ABSTRACT OF DISSERTATION

Luke Elden Dodd

The Graduate School

University of Kentucky

2010

FOREST DISTURBANCE AFFECTS INSECT PREY AND THE ACTIVITY OF BATS IN DECIDUOUS FORESTS

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A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By
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Lexington, Kentucky

Director: Dr. Lynne K. Rieske-Kinney, Professor of Entomology

Lexington, Kentucky

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The use of forest habitats by insectivorous bats and their prey is poorly understood. Further, while the linkage between insects and vegetation is recognized as a foundation for trophic interactions, the mechanisms that govern insect populations are still debated. I investigated the interrelationships between forest disturbance, the insect prey base, and bats in eastern North America.

I assessed predator and prey in Central Appalachia across a gradient of forest disturbance (Chapter Two). I conducted acoustic surveys of bat echolocation concurrent with insect surveys. Bat activity and insect occurrence varied regionally, seasonally, and across the disturbance gradient. Bat activity was positively related with disturbance, whereas insects demonstrated a mixed response. While Lepidopteran occurrence was negatively related with disturbance, Dipteran occurrence was positively related with disturbance. Shifts in Coleopteran occurrence were not observed. Myotine bat activity was most correlated with sub-canopy vegetation, whereas lasiurine bat activity was more correlated with canopy-level vegetation, suggesting differences in foraging behavior. Lepidoptera were most correlated with variables describing understory vegetation, whereas Coleoptera and Diptera were more correlated with canopy-level vegetative structure, suggesting differences in host resource utilization.

I assessed the food habits of bats captured in mist nets. Morphological identification of prey suggested consumption of insect taxa varies across bat species and, at least for the most commonly-captured species, *Myotis septentrionalis,* across the region (Chapter Three). Trophic connections were further delineated between *M. septentrionalis* and its prey by sequencing C*OI* fragments of insect prey obtained from fecal samples. Prey identities were inferred for C*OI* fragments using web-based searches (Chapter Four), as well as tree-building analyses (Chapter Five). Lepidoptera were detected most frequently in all prey identification procedures, though prey detection varied with procedure thus suggesting methodological bias. Prey species were identified using the Barcode of Life Database; the wingspan of prey consumed by *M. septentrionalis* was smaller than that reported for other sympatric species.

My research demonstrates regional variation in bat activity, bat foraging, and prey occurrence across a gradient of forest disturbance. Conservation efforts should consider the importance of vegetation structure and plant species richness to sustain populations of both bats and their insect prey.

KEYWORDS: foraging ecology, predator-prey interactions, food habits, forest succession, Appalachia

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CHAPTER ONE: INTRODUCTION

Statement of Issue

Beyond natural disturbance processes, human land use and resource extraction widely involves disturbance of forest vegetation and the broader ecosystem (Jones *et al.* 1999; Feldhake and Schumann 2005). In eastern North America, and Appalachia specifically, forests are fragmented and parceled; little remained unutilized by humans during the 19th and 20th centuries (Constanz 2000; Gragson and Bolstad 2006). Much of the land that was cleared for agriculture, and much of the land from which timber was harvested, has reverted to forestland (Jones *et al.* 1999; Gragson and Bolstad 2006). Of the human land use practices occurring in Appalachia, few are as prevalent as timber harvesting (Feldhake and Schumann 2005; Gragson and Bolstad 2006). Given this, an understanding of how silvicultural and other land-use practices impact forests is central to understanding the ecology and communities of forests in Appalachia and, more broadly, eastern North America.

A basic ecological understanding of vertebrate, invertebrate, and floral communities is fundamental to achieve goals for both ecological stewardship and for resource extraction (Guldin *et al.* 2007; Homyock and Haas 2009). Bats form an understudied but important assemblage of vertebrate predators in forests in North America (Fenton 2003; Brigham 2007). In recent years research on bat ecology has moved toward an investigation of how bats use their forest environments and how anthropogenic forces may affect them (Brigham 2007). Even so, relatively few studies have concurrently studied the land use and occurrence of bats and their insect prey base. Consequently, the use of forest habitats by foraging bats, and how this habitat use is

influenced by the insect prey base, is poorly understood (Brigham 2007; Lacki *et al.* 2007a). Although the linkage between insects and vegetation is widely recognized as a foundation for trophic webs in forested systems, the role that bottom-up processes play in governing insect populations in forest systems is still largely debated (Ober and Hayes 2010). Studies that have considered the impacts of disturbance on faunal communities have more commonly compared the impact of a single level of silvicultural harvest with a non-harvest condition; assessment of the impact of such disturbance across a gradient of intensities is less common and is in need of further study (Homyock and Haas 2009).

I investigated the interrelationships between bats, nocturnal flying insects, and forest disturbance at two levels. First I addressed these interrelationships from a broad community level by comprehensively surveying predator and prey assemblages across a disturbance gradient. I then address these interactions from a more intimate predator-prey level by investigating the specific prey consumed by a model bat species, the northern bat (*Myotis septentrionalis* Trouessart). In addressing this interaction on this level, I present higher-resolution data than was previously attainable. Lastly, I investigate the foraging behavior of a predator in the context of its prey occurrence across the landscape.

Objectives and Hypotheses

Though the actual availability of insect prey to different bat species is a consequence not only of prey occurrence in the external environment, but also the ability of the bat species to detect and capture prey (e.g., differences in echolocation and wing morphology across species), broad surveys do provide an indication of insect abundance and, hence, relative availability (Barclay and Brigham 1991; Whitaker 1994; Houston *et al.* 2004). Further, when stratified across an environmental gradient, such broad surveys

illuminate relationships between the environment and insect populations (Okland 1996; Deans *et al.* 2004). I used an acoustic detection system to assess bat activity in tandem with standard techniques for sampling insects. The Anabat II system has become a common research tool due to its cost-efficiency and ease of use (Weller *et al.* 1998; Britzke *et al.* 1999). Such acoustic detection can provide a relative index of activity (e.g., Law and Chidel 2002; Scott *et al.* 2010) and is used for identification of species assemblages found in the temperate forests of North America (e.g., Britzke *et al.* 2004; Brooks and Ford 2005). By assessing predator and prey concurrently, I draw inferences about the effects that spatiotemporal variation of prey holds for predators and how the forest environment influences prey occurrence.

These data, presented in Chapter Two, address the hypotheses that the abundance and composition of nocturnal insect assemblages vary in response to forest disturbance, regional location, and time during the growing season, all of which are consequences of changes in the host plant base across the disturbance gradient. I generate data to address my hypotheses that forest bat activity varies in response to forest disturbance, as well as within the treated areas, in a manner consistent with the bat species' ecomorphology (i.e., the biological context associated with a species' morphology, *sensu* Karr and James 1975). My data demonstrate regional trends in bat activity and prey occurrence across a disturbance gradient, but the associations of predator and prey with vegetation attributes were not consistent.

An understanding of the food habits of a predator requires characterization of the interactions between predator and prey. Traditional analysis of the diets of bats has relied upon identification of undigested, chitinous bits of insect exoskeleton present in feces or

the digestive tract, or the collection of insect body parts culled by the bat when feeding (Whitaker 1988). Morphological identification of prey items contained in feces is the most frequently used method for investigating the diet of bats and has numerous biases (Lacki *et al.* 2007a). Integration of molecular analyses into my research provided a mechanism to directly link specific prey species with predation by specific bat species (Brigham 2007). Application of molecular techniques has been limited in the field of bat ecology (McCracken *et al.* 2005; Carter *et al.* 2006; Clare *et al.* 2009), but has become increasingly commonplace in other ecological disciplines (Symondson 2002; Sheppard and Harwood 2005; Greenstone 2006). In addition to basic dissection procedures, I developed and implemented a standard technique for extracting and amplifying DNA from field-collected fecal samples from bats. I compare and contrast multiple approaches to inferring prey identity from standard "barcode" sequences, and I compare the molecular approaches to the traditional approach of evaluating prey consumption.

Chapters Three and Four address the hypothesis that the dietary specialization of bat species varies in a manner consistent with individual species ecomorphologies. Chapter Three is an investigation of assemblage and region-wide food habits at a relatively course resolution. Chapter Four is a highly-resolved assessment of the dietary niche of a model predator in comparison with the rest of the bat assemblage. My data suggest *M. septentrionalis* consumed prey that were rarely the most abundant and presumably not the most available. Further, my data suggest that *M. septentrionalis* consumed prey which were smaller in size relative to those eaten by other bat species that are more exclusively gleaners or aerial-hawkers in the continuum of foraging behavior (i.e., gleaning being the behavior of taking prey directly from a surface and hawking

being the behavior of taking prey directly from the air while in flight, *sensu* Jones and Rydell 2003). As a complement to Chapter Four, I present further documentation of my exploratory molecular approach in Chapter Five, where I collate a DNA sequence library from field-collected forest Lepidoptera. Using this sequence library in conjunction with sequences from GenBank I lay the groundwork for novel analyses that may prove fruitful for assessing trophic linkages.

Management Implications

My data demonstrate varied responses between predator and prey (Chapter Two), and show that the prey base consumed by forest bats is not static even at a coarse resolution (Chapter Three). Thus broad implementation of forest management practices must be tempered by site conditions and local faunal communities (e.g., the presence of any critical habitat or sensitive species). Despite the necessity of localized management prescriptions, my data point to generalizations that can be broadly integrated into forest management plans. Common insect assemblages form the majority of the diets of the forest bats studied (Chapters Three through Five), my data suggests that management of foraging habitat for forest bats would benefit from a coarse, landscape approach as opposed to a finer species-level approach (Samways 2007). Given the widespread consumption of Lepidoptera, and the overlap in family-level correlations with vegetation metrics, my data suggest that focused management efforts will likely allow for simultaneous management of a wide diversity of Lepidoptera.

Management of upland foraging habitat for bats should focus on Coleoptera and Lepidoptera. Given the correlation of common Lepidoptera with understory vegetation, and the broad correlation of Coleoptera with tree diameter, management prescriptions

should foster a diverse, well-thinned sub-canopy with a canopy of larger than average trees. This management approach will complement the needs of foraging bats suggested by my data; reduction of clutter within a forest will promote increased bat activity (Chapter Two). Further, maintenance of small patches of moderate silvicultural disturbance dispersed across the landscape will increase structural complexity and diversity of habitats, thus promoting landscape-level insect biodiversity and facilitating bat activity (Dodd 2006; Guldin *et al.* 2007; Samways 2007).

