Foraging ecology of a bat assemblage

by

Marisa Reese Arh, H.B.Sc.

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Department of Ecology and Evolutionary Biology

University of Toronto

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ABSTRACT

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Marisa Reese Arh, Masters of Science, 2009
Ecology and Evolutionary Biology, University of Toronto

Here I examine five non-migratory sympatric bat species that are similar in their morphology and general ecology: *Eptesicus fuscus*, three myotid bats, *Myotis leibii*, *M. lucifugus*, *Myotis septentrionalis*, and *Pipistrellus (= Perimyotis) subflavus*. By examining echolocation call structure, wing and jaw morphology and diet, I defined finer niche differences between species. *M. lucifugus* and *M. septentrionalis* differ in the relative abundance of moths in their diet. My results on skull morphology suggest *M. lucifugus* consumes harder insects than *M. septentrionalis*. Conversely, my data suggests *M. leibii* is not as efficient within a cluttered habitat but is capable of foraging in edge habitats. *Eptesicus fuscus* is capable of efficiently consuming larger insects whereas *P. subflavus* has echolocation and jaw characteristics that indicate specialization in smaller insects. Significant differences amongst these 5 species are evident based on my data of overall morphology and diet.
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INTRODUCTION

Bats have evolved to exploit night skies through a unique combination of two mammalian adaptions: flight and echolocation (Kalko 1995). These two characteristics constrain bat foraging on a broad scale such that individual bat species are not able to efficiently and successfully hunt all available prey in all available foraging habitats. Foraging behaviour, partially as determined by morphology, predicts these fine-grained niches (Siemers & Schnitzler 2004; Nicholls & Racey 2006b; Costa et al. 2008; Lloyd 2008). Ecomorphological studies predict that the foraging behaviour of a bat species should relate to its wing morphology and observed echolocation call designs (Norberg & Rayner 1987; Brigham et al. 1997; Ratcliffe & Dawson 2003; Stoffberg & Jacobs 2004). In turn, this should allow us to determine likely foraging strategies and habitats for a particular species (Norberg & Rayner 1987). Usually this study involves the comparison of bat species based on their call structure and wing morphology (Ratcliffe et al. 2005; Dietz et al. 2006; Zhang et al. 2007). Those differences can then be related to their ecology to discern interspecific relationships.

Outside of the tropics and subtropics, however, bat species tend to belong to the same or similar guilds with less diversity (Arlettaz 1999; Stevens & Willig 2002). Species that make up a local temperate bat community tend to be quite similar morphologically (Patten 2004). These similar sympatric species often do not differ to the degree used to define them with respect to their general ecomorphology and small differences between species will often lead them to be classified in the same coarse grain foraging guild (Arlettaz 1999). This makes it difficult to ascertain how such a community can successfully occupy a habitat where it consists of similar species with similar gross niches. In a bat community consisting of species with similar morphology and echolocation characteristics, there must be a more information on detailed
aspects of ecology and morphology to ascertain the differences between species (Siemers & Schnitzler 2004). For example, two open habitat aerial hawking bats likely do not coexist in the same area without some level of difference in niche characteristics. Here aerial hawking is defined as a foraging behaviour where a flying bat will hunt and capture a flying insect, compared to gleaning where a bat will hover and capture an insect resting on a substrate. However, if there is a minimum level of difference (which may not be evident through basic morphology and echolocation analyses) such as temporal, spatial, or resource variances, then two seemingly similar species may occupy the same habitat while occupying individual niches. Where two sympatric species appear to have the same niche, there must be further investigation to determine what differences do allow for sympatry.

Bat echolocation calls vary in duration (10-100ms), frequencies (10-212 kHz) and intensities (40-130dB). Some echolocation call designs are better suited to specific habitats than others and the designs available to particular bat species tend to reflect where and upon what a species will forage (Neuweiler 1984; Schnitzler & Kalko 2001). Certain call designs are better suited to cluttered environments whereas others allow for successful foraging in open spaces. In a cluttered environment, a bat must differentiate between clutter echoes and echoes generated by would-be prey (Siemers et al. 2001). Shorter duration calls (<10ms) minimize call-echo overlap in a cluttered environment. Bats which primarily hunt airborne insects in open habitats do not normally face these same demands as any echo returned is typically indicative of a flying insect. Further, broader bandwidth and higher peak frequency calls (in my study greater than 25 kHz and 50 kHz respectively) should improve the discrimination of prey from background clutter (Siemers et al. 2001). On the other hand, lower frequency, narrower bandwidth calls are ideal for long range sensing due to less acoustic attenuation but less suitable for the location of smaller
insects due to their long wavelengths (Lawrence & Simmons 1982; Holderied & von Helversen 2003). Interspecific differences are associated with sensory constraints, and opportunities, and should relate to the habitats species are found (Faure & Barclay 1994). Many insectivorous gleaning bats use prey-generated sounds, and perhaps even visual cues, to detect and localize potential prey (Bell 1985; Schnitzler & Kalko 2001; Hubner & Wiegrebe 2003; Rydell & Eklof 2003; Russo et al. 2007).

For insectivorous bats, wing morphology plays a key role in constraining and allowing for specific foraging behaviours governed by manoeuvrability and sometimes, the ability to hover. Finding food requires efficient flight and flight costs increase with body size so bats may have had selective pressures over time to minimize their mass (Dumont et al. 2005). Foraging behaviour and habitat preference can be predicted by wing morphology and vice versa, suggesting a tight correlation between morphology and ecology. Wing morphology in a given species tends to be correlated with primary foraging behaviours, habitat type, migratory habits, and prey size range (Norberg 1995). Wing morphology strongly influences flying ability and, along with call structure, may restrict species to certain habitats (Stockwell 2001). Aspect ratio describes the shape of the wing (higher ratio describes a narrow wing). Wing loading is the ratio of mass to surface area where a higher wing loading should equate to greater maximum flight speeds and lower maneuverability. High wing loading and high aspect ratio decrease the maneuverability of a bat, making it poorly suited to a cluttered environment (Findley 1972; Saunders & Barclay 1992).

Correlations exist between cranial morphology and dietary niche that can allow comparisons in diet composition and niche breadth (Freeman 2000; Metzger & Herrel 2005). A diet with harder insects would require jaw and dental components to efficiently capture and
consume food that would be more difficult to handle and process. It would be beneficial to
exploit the ability to consume harder foods than sympatric species since this would allow a
broader niche breadth than those species limited to only softer foods. Skulls are optimized to
meet the stresses and demands of holding and masticating food (Dumont et al. 2005). Larger
muscles and a wider jaw allow the stronger bite force necessary for the consumption of harder
foods. This would lead to selection for larger skulls that can attach these larger muscles.
Additionally, larger and wider jaws will have larger gapes without a correlative loss in maximum
bite force (Barlow et al. 1997). Skull shape (including mandible and cranial length and width) as
well as relative size will differ between species based on the pressures of food exploitation (York
& Papes 2007).

Mechanical advantage allows a measurement of how powerful the bite of the bat is based
on the linear measurements of the jaw. Mechanical advantage is ratio of the moment arm of the
muscle force to the moment arm of the bite force and it indicates how much muscle tension is
applied to generate force. The higher the mechanical advantage, the higher the bite force where
a bat with a higher mechanical advantage would be able to more efficiently consume harder prey
items than a bat with a lower mechanical advantage (Garcia-Morales et al. 2003). Different
predictions for differences in diet based on cranial morphology can be tested against field studies
by comparing morphology to diet. For example, analysis of fecal components will describe the
types of prey consume and in what differences of abundance thus determining if a given species
selectively forages which may be related to its morphology (Agosta et al. 2003).

Harder prey can be consumed with a more powerful bite, stronger jaws, and longer
canines (Freeman 1979a; Evans & Sanson 1998; Dumont 2007). How an animal bites or chews
food must remain flexible with changing hardness of captured food. Species will differ in how
they bit and chew food which is evident in preferred gape angles and bite points (VanValkenburgh 1996). The gape angle is the degree of the mouth opening and bite point is the point along the tooth row where force is transferred to a food item (Dumont & Herrel 2003). Species may or may not be selective in their diet but they must be able to consume different types of prey since availability will change (Agosta et al. 2003). Bats that consume a higher proportion of beetles have a robust jaw, well-developed sagittal crests and fewer but larger teeth than do those bats that eat primarily softer bodied insects (Freeman 1979b; Freeman & Lemen 2007). A broader availability of prey items is beneficial since it allows more choice (especially when the moth availability decreases), however this does come with the cost of having heavier jaws that requiring more energy and which may be more costly as flight should select for lower mass.

