noctivagans	*P*= 0.64	*P*= 0.84	*P* < 0.001 2017<2007/8	*P*= 0.75	*P*= 0.50	*P* < 0.001 2017<2007/8
M. leibii	*P*= 0.60	*P*= 0.38	*P* < 0.001 2017<2007/8	*P*= 1	*P*= 0.69	*P* < 0.001 2017<2007/8
M. lucifugus	*P* < 0.002 2017<2007/8	*P* < 0.02 2017<2007/8	*P* < 0.001 2017<2007/8	*P* < 0.001 2017<2007/8	*P* < 0.001 2017<2007/8	*P* < 0.001 2017<2007/8
M. septentrionalis	*P* = 0.03 2017<2007/8	*P* = 0.03 2017<2007/8	*P* < 0.001 2017<2007/8	*P*= 0.50	*P*= 0.50	*P* < 0.001 2017<2007/8
P. subflavus	*P*= 0.17	*P*= 0.32	*P* < 0.005 2017<2007/8	*P*= 0.38	*P*= 0.73	*P* < 0.001 2017<2007/8
E. fuscus / L. noct.	*P*= 0.22	*P*= 0.22	*P* < 0.001 2017>2007/8	N/A	N/A	N/A

Table 3.

Species	Passes	Prop. passes	Reg. coeff. passes	Buzzes	Prop. buzzes	Reg. coeff. buzzes
E. fuscus	$P < 0.008$ 2017>2007/8	$P = 0.10$	$P = 0.18$	$P = 0.50$	$P = 0.50$	$P < 0.001$ 2017>2007/8
L. borealis	$P < 0.004$ 2017>2007/8	$P < 0.004$ 2017>2007/8	$P < 0.001$ 2017>2007/8	$P = 0.25$	$P = 0.25$	$P < 0.001$ 2017>2007/8
L. cinereus	$P < 0.004$ 2017>2007/8	$P < 0.004$ 2017>2007/8	$P < 0.001$ 2017>2007/8	$P = 1$	$P = 1$	$P < 0.001$ 2017>2007/8
L. noctivagans	$P = 0.03$ 2017>2007/8	$P = 0.03$ 2017>2007/8	$P < 0.001$ 2017>2007/8	$P = 0.50$	$P = 0.50$	$P < 0.001$ 2017>2007/8
M. leibii	$P = 0.38$	$P = 0.63$	$P = 0.01$ 2017>2007/8	$P = 0.50$	$P = 0.50$	$P < 0.001$ 2017>2007/8
M. lucifugus	$P = 0.47$	$P = 0.55$	$P = 0.38$	$P = 1$	$P = 1$	$P = 0.14$
M. septentrionalis	$P = 0.75$	$P = 0.88$	$P < 0.001$ 2017<2007/8	N/A	N/A	N/A
P. subflavus	$P = 0.25$	$P = 0.25$	$P < 0.001$ 2017<2007/8	$P = 1$	$P = 1$	$P < 0.001$ 2017>2007/8
E. fuscus / L. noct.	$P = 1$	$P = 1$	$P < 0.001$ 2017>2007/8	N/A	N/A	N/A

Supplementary Table 1.

Sedentary Species - 2007/8 Data

		E. fuscus			*M. leibii*			*M. lucifugus*			*M. septentrionalis*			*P. subflavus*		
		Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope
Pass Data	Open field	162	0.237	0.024	16	0.029	0.016	303	0.327	0.028	9	0.062	0.015	55	0.047	0.017
	Clutter/edge	2	0.019	0.008	3	0.036	0.01	8	0.063	0.01	4	0.073	0.007	0	0	0
	Water	16	0.012	0.026	3	0.009	0.011	297	0.812	0.037	3	0.024	0.008	73	0.102	0.036
Buzz Data	Open field	11	0.062	0.016	4	0.03	0.011	86	0.343	0.024	2	0.004	0.007	15	0.043	0.011
	Clutter/edge	0	0	0	0	0	0	1	0.048	0.005	0	0	0	0	0	0
	Water	1	0.004	0.007	0	0	0	47	0.439	0.03	0	0	0	12	0.116	0.021

Sedentary Species - 2017 Data

		E. fuscus			*M. leibii*			*M. lucifugus*			*M. septentrionalis*			*P. subflavus*		
		Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope
Pass Data	Open field	279	0.339	0.019	10	0.024	0.007	34	0.122	0.01	0	0	0	20	0.039	0.008
	Clutter/edge	18	0.052	0.01	8	0.022	0.006	13	0.038	0.012	2	0.05	0.004	5	0.011	0.01
	Water	33	0.146	0.033	3	0.016	0.014	105	0.136	0.041	0	0	0	6	0.014	0.017
Buzz Data	Open field	18	0.269	0.013	4	0.042	0.005	0	0	0	0	0	0	5	0.077	0.004
	Clutter/edge	2	0.071	0.004	2	0.048	0.003	1	0.048	0.003	0	0	0	1	0.048	0.004
	Water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Table 2.