CHAPTER TWO: BAT ACTIVITY AND INSECT OCCURRENCE VARIES ALONG A GRADIENT OF DISTURBANCE

Introduction

The population-level relationships between insects and their predators are important for both ecological and conservation reasons, primarily as a consequence of the abundance and diversity that insects serve as a basal trophic level. Despite the role that bats play as the primary vertebrate predators of nocturnal insects (Fenton 2003), relatively few studies have examined land use and bat and insect activity concurrently. Consequently, the use of forested habitats by foraging bats, and how habitat use of bats is influenced by the availability of insect prey, remains poorly understood (Jones and Rydell 2003; Brigham 2007). Just as the bat-insect interaction has proven a fruitful system for the study of predator and prey at the individual (i.e., behavioral) level, the interaction between these fauna at population level may further illuminate broad trends in predator-prey ecology (Waters2003; Brigham 2007).

The prey base of insectivorous bats varies within and among landscapes (Burford *et al.*1999; Wickramasinghe *et al.* 2004; Dodd *et al.* 2008), and some studies on foraging behavior and habitat use of forest-dwelling bats show correlations with occurrence of insect prey (Ober and Hayes 2008; Lacki *et al.* 2009; Morris *et al.* 2010). Even so, bat activity and habitat use is variable at the forest level, and studies addressing forest disturbance are not consistent nor necessarily in agreement. Elevated levels of bat activity have been associated with mature forests (Lacki *et al.* 2007a), forest edges (Hogberg *et al.* 2002) and corridors (Zimmerman and Glanz 2000), along with silvicultural practices such as thinning (Erikson and West 1996; Humes *et al.* 1999) and patch harvesting

(Menzel *et al.* 2002). The majority of bats in eastern North America have a wing morphology and echolocation ability well-suited for feeding in complex forest environments, i.e., amidst tree canopies and 'clutter' of vegetation, though exceptions include lasiurine species such as the hoary bat (*Lasiurus cinereus*) and, to a lesser degree, the eastern red bat (*L. borealis*) (Lacki *et al.* 2007a).

Prey abundance and availability also influences bat activity and habitat use across the forest landscape. Bats face two fundamental decisions when foraging: where to forage and which prey to consume (Whitaker 1994). Identifying which insects are preyed upon by bats is integral to understanding the relationships between bat ecomorphology (the biological context associated with species' morphology) (*sensu* Karr and James 1975), foraging behavior, and prey availability; however, a broader understanding of foraging ecology is ultimately dependent on the spatial and temporal occurrence of prey and their ease of capture among habitats (Whitaker 1994). Thus, an understanding of how insects vary seasonally across the landscape is essential to achieve a more complete understanding of the foraging behavior of forest-dwelling bats.

Insect assemblages vary somewhat predictably across multiple spatial scales in temperate forests (Okland 1996). Insect abundance and diversity correlates with plant richness and abundance, both taxonomically and functionally (Strong *et al.* 1984; Marques *et al.* 2000; Haddad *et al.* 2001). Not surprisingly, nocturnal sampling supports this theoretical framework in agricultural systems (organic vs. conventional farms; Wickramasinghe *et al.* 2004), managed forests (clearcut vs. interior forest; Grendal 1996), and in comparisons between systems (pastureland vs. forest; Leslie and Clark 2002). Lepidoptera, some of the most ubiquitous nocturnal aerial insects in eastern North

America, have been shown to respond to site-level patterns of disturbance (Burford *et al.* 1999; Summerville and Crist 2002; Summerville and Crist 2003) and also to variation in available habitats at the landscape and regional scale (Hammond and Miller 1998; Hill 1999; Summerville *et al.* 2001; Summerville *et al.* 2003). This variation in Lepidoptera is likely a consequence of floristic variation; forest structure is essentially a "snap shot" of forest succession, and shifts in lepidopteran occurrence arise when disturbance in a forest system (e.g., harvest) surpasses a "threshold" of floristic change (Summerville and Crist 2002; Summerville and Crist 2003; Dodd *et al.* 2008). Intense disturbance such as clear-cuts or seed tree harvests decreases lepidopteran diversity (Summerville and Crist 2002). Regardless, even when species richness of Lepidoptera is depressed in clear-cut stands, richness does not vary greatly between regenerating and unharvested stands and is little affected by less intensive management (Summerville and Crist 2002). Lepidopteran abundance or richness was not affected by selective harvest, stand size, or stand age in eastern North America (Burford *et al.* 1999; Summerville and Crist 2002; Dodd *et al.* 2008), but the occurrence of lepidopteran families varies considerably among different stand conditions (e.g., species composition, age and size classes of timber) (Burford *et al.*; Dodd *et al.* 2008).

Forest-dwelling bats are an ecologically sensitive predator group facing a multitude of threats in North America (Brigham 2007; Blehart *et al.* 2009; Cryan and Barclay 2009); a clear understanding of how forest disturbance and land use affects their foraging habitats is critical to developing sound stewardship practices focusing on bat preservation. My study compared the co-occurrence of insect prey with activity levels of forest-dwelling bats, and investigated how predator and prey responded to silvicultural

disturbance across the Central Appalachian region (USA) of eastern North America. I hypothesize that morphologically distinct bats should be associated with different habitat conditions across this disturbance gradient. I focus my study on two bat ensembles: lasiurine bats (migratory tree bats; *Lasiurus* spp. and *Lasionycteris* spp.) and myotine bats (mouse-eared bats; *Myotis* spp.). These two groups broadly represent major suites of morphological characters found in North American bats. Those species which both hawk and glean prey (myotines) are better adapted to cluttered habitats whereas species that more exclusively hawk prey (lasiurines) are better adapted to more open habitats (Norberg and Raynor 1981; Patterson *et al.* 2003). I also hypothesize that the abundance and composition of nocturnal insect assemblages varies both regionally and temporally with silvicultural disturbance as a consequence of changes in host plant availability.

Methods

Study Areas and Disturbance

My study sites were located in mixed-age upland hardwood forests in the Central Appalachian region of North America (Appendix A) in the Daniel Boone National Forest, Kentucky (Lat. 38°2′ N, Long. 83°35′ W); the Raccoon Ecological Management Area, Ohio (Lat. 39°11′ N, Long. 82°22′ W); the Royal Blue Wildlife Management Area, Tennessee (Lat. 39°11′ N, Long. 82°23.′ W); and commercial timberland in Wyoming County, West Virginia (Lat. 37°30′ N, Long. 81°36′ W). A gradient of silvicultural disturbance was established at each site during the dormant season of 2006-2007 (Beachy 2007). Four plots randomly received one of four treatments covering ca. 10 ha each, resulting in a gradient of disturbance intensity that included: 1) seed tree harvest (7.7 ± 2.1 m^2 per ha residual basal area), 2) shelterwood harvest (18.0 ± 0.9 m^2 per ha residual

basal area), 3) single tree harvest (21.9 ± 1.0 m^2 per ha residual basal area), and 4) undisturbed forest (control) (26.8 ± 0.9 m^2 per ha basal area). Bat activity and insect occurrence were concurrently monitored in each plot across four sampling intervals (May, June, July, August) during the growing seasons of 2007 and 2008.

Bat Activity

I used the Anabat II system (Titley Electronics, Ballinia, Australia) to record echolocation calls. Detection systems were powered by a 12 V gel-cell battery, housed in plastic containers to protect equipment from inclement weather (O'Ferrell 1998), and mounted on 1.6-m camera tripods (Appendix B). Detection systems were regularly calibrated using an ultrasonic insect repeller (Hayes 2000; Larson and Hayes 2000); no difference in detection capability was observed within or among my Anabat II systems over the course of the study.

Detection systems were simultaneously placed at a fixed point at the interior and edge of each plot within a study site (n = 8) to ensure concurrent monitoring at all plots within a site (Scott *et al.* 2010). Interior detection systems were >50 m from plot boundaries. Detection systems placed at the edges were positioned so the detection cone followed the plot boundary for >50 m. Acoustic surveys spanned ≥2 nights during each sampling interval to account for nightly variation, and occurred concurrently with insect sampling.

Insect Occurrence

To compensate for the bias introduced by any single approach, I used two techniques to assess prey occurrence (Kunz 1988; Krebs 2000) (Appendix C). Nocturnal phototactic insects were surveyed using a 10 W blacklight trap (Universal Light Trap,

Bioquip, Rancho Dominguez, CA) suspended at 2.5 m. A cotton wad soaked in ethyl acetate was placed in each trap to kill captured insects. Malaise traps ('Square Configuration' Malaise Trap, Bioquip, Rancho Dominguez, CA) placed at ground level were used to survey insects not typically captured in blacklight traps. Collection jars containing a ca. 2×6 cm Dichlorvos-based 'pest strip' as a killing agent were affixed to the traps at dusk so as to capture only nocturnal insects. Insects were removed the following day and stored in 70% ethanol.

Fixed sampling locations were established for insect trapping in both interior and edge locations within each plot, chosen to represent disturbance intensity, potential for use by predator and prey (i.e., flyways and corridors), and accessibility. Traps were spaced far enough apart to ensure no interference between trap types (Muirhead-Thomson 1991). Interior sampling locations were >50 m from treatment boundaries and edge sampling locations were located on plot boundaries. Insects were surveyed on a single night in each sampling interval, concurrent with acoustic surveys for bats.

Insects were identified using available keys (Holland 1903; Borer and White 1970; Covell 2005; Triplehorn and Johnson 2005) and reference collections at the University of Kentucky. Insects ≥10 mm in length captured in light traps were identified to the lowest taxon practical; Lepidoptera were identified to species and other insects to the family level. Smaller insects (<10 mm) captured in light traps were combined, dried and weighed to estimate biomass per trap. All insects captured in malaise traps were identified to the lowest taxon practical (generally family level; Borer and White 1970; Triplehorn and Johnson 2005).

Vegetation Assessment

I measured vegetation data with 11.3-m-radius plots (0.04 ha) at randomly selected points within each larger 10-ha plot using the random-point generator extension (Jenness 2005) in ARCVIEW, version 3.2 (ESRI, Redlands, California); all points were located ≥25 m apart. Vegetation was assessed in early to mid-June of 2007 and 2008 from 7 to 40 vegetation plots per treatment plot (Beachey 2007). An ocular tube was used (James and Shugart 1970) to determine percent cover of saplings and shrubs. Ocular-tube readings were averaged over 20 points within the plot; observers recorded the presence of both sapling and shrub cover when looking through the ocular tube downward from the line of sight at a 45° angle and straight up at each point (Bulluck and Buehler 2008). Individual saplings within plots were identified within plots and counted. The basal area of canopy trees (>10 cm diameter at 1.3 m) was estimated at each plot center; delineated trees were identified and their diameters measured.