Prey characteristics determine the production of force and absorption of stress necessary to efficiently process a given food item (Freeman & Lemen 2007). Examination of the diet of a species should relate to its morphology; however, it is important to note that morphology alone will not give detailed information of diet since measures of hardness and size must be considered in an ecological context; two insects of similar size and hardness may differ in availability and ability to escape predation. Ideally, a survey of the insect composition of a given habitat would give information of all the resources available to a given species. Examination of the morphology would then allow predictions as to which food items the species is capable of efficiently consuming.

Within any community there will likely be niche-partitioning occurring to some degree. Spatial or temporal partitioning of relative diets may also allow for sympatry. For example, in Britain *Pipistrellus* and *P. pygmaeus* are cryptic, sympatric species that have only a 5% overlap
in their foraging areas (Nicholls & Racey 2006a). Significant differences in food may be due to behaviour- a larger species may be able to subdue and consume larger and thus more energetically beneficial prey. Differential access or consumption of prey may also be due to morphology that would allow more efficient processing of food (one species may be able to better crush a harder prey item than another species). There may be a combination of these factors determining niche partitioning; this is a simplified example since most bat communities consist of more than two species and in some locations approach 100 species. Therefore, when looking at a bat assemblage, morphology is only one factor of many that will allow and explain the successful coexistence of various species. These tradeoffs have allowed for bats to be divided into guilds based on their preferred habitat and foraging behaviour (Siemers & Schnitzler 2004). Ratcliffe et al. (2006) describe four groups for predatory bats: ground gleaning bats (forage stationary insects off a substrate), behaviourally flexible bats, clutter-tolerant aerial hawking bats and open spacing aerial hawking bats. For my thesis I will examine the importance of echolocation and wing and jaw morphology in identifying the complexity and components of a bat assemblage. Where species belong to the same group, I hypothesize that there will be biologically meaningful, identifiable differences between species that will help to explain the composition of this bat assemblage. Furthermore, I hypothesize that the foraging ecologies of the aforementioned bat species can be determined through a body of data that includes diet selection, call structure, foraging strategies, and skull and wing morphology.

All five residential species in southern Ontario are insectivorous bats belonging to the family Vespertilionidae (Fenton & Barclay 1980; Fujita & Kunz 1984; Kurta & Baker 1990; Best & Jennings 1997; Caceres & Barclay 2000). The eastern pipistrelle, Pipistrellus subflavus and the small-footed myotid, Myotis leibii are the smallest species, followed by both the little
brown bat, *Myotis lucifugus*, and the northern long-eared bat, *Myotis septentrionalis*. The largest species is the big brown bat, *Eptesicus fuscus* (respective weights of 5-7g, 3-5g, 4-11g, 4-7g, 10-21g; Fraser *et al.* 2007). These five residential vesperilionids are sympatric throughout southern Ontario (see Figure 1) and are morphologically similar on a coarse scale. Norberg and Rayner (1987) used wing morphology as a means of predicting foraging behaviour (aerial hawking, trawling, or gleaning) and foraging habitat (clutter, open, edge). Based on Norberg & Rayner (1987), wing morphology alone predicts that the three myotids should be capable of foraging in cluttered habitat (due to average wingspans and low aspect ratios) whereas *E. fuscus* and *P. subflavus* are classified as more open space aerial hawkers that should use less cluttered habitats. Both have low wing loading but *P. subflavus* is documented as having longer wings with lower wing loading than *E. fuscus* (therefore less maneuverable). Ratcliffe & Dawson (2003) showed that *M. lucifugus*, in addition to *M. septentrionalis*, is capable of gleaning in a cluttered environment. Thus, the broad niches of these sympatric species may overlap to varying degrees but there must be a minimum degree of difference on the fine grained niche scale to prevent competitive exclusion. By looking solely at wing morphology and echolocation characteristics, some other fine grained niche differences may be overlooked. In my thesis I describe how I use echolocation, wing morphology, diet, and jaw morphology to determine how niche partitioning occurs between species.

My study demonstrates that coarse grain differences in either wing morphology or echolocation call structure are not reliable indicators of niche differentiation in insectivorous bats (Arlettaz 1999); or that insectivorous bats do not experience much in the way of interspecific competition for resources.
MATERIALS AND METHODS

Animals and Trapping

All experiments were conducted at the Queen’s University Biological Station (QUBS) near Chaffey’s Lock, Ontario, Canada (44°34´N, 79°15´W) from late May to early September in 2006, 2007, and 2008. Fecal sampling was conducted in the last two years only and insect surveys were only conducted in 2008. Bats were captured in modified harp traps (Tuttle 1974). Trapping occurred at two abandoned mica test mines (one facing out onto a field and the other within a mixed deciduous old growth forest) and a culvert facing out to a beaver pond (see Figure 2). The sites common names are East Field, Eel Bay, and ‘Frank’s Hole’ respectively. Captured bats were housed in wooden bat boxes where they had access to water ad libitum and were fed mealworms, Tenebrio molitor, by hand.

Echolocation behaviour and foraging strategy

During the evenings bats were tested, which occurred within two nights of capture, each bat was kept in an individual cloth bag. Each bat was released at point of capture after their trials were completed. Moths and other insects were captured from light traps at QUBS. I used a flight room (9.14x3.66x3.66m) on a small glade in a mixed temperate forest where the canopy was intact and designed to mimic a cluttered habitat to assess echolocation flexibility (Ratcliffe & Dawson 2003). The walls and the ceiling of the flight room were framed in spruce, Picea glauca (3.66x0.1x0.05m studs spaced 0.61m apart on centre) and the entire structure was covered with 1 mm black fiberglass screen. For each trial one individual bat (either E. fuscus, M. leibii or P. subflavus) was introduced into the flight room and presented with a live moth that was tethered
but free flying or pinned to a substrate to simulate foraging situations that would demand either aerial hawking or substrate gleaning, respectively. One trial equaled the presentation of a single prey item followed by a successful or unsuccessful attempt at capture. Trial results for *Myotis lucifugus* and *Myotis septentrionalis* were taken from a 2003 study completed in this same flight room (Ratcliffe & Dawson 2003). Data was taken from this study since the same methodology was followed therefore it was not necessary to repeat the same trials for these two species.

I tested each bat’s ability to capture flying moths in the flight room by tethering a moth to the end of a 0.2 mm diameter black cotton thread, 1-2m long, hung approximately in the centre of the room. Throughout trials, the bat was free to fly and perch in the room. Due to a lack of success with moths, I used midges (Diptera: Chironomidae) for trials using *P. subflavus*, which they successfully hunted. Chironomids were collected at dusk using an insect collection net. Upon the start of a trial, the net was emptied into the flight room and the recordings monitored for feeding buzzes indicative a bat capturing an insect.

I tested the gleaning ability of each bat to capture a moth that was pinned to a cotton cloth covered trellis (as described in Ratcliffe & Dawson 2003; ter Hofstede *et al.* 2008). The trellis (1x1.5m) was constructed with a framework of spruce. The wooden frame of the trellis was nailed with Velcro pieces at the corners to which the black cloth was attached so it hung vertically on the trellis. The middle and each corner of the cloth had 5x5cm square portions of black fiberglass screen to allow a microphone behind the trellis to record sequences. Moths were placed dorsum up and pinned to a screen portion through the thorax so that the moth fluttered in place. Moths were only used when they were moving so as to create the prey-generated sounds that a gleaner will often use to facilitate foraging. The thread was not knotted to the cloth to accommodate the insect’s easy removal by the bat.
Flight room call recording and analysis