Migratory Species - 2007/8 Data

		L. borealis			*L. cinereus*			*L. noctivagans*		
		Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope
Pass Data	Open field	51	0.046	0.019	9	0.004	0.013	135	0.045	0.03
	Clutter/edge	0	0	0	0	0	0	0	0	0
	Water	27	0.04	0.031	0	0	0	1	0.008	0.004
Buzz Data	Open field	6	0.019	0.008	3	0.004	0.007	6	0.046	0.013
	Clutter/edge	0	0	0	0	0	0	0	0	0
	Water	4	0.025	0.014	0	0	0	0	0	0

Migratory Species - 2017 Data

		L. borealis			*L. cinereus*			*L. noctivagans*		
		Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope	Total Count	Average Proportion	Slope
Pass Data	Open field	219	0.259	0.008	93	0.095	0.023	9	0.009	0.008
	Clutter/edge	48	0.168	0.01	18	0.095	0.008	26	0.103	0.015
	Water	30	0.025	0.03	10	0.025	0.021	2	0.015	0.012
Buzz Data	Open field	9	0.077	0.006	1	0.006	0.005	1	0.005	0.004
	Clutter/edge	3	0.095	0.007	1	0.048	0.004	2	0.071	0.008
	Water	0	0	0	0	0	0	0	0	0

Chapter Five

Concluding Remarks

Chapter 5: Concluding Remarks

Sensory ecology explores how environmental information is obtained, processed, and used by different organisms. Using this information to optimize foraging behaviours illustrates how sensory systems can relate directly to the effective acquisition of energy. Echolocation is a relatively rare sensory system employed by a handful of vertebrate groups (Fenton 1984). Of all echolocators, only laryngeally echolocating bats and toothed whales use echolocation to hunt and, correspondingly, are the only echolocators known to produce landing and / or feeding buzzes (Madsen and Surlykke 2013). These buzzes are extreme examples of acoustic signal production and control and, when combined with associated motor outputs, make bats and toothed whales ideal model systems for exploring the flexibility and limitations of sensorimotor integration.

In this thesis, I first explored whether bats and / or toothed whales adjust their acoustic behaviour when hunting in the presence of conspecifics. Next, I investigated the development of the landing buzz in big brown bat pups from acoustic, flight, and morphological perspectives and identified correlations between different measures of juvenile bat growth. Finally, I analysed one of the most robust and comprehensive data sets of acoustic monitoring information for pre- and post- white-nose syndrome bat activity in South Eastern Ontario and identified the influence of species-specific population declines on relatively unaffected remaining species. Together, these projects will support further research and promote an inclusive understanding of sensorimotor integration, sensory system flexibility, and the natural behaviours of mammalian echolocators.

Each of my data chapters uses echolocation signals to extrapolate information regarding additional behaviours. In my first project, for example, I use changes in spectral, temporal, and directional signal information to identify behavioural responses to acoustic clutter (Moss and Surlykke 2010; Surlykke et al. 2009). In my second project, these spectral and temporal parameters were used to define the appearance and characteristics of a stereotypical landing buzz as well as its precursors (Melcón et al. 2007; Ratcliffe et al. 2013). Finally, in my third project, echolocation signals were used to identify species presence between habitats (where different species are located) as well as hunting activities (whether those species are hunting there). In addition to being behaviourally informative, acoustic recordings of echolocating mammals also provide a glimpse into a world that is otherwise unavailable to us for study. Much of the information emitted by echolocating mammals, specifically vespertilionid bats, is ultrasonic and therefore inaudible to humans without sophisticated recording and analysis technology (Jones 2005). As the study of bioacoustics continues, these data acquisition and analysis programs will likely become more advanced and allow future research to delve further into the intricacies of signal production and processing.

My thesis explores echolocation and associated activities (e.g. hunting, locomotion, and landing) through multiple ethological perspectives and using multiple methodologies. I have combined experimental studies with natural observations to develop a comprehensive and robust analysis of the flexibility and ecological limitations of sensorimotor integration in echolocating mammals. As with all research, however, my data chapters allow for further analysis and study. Bat and porpoise foraging data (from

Chapter 2) can be processed for flight and swim speeds to consider the effects of food competition on motor activity outputs. Additionally, performing this study under conditions that are more representative of natural hunting conditions may provide more accurate and reliable results, such as using multiple conspecifics and mobile prey.