Analyses

For acoustic surveys, Anabat sequence files were downloaded using Analook, version 4.8j. A program filter followed by visual inspection was used to remove extraneous acoustic data from the surrounding environment. The 'countscan' function was used to count the total number of echolocation pulses per night as a measure of overall bat activity. This variable is opposed to density, which cannot be known (Hayes 2000). Those sequences with ≥5 echolocation pulses were then retained for subsequent species-group analysis. These data were compared to a reference library of echolocation sequences of known species, and the sequences classified to species group using Fisher's linear discriminate function analysis (Britzke 2003; Lattin *et al.* 2003; Wolf *et al.* 2009). I

then counted the resulting number of echolocation pulses per night identified as belonging to either the lasiurine or myotine species groups.

Suites of response variables were evaluated across my three survey approaches for predator and prey. Response variables for bat activity included total pulses per night, lasiurine pulses per night, and myotine pulses per night. Response variables for insect occurrence included abundance of focal insect orders (Lepidoptera and Coleoptera were the focus of blacklight traps and Diptera were the focus of malaise traps), the Shannon index of diversity ($H' = -\Sigma p_i \ln p_i$) of families within each order (Magurran 1988; Allgood *et al.* 2009) and, in the case of blacklight trap surveys, biomass of insects < 10 mm. All response variables were tested for homogeneity of variance using Variance Ratio $F\text{-}_{MAX}$ tests, with analyses based on log-transformed values when variances were heterogeneous (Sokal and Rohlf 1969).

Annual variation was assessed using one-way analyses of variance (ANOVAs). If data varied between years, this variation was partitioned out in subsequent analysis as a covariate. If not, data for both years of sampling were pooled. Multivariate analyses of variance (MANOVAs) were performed for each suite of response variables (echolocation surveys, blacklight traps, malaise traps). Main effects in these analyses included disturbance and study site. Sampling interval was incorporated as a nested (hierarchical) effect within study site due to repeated surveys of the same physical location (Zar 1999). Plot position (i.e., interior versus edge) was incorporated as a nested (hierarchical) effect within the disturbance effect. The interactions between the main effects of disturbance and study site were also examined. When global MANOVA and subsequent ANOVAs

were significant, I used Tukey's Honestly Significant Difference means separation procedures to evaluate effects (Zar 1999).

Canonical correspondence analysis (CCA) was performed on data from each survey approach with vegetation variables collected across study plots in Kentucky, Tennessee, and West Virginia (Lattin *et al.* 2003). Response variables for acoustic surveys included the number of echolocation pulses per night for both lasiurine and myotine species groups. Order-level abundance and the Shannon index (H') at the family level were considered for analyses of Coleoptera and Diptera, respectively. Finally, the most abundant lepidopteran families were analyzed separately; abundance and species richness within families were considered as response variables. Explanatory variables from vegetation assessments included sapling density (stems/ha), sapling cover (mean %), sapling species richness (n), shrub cover (mean %), mean diameter of canopy trees (cm), basal area of canopy trees (m^2/ha), and canopy tree species richness (n). Percent frequency data were arcsine-square root transformed prior to analysis (Zar 1999). Because sample points for vegetation variables were randomly chosen and were not related to sample points for either predator or prey, vegetation data were randomly sampled with replacement from among the data set within each plot.

The delineation of values from CCAs used for interpretive purposes was made *a posteriori*. Variables were considered significant for a canonical axis when possessing both a standardized canonical coefficient ≥ 0.40 and a correlation ≥ 0.20 with the opposing dataset. In this way I interpreted variables that contributed a relatively large amount of variation to my analyses and also suggested an association between flora and fauna.

Results

Bat Activity

Four survey intervals were completed for each growing season, resulting in acoustic surveys spanning 94 nights (n = 696 survey nights). I recorded a total of 58,428 echolocation files. From these data, I counted 1,037,274 echolocation pulses. Of these, 459, 753 pulses were identified; 59,886 pulses (13%) were lasiurine species and 69,990 pulses (15%) were myotine species. The remaining 72% were identified as other species (*Eptesicus fuscus* Beauvois, *Nycticeius humeralis* Rafinesque, *Perimyotis subflavus* Cuvier). No difference was detected between survey years for total pulses, lasiurine pulses, or myotine pulses; however, all global models were significant (Table 2.1). Multivariate analyses were significant for disturbance, plot position, study site, and sampling interval. Subsequent univariate analyses were all significant. Main effects were significant, as well as their interaction.

Total pulses, lasiurine pulses, and myotine pulses exhibited similar patterns. Total pulses and lasiurine pulses were lowest in undisturbed forests and highest in the most intensely disturbed plots (seed tree). Similarly, myotine pulses were lowest in undisturbed forests, but there was no difference in myotine pulses among disturbed plots. Distinct regional differences were also evident. The greatest number of total pulses per night was recorded in Tennessee, followed by Ohio, Kentucky, and West Virginia. Lasiurine pulses per night were greatest in Ohio and Tennessee, followed by West Virginia and Kentucky. The least number of myotine pulses per night was recorded in West Virginia; the remaining sites did not differ. The nested effect of plot position was not significant for any echolocation response variable. The nested effect of sampling

interval over the growing season was significant for total pulses, which increased over the growing season, and was lowest in May and highest in August.

Variation in bat activity corresponded with vegetation variables; canonical eigenvalues of both ordination axes of my CCA were significant (Table 2.2). The first axis accounted for over 58% of the variation in the data. For the first axis, variation in vegetation was associated with sapling richness and shrub cover, whereas variation in bat activity was associated with myotine pulses per night; this was inversely correlated to sapling richness and shrub cover. On the second axis, variation in vegetation was associated with canopy tree richness, and variation in bat activity was associated with lasiurine pulses per night, which was inversely correlated with canopy tree richness.

Insect Occurrence

I surveyed insects over 32 nights during two growing seasons. My blacklight traps (n = 248 samples) yielded 35,566 insects across 13 orders, of which 29,066 (82% total insects) were Lepidoptera from 24 families. Noctuidae were most abundant, with 9,507 individuals captured. Other abundant families (n > 100 individuals) included Geometridae (n = 5,324), Arctiidae (n = 5,236), Notodontidae (n = 2,859), Pyralidae (n = 2,208), Lasiocampidae (n = 794), Saturniidae (n = 869), Sphingidae (n = 124), Oecophoridae (n = 485), Limacodidae (n = 378), Tortricidae (n = 191), Lymantriidae (n = 179), and Yponomeutidae (n = 100). There were also 5,245 Coleoptera in my blacklight trap samples (15% total insects) from 32 families. Carabidae and Scarabidae were most abundant, with 2,835 and 1,160 individuals captured, respectively. Other abundant families (n > 100 individuals) included Elateridae (n = 485) and Silphidae (n = 107). In

total, Lepidoptera and Coleoptera comprised 97% of the insects captured in blacklight traps.

Global models were significant for data from blacklight traps (Table 2.3). Lepidoptera were more abundant during the second field season, but there was no difference in diversity of families between years. Coleoptera were more abundant and also more diverse during the second field season. Biomass of insects (<10 mm) captured in blacklight traps did not vary annually. Multivariate analyses were significant for disturbance, sampling interval, and study site, but not for plot position. Subsequent univariate analyses were significant across the entire suite of response variables for blacklight traps, including lepidopteran abundance, lepidopteran diversity, coleopteran abundance, coleopteran diversity, and biomass of insects <10 mm.

Lepidopteran abundance and diversity varied temporally and spatially (Table 2.3). The main effects of disturbance and study site were significant, but the interaction was not. Lepidopteran abundance was higher in undisturbed plots compared to plots with seed tree harvests. Diversity, however, was lowest in the highly disturbed seed tree harvests compared to remaining disturbance levels. Regional differences were also evident; more Lepidoptera were captured at plots in Ohio versus plots in Kentucky and Tennessee. A similar trend was evident for diversity. As the nested effect of plot position was not significant in the MANOVA, the significance of this effect was not interpreted at the univariate level. The nested effect of sample interval was significant; fewer and less diverse Lepidoptera were captured in May compared to subsequent months.

Occurrence of Lepidoptera corresponded with vegetation variables; canonical eigenvalues of both the first and second ordination axes were significant and explained

nearly 60% of the variability in the data (Table 2.2). The first axis accounted for over 33% of the variation, and the second axis accounted for over 25%. For the first axis, variation in vegetation was associated with sapling richness. Variation in lepidopteran occurrence on the first axis was associated with arctiid, noctuid, and notodontid abundance, and notodontid richness; all variables were positively correlated with sapling richness. For the second axis, variation in vegetation was associated with sapling density. Correlation of lepidopteran variables was weak and less than my 0.20 threshold; even so, noctuid abundance was most correlated with sapling density.

Coleopteran occurrence and the biomass of insects <10 mm varied less than that demonstrated for Lepidoptera (Table 2.3). For all explanatory variables the effect of study site was significant while the effect of disturbance was not. Coleopteran abundance was higher in Ohio than in Tennessee and West Virginia; abundance in Kentucky was intermediate. Conversely, Kentucky blacklight captures were more diverse than either Ohio or Tennessee; West Virginia was intermediate. Biomass of insects <10 mm was three times higher in plots in Ohio than in other study sites. Nested effects were not significant for these response variables.

Occurrence of Coleoptera corresponded with vegetation variables; the canonical eigenvalue of the first ordination axis was significant and accounted for over 86% of the variation in the data (Table 2.2). Variation in vegetation was associated with canopy tree diameter. Variation in Coleoptera was associated with abundance and not diversity; abundance was positively correlated with canopy tree diameter.

My malaise trap samples (n = 248) yielded 31,122 insects across 11 orders, of which 25,575 (82%) were Diptera from 33 families. Cecidomyiidae was the most

abundant dipteran family, with 19,610 individuals. Other abundant families (n > 100 individuals) included Sciaridae (n = 1,696), Phoridae (n = 971), Mycetophilidae (n = 517), Psychodidae (n = 383), Tipulidae (n = 376), Dolichopodidae (n = 364), Chironomidae (n = 299), and Muscidae (n = 231). Aside from Diptera, other abundant orders (n > 100 individuals) captured in malaise traps included the Hemiptera (n = 2,154), Lepidoptera (n = 2,088), Hymenoptera (n = 1,021), and Coleoptera (n = 202).

Global models were significant for data from malaise traps (Table 2.4). Neither abundance nor diversity of Diptera varied between years. Multivariate analyses were significant for disturbance, plot position, sampling interval, and study site. Subsequent univariate analyses were significant for both dipteran abundance and diversity.