Call sequences during foraging trials were recorded using an Ultrasound Gate condenser microphone capsule CM16 attached to Ultrasound Gate 116-200 digitizing board with a 44.1 kHz sampling rate (Avisoft Bioacoustics, Berlin, Germany). During the aerial hawking trials, the microphone was attached to a tripod approximately 1 m high placed 3 m away from the resting position of the moth. During the gleaning trials, the same tripod was kept in the same location, directly behind the trellis. Audio (aforementioned microphone) and video recording (infrared camera placed on ground directly below moth) began before the bat was released. A sequence was determined to be a successful capture when the moth was removed from the thread or the trellis by the bat, as shown by the video recording. Attack sequences were analyzed using Avisoft SasLab Pro (Avisoft Bioacoustics, Berlin, Germany). Interpulse intervals (interval from the end of one call to the beginning of the next call) were measured using oscillograms. Each sequence was band pass filtered using Butterworth filter type between 8 and 200 kHz. A 1024 FFT size Hanning window was used to analyze calls. Peak frequency (frequency of maximum intensity) was determined from power spectra. Bandwidth (kHz) was calculated at 6 dB and 15 dB below peak frequency using Avisoft SasLab Pro. Calls were divided into phases using terminology and methodology of Griffin (1960) and Surlykke and Moss (2000). I designated calls greater than 50ms as ‘search calls’, those between 10ms and 50ms as ‘approach calls’ and those less than 10ms as ‘buzz calls’. I removed the first and last two calls of each phase to prevent the inclusion of transitional call types before averaging the values for call duration, bandwidth, and peak frequency for each of the three phases (Ratcliffe & Dawson 2003).
Ecological morphology

Wing morphology

Prior to each trial, digital photographs of each bat were taken (Nikon, model Coolpix 5700). Measurements were made of wingspan, wing area, arm wing area, hand wing area, arm length, hand length, and weight following Norberg and Rayner (1987) as shown in Figure 3. Length and area measurements were made using ImageJ (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA). Aspect ratio and wing loading were also calculated for each individual (see Norberg and Rayner 1987 for methods). Wingspan, wing area, aspect ratio, and wing loading were compared using non-parametric statistics because our data violated the assumptions of parametric tests (Zar 1999). Within each species, data was averaged for each character to allow comparison with Norberg and Rayner (1987).
**Jaw morphology**

Jaw morphology data collection was done at the Royal Ontario Museum (ROM) in Toronto, Ontario, Canada. Any available skulls and mandibles from *Myotis lucifugus, M. septentrionalis, M. leibii, Pipistrellus (=Perimyotis) subflavus,* and *Eptesicus fuscus* taken from Ontario, Quebec, or New York were used. In four cases, to increase sample size, specimens from other regions were used after testing of various measurements determined there was no consistent, quantifiable difference between sites. Of these additional skulls three were for *Myotis leibii* (n=3, Mann-Whitney U, p=0.143) and one was for *Myotis septentrionalis*. A total of 61 adult skulls from the 5 species were sampled (Table 1).

Mandible measurements were taken in March 2008 using digital calipers (Fisherbrand, Fisher Scientific). Each measurement was made three times, each on separate days with the average taken as the measurement. These were of the moment arm of the masseter (measured from the jaw joint to the masseteric depression), the moment arm of the temporalis (measured from the top of the coronoid process to the jaw joint), and the outlever (with the canine as the bite point), as shown in Figure 4.

Digital photos of the lateral view of the mandible and skull as well as the ventral view of the skull were taken in January 2009 using a Nikon D40x digital camera. Pictures were analyzed using Geomorph (Free Software Foundation, Boston MA), a computer program that uses 2D geometric morphometrics, combining multivariate statistics, and graphics (Thompson 1917). This method captured the morphology of objects using coordinates of topographically corresponding points that are homologous among individuals. The landmarks used for this procedure are documented in Figure 5 and Table 2. The landmarks were chosen from photographs of all specimens in the ventral view (12 landmarks, n=60) and digitized using
tpsDig (Rohlf 2005). Digital photographs of all views from each skull were taken twice and on separate days. Data for each individual were then averaged across these repeatedly measured photographs to minimize measurement error. Bilaterally homologous coordinates were also averaged to avoid inflating degrees of freedom; because some landmarks were not visible on both sides of all specimens, averaging was done by reflecting one side across the midline of the skull (specified by a baseline extending from landmark 1 to landmark 2, Figure 5).

Landmarks were superimposed to remove variation in scale, position and orientation by a generalized least-squares Procrustes superimposition (Rohlf & Slice 1990). Centroid size, which is the square root of the summed squared distances from each landmark to geometric center of the object, was used as our indicator of skull size (Bookstein 1996; Zelditch et al. 2004). A Canonical Variate Analysis (herein called CVA) was used to find the set of variables that allowed the greatest discrimination between the five species using CVAGen. The significance of the CVA was tested using a MANOVA. A jackknifing procedure was also used to test how well each specimen was assigned to the CVA. ANOVAs were used to look at the differences in centroid size and mechanical advantage at the masseter and the temporalis.

**Insect survey**

Insect surveying was performed during the summer of 2008 once each week. Surveying was similar to Burles et. al. (2008) although less surveying nights were performed due to logistical constraints. Two 160 watt mercury vapour lights (Bioquip, model 2818) were set up, one in a forest site and one in a field site, both on the QUBS property. These two sites were approximately 50 metres apart. The lights were kept on timer to run from 10pm to 4am. Within two feet of each light, a 5 gallon white bucket was suspended containing a jar of acetone covered with filter paper. A black plastic car funnel was used to cover each bucket. At 4am, the contents
of each bucket were emptied into a canning jar with 90% ethanol. All insects within each jar were counted and identified to order. Insects were identified to order, as for diet as well (see below).

*Diet*

Fecal analyses were performed during the summers of 2007 and 2008 according to Whitaker (1988). All bats trapped during this time were weighed (Ohaus digital scale, Fisher Scientific), sexed, aged using epiphyseal plates; forearm length was also recorded (Fisher brand digital calipers, Fisher Scientific). Each bat was given an identification number and placed individually into a clean cloth bag for approximately an hour. Whitaker (2004) found 10 minutes was sufficient time but here I chose to extend the time to ensure a sample due to less bats being available than in that study. Bats were then removed and released to the site of capture. Fecal material was placed in individual glass vials with 90% ethanol and labeled with the identification number. All fecal analysis was done using this identification number by someone who had not done the labeling and was therefore blind to species identity. Fecal analysis followed Whitaker (1988) and insect parts were identified to order whenever possible. Percentage volume for each order was determined for each sample. Where no material was identifiable in a portion of a sample, it was recorded as unknown for statistical comparison to samples that contained at least one identifiable part. By not containing identifiable parts these samples may consist of mainly soft bodied insects and thus these samples are still of interest for the diet analysis. Kruskal Wallis tests were performed using SPSS on the percentage volume to look for differences in proportional diets.
RESULTS

Echolocation

A sample size of six individuals per species was used for all statistical testing of echolocation call structure and behaviour to aerial hawking. Only one *M. leibii* and one *E. fuscus* attempted to glean and no gleaning attempts were made by *P. subflavus*. Without chironomids in the room, *P. subflavus* did not emit calls indicative of insect capture (showing a buzz phase of calls less than 10ms apart). Gleaning and aerial hawking data for *M. lucifugus* and *M. septentrionalis* were taken from a previous data set taken from Ratcliffe and Dawson (2003) that indicated that both species reliably glean and hawk prey but that *M. septentrionalis* was better adapted to gleaning than was *M. lucifugus*. No gleaning analyses of echolocation call structure were done due to the lack of substantial new gleaning data. A multivariate ANOVA was completed in SPSS with a Bonferroni post hoc test after the data was confirmed to be normally distributed. The multivariate test to allow all call characteristics and phases to be compared in the same test since all measurements were taken from the same calls and are related. There were significant differences amongst species regarding call characteristics (MANOVA, df=36, p<0.001), a summary of these differences is shown in Table 3. Furthermore, a visual representation is shown in Figures 4 and 5 for bandwidth and peak frequency respectively.

Echolocation call duration was statistically similar for all species in all three phases (search, approach, buzz). There were significant differences between all three phases for bandwidth (MANOVA, df=4, p=0.037_{Search}, p<0.006_{Approach}, p=0.002_{Buzz}). For the search phase, *Myotis septentrionalis* had a bandwidth significantly higher than *Myotis leibii*. During the approach phase *Eptesicus fuscus* was significantly lower in bandwidth than all other species.
except *Myotis lucifugus*. *Pipistrellus (=Perimyotis) subflavus* was significantly higher than all other species with regards to bandwidth during the buzz phase.