With respect to my third chapter, future studies should consider whether the development of feeding buzzes occurs simultaneously with the landing buzz. Developmentally, it seems reasonable that pups experience surface landings prior to actively hunting moving prey. As indicated in my Chapter 3 discussion, captive adult bats are generally larger than wild adult bats, due to consistently available resources, reduced flight time, and constant temperatures / environmental conditions (Mayberry and Faure 2015). While my growth rates and developmental trajectories of captive animals were similar to those in the literature of wild juveniles (Kurta and Baker 1990), it is still possible that due to different environmental conditions, captive pups may not fully represent pup development (including landing buzzes) observed in the wild. Supplementing laboratory studies with data from wild animals, then, may help to confirm my results.

Finally, my final data chapter (Chapter 4) could be augmented with mist net capture data to confirm species presence in different habitats, as well as insect capture surveys to identify whether the prey in different habitats is more suitable for certain species. Additional recording nights over water sites would also be helpful to assess the potential changes in water activity pre- and post- white-nose syndrome introduction. Analyses would also be improved with more matched evenings for comparison overall. Since it is not possible to retroactively acquire pre- white-nose syndrome information in

South Eastern Ontario, future studies comparing pre-and post- white-nose syndrome bat activity should be conducted immediately in areas that (*i*) are currently not affected by white-nose syndrome and (*ii*) match the ecological conditions under which white-nose syndrome can flourish (i.e. high bat abundance, susceptible hibernating species, hibernation temperatures). Although ecologists hope that white-nose syndrome does not spread into currently unaffected areas, I cannot be naïve enough to assume this disease will not continue to spread. Bats do a good job of transmitting the fungus even without accidental human assistance, and *Pseudogymnoascus destructans* infection across North America seems, unfortunately, likely.

Referring back to the four questions of ethology (Tinbergen 1963), my thesis confirms the importance of approaching a topic from multiple perspectives. By exploring the influence of conspecifics, I approached echolocation from both evolutionary and mechanistic perspectives. I provided information regarding (*i*) how natural selection experienced by different species has influenced echolocation with respect to acoustic clutter and (*ii*) the physiological basis for acoustic adjustments observed under different environmental conditions. In my second project, I took an ontogenetic approach to natural echolocation behaviours and identified (*iii*) the sequence of developmental traits that bring about the landing buzz during a pup's infancy, and (*iv*) how both vocal and flight abilities change throughout sequential life stages, including potential underlying morphological mechanisms. Finally, my third data chapter exploring foraging niche release approached echolocation from the perspective of its current adaptive function. From this study, I identified (*v*) how variations in echolocation, physiology, and flight ability interact with habitat to influence species-specific foraging efficiency, and (*vi*) how

bat sensorimotor integration is limited by the differences between species-specific realized and fundamental, or potential, foraging niches.

This conceptual overview of echolocation and associated sensorimotor integration has provided additional insight into animal communication, sensory ecology, and behavioural ecology. By using laryngeally echolocating bats and toothed whales specifically, I have examined natural echolocation behaviour at an extreme physiological level. Through mechanistic, developmental, evolutionary, and functional perspectives, I have provided evidence supporting the limited flexibility of echolocation behaviours and identified areas of sensory and foraging ecology that would benefit from further study.

References

Fenton, M. B. (1984). Echolocation: implications for ecology and evolution of bats. *The Quarterly Review of Biology*, *59*, 33-53.

Jones, G. (2005). Echolocation. *Current Biology*, *15*, R484-R488.

Kurta, A., & Baker, R. H. (1990). *Eptesicus fuscus*. *Mammalian Species*, (356), 1-10.

Madsen, P. T., & Surlykke, A. (2013). Functional convergence in bat and toothed whale biosonars. *Physiology*, *28*, 276-283.

Mayberry, H. W., & Faure, P. A. (2015). Morphological, olfactory, and vocal development in big brown bats. *Biology Open*, *4*, 22-34.

Melcón, M. L., Denzinger, A., & Schnitzler, H. U. (2007). Aerial hawking and landing: approach behaviour in Natterer's bats, *Myotis nattereri* (Kuhl 1818). *Journal of Experimental Biology*, *210*, 4457-4464.

Moss, C. F., & Surlykke, A. (2010). Probing the natural scene by echolocation in bats. *Frontiers in Behavioral Neuroscience*, *4*, 33.

Ratcliffe, J.M., Elemans, C.P.H., Jakobsen, L., & Surlykke, A. (2013) How the bat got its buzz. *Biology Letters*, 9, 2012103.

Surlykke, A., Ghose, K., & Moss, C. F. (2009). Acoustic scanning of natural scenes by echolocation in the big brown bat, *Eptesicus fuscus*. *Journal of Experimental Biology*, *212*, 1011-1020.