Dipteran abundance and diversity varied spatially (Table 2.4). Main effects were significant, as well as their interaction. Dipteran abundance was higher in the plots disturbed by shelterwood harvests as compared to the less intensively disturbed single-tree harvests and undisturbed plots. Dipteran diversity differed across the disturbance gradient. Undisturbed plots were more diverse than single-tree harvests; diversity in the other disturbance levels was intermediate. Regional differences were also evident. More Diptera were captured in plots in West Virginia than in Kentucky, but dipteran diversity was greater in Ohio than either Tennessee or West Virginia. Neither the nested effect of plot position nor sample interval was significant.

Occurrence of Diptera corresponded with vegetation variables; the canonical eigenvalue of the first ordination axis was significant and explained more than 67% of the variation in the data (Table 2.2). Variation in vegetation was associated with canopy tree

richness. Variation in Diptera was associated with abundance but not diversity; abundance was inversely correlated with canopy tree richness.

Discussion

My data demonstrate variation in response to silvicultural disturbance between forest-dwelling bats and their insect prey, and also demonstrate variation in response among prey assemblages (Figure 2.1). Though both bat ensembles exhibited consistent trends in activity in relation to disturbance, responses varied across the three major prey assemblages: Coleoptera, Diptera, and Lepidoptera. In total, my CCAs suggest varied vegetation characteristics underpin the results generated by my hypothesis-driven analyses (i.e., MANOVAs, ANOVAs, and means separation procedures). Whereas the relationships between both bat ensembles and vegetation support the importance of habitat structure in influencing predator activity patterns, the relationships of prey assemblages with vegetation also vary, suggesting differences in host resource utilization.

My results indicate an overall increase in bat activity in disturbed habitats (Table 2.1) comparable to other studies (Grindal and Brigham 1998; Owen *et al.* 2004; Brooks 2009). I anticipated lower activity of myotine bats in more heavily disturbed plots based upon wing morphology and echolocation characteristics (Lacki *et al.* 2007), but my results did not support this hypothesis. Activity of myotine bats has been negatively related to open and thinned stands in coniferous systems, whereas lasiurine species foraged in both thinned and unthinned stands (Morris *et al.* 2010). My results demonstrated that regardless of differences in ecomorphology between these ensembles, both groups of bats were more active in areas with silvicultural harvest.

There are multiple approaches to identify echolocation calls of bats (Vaughan *et al.* 1997; Parsons 2001; Milne 2002; Wolf *et al.* 2009). The statistical technique used in this study is more objective than identification approaches that rely upon simple visual interpretation of sonograph characteristics (Milne 2002). Further, the call library I used is robust and consists of multiple echolocation calls collected over the distributions of species (over 23,000 individuals recorded across eastern North America) (E. Britzke, pers. comm.). Even so, call characteristics and short sequences of echolocation pulses are not diagnostic for most species in eastern North America (E. Britzke, pers. comm.). Because of this, and the large sample size of the study, I judged it important to try to maximize power of my identification approach and only identified echolocation calls that occurred in series of ≥5 pulses (Britzke *et al.* 2004). In doing so, I discounted nearly half the echolocation calls recorded, but retained a high degree of confidence that the calls that I have identified as belonging to either the lasiurine or myotine ensembles truly are of these groups (i.e., avoiding Type II error).

Drawing conclusions about relative differences in the activity levels of different ensembles of bat species is difficult because the probability of detecting echolocation calls differs among bat species (Britzke 2003). Even so, my exploratory analyses suggest differences between myotine and lasiurine bat activity in relation to vegetation variables (Table 2.2). Thus, while lasiurine and myotine bats both exhibit similar patterns along the disturbance gradient, varied characteristics underpin these respective patterns. My data indicate that myotine species are more affected by the sub-canopy vegetation layer. Given the gradient of disturbance considered in my study, I suggest that the reduction in sub-canopy clutter by disturbance increases the opportunity for foraging by myotine bats

using both hawking and gleaning strategies (Ratcliffe and Dawson 2003). While gleaning as a foraging strategy may be conceptualized behaviorally as "predatory cheating" (Faure *et al.* 1993; Lacki *et al.* 2007a), these gleaning bats may "cheat" similarly in time and space by being less constrained in where they can forage. My data suggest that disturbance of any intensity increases activity of myotine bats. In contrast, lasiurine species were negatively correlated with canopy tree richness. The most intensely disturbed plots with the least cluttered overstory generally possessed the highest activity for this group. I suggest that a reduction of clutter associated with the overstory resulted in a positive response by lasiurine bats, which primarily hawk prey in flight (Lacki *et al.* 2007a; Morris *et al.* 2010). Thus, my results demonstrate varied interactions between vegetation structure and ensembles of bats.

Though bat activity varied across the gradient of disturbance, it did not vary between plot interiors and plot edges (Table 2.1). Other studies have suggested bats have a propensity to use forest edges and corridors, corresponding toareas of increased abundance of insects (Walsh and Harris 1996; Grindal and Brigham 1999; Hogberg *et al.* 2002; Morris *et al.* 2010). Whereas myotine bats have been reported to forage within the interior of less intensively managed stands and to avoid edges, lasiurine bats are more ubiquitous in their use of such habitats (Patriquin and Barclay 1990; Owen *et al.* 2003; Morris *et al.* 2010). The edges of silvicultural harvests in my study were variable, and ranged from gradual to drastic shifts in the density of both canopy and sub-canopy strata of vegetation. My data suggest that bat activity across a gradient of edge contrast (*sensu* Ries *et al.* 2004) does not vary in comparison with interior of disturbed forest patches at the scale I evaluated in upland hardwoods of the Central Appalachians. I suggest that the

limited difference in bat activity in my study is constrained, in part, by the relatively small patch size of disturbance (ca. 10 ha), rendering plot position irrelevant. Bats in my study areas were foraging within large forest gaps with reduced clutter and not flying along a defined landscape contour. The uniformity in insect activity between edge and interior habitat conditions may further explain the lack of differences observed for bat activity in this study, and suggests that bats of both ensembles may be able to adapt to local conditions on a limited spatial scale. Even so, forest bats use other edges that were not assessed in my study, e.g., along the top of tree and forest canopies (Menzel *et al.* 2000; Kalcounis *et al.* 1999).

In contrast with the responses of both bats and the other prey assemblages, broad shifts in Coleoptera were not observed with disturbance (Table 2.3), likely due to varied responses across coleopteran taxa (Okland *et al.* 2008). While studies in Appalachia have demonstrated an increase in the richness of some Coleoptera with disturbance (Lenski 1982), and while coleopteran diversity has also been shown to correlate with more mature forest systems (Butterfield *et al.* 1995), disturbance has more generally been shown to induce broad shifts in coleopteran species occurrence, particularly for Carabidae (Werner and Raffa 2000; Koivula *et al.* 2002; Work *et al.* 2010) , which were the most commonly captured coleopterans in my study. Such observations do not necessarily impact broad measures of abundance, nor richness. My observations indicate overall coleopteran abundance and diversity remain the same across the disturbance gradient, but my canonical correspondence analysis indicates that coleopteran abundance was positively correlated with canopy tree diameter. This suggests the Coleoptera in my study were

most affected by canopy-level vegetation and, specifically, positively correlated with larger diameter timber identified with more mature, later seral stage habitats (Table 2.2).

Diptera responded positively to disturbance (Table 2.4), and was negatively correlated with canopy tree richness (Table 2.2). As suggested for deciduous and coniferous habitats in western North America (Hughes *et al.* 2000), my data demonstrate Diptera are influenced by forest habitat and structure. This trend is likely driven by the Cediomyiidae, the most abundant dipteran family captured. A correlation between cecidomyiids and habitats with denser canopy cover has been noted in coniferous forests (Allgood *et al.* 2009), where overall abundance was balanced as members of the dipteran community changed with stand age and harvest. Similarly, my data suggest that in hardwood forests dipteran abundance was higher in plots with lower canopy richness; a vegetation trait associated with silvicultural disturbance. My data, coupled with that of Allgood *et al.* (2009), suggest similarities in the occurrence of the dipteran prey base for bats between deciduous and coniferous forests of eastern North America.

Lepidopteran occurrence in my study was inversely related to disturbance (Table 2.3), corroborating results from other studies (Summerville and Crist 2008). This is likely a reflection of the dependence of many Lepidoptera on the foliage of dominant canopy-tree species for development (Covell 2005; Tallamy and Shropshire 2009). Even so, my canonical correspondence analysis indicates a link between the sub-canopy vegetation layer and multiple lepidopteran families. This assemblage is reliant on a forested habitat defined by the richness and structure of vegetation.

Lepidoptera are the most consistently and heavily consumed prey for both the lasiurine and myotine ensembles (Lacki *et al.* 2007a). Thus, my data for Lepidoptera are

particularly relevant for stewardship and conservation efforts, and point to a paradoxical relationship between forest bats and their nocturnal prey. While my data demonstrate bat activity positively correlates with disturbance, lepidopteran occurrence negatively correlates with disturbance. Morris *et al.* (2010) suggest that habitat structure is more important than prey occurrence in determining spatiotemporal foraging patterns of bats in coniferous forests. My data supports this supposition in the upland hardwood systems of eastern North America. Although disturbance may reduce clutter and stem density, thus facilitating bat flight and habitat usage, disturbance also shifts the quality and quantity of vegetation, reducing the abundance and diversity of the available lepidopteran prey base. Thus, my observations of Lepidoptera may have two explanations: (1) disturbance directly impacts Lepidoptera by reducing host resources or (2) disturbance indirectly impacts Lepidoptera by increasing susceptibility to predation, resulting in either predator avoidance or population regulation. Regardless, given the importance of floral diversity in maintaining the biodiversity of forest Lepidoptera (Summerville and Crist 2008), managers and stewards should account for predator, prey, and the host plant base. Land managers should maximize floral diversity when working toward conservation goals for forest dwelling bats to maximize the occurrence of Lepidoptera and provide a reliable prey base for foraging bats (Panzer and Schwartz 1998; Lacki and Dodd *In Press*).

Beyond the responses of predator and prey to disturbance, my data further suggest broad regional and temporal differences in both bat and insect assemblages. Regional differences in bat activity are likely related to differences in composition of bat assemblages (Barbour and Davis 1969; Harvey *et al.* 1999). Not surprisingly, the site that possessed the highest observed activity (Tennessee) (Table 2.1) also supports the richest

bat assemblage (Barbour and Davis 1969). Similarly, shifts in forest insect biodiversity are readily apparent at scales with discrete assemblages (e.g., Summerville *et al.* 2001), but shifts are seen as well at scales fine enough to possess sympatric assemblages (Hughes *et al.* 2000; Rieske and Buss 2001). Though more studies have assessed the effects of forest management practices on the biodiversity of insects at and within a landscape level, variation at a broader scale is clearly evident; management considerations must take coarse scale in to consideration to achieve/maintain biodiversity goals (Werner and Raffa 2000; Samways 2007). My data demonstrate strong regional effects for forest insects; differences were found for all common prey taxa. These data thus underpin the importance of landscape-level and regional variation on determining patterns of insect diversity and, thus, site-level management of foraging habits for bats.