There were significant differences in peak frequency between all phases (MANOVA, df=4, p<0.001 for all 3 phases). During the search phase, the three myotid species all had high frequencies with *M. lucifugus* being lower than *M. leibii*. *Pipistrellus subflavus* had a peak frequency lower than *Myotis leibii* and *Myotis septentrionalis*. *Eptesicus fuscus* had a peak frequency lower than all other species for both the search and approach phases. For the buzz phase, *P. subflavus* was higher in peak frequency than all other species except for *M. leibii*. There were no other differences in the buzz phase for peak frequency.
Ecological morphology

Wing morphology

A sample size of ten bats per species was used for the wing morphology analyses with data for *M. lucifugus* and *M. septentrionalis* taken from Ratcliffe and Dawson (2003). Data was normally distributed and I conducted a MANOVA with Bonferroni post hoc test was used to compare various wing morphology characteristics between species. A multivariate analysis was chosen to allow comparison of all wing characteristics and weight in the same test since all characteristics relate to each other and all measurements were taken off the same photo for each bat. Table 4 summarizes the results. There were significant differences between species for mass, wingspan, wing area, aspect ratio, wing loading, and tip index (MANOVA, df=4, p<0.001 for all). *Eptesicus fuscus* was heavier than all other species and had greater wingspan, larger wing area, and higher aspect ratio than all other species. *M. leibii* was lighter than *M. lucifugus*. *Pipistrellus subflavus* had a wing area lower than *M. lucifugus* and *M. septentrionalis*. The aspect ratio of *M. leibii* was significantly lower than *M. lucifugus* and *P. subflavus*. The only significant result seen for wing loading was for *E. fuscus* which was higher in this species than in all other species. Tip index was significantly higher for *E. fuscus* as compared to *M. leibii* and *M. septentrionalis*.

Skull morphology

The CVA analysis showed that shape change along the first canonical variate axis is characterized by a relative lengthening and widening of the palate and a relative increase in the length and width of the base of the posterior region of the skull as detailed below (Figure 8, Table 5). Additionally, the first premolar is moving more anterior and lateral relative to the canine. Along the second axis change in the shape is characterized by a relative increase in
length of the base of the posterior region of the skull and a shortening and widening of the palate.
Change in shape from *E. fuscus* to *M. septentrionalis* is characterized by a widening of the base
of the posterior region of the skull and a shortening and widening of the anterior region of the
palate. The changes in shape from *E. fuscus* to *M. septentrionalis* are exemplified in Figure 9 by
a lengthening and narrowing of the palate. *E. fuscus* to *M. leibii* shows a slight lengthening of the
palate with the same feature being shown in *M. lucifugus* along with a lengthening of the base of
the posterior region of the skull. Due to non parametric data, a Kruskal Wallis was performed to
distinguish differences for skull size and mechanical advantage. There was a significant
difference in skull size as shown in Figure 10 (Kruskal-Wallis, H=53.72, p<0.0001), where all
skulls were different from each other. *E. fuscus* was the largest, followed by *M. septentrionalis*,
*M. lucifugus*, and *M. leibii*. *Pipistrellus subflavus* was the species with the smallest skull size.

The mechanical advantage of the masseter was significantly different between species
(Kruskal-Wallis, H=15.82, p=0.0033). The statistical results are also represented in Figure 11.
*Post hoc* tests were performed using t-tests on species pairs. *M. leibii*, *E. fuscus*, and *P.
subflavus* have similar mechanical advantages and *M. lucifugus* was significantly lower from *M.
leibii* and *P. subflavus*. The species with the lowest masseter mechanical advantage was *M.
septentrionalis* which was significantly lower than all other species.

The mechanical advantage (ratio of the moment arm of the muscle force to the moment
arm of the bite force) of the temporalis varied in its results from the masseter. The Kruskal
Wallis test was also significant (Kruskal-Wallis, H=35.04, p<0.0001, Figure 12) with *E. fuscus*
having a higher mechanical advantage than all other species. *Pipistrellus subflavus* and *M.
lucifugus* had high mechanical advantages that were statistically similar. *Myotis septentrionalis*
and *Myotis leibii* both had the lower mechanical advantages of the temporalis than the other three species, with *M. leibii* being significantly lowest.

When are data did not conform to the assumptions of parametric tests, equivalent nonparametric test were used.

*Assemblage structure*

There was a significant difference amongst months (6 months across two years) for sex ratio (Kruskal Wallis, df=5, p=0.003), age ratio, weight, and species ratio (all Kruskal Wallis, df=5, p<0.001). June had significantly less juveniles than July or August; furthermore July also had significantly less juveniles than August in 2007 however there were no differences in any of the months with regards to age ratios for 2008. The sex ratio was more even in August than in June of both years, where I caught significantly more males than females in these early months. All bat species were heavier in August than in July for both years. The number of individuals trapped per species was similar except for *P. subflavus* been caught in significantly lower numbers. Table 6 shows the distributions of species across months. Figure 13 shows the total number of individuals captured per month across the two years, clearly showing an increase in bats caught from June to August.

*Insect surveying*

Surveys were completed only in July and August of 2008. More Lepidoptera were captured in July than in August (Kruskal Wallis, df=1, p=0.002) and the same result was found for Coleoptera (Kruskal Wallis, p=0.018), Trichoptera (Kruskal Wallis, p=0.005) and Hymenoptera (Kruskal Wallis, p=0.027), see Figure 14. There was no difference in orders captured between the forest site and the open field site.
**Diet analysis**

Only four species were compared since no samples were obtained for *P. subflavus*. There were significant differences between months for unidentifiable samples (Kruskal Wallis, df=4, p=0.001) and Lepidoptera (Kruskal Wallis, df=4, p=0.009) such that August had a higher number unidentifiable samples though and fewer samples of Lepidoptera than were recorded in June or July. There was also a difference between years for both unidentifiable samples and Lepidoptera (both Kruskal Wallis, df=1, p<0.001) where *Lepidoptera* was higher in 2007 diets for all species except *Myotis lucifugus* and there were more unidentifiable samples in 2008 diets for all species (in 2007 all insects were identifiable to order in all samples but *M. leibii*).

Fecal results between species and months are detailed in Figures 13 through 16. Amongst species, there were significant differences for samples with no identifiable parts indicative of soft bodied prey items (Kruskal Wallis, df=3, p=0.17), Lepidoptera (Kruskal Wallis, df=3, p=0.11), Hemiptera (Kruskal Wallis, df=3, p=0.007). There were no differences in diet between *M. lucifugus* and *M. septentrionalis* however *M. septentrionalis* consumed significantly more Lepidoptera and had more samples with unidentifiable parts than *E. fuscus* whereas there was no difference between *E. fuscus* and *M. lucifugus* for these orders. *E. fuscus* consumed more Hymenoptera and Hemiptera than *M. lucifugus* and *M. septentrionalis*. *M. leibii* had more samples with unidentifiable parts than *M. septentrionalis* but no differences in diet with the other two species.
DISCUSSION

With several bat species coexisting in southern Ontario, there should be a minimum difference in foraging strategies between species (Pianka 1974). Siemers and Schnitzler (2004) suggested that successful niche partitioning occurs amongst 5 sympatric European vespertilionid species all belonging to the same guild and with similar morphology. For these species, however, aerial hawking capture success varied relative to the distance of capture from clutter and these differences were reflected in call structure, thus allowing a description of within guild niche differentiation. In my study, I find a similar though more complex structure of within guild niche differentiation between the five Ontario species. Here I describe how each species occupies a unique niche in this assemblage, as visualized in Figure 19.

_Myotis lucifugus and Myotis septentrionalis_

Broders _et. al._ (2004) looked at _M. septentrionalis_ and _M. lucifugus_ to see how their foraging patterns were different considering they are both of similar size. They found that the echolocation call structure was different among clutter categories with _M. lucifugus_ foraging in a wider variety of habitats. _M. septentrionalis_ would rarely forage in open areas, foraging mainly in forest interiors. The assemblage of bats that Broders et al. (2004) investigated (in Nova Scotia) consists of these two species alone. With both _M. septentrionalis_ and _M. lucifugus_ gleaning and capable of foraging in a cluttered habitat, this raises the question (which I will here answer) of how these two species can successfully coexist if they occupy the same foraging habitat.