Although I detected striking increases in the abundance of both Lepidoptera and Coleoptera during the second year of my study, bat activity did not differ substantially between years. Lepidoptera and Coleoptera broadly utilize different host resources; lepidopteran caterpillars eat live vegetation and beetle larvae eat both living and dead flora and fauna. In the case of Coleoptera, an interesting interplay between abundance and measures of diversity plays out as a forest matures following disturbance (Koivula *et al.* 2002). In coniferous systems, it is thought that flushes in Carabid species richness correlates with invasion by "open habitat" species, lasting 20-30 years post-harvest (Niemala *et al.* 1993; Koivula *et al.* 2002). Disturbance impacts are complex and may take more than a single growing season to come to fruition (Taki *et al.* 2010). My inability to detect between-year differences in bat activity provides weak evidence for opportunistic prey-switching, or a lack of significant top-down pressure across broad

taxonomic groups. I suggest that bat assemblages have less of an opportunity to shift in response to disturbance relative to the insect prey on the temporal scale evaluated in this study. Bats are long-lived and lack the reproductive capacity of insects. Consequently, insects are more sensitive to local habitat changes and can provide a rapid assessment of the effects environmental change (Hill *et al.* 1995; Kitching *et al.* 2000; Werner and Raffa 2000; Summerville *et al.* 2004).

Seasonal differences within the growing season were also readily apparent and illustrate changes in prey abundance and availability. My data demonstrate that the lepidopteran prey base is less abundant early in the growing season, which is reflected in the amount of foraging activity of its primary predator. Lepidoptera, my most commonly-captured prey taxon, are known to peak throughout early June to late August in temperate forests (Rings *et al.* 1992; Thomas and Thomas 1994; Thomas 2001). My data demonstrate a synchrony between predator and prey.

In summary, my data provide an indication that both forest-dwelling bats and their insect prey vary broadly and predictably in response to forest structure across the Central Appalachian region of eastern North America. My data corroborate that of Morris *et al.* (2010), that habitat structure takes primacy in determining activity patterns (i.e., foraging) of bats versus patterns in prey occurrence. Even so, relationships between prey assemblages and the host plant base suggest a paradox if using silvicultural disturbance as a management tool for both predator and prey. My study also suggests strong differences in the occurrence of major prey assemblages both regionally and temporally. I recommend further studies across a diversity of disturbance regimens and regions as a means of testing the validity of trends across broader spatial and temporal gradients. Until

relationships are resolved across forest systems, patches of varied disturbance at moderate levels across the landscape are a useful tool to achieve preferred biodiversity goals for forest-dwelling bats and forest insects and maintain endemic species on a regional scale (Taki *et al.* 2010; Werner and Raffa 2000; Work *et al.* 2010). Given the ephemeral nature of insect occurrence as forests mature, future studies should focus on better understanding the long term changes that arthropod communities exhibit following forest disturbance.

Table 2.1. Variation in bat activity in Central Appalachia, 2007-2008. Different letters within a column indicate significant differences (P<0.05). The interaction between disturbance and study site was significant for all response variables.

Explanatory Variable	Level (n)	Mean Pulses per Night (SE)		
		Total ($F_{31, 411} = 10.01$)	Lasiurine ($F_{31, 411} = 8.42$)	Myotine ($F_{31, 411} = 7.27$)
Year	2007 (224)	1430.1 (207.0)	152.0 (27.4)	138.9 (25.6)
	2008 (222)	1552.6 (255.8)	116.4 (22.2)	174.3 (62.5)
Disturbance	Undisturbed (59)	223.9 (53.8) c	11.4 (3.9) c	32.7 (5.7) b
($\lambda_{9, 996} = 10.68$)	Single-Tree (133)	1538.5 (248.7) b	132.9 (34.9) b	152.4 (34.2) a
	Shelterwood (132)	1696.3 (229.7) b	131.7 (27.7) b	159.3 (44.0) a
	Seed Tree (122)	2688.9 (611.9) a	197.9 (41.7) a	217.9 (107.0) a
Plot Position	Edge (354)	1345.2 (224.1)	148.3 (32.4)	150.8 (30.1)
($\lambda_{12, 1082} = 4.0$)	Interior (342)	1640.6 (239.6)	121.8 (17.0)	161.6 (57.6)

Table 2.1. (continued)

Study Site	Kentucky (112)	816.3 (135.4) c	48.3 (9.8) b	93.6 (40.0) a
($\lambda_{9, 996} = 10.13$)	Ohio (126)	1840.3 (202.7) b	211.9 (43.8) a	176.5 (35.2) a
	Tennessee (141)	2913.7 (575.1) a	167.4 (36.4) a	252.8 (94.8) a
	West Virginia (67)	325.2 (53.5) d	61.9 (17.0) b	21.5 (4.6) b
Sample Interval	May (99)	616.6 (100.3) c	102.0 (20.6)	79.8 (33.5)
($\lambda_{36, 1209} = 4.51$)	June (127)	1433.1 (343.9) b	131.0 (37.8)	86.5 (26.2)
	July (117)	1487.1 (206.5) b	151.9 (44.2)	171.8 (45.8)
	August (103)	2342.6 (479.8) a	149.3 (27.7)	299.3 (127.6)

Table 2.2. Canonical correspondence analyses of the relationships between bat activity and vegetation attributes and insect occurrence and vegetation attributes in Central Appalachia, 2007-2008. Values in this table are not exhaustive and only include those considered for interpretation; variables were considered significant for a canonical axis when possessing both a standardized canonical coefficient ≥ 0.40 and a correlation ≥ 0.20 with the opposing dataset. See Appendices D - G for comprehensive results.

CCA Analysis	First Axis (*F*-Score)		Second Axis (*F*-Score)	
(Wilks λ Score)	Standardized Coefficients	Correlation of Datasets	Standardized Coefficients	Correlation of Datasets
Bat Activity ($\lambda_{14, 622} = 4.86$)	($F_{14, 622} = 4.86$)		($F_{6, 312} = 4.71$)	
Canopy Tree Richness	1.1241	0.1084	0.6285	0.2607
Sapling Richness	0.5922	0.2388	-0.4517	-0.0778
Shrub Cover	0.4724	0.2179	-0.0189	-0.0502
Lasiurine Pulses	-0.1635	-0.2146	-1.1604	-0.2225
Myotine Pulses	-0.9050	-0.3345	0.7446	0.0402

Table 2.2. (continued)

Lepidopteran Occurrence ($\lambda_{70, 951} = 1.79$)	($F_{70, 951} = 1.79$)		($F_{54, 836} = 1.55$)	
Sapling Density	-0.8353	-0.0337	0.8258	0.3641
Sapling Richness	0.7765	0.2474	-0.1700	0.2211
Arctiid Abundance	0.7237	0.2769	0.8532	0.0482
Noctuid Abundance	0.7051	0.2645	1.0878	0.1620
Notodontid Abundance	-0.4679	0.2226	-0.9391	-0.0959
Notodontidae Richness	0.8839	0.2512	-0.3575	-0.0509
Coleopteran Occurrence ($\lambda_{14, 354} = 2.53$)	($F_{14, 354} = 2.53$)			
Canopy Tree Diameter	0.6757	0.2728		
Coleopteran Abundance	1.7407	0.3231		

Table 2.2. (continued)

Dipteran Occurrence ($\lambda_{14, 350} = 2.66$)		($F_{14, 350} = 2.66$)
Canopy Tree Richness	-1.0052	-0.2508
Dipteran Abundance	0.9134	0.3193

Table 2.3. Variation in insects captured in blacklight traps in Central Appalachia, 2007-2008. Different letters within a column indicate significant differences ($P<0.05$).

Explanatory Variable	Level (n)	Mean per Trap (SE)				
		Lepidoptera Abundance ($F_{32, 245} = 7.31$)	Lepidopteran Diversity (H') ($F_{32, 245} = 4.05$)	Coleopteran Abundance ($F_{32, 245} = 13.92$)	Coleopteran Diversity (H') ($F_{32, 245} = 9.48$)	Biomass (g) ($F_{32, 245} = 5.29$)
Year	2007 (122)	93.0 (8.6) b	1.44 (0.05)	2.71 (0.70) b	0.18 (0.04) b	1.27 (0.16)
	2008 (126)	140.7 (10.8) a	1.50 (0.03)	39.00 (6.45) a	0.84 (0.05) a	1.06 (0.17)
Disturbance ($\lambda_{15, 577} = 1.69$)	Undisturbed (64)	166.1 (18.7) a	1.48 (0.06) a	14.28 (3.29)	0.57 (0.09)	1.46 (0.25)
	Single-Tree (63)	110.7 (11.1) ab	1.51 (0.06) a	16.84 (4.07)	0.53 (0.07)	0.95 (0.12)
	Shelterwood (62)	100.1 (12.5) ab	1.50 (0.05) a	19.32 (5.19)	0.51 (0.07)	0.91 (0.15)
	Seed Tree (59)	89.1 (10.2) b	1.39 (0.06) b	35.12 (12.30)	0.44 (0.07)	1.35 (0.36)
Plot Position	Edge (125)	142.0 (11.7)	1.50 (0.04)	23.94 (5.67)	0.56 (0.05)	1.32 (0.18)
	Interior (123)	92.0 (7.3)	1.45 (0.04)	18.32 (4.03)	0.47 (0.05)	1.01 (0.14)

Table 2.3. (continued)

Sample Interval	May (60)	61.7 (9.2) b	1.08 (0.07) b	4.83 (0.94)	0.31 (0.05)	0.44 (0.16)
($\lambda_{60, 982} = 5.35$)	June (62)	139.8 (17.9) a	1.57 (0.05) a	34.37 (10.14)	0.87 (0.08)	1.57 (0.30)
	July (63)	138.6 (13.9) a	1.72 (0.03) a	24.98 (6.31)	0.56 (0.08)	1.12 (0.16)
	August (63)	126.4 (11.9) a	1.50 (0.04) a	19.84 (6.57)	0.31 (0.06)	1.49 (0.26)
Study Site	Kentucky (59)	105.5 (13.0) b	1.54 (0.07) ab	15.03 (2.67) ab	0.64 (0.08) a	0.85 (0.13) b
($\lambda_{15, 577} = 6.56$)	Ohio (61)	164.8 (16.3) a	1.59 (0.05) a	50.25 (12.66) a	0.48 (0.07) b	2.45 (0.40) a
	Tennessee (64)	62.2 (7.8) b	1.40 (0.05) b	11.78 (3.54) b	0.40 (0.07) b	0.60 (0.08) b
	West Virginia (64)	137.7 (15.0) ab	1.37 (0.06) b	8.42 (1.76) b	0.55 (0.08) ab	0.78 (0.09) b

Table 2.4. Variation in Diptera captured in malaise traps in Central Appalachia, 2007-2008. Different letters within a column indicate significant differences ($P<0.05$).