My fecal analysis shows that _M. septentrionalis_ consumed more Lepidoptera than any of the other species. Though there was no difference between the forest site and the open site for
Lepidoptera during the insect survey, the difference in diet between these two species show that there may be resource partitioning occurring where *M. septentrionalis* can more efficiently consume moths than *M. lucifugus*. The differences seen in skull morphologies attest to the likely prey types being hunted. *Myotis septentrionalis* had significantly larger skulls but less mechanical advantage for both the masseter and the temporalis compared to *M. lucifugus*. Large masseter muscles are indicative of powerful bites (Freeman 1984). The greater development of the masseter muscle in herbivores shows in a similar fashion for harder diet bats where hard foods must be broken down in a grinding fashion (Freeman 1979a). *Musculus temporalis* plays a factor in bite force since bite force appears to correlate with an increase in the cross sectional area of this muscle (Herrel *et al.* 2008).

Due to these two species being closely related phylogenetically (Stadelmann *et al.* 2007), it is more understandable as to why my results do not show differences in all their morphologies. These two species appear to be capable of foraging in the same habitats with the same behaviours and hunting the same prey types. The differences in skull morphology may show a difference in preference of efficient foraging that is evident during times of the summer where preferred prey types are more abundant. During months where there is a decrease in preferred insect type, such as Lepidoptera for *M. septentrionalis*, there may be diet overlap. In this study insect surveys were only performed in July and August though significantly more insects (including Lepidoptera) were captured in July than August. The similarities in wing morphology do not necessarily dictate a similarity in diet but more likely this may be related to the similarity in foraging habitat (clutter capabilities) and hunting behaviour (both gleaning and aerial hawking) (Hodgkison *et al.* 2004; Stoffberg & Jacobs 2004).
Further evidence of niche partitioning between species may also have to do with their specific foraging grounds and not just their means of foraging. *Pipistrellus pygmaeus* and *P. pipistrellus* are two cryptic species in Britain that decrease interspecific competition through habitat partitioning by having discrete foraging sites ((Nicholls & Racey 2006b). The myotids in southern Ontario are not sister species (meaning these two species are not the closest related to each other), however *M. lucifugus* and *M. septentrionalis* have a more recent common ancestor and *M. leibii* is more distantly related (Stadelmann et al. 2007).

**Eptesicus fuscus**

Although *E. fuscus* is larger than the other species, leading to the assumption that it is capable of consuming larger, harder species, the difference in diet was less than expected. My results indicate that this species consumes insects harder than *M. lucifugus* (more Hymenoptera and Hemiptera) and *M. septentrionalis* (less Lepidoptera). This species had greatest differences regarding wing morphology. *Eptesicus fuscus* is heavy, with a long wingspan, large wing area, high aspect ratio, high wing loading, and high tip index. All these characteristics are indicative of a bat that has low maneuverability and would not be able to forage in a clutter environment (Stockwell 2001). This was further supported by echolocation characteristics showing a low bandwidth and low peak frequency that would make it difficult to efficiently detect small prey items or discriminate prey items from a cluttered background (Schnitzler & Kalko 2001; Siemers et al. 2001). The calls of this species would be most suitable to larger prey items in an open environment where the calls would not be as readily attenuated in an open field. Furthermore, my wing morphology results indicate that *E. fuscus* would not be able to hunt in a cluttered environment such as a forest. What is intriguing to note is that one of the bats did glean during the foraging behaviour gleaning trials. It successfully picked off a moth off of the trellis several
times, though it landed rather than hovered. For the duration of the summer, additional *E. fuscus* bats (approx. 20) were tested for gleaning (even after the sample size had been reached for the project) and no other bats gleaned. I therefore suggest that this is likely a chance encounter rather than being indicative of the foraging behaviour of this species. Acoustic surveying of specific foraging habitats will perhaps support or refute whether this species is capable of foraging in a cluttered habitat where gleaning would occur.

*Eptesicus fuscus* has a short, wide skull that is indicative of a harder diet. The skull is larger than those of other species which is expected based on size and weight differences alone. The mechanical advantage of the masseter was greater than that of *M. septentrionalis*; however the mechanical strength of the temporalis was significantly higher than in all other species. Thus *E. fuscus* may not have greater strength to chew a hard insect such as Coleoptera (explaining the lack of difference in diet for this insect order compared to other species) but it would have greater strength to bite through a thick exoskeleton. Whitaker & Barnard found that majority of *E. fuscus*’s diet was Coleoptera (57.7%), showing evidence that this species is capable of consuming large amounts of hard beetles (2005). My study adds to the understanding of this species by showing it is the piercing ability not chewing ability that enable consumption of Coleoptera. The larger skull along with echolocation characteristics indicates an ability to successfully hunt larger prey items. The aforementioned mandible characteristics suggest there would be an advantage over other species for efficiently consuming hard insects, by being able to quickly pierce the exoskeleton, however my results do not show any advantage at chewing harder insects.

I conclude therefore that the combination of echolocation, jaw, and wing morphology indicate that this species is an open forager that is more likely to pursue larger, faster flying prey
items. A recent study (Clare et al. 2009) identified fecal samples from *Lasiurus borealis* using DNA fragments, thus allowing a detailed list of the species consumed. This bat is similar in size to *E. fuscus* and also has robust jaws, however the diet analysis found that majority of the species consumed were soft bodied Lepidoptera and 60% of the consumed prey species were capable of hearing (Clare et al. 2009).

**Pipistrellus subflavus**

Whereas the predictions made for *E. fuscus*’s foraging behaviour appear to be confirmed, *P. subflavus* is somewhat more complex. This species’ long, narrow wings are indicative of an open space aerial hawker however it had significantly broader bandwidth and higher peak frequency calls than other species, even the myotids capable of gleaning in clutter. I predict this species is capable of edge foraging but the call characteristics here may be more indicative of diet rather than habitat. Broader bandwidth calls of higher frequency allow smaller prey items to be discriminated from a background (Schnitzler & Kalko 2001). This tends to be of use in a cluttered setting simply to discriminate any prey item from the cluttered background but it may be of use in an open setting for particularly small prey items. There were no fecal samples that were collected for *P. subflavus* (bats were caught but none provided samples) so it is difficult to ascertain the diet of this species. In the flight room it was determined that *P. subflavus* would not successfully aerial hawk Lepidoptera but when high amounts of chironomids where placed in the flight room, numerous buzz calls were recorded indicating aerial hawking (which was also recorded on the infrared CCTV system). The two open field aerial hawking species in this bat assemblage, *E. fuscus* and *P. subflavus* likely differ in their diet with regards to the prey items they pursue, based on jaw morphology and echolocation. The resource partitioning seen in both
the clutter capable species and open space species allow successful coexistence of sympatric species that otherwise appear to occupy the same niche.

*Pipistrellus subflavus* is thought to be an open space or around clutter forager (Fujita & Kunz 1984; Loeb & O'Keefe 2006), however in this study I found high peak frequencies and broad bandwidths that would allow identification of small prey from cluttered background (Schnitzler & Kalko 2001). My results on this species are important to note as they show that this species may be capable of more flexible foraging than previously thought (Macdonald *et al.* 1994; Broders *et al.* 2001); such high peak frequencies and bandwidths would not be expected for a bat that forages in open environments. The wing morphology of this species shows that it is unlikely to be capable of efficiently maneuvering and foraging in a cluttered environment like a forest. The echolocation characteristics show a greater ability to detect smaller insects rather than being used for clutter discrimination. In other areas (Whitaker 2004) there have been records of Lepidoptera being a part of the eastern pipistrelle’s diet and the lack of success with moths in the flight-room may be due to the size of the moths used or an artifact of the flight room size being too cluttered for this species. *Eptesicus fuscus* trials did not have any problems however, as mentioned in my results, it employs lower frequency calls compared to the high frequency of *Pipistrellus subflavus* calls. The skull morphology for this species was surprising, the mechanical advantage of the masseter was higher than *E. fuscus*. I predict that there may not be a great difference in the ability to efficiently consume harder prey items. The difference in skull size along with differences in echolocation call structure (*E. fuscus* having broader calls of lower frequency and *P. subflavus* having high frequency, high bandwidth calls) suggests resource partitioning occurring not based on insect hardness but on insect size. The echolocation differences show *P. subflavus* being more adept at detecting smaller insects and the difference in
weights and skull sizes suggest that *E. fuscus* would be better capable of handling and consuming larger insects. Also the size difference would make larger insects more efficient energy wise for *E. fuscus*. To adapt to successfully hunt in an open space, likely the wing and echolocation adaptations of these two species prevent them from efficiently hunting in cluttered habitat. The differences in weight, jaw morphology, and wing morphology show that there is partitioning occurring at the resource level between smaller (*P. subflavus*) and larger (*E. fuscus*) airborne insects.