Explanatory Variable	Level (n)	Mean per Trap (SE)	
		Dipteran Abundance ($F_{31, 247} = 2.84$)	Dipteran Diversity (H') ($F_{31, 247} = 3.62$)
Year	2007 (120)	0.73 (0.04)	104.7 (10.4)
	2008 (128)	0.75 (0.04)	101.7 (8.5)
Disturbance	Undisturbed (63)	85.6 (10.3) b	0.81 (0.06) a
($\lambda_{6, 430} = 4.90$)	Single-Tree (62)	72.6 (8.2) b	0.66 (0.05) b
	Shelterwood (63)	136.0 (14.5) a	0.72 (0.06) ab
	Seed Tree (60)	118.6 (17.5) ab	0.78 (0.05) ab
Plot Position	Edge (124)	104.6 (9.8)	0.69 (0.04)
($\lambda_{8, 430} = 3.04$)	Interior (124)	101.6 (9.2)	0.79 (0.04)
Sample Interval	May (60)	104.7 (14.0)	0.63 (0.05)
($\lambda_{24, 430} = 2.89$)	June (62)	132.0 (17.5)	0.84 (0.06)
	July (63)	102.6 (10.6)	0.81 (0.05)
	August (63)	73.7 (9.3)	0.69 (0.05)

Table 2.4. (continued)

Study Site	Kentucky (58)	76.9 (11.1) b	0.80 (0.06) ab
($\lambda_{6, 430} = 7.39$)	Ohio (63)	105.2 (14.4) ab	0.93 (0.05) a
	Tennessee (64)	108.4 (13.7) ab	0.63 (0.05) b
	West Virginia (63)	119.8 (13.4) a	0.60 (0.05) b

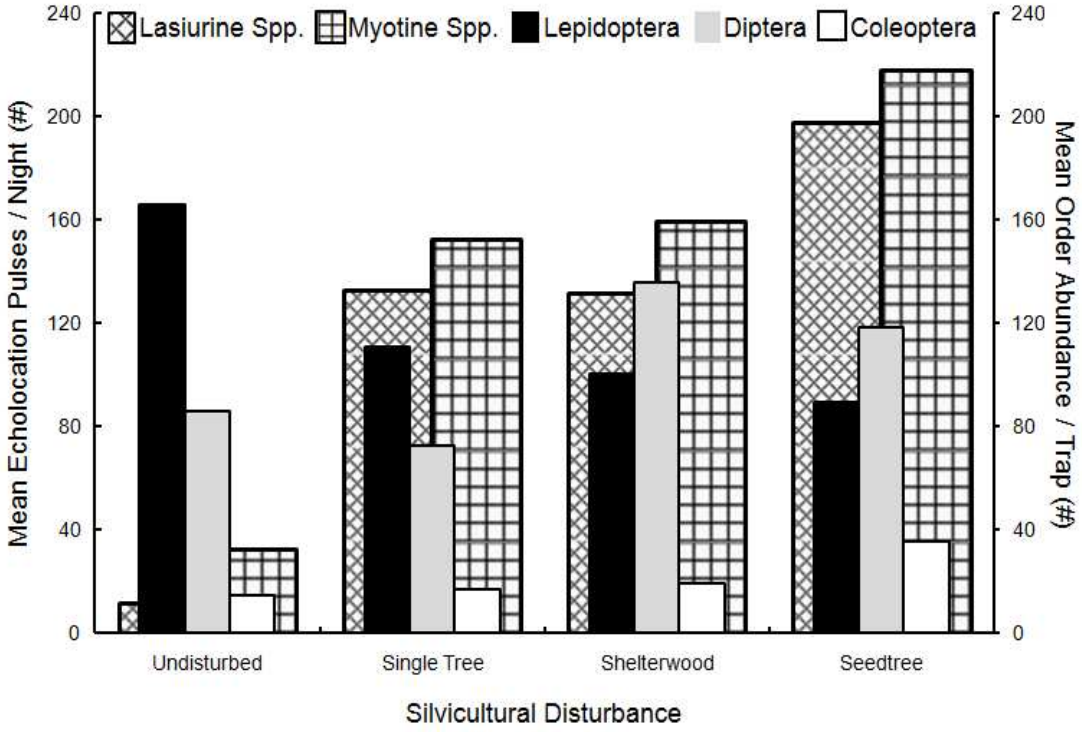


Figure 2.1. Synthesis of bat activity and insect occurrence across a gradient of forest disturbance in Central Appalachia, 2007-2008. The left axis depicts surveys of bat activity (via Anabat II system) and the right axis depicts surveys of insect occurrence (Coleoptera and Lepidoptera via blacklight traps; Diptera via malaise traps).

CHAPTER THREE: REGIONAL VARIATION IN THE FOOD HABITS OF BATS IN CENTRAL APPALACHIA

Introduction

Eastern North American bats are exclusively insectivorous, but the relative consumption of different insect taxa varies between species and may also vary over their distribution (Jones and Rydell 2003; Lacki *et al.* 2007b). Most forest bats, however, do demonstrate moderate selection (>40% of diet) for one or more insect orders (Lacki *et al.* 2007a). Plecotine bats (*Corynorhinus* spp.) prey heavily upon moths (>80% of diet); consequently, these gleaning bats are generally accepted as "foraging specialists" (Lacki *et al.* 2007a). More variably, the big brown bat (*Eptesicus fuscus* Beauvois) selectively preys on Coleoptera in parts of its distribution (Storm and Whitaker 2008). This selectivity suggests specialization and exploitation of a prey resource absent or underrepresented in the diet of other conspecific bat species. The dietary variation for conspecific species such as the eastern red bat (*Lasiurus borealis* Müller), northern bat (*Myotis septentrionalis* Trouessart), and tri-colored bat (*Perimyotis subflavus* Cuvier) suggests these bats may be "foraging opportunists," as these bats prey heavily on locally abundant insect taxa (Lacki *et al.* 2007a).

Foraging bats face two decisions: where to forage, and what prey to eat (Whitaker 1994). While most studies address what insects are eaten (Lacki *et al.* 2007a), a broad understanding of which taxa are consumed within the landscape-level arthropod assemblage is largely lacking due to food habits analyses focused on limited numbers of species. Further, there is a paucity of data regarding how prey consumption varies over

the distribution of different bat species (Lacki *et al.* 2007b). Even so, regional variation in food habits has been demonstrated for other flying vertebrate predators, i.e. birds, in a variety of ecosystems) (e.g., Duffy *et al.* 1987; Boshoff *et al.* 1990), so regional variation of the dietary niches of bats in eastern North America is likely.

Community-level food habit studies do exist for bats in North America (Whitaker 1972; Fenton and Bell 1979; Lacki *et al.* 2007a; Lacki *et al.* 2007b; Feldhammer *et al.* 2009) and other continents (Feldman *et al.* 2000; Rakotoarivelo *et al.* 2007), but few studies have outlined the food habits of bat assemblages in Appalachia (but see Griffith and Gates 1985; Carter *et al.* 2003) and none have investigated food habits on a regional level. This is merited, however, as an understanding of this will permit both 1) insight into how a major group of vertebrate predators partition their dietary niches, and thus 2) contribute to more effective management of this predator group, of which the populations of many members are in decline (Pierson 1998).

My data address the need for a more comprehensive understanding of bat food habits at the community level. My broad hypothesis is that the food habits of different bat species will correspond with ecomorphological characteristics (*sensu* Karr and James 1975) such as cranial structure and wing morphology (Freeman 1981; Norberg and Raynor 1987). Plecotine species are adept at gleaning prey; I expect these species (e.g., *C. rafinesquii* Lesson) to primarily consume Lepidoptera (Lacki and Dodd, *In Press*). Serotine species hawk prey from the air and possess a robust cranial structure and relatively large body size; I expect these species (e.g., *E. fuscus*) to consume larger, harder-bodied prey (i.e., Coleoptera and Hemiptera) (Storm and Whitaker 2008). Smaller-sized bats with more delicate cranial structures should consume more soft-

bodied, smaller-sized prey; I expect to find this for *P. subflavus* and smaller myotine species (Carter *et al.* 2003). Further, I investigated the extent to which food habits varied across the Central Appalachian region. I hypothesized that those bat species with "intermediary" characteristics (i.e., larger myotine species and the lasiurine species) will be more variable in their capacity to take different types of prey and, thus, was more likely to exhibit regional variation in diet.

Methods

Study Areas and Field Collection

Fecal samples were from bats collected regionally across the Central Appalachians of eastern North America (Appendix A), including the Daniel Boone National Forest, Kentucky (Lat. 38°2′ N, Long. 83°35′ W), the Raccoon Ecological Management Area, Ohio (Lat. 39°11′ N, Long. 82°22′ W), the Royal Blue Wildlife Management Area, Tennessee (Lat. 39°11′ N, Long. 82°23.′ W), and commercial timberland in Wyoming County, West Virginia (Lat. 37°30′ N, Long. 81°36′ W). Field collections took place in a matrix of upland hardwood forestland actively managed for timber production and used for scientific research.

Bats were captured throughout their active periods (March-September) across my study areas from 2006 to 2008. Monofilament nylon mist nets (2.6, 6, 9 m in length; 6.8 m^2, 15.6 m^2, 23.4 m^2 in area) (Avinet, Dryden, NY, USA) were placed throughout the study areas over flyways formed by roads and road-ruts with pooled water, small streams, trails, and ridgelines. Captured bats were handled in accordance with the University of Kentucky Institutional Animal Care and Use Committee (#01019A2006) and state and federal collection permits. Bats were held separately in single-use, disposable cotton bags

(20 × 30.5 cm) (Avinet) for ca. 20 min to allow defecation. Fecal samples from each individual were then collected into 1.5 mL microcentrifuge tubes, placed on ice or in a mobile freezer (ca. 0°C) (MT17, Engel USA, Jupiter, FL, USA), and transferred to long-term freezer storage (−80°C) immediately upon return to the laboratory.

Dissection Procedure and Analysis

Pellets were dissected as described by Whitaker (1988) and prey remains were identified to order (Whitaker 1988; Triplehorn and Johnson 2005). In contrast to most previous food habit studies of bats, my identification of prey items in the order Hemiptera includes the suborder Auchenorrhyncha (i.e., previously a distinct order, Homoptera) (Lacki *et al.* 2009). I visually estimated frequency (%) of prey items in the diet of each bat species, and also estimated the volume (%) of prey items in pellets from each individual bat to the nearest five percent. Up to three pellets were dissected from each bat, and average values across pellets were used in determining percent volumes of prey in the diet (Lacki *et al.* 2007b). Fecal samples from *M. septentrionalis* from study areas in Kentucky, Ohio, and Tennessee were subsequently preserved in ca. 1.5 mL 95% ethanol and placed in freezer storage (−80°C) until subsequent DNA-based analysis and consideration in a comparative analysis of methods (Chapter Three).

I compared both the frequency and volume of prey taxa consumed across the bat assemblage to investigate trends in prey selection. I assessed regional differences in diet using nonparametric Kruskal-Wallis tests (Noether 1990) for the two most commonly captured bat species, *L.borealis* and *M. septentrionalis*. The response variables tested included volume of Coleoptera, Diptera, and Lepidoptera, which are the prey taxa most commonly consumed by bats in eastern North America (Whitaker 2004; Lacki *et al.*

2007a). If overall tests were significant ($P \leq 0.05$), a non-parametric means comparison procedure was carried out to discern differences (C. Srinivasan, pers. comm.).

Results

I collected and dissected 318 pellets from 132 bats of the total 222 bats captured (Table 3.1). Fecal dissections were performed for seven species: *M. septentrionalis* (n = 81), *L. borealis* (n = 35), *E. fuscus* (n = 9), *P. subflavus* (n = 4). *C. rafinesquii* Saint-Hilaire (n = 1), *Lasionycteris noctivagans* Peters (n = 1), and *M. leibii* Audobon and Bachman (n = 1). Seven insect orders were identified, as well as evidence for consumption of the Arachnida in *M. septentrionalis*. Coleoptera and Lepidoptera were the most frequently consumed insect orders. Coleoptera were found in the diet of all bats assessed. Lepidoptera were found in the diet of nearly all bats, with the exception of two *E. fuscus* individuals.

Prey composition at the ordinal level within fecal pellets varied across bat species (Table 3.1). *Myotis septentrionalis* consumed a high volume of Lepidoptera, followed by Coleoptera, with a much smaller component of Diptera. The remaining six prey orders comprised <10% of the pellet contents for *M. septentrionalis*. *L. borealis* similarly consumed a high volume of Lepidoptera, but consumed a greater volume of Coleoptera than that observed for *M. septentrionalis*. Remaining prey orders comprised <10% of the pellet contents for *L. borealis*. The diet of *E. fuscus* differed from either *M. septentrionalis* or *L. borealis*; Coleoptera and Hemiptera formed >80% of the pellet contents of this species. Lepidoptera comprised 14% of pellet contents of *E. fuscus* and minor amounts were recorded for Diptera and Trichoptera. In contrast, while Lepidoptera also formed the bulk of the diet of *P. subflavus,* pellets of this species contained a higher

volume (33%) of Diptera than any other bat species assessed. Although trace amounts of Coleoptera were identified in *C. rafinesquii* pellets, the entire volume of pellets from *C. rafinesquii* were from Lepidoptera. The dietary composition of *L. noctivagans* was more balanced, with five different orders of prey documented within the pellets of the single individual I assessed. This individual consumed a higher volume of Coleoptera in comparison with *L. borealis*, the other lasiurine species. The single *M. leibii* I assessed consumed a high volume of Lepidoptera, but unlike its congener *M. septentrionalis*, it consumed a higher volume of Diptera than Coleoptera. Trichoptera was the only remaining component in the diet of *M. leibii*.

Consumption of common insect orders by *M. septentrionalis* (N = 82 bats) varied across study sites (Table 3.2), but no differences were detected across sites for *L. borealis* ($P > 0.05$). Lepidoptera comprised a higher volume of the diet of *M. septentrionalis* in Ohio versus Tennessee ($\chi^2 = 9.4$; $P = 0.02$), with values in Kentucky and West Virginia intermediate. In contrast, Coleoptera formed a higher volume of the diet of *M. septentrionalis* in Tennessee versus West Virginia ($\chi^2 = 7.6$; $P = 0.05$).

Discussion

My data provides evidence that consumption of arthropod taxa varies across bat species and varies regionally for the most commonly-captured species within my study areas. However, the inferences drawn from this dataset must be tempered due to the small sample sizes that limit my statistical power (Hayes and Steidl 1997).

Consumption patterns in my study underpin the importance of both Coleoptera and Lepidoptera to the lasiurine and myotine species in Appalachia (Carter *et al.* 2004). Nevertheless, my data suggest dietary differences between these ensembles, likely due to

differences in ecomorphology. The lasiurine *L. borealis* appears to consume Coleoptera more frequently than *M. septentrionalis*, thus illustrating the importance of Coleoptera to opportunistic hawking species (Carter *et al.* 2003; Carter *et al.* 2004). In contrast, my data supports the suggestion that *M. septentrionalis* consumes Arachnids and other terrestrial prey via gleaning (Faure *et al.* 1993; Whitaker 2004). Differences in prey consumption correlate with broad trends in ecomorphology across bat species, and corroborate data from more easterly portions of Appalachia (Woods *et al.* 1999; Carter *et al.* 2003).

My data also concur with other studies of the food habits of bats that exist on either end of the body-mass continuum in eastern North America. On the heavier end of this continuum, *E. fuscus* is acknowledged as a foraging specialist able to consume hard-bodied prey due to a large body mass and robust cranial morphology (Freeman 1981; Agosta *et al.* 2003; Storm and Whitaker 2008). My data support this hypothesis based on the observed consumption pattern for Coleoptera, as well as with the relatively high incidence of Hemiptera. In contrast, the fecal pellets from the smaller-sized myotine species, *M. leibi*, and the other small-sized bat considered, *P. subflavus*, suggest a heavier reliance on Diptera. Consumption patterns emphasizing softer-bodied prey for these species have been previously noted and attributed to small body mass and cranial morphology (Freeman 1981; Carter *et al.* 2003). Beyond the ecomorphological relationship between prey hardness and predator size, however, my data may further illustrate differences in prey detection between larger and smaller-sized bat species. While *E. fuscus* is a larger species and, thus, is able to consume both larger and harder-bodied prey than smaller-sized conspecific species such as *M. leibii* and *P. subflavus*, it is likely constrained by echolocation. The relatively large size of *E. fuscus* contributes to

relatively lower echolocation frequencies (Kurta and Baker 1990) and, thus, likely contributes to non-detection of smaller-sized insects which are detected by smaller bats with higher frequency echolocation calls (Fenton 1990).

Data collected for single bats of different species largely agree with past studies. The fecal pellets I assessed for *C. rafinesquii* suggest specialization on Lepidoptera, consistent with previous observations for this species, as well as other plecotine bats (Lacki and Dodd *In Press*). In contrast, the single *L. noctivagans* I assessed possessed a relatively diverse diet. Even so, the food habits of the individual in this study differs from the data presented in other studies (i.e., greater consumption of either Diptera or Lepidoptera) (Carter *et al.* 2003), suggesting *L. noctivagans* is a generalist and opportunistic species throughout its distribution. In total, bats in the forests of Central Appalachia exhibit a broad breadth of food habits.

A robust sample size allowed detection of regional variation for *M. septentrionalis*. Though the components within the diet of this bat are not truly orthogonal (i.e., autocorrelation between percent data), my data does reflect true differences across the region and supports hypotheses that *M. septentrionalis*, and other similarly-sized myotine species, are adaptable predators with varied food habits, likely capitalizing on locally abundant insect taxa (Lacki *et al.* 2007a). Even so, consideration of these data in tandem with insect abundance data presented in Chapter Two suggests an intriguing relationship. Consumption patterns of Lepidoptera by *M. septentrionalis* generally correspond with the trends in relative abundance across study sites (Figure 3.1). Lepidoptera captured in blacklight traps were more abundant in Ohio versus in Kentucky and Tennessee; correspondingly, lepidopteran consumption was higher in Ohio versus

Tennessee. Coleopteran abundance in blacklight traps was higher in Ohio than in Tennessee and West Virginia, although the consumption of this insect order was higher in Tennessee versus West Virginia. In tandem, these data suggest consumption of Coleoptera as alternative prey, likely due to the lower availability of Lepidoptera. Similar tradeoffs in dietary composition of Coleoptera and Hemiptera in areas with varied prey abundances have been suggested for *E. fuscus* (Agosta *et al.* 2003). My data suggests a similar relationship for *M. septentrionalis* on a regional scale. Thus, this regional variation of *M. septentrionalis* suggests this species is may be less constrained by ecomorphology than other species for which I collected data; this is a highly maneuverable species capable of both gleaning and hawking prey (Ratcliffe and Dawson 1993) and possesses a cranial structure and body size intermediate to many bat species in eastern North America (Caceres and Barclay 2000).

In summary, this study presents a baseline of regional data for the diets of Central Appalachia, particular for upland habitats. Failing to find regional differences in the diet of *L. borealis* may indeed reflect a lack of variation in diet, but it is worth noting that the sample size of this bat species (n = 35) was more limited than that for *M. septentionalis* (n = 81), and sample effort was heavily skewed for the Kentucky site (77% of samples). Given this, I suggest that subsequent analyses seek a more robust sample size when considering regional analysis. Further, a more thorough sampling effort should be put forth to assess the food habits of bat species less frequently captured or absent from this study. This would be best accomplished by stratifying survey efforts across a greater diversity of habitats. Surveys conducted in this study were in either (1) upland habitats or (2) along smaller, ephemeral bodies of water. Sampling larger, perennial water sources,

as well as likely flight corridors and natural landscape contours (e.g., bluff lines) would increase the likelihood of capturing of species that eluded capture in this study.

Table 3.1. Prey identified in fecal samples of bats in Central Appalachia, 2006-2008. Data are expressed as percent volume (percent frequency) per bat. Numbers of individuals per bat species are indicated in brackets. "Tr." denotes a trace volume estimate (i.e., prey present but <5% volume in all pellets). "Other" includes hair, plant material, and unidentified items.