**Myotis leibii**

In southeastern Ontario, the bat assemblage (described in this study and previously) is composed of primarily aerial hawking species (Fenton & Barclay 1980; Fujita & Kunz 1984; Kurta & Baker 1990; Best & Jennings 1997; Caceres & Barclay 2000). *M. septentrionalis* both aerially hawks and gleans prey in the wild, while the aerial hawking *M. lucifugus* also gleans prey in our flight room. My results show *Myotis leibii* to be an aerial hawker with some evidence of gleaning ability but to a lesser extent than its two sympatric congeners. Of 16 *M. leibii* tested, only one attempted to glean, showing that this species is more successful at foraging airborne prey.

My results indicate that *M. septentrionalis* and *M. lucifugus*, being more closely related, are behaviourally flexible species and *M. leibii* is an aerial hawker capable of hunting in and around clutter (Stadelmann *et al.* 2007). Its diet was not significantly different from other species but there was a trend towards softer insects based on the number of samples with unidentifiable parts. The small skull size, average masseter mechanical advantage (relative to other species in this study) and low temporalis mechanical advantage support the conclusion of this species more efficiently consuming softer prey types. *Myotis leibii* did not overall differ from other species,
thus it appears to be quite flexible in being capable of foraging in all habitat types. It is not as open restricted as *E. fuscus* and *P. subflavus* nor is it as successful gleaning in a cluttered habitat as the other myotids. Likely the specific foraging ecology of this species is dictated by the specific area, which other species are present and where given prey types are present. Rather than being restricted by habitat type it is likely more restricted by interspecific competition or prey availability.

*P. subflavus* has a higher aspect ratio than *M. leibii* suggesting *M. leibii* should be more maneuverable in a cluttered environment, though there was no difference in wing loading. This correlates to expected differences in mechanical constraints related to adaptation for foraging in clutter. Respective mean weights of 5.5 g and 4.4 g means these two species are both smaller than the other species found in the area (see Fenton & Barclay 1980; Kurta & Baker 1990; Caceres & Barclay 2000; Ratcliffe & Dawson 2003). My study shows similar adaptation to separate habitats where *M. leibii* is more adapted to clutter edge, both in terms of its echolocation and morphology, than is *P. subflavus*. So in this area where there are species of a similar size, there is niche partitioning of foraging habitat by echolocation differences.

**Other factors**

*Interspecific insect variances*

Goldman and Henson (1977) reported that some bats cannot eat certain prey even though they are capable of physically capturing the insect. This could be due to palatability or toxicity (i.e. tiger moths (Arctiidae) which have a range of palatability due to ingested biological chemicals (Hristov & Conner 2005). It could also be due to the inability or inefficiency to pierce the cuticle of captured insects that must be quickly subdued (Freeman 1992). Further study could assess insect surveys at a species level to see if larger moths or more moth species with hearing
are present in August relative to other months. The high number of hearing prey items is indicative that evasiveness is not a factor for this particular moth species. Furthermore, it is difficult to determine hardness or intractability at an ordinal level since this will vary between species. Here intractability describes the amount of force needed to pierce through an insect (Freeman 1981).

When using a taxonomic level such as order, differences must be generalized, such as stating that moths are softer and more pliable than beetles. Although comments about hardness are useful, for most studies it is sufficient to follow Freeman’s (1981) classification since the description of diets are generally in proportional measurements at a taxonomic level higher than species (usually order, for example: Aldridge & Rautenbach 1987; Saunders & Barclay 1992; Arlettaz et al. 1997; Siemers & Swift 2006). Recently, diet analyses have been done using DNA bar coding allowing identification of prey items to the species level (Clare et al. 2009). Lepidoptera is evident in a fecal sample due to the presence of scales, however there are differences in moths based on size and ability to evade bats (based on if a given species can hear). Ordinal level analysis of diet requires generalizations about hardness across species rather than intractability within species for individual insects. It also may not be prey hardness that affects diet but prey size which also differs between species (Burles et al. 2008). Fecal surveys were done at the order level thus to complete insect surveys at an order level is suitable, but the interspecific differences of hardness, intractability, size, and evasiveness would explain the lack of continuity between the fecal and insect surveys.

A further example of behavioural variations being represented in morphology would be the foraging methods of different insectivorous bats. Reduker (1983) compared Myotis evotis and M. volans to explain how some of the morphologically distinct characteristics between these two
species are due to the former being a substrate gleaner (taking insects off substrates) and the latter being an aerial hawker (catching prey on the wing). Although *M. evotis* can produce a more forceful bite at a wider gape, *M. volans* has the potential for faster jaw closure and the ability to produce a series of rapid nipping motions; faster jaw closure and nipping motions for an aerial hawker would be adaptations to quickly capture, subdue, and consume prey while in flight. Additionally, *M. evotis* had a significantly larger skull and higher coronoid process, which increases the area for muscle attachment. *M. volans* is a heavier bat but *M. evotis* had larger muscles (such as in the masseter muscle) which are indicative of powerful bites (Freeman 1984). Furthermore, these results fit in with their diet as *M. evotis* consumes more substrate resting Coleoptera and *M. volans* consumes more flying Lepidoptera. Faure and Barclay (1994) show that *M. evotis* is capable of both gleaning and aerial hawking however Redeker (1983) suggests that the cranial morphology differences in these two species directly relate to pressures from different foraging strategies although it is unclear how big of a role the proportional diet plays in the morphology.

*Myotis lucifugus* and *Myotis septentrionalis* present a similar relationship in my south eastern Ontario bat assemblage. *Myotis septentrionalis* consumes more Lepidoptera (based on diet results) and *Myotis lucifugus* has a stronger bite where it would be able to efficiently consume harder prey items such as Coleoptera. A similar study in British Columbia between *Myotis lucifugus* and *Myotis keenii* found selective foraging occurring based on prey size (rather than prey taxa), however in this assemblage *M. lucifugus* was consuming larger prey items and more Lepidoptera (Burles *et al.* 2008). This is further evidence of the need to add other empirical data, such as information on foraging strategies and bite behaviour, to determine diet. Further studies with behaviour and morphology data will give a better understanding of how behaviour...
affects morphology and how strong the relationship is between morphology and foraging ecology.

Jaw and wing morphology, respectively, likely predisposes individuals to feed on certain prey types or employ certain foraging strategies (Saunders & Barclay 1992; Dumont et al. 2005). However, those margins may be further constrained by interspecific competition. Following the assumptions of the competitive exclusion principle (Moore 1990), sympatric species must differ in at least one niche dimension. For myotids, my results indicate it to be their level of gleaning and ability to forage in clutter (Siemers & Swift 2006). As a whole, the assemblage may be resource partitioning at a spatial level. Saunders and Barclay (1992) found that *M. lucifugus* and *M. volans* were both capable of foraging in moderate clutter however it appears they both forage in distinct, separate open areas. This was believed to be due to the availability of prey or resource partitioning by these two morphologically similar species.

**Future studies**

**Bite point**

In my study there was no information collected about preferred biting style, such as the preferred bite point. Bite force is the easiest measure; however, it is difficult to determine if the bite produced when measured is an accurate representation of bites used in foraging. The bite force measured may simply be defensive bites due to being handled and having a bite measurement device forced into the bat’s mouth (Dumont & Herrel 2003). All skull analyses were done using the canine as the bite point to then calculate the relative mechanical advantage of the masseter and temporalis. Species will differ in how they bite and chew similar food and this is evident when comparing gape angles and bite points (VanValkenburgh 1996). The gape angle is the degree of the mouth opening and bite point is the point along the tooth row where
force is transferred to a food item (Dumont & Herrel 2003). There is a trade-off between gape angle and mechanical advantage where larger gape angles require muscles to stretch the jaw and thus have negative impacts on the mechanical advantage of those muscles (Herring 1974). There is an optimal gape angle for a given jaw shape which produces the maximum bite force however generally the bite force decreases at increasing gape angles (Dumont & Herrel 2003). Bite force increases as the bite point moves posterior in the mouth so for a given species there is an ideal bite point and gape angle to produce the desired bite force (Vaneijden 1991; Dumont & Herrel 2003).

There are species specific variation in the preference of bite points which is likely due to differences in jaw morphology which affect gape angles and the type of preferred prey items thus determining the ideal bite force needed for efficient processing (Dumont 1999). Here by using the same bite point for all five species I am able to see differences in mechanical advantage however this may not be reflective of the true power of a given species. For example, *E. fuscus* and *P. subflavus* lack a differences in their mechanical advantages based on analyses done using the canine. It may be possible that one of these species uses a different bite point majority of the time in the field that may lead to a different mechanical advantage that would have instead shown a difference between these two species that would have then related to a difference in insect hardness. Dumont *et al.* (2005) looked at differences in stress from different types of bites made by *Artibeus jamaicensis* and found that the typical bite points and bite types used produced the lowest stress than bites not typically used. A future experiment could determine the preferred bite points for each species and analyzing bite force using those specific bite points.
Acoustic surveying

Data collected for my thesis, though not present herein, will be analyzed to see how spatial partitioning may be playing a role in this assemblage (see Appendix). Nicholls and Racey (2006b) used radio tracking in Scotland of two myotid species, with similar echolocation repertoires, to determine that spatial partitioning was key in their coexistence. Species that may be capable of foraging in a wide array of habitats, such as the myotids in my study, may in fact have a more narrowed foraging habitat based on spatial partitioning with other species. This spatial partitioning would be evident through acoustic monitoring.

The insect surveying included in my thesis did not find a difference between the open and field site which may be due to the small distance between these two sites. More strenuous insect surveying at more sites may show differences that could then be related to any differences between species shown through acoustic surveying to ascertain whether foraging sites are related to insect abundances.

Future work will show presence and frequency of calling by species in an open field and a cluttered forest. This will add to the body of work on my assemblage to further explain the fine grained niches of these species.

Conclusions

My results add to the understanding of the echolocation and foraging behaviour of all 5 resident bat species of southeastern Ontario and how these species interact within this assemblage. Their echolocation characteristics and wing morphologies allows us to understand some of the limitations of their foraging abilities.
My study has shown that it is unlikely that any one method of analysis will directly correlate with diet. For example, echolocation will allow the determination of likely prey that can be detected and the habitat in which the bat is likely foraging however empirical data has shown that actual habitats may differ from hypothesized habitats due to the influence of prey availability (Saunders & Barclay 1992). This applies to examining morphology as well where empirical data may show differences from predictions due to other factors such as prey availability and behaviour. Foraging ecology is complex and with more information, there is a stronger description for a given species.

The trapping data did not reveal a difference in the abundance of species except for *P. subflavus* that appears to be lower in numbers than other species. Analyzing the trapping data will assist in future work of acoustic surveying by relating abundance to frequency of calls recorded. Furthermore, this assists in the continued assessment of the health of this assemblage. There was a relative increase overall in the species captured from June to August. As the summer progresses, maternal colonies dissolve and juveniles disperse. The increase in weights from June to August is also expected due the need to increase fat reserves for the upcoming hibernation (Agosta *et al.* 2005).

The five species studied in this paper show the difficulty in defining subtle differences in the ecology behind a group of species. Although all of these species are quite similar in a broad sense of guild classification, differences in various characteristics allow niche partitioning. By looking at morphology, fine niche differences are evident between species that occupy the same habitat and use the same foraging methods. Bats are unique as mammals by being able to fly and echolocate adds additional dimensions to this analysis. Looking at wing morphology and echolocation alone fails to explain the coexistence of these five species that are so similar on a
gross level. Through the above mentioned analyses there can be better understanding of how these species coexist based on resource and spatial partitioning.

Figure 1. General distribution of study species a) *Eptesicus fuscus*, b) *P. subflavus*, c) *M. septentrionalis*, d) *M. leibii*, and e) *M. lucifugus*. Maps are taken from Mammalian species accounts for each species.

**Figure 2.** Map showing the location of trapping sites used in this project, a) general location of study sites relative to Kingston, Ontario (red circle) and b) the three trapping sites in Frontenac county (1 being the open field abandoned mine, 2 being the cluttered forest abandoned mine and 3 being the man made culvert). Both maps were created in Google Maps.
Figure 3. Visual description of how wing measurements were taken where B represents wingspan, l represents length, and S represents area. T₁ and T₂ define tip length and tip area ratios and I calculates wingtip shape index. Figure taken from Norberg and Rayner (1987).
Table 1. Inventory of skulls from ROM used for skull morphology analysis showing species, identification number and location found.

<table>
<thead>
<tr>
<th>Species</th>
<th>ID</th>
<th>Location</th>
</tr>
</thead>
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<td><em>E. fuscus</em></td>
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</tr>
<tr>
<td><em>E. fuscus</em></td>
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<td>Brantford</td>
</tr>
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<td><em>E. fuscus</em></td>
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<td>Ottawa</td>
</tr>
<tr>
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<td>York</td>
</tr>
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<td>York</td>
</tr>
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<td>York</td>
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<td><em>P. subflavus</em></td>
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Figure 4. Generic mandible showing measurements for moment arm of the masseter in blue (measured from the jaw joint to the masseteric depression), moment arm of the temporalis in green (measured from the top of the coronoid process to the jaw joint), and outlever in red (with the canine as the bite point).
Figure 5. 2D Landmarks chosen on specimens of each species in the ventral view. See Table 2 for description of each landmark. Picture from Barbour & Davis (1969).
**Table 2.** Description of landmarks used in this study.

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Anterior extent on the midline suture of the premaxilla</td>
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<tr>
<td>2</td>
<td>Posterior extent on the midline of the ventral edge of the foramen magnum</td>
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<td>3</td>
<td>Lingual and posterior edge of the first incisor</td>
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<td>4</td>
<td>Lingual edge of the canine</td>
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<tr>
<td>5</td>
<td>Lingual and posterior edge of the first premolar</td>
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<td>6</td>
<td>Posterior extent on the midline of the palatal</td>
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<td>7</td>
<td>Labial and posterior edge of the first molar</td>
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<td>8</td>
<td>Posterior extent of the third molar</td>
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<td>9</td>
<td>Medial edge of the mandibular fossa</td>
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<td>10</td>
<td>Lateral edge of the mandibular fossa</td>
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<td>11</td>
<td>Lateral extent of the skull posterior to the auditory meatus</td>
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<tr>
<td>12</td>
<td>Posterior extent of the occipital condyle</td>
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Table 3. Echolocation results for five species with results for *M. lucifugus* and *M. septentrionalis* from Ratcliffe & Dawson (2003). Range represents the interquartile range. Also shown are the F and p values from ANOVAs completed across the five species for each of the echolocation characteristics within each phase. Each species had a sample size of 6.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase</th>
<th>M. lucifugus</th>
<th>M. septentrionalis</th>
<th>M. leibii</th>
<th>E. fuscus</th>
<th>P. subflavus</th>
<th>F</th>
<th>p</th>
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<td>Median</td>
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<td>Median</td>
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<td>23.98-30.35</td>
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Figure 6. Bandwidth (kHz) at -15 dB from peak frequency for the five species at search, approach, and buzz phases with standard error bars. Species listed from left to right are *E. fuscus, P. subflavus, M. leibii, M. lucifugus, M. septentrionalis*. In the search phase *Myotis septentrionalis* had a bandwidth significantly higher than *Myotis leibii*. During the approach phase *Eptesicus fuscus* was significantly lower in bandwidth than all other species except *Myotis lucifugus*. *Pipistrellus subflavus* was significantly higher than all other species with regards to bandwidth during the buzz phase. Same letters connect bars of insignificant differences, each echolocation phase analysed separately.
Figure 7. Peak frequency for the five species at search, approach, and buzz phases with standard error bars. Species listed from left to right are E. fuscus, P. subflavus, M. leibii, M. lucifugus, and M. septentrionalis. Myotis lucifugus was lower than M. leibii. Pipistrellus subflavus had a peak frequency lower than Myotis leibii and Myotis septentrionalis. Eptesicus fuscus had a peak frequency lower than all other species for both the search and approach phases. For the buzz phase, P. subflavus was higher in peak frequency than all other species except for M. leibii. Same letters connect bars of insignificant differences, each echolocation phase analysed separately.
Table 4. Wing morphology characteristic and mass results using a MANOVA and Bonferroni *post hoc* tests.

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<thead>
<tr>
<th></th>
<th><em>Eptesicus fuscus</em></th>
<th><em>Perimyotis subflavus</em></th>
<th><em>Myotis leibii</em></th>
<th><em>Myotis lucifugus</em></th>
<th><em>Myotis septentrionalis</em></th>
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<th>p</th>
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<tr>
<td>Mass (g)</td>
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<td>Wingspan (cm)</td>
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<td>Tip shape index</td>
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</tbody>
</table>
Figure 8. Canonical variates axes 1 and 2 for five species, *E. fuscus* (black triangles), *P. subflavus* (red stars), *M. septentrionalis* (black circles), *M. leibii* (blue crosses), and *M. lucifugus* (purple squares).
Table 5. Results from the CVA and MANOVA.

<table>
<thead>
<tr>
<th>Axis</th>
<th>Wilk’s λ</th>
<th>Chi²</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0000</td>
<td>465.11</td>
<td>152</td>
<td>&lt; 0.000001</td>
</tr>
<tr>
<td>2</td>
<td>0.0005</td>
<td>288.19</td>
<td>111</td>
<td>&lt; 0.000001</td>
</tr>
<tr>
<td>3</td>
<td>0.0324</td>
<td>128.64</td>
<td>72</td>
<td>0.00005</td>
</tr>
</tbody>
</table>
Figure 9. Deformation grids illustrating the shape change from *Eptesicus fuscus* to a) *P. subflavus*, b) *M. septentrionalis*, c) *M. leibii*, and d) *M. lucifugus*.
Figure 10. Centroid size from the geometric morphometrics by species where a Kruskall-Wallis ANOVA was performed followed by post hoc t-tests (results in graph). All skulls were significantly different in size from each other.
Figure 11. Mechanical advantage of the masseter by species with Kruskal Wallis results written in the figure. *M. leibii*, *E. fuscus*, and *P. subflavus* have similar mechanical advantages and *M. lucifugus* was significantly lower from *M. leibii* and *P. subflavus*. The species with the lowest masseter mechanical advantage was *M. septentrionalis* which was significantly lower than all other species.
Figure 12. Mechanical advantage of the temporalis with results written in the figure. *E. fuscus* had a higher mechanical advantage than all other species. *Pipistrellus subflavus* and *M. lucifugus* had high mechanical advantages that were statistically similar. *Myotis septentrionalis* and *Myotis leibii* were significantly lower than the other three species with *Myotis leibii* being the lowest.
Table 6. Trapping results from 2007 and 2008 showing totals per species and per year.

<table>
<thead>
<tr>
<th>Species</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
<td>July</td>
</tr>
<tr>
<td><em>M. lucifugus</em></td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td><em>M. leibii</em></td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td><em>M. septentrionalis</em></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><em>E. fuscus</em></td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td><em>P. subflavus</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>19</td>
<td>34</td>
</tr>
</tbody>
</table>
Figure 13. Comparison of total bat calls recorded during acoustic surveys (black bars, right axis) and total bats caught by harp trapping (grey bars, left axis) over both summers.
Figure 14. Insect survey results for July (black) and August (grey) with standard error bars. Trichoptera and Diptera values are shown on the secondary axis due to high numbers.
Figure 15. Fecal sampling results for *Eptesicus fuscus* showing percentage volume of average sample per order (U=unidentifiable, Lep=Lepidoptera, Col=Coleoptera, Hym=Hymenoptera, Ara=Aranaea, Trich=Trichoptera, Neur=Neuroptera, Hemi=Hemiptera, Orth=Orthoptera, Dip=Diptera, Homo=Homoptera). Bar graphs with standard error bars represent months where samples were collected (June 2007, July 2007, June 2008, July 2008 from left to right).
Figure 16. Fecal sampling results for *Myotis leibii* showing percentage volume of average sample per order (U=unidentifiable, Lep=Lepidoptera, Col=Coleoptera, Hym=Hymenoptera, Ara=Aranaea, Trich=Trichoptera, Neur=Neuroptera, Hemi=Hemiptera, Orth=Orthoptera, Dip=Diptera, Homo=Homoptera). Bar graphs with standard error bars represent months where samples were collected (June 2007, July 2007, June 2008, July 2008 from left to right).
**Figure 17.** Fecal sampling results for *Myotis lucifugus* showing percentage volume of average sample per order (U=unidentifiable, Lep=Lepidoptera, Col=Coleoptera, Hym=Hymenoptera, Ara=Aranaea, Trich=Trichoptera, Neur=Neuroptera, Hemi=Hemiptera, Orth=Orthoptera, Dip=Diptera, Homo=Homoptera). Bar graphs with standard error bars represent months where samples were collected (June 2007, July 2007, June 2008, July 2008 from left to right).
Figure 18. Fecal sampling results for *Myotis septentrionalis* showing percentage volume of average sample per order (U=unidentifiable, Lep=Lepidoptera, Col=Coleoptera, Hym=Hymenoptera, Ara=Aranaea, Trich=Trichoptera, Neur=Neuroptera, Hemi=Hemiptera, Orth=Orthoptera, Dip=Diptera, Homo=Homoptera). Bar graphs with standard error bars represent months where samples were collected (June 2007, July 2007, June 2008, July 2008 from left to right).
Figure 19. Summary of skull characteristics as they directly relate to prey hardness for species *Myotis septentrionalis* (blue diamond), *Myotis lucifugus* (red square), *Myotis leibii* (green triangle), *Eptesicus fuscus* (grey X), and *Pipistrellus subflavus* (black star). Higher values indicate capability of harder prey species for that particular skull characteristic.
REFERENCES CITED


APPENDIX

Acoustic surveying

Recording

For the second and third summer, acoustic surveys were performed at 5 chosen sites near the Queens University Biological Station property. Three sites were chosen in the first summer that were all mixed forest facing out onto a grass field. One site was not surveyed in the second summer due to being unavailable by the owner. Another field site was added as well as a lakefront site that backed onto a forest. Each site was surveyed once every five days from 10pm to 4am. One microphone was placed 3 meters in from the tree line (facing into the forest) and another microphone was placed 3 meters out from the tree line (facing out to the field). Both microphones were tilted at a $45^\circ$ angle for maximum attenuation. Call sequences were recorded using Ultrasound Gate condenser microphone capsule CM16 attached to Ultrasound Gate 116-200 (Avisoft Bioacoustics, Berlin, Germany). Recording was triggered by any pulse greater than 20 kHz and digitized using Avisoft SasLab Pro (Avisoft Bioacoustics, Berlin, Germany). Due to the sensitivity of the microphones, recording would stop during rain and an acoustic survey would be cancelled and repeated on another evening if the recording ceased for more than 15 minutes.

Analysis

Callviewer18, an application in Matlab (Mathworks Inc), was used to identify calls and calculate various call characteristics. Only the characteristics of maximum frequency, call duration, energy, frequency at the 50th percentile, and frequency at maximum energy were chosen to train the discriminate function analysis. All calls were manually filtered to remove higher harmonics,
any calls greater than 90 kHz at maximum frequency, and calls with an interpulse interval less than 10ms (indicative of being a terminal buzz sequence). Buzz calls were removed due to having significantly different characteristics of search calls which would make it difficult to complete a discriminate function analysis. Known calls were taken from search calls recorded in the flight room using the same equipment and method aforementioned in the flight room section. Recordings of known search calls were completed in August 2008 and were subjected to the same filtering process as the survey calls. Known calls were run through a multivariate ANOVA in SPSS 16.0 to confirm statistically different groups of the five species (Myotis leibii, M. lucifugus, M. septentrionalis, Eptesicus fuscus, Pipistrellus subflavus). An initial discriminate function analysis using all known calls gave 62.9% accuracy for correct group membership. A discriminate function analysis was then performed using the known calls with the set of unknown calls divided by evening and microphone. Each analysis gave likely group membership and percentage of likelihood of membership across all five species for each individual call. Presence or absence was determined by whether there were at least three consecutive calls with greater than 40% likelihood of membership in a specific group. In addition, overall number of calls and number of calls per species were calculated.