Food Item	*Myotis sept.* [81]	*Lasiurus borealis* [35]	*Eptesicus fuscus* [9]	*Perimyotis subflavus* [4]	*Corynorhinus rafinesquii* [1]	*Lasionycteris noctivagans* [1]	*Myotis leibii* [1]
Arachnida	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Coleoptera	37 (100)	43 (100)	66 (100)	19 (100)	Tr. (100)	55 (100)	20 (100)
Diptera	6 (63)	1 (46)	2 (22)	33 (100)	0 (0)	22 (100)	23 (100)
Hemiptera	2 (44)	4 (60)	14 (67)	6 (100)	0 (0)	13 (100)	0 (0)
Hymenoptera	1 (10)	1 (6)	0 (0)	2 (25)	0 (0)	0 (0)	0 (0)
Lepidoptera	49 (100)	48 (100)	14 (78)	38 (100)	100 (100)	10 (100)	53 (100)
Neuroptera	1 (16)	2 (20)	0 (11)	2 (25)	0 (0)	Tr. (100)	0 (0)
Trichoptera	2 (30)	1 (11)	1 (22)	2 (75)	0 (0)	0 (0)	5 (100)
Other	3 (27)	3 (14)	3 (56)	0 (0)	0 (0)	0 (0)	0 (0)

Table 3.2.Percent volume (percent frequency) of Coleoptera and Lepidoptera consumed by *Myotis septentrionalis* across study sites in Central Appalachia, 2006-2008. Different letters within a column indicate significant differences in volume data ($P<0.05$).

Study Area	N	Lepidoptera	Coleoptera	Diptera
Kentucky	40	50 (100) ab	39 (100) ab	6 (68)
Ohio	19	57 (100) a	33 (100) ab	3 (47)
Tennessee	18	40 (100) b	41 (100) a	8 (72)
West Virginia	4	48 (100) ab	23 (100) b	15 (50)

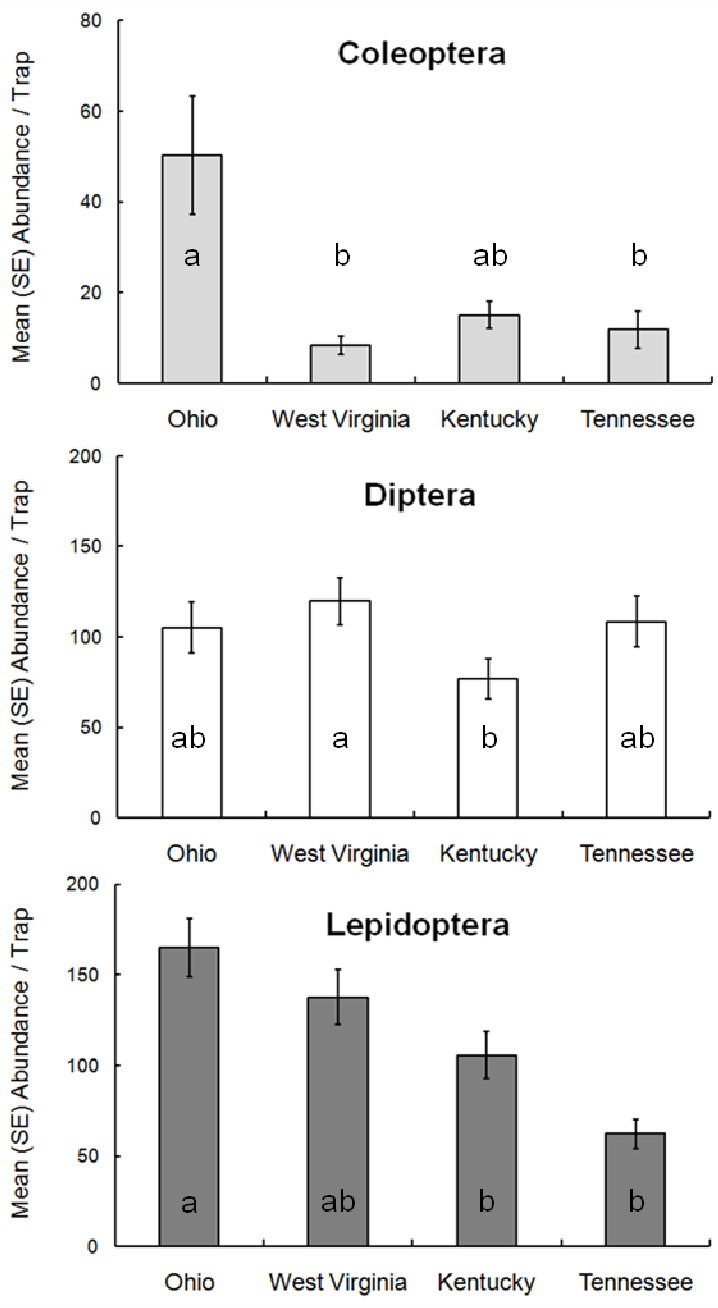


Figure 3.1. Variation in prey abundance across Central Appalachia, 2007-2008, as assessed by blacklight traps (Coleoptera and Lepidoptera) and malaise traps (Diptera). Different letters indicate significant differences across study areas ($P<0.05$). Collection and analysis of these data were presented in Chapter Two.

CHAPTER FOUR: DNA-BASED TECHNIQUES ALLOW A HIGH RESOLUTION ANALYSIS OF PREY SELECTION BY A FOREST-DWELLING BAT (*MYOTIS SEPTENTRIONALIS*)

Introduction

The molecular delineation of individual trophic linkages between predators and prey necessarily underpins a more cohesive understanding of how species interact at the population level (Symondson 2002; Sheppard and Harwood 2005; Weber and Lundgren 2009). In particular, understanding the foraging ecology of a predator is integral to elucidating its role in regulating prey populations and, conversely, how prey availability potentially affects predator density and distribution (Holling 1961). Even so, predators are often cryptic; thus, direct observations of predation events may not be possible (Sheppard and Harwood 2005) and even when they are, the frequency with which a predation event is observed can be extremely low (e.g., Jackson 1977; Reddy and Fenton 2003). Insectivorous bats are such an example because their aerial foraging activity and nocturnal habits make them an especially elusive group in which to evaluate predator-prey relationships and fully elucidate the strength of specific trophic linkages (Jones and Rydell 2003).

Bats are among the most globally diverse mammalian taxa, representing over 1,100 species and occupying a variety of foraging niches (Patterson *et al.* 2003; Simmons and Conway 2003). Over 25 species are found throughout forests of North America (O'Shea and Bogan 2003; Brigham 2007), however many species are endangered or of concern with populations in decline (Pierson 1998; Racey and Entwhistle 2003; Lacki *et al.* 2007a). Human disturbance through manipulation of natural resources, land

development, and habitat fragmentation contributes to the loss of roosting and foraging habitat (Brigham 2007). Compounding this, an emerging pathogen is decimating entire hibernating colonies of cave-roosting myotine species (mouse-eared bats) in eastern North America (Blehart *et al.* 2009; Gargas *et al.* 2009), and the proliferation of wind turbines is correlated with widespread mortality of lasiurine species across North America (migratory tree bats) (Kunz *et al.* 2007; Cryan and Barclay 2009). More fundamentally, the relationships among foraging behavior, habitat use, and prey occurrence remain unclear for insectivorous bats (Tibbels and Kurta 2003; Lacki *et al.* 2007a; Dodd *et al.* 2008). Bats may exert top-down regulation of insect populations (Kalka *et al.* 2008; Williams-Guillen *et al.* 2008), as well as serve as economically-important biological control agents in agroecosystems (Cleveland *et al.* 2006). However, quantitative evidence of their effects is lacking (Jones and Rydell 2003) and studies in the forests of North America are nonexistent. This is because there has been no rigorous demonstration of top-down regulation (i.e., a concurrent assessment of both predator and prey across structured treatment levels).

Most forest-dwelling bats in North America are insectivores and demonstrate moderate selection for one or more insect orders (>40% of diet; Lacki *et al.* 2007a). The relative consumption of different insect taxa varies across species and may vary geographically (Jones and Rydell 2003; Brigham 2007; Lacki *et al.* 2007a). Despite evidence of specialization and variation, knowledge of prey selection by insectivorous bats is largely limited to the ordinal level as most studies have relied upon morphological identification of undigested, chitinous fragments of exoskeleton present in feces or the digestive tract (Whitaker 1988; Jones and Rydell 2003; Lacki *et al.* 2007a). However, for

plecotine species (big-eared bats) differential selection of prey has been documented at finer taxonomic resolution through the collection of prey remnants that these bats have dropped during feeding (Lack and Dodd *In Press*). Given that the prey base of insectivorous bats varies within and among landscapes (Grendal 1996; Burford *et al.* 1999; Wickramasinghe *et al.* 2004; Dodd *et al.* 2008; Morris *et al.* 2010), and that foraging behavior and habitat use are correlated with bat morphology (Freeman 1981; Norberg and Raynor 1987; Arlettaz *et al.* 1997; Arlettaz 1999), it is likely that bat species select prey in relation to their size, availability (abundance and ease of capture) and predilection (their likes and dislikes) (Charnov 1976; Barclay 1991; Lacki *et al.* 2007a).

Lepidoptera are a prey group often consumed by bats in North America (Lacki *et al.* 2007a). The distribution patterns and preferred habitats of Lepidoptera vary across taxa (Covell 2005), presumably in response to changes in the host plant base that is often linked to forest management and disturbance (Summerville and Crist 2008). A more resolved understanding of which taxa are consumed by bats is needed to better comprehend prey selection, and to gain insight into the trophic linkages that may be vulnerable to perturbations in changing forested ecosystems (Brigham 2007; Lacki *et al.* 2007a).

Prey consumption can be determined from morphological analysis of predator gut contents, fecal samples, or culled remains of prey items (e.g., collected from a cave floor). However, these remains are often suboptimal for truly identifying the components of bat diets (Whitaker 1988). Post-consumption, prey items are degraded and difficult to identify. Using polymerase chain reaction (PCR) to probe a predator's gut or fecal contents for prey DNA fragments that are useful for species identification (e.g., DNA

barcodes) has the potential to identify specific predator-prey interactions. The viability of DNA-based techniques has been demonstrated across numerous systems and has provided valuable insights into cryptic trophic linkages between predators and prey (or parasitoids and hosts - see Greenstone 2006) in terrestrial (e.g., Read *et al.* 2006; Harwood *et al.* 2007; Lundgren *et al.* 2009) and marine (e.g., Deagle *et al.* 2007; Deagle *et al.* 2009) systems. The use of DNA-based techniques to investigate trophic linkages has been limited in the field of bat ecology. A foundation has been laid for both sanguinivorous (Carter *et al.* 2006) and insectivorous species (Clare *et al.* 2009), but further efforts to integrate these techniques into the discipline are warranted, as traditional means have limited resolution and inherent biases. Traditional dissection of feces or digestive tracts potentially under-represents soft-bodied prey and, further inferences of prey identity are limited and vary across orders (Whitaker 1988; Lacki *et al.* 2007a).

I used DNA-based techniques to broadly investigate the prey consumed by the northern bat (*Myotis septentrionalis* Trouessart; Chiroptera: Vespertilionidae). *Myotis septentrionalis* is a commonly encountered tree-roosting species in the Central Appalachian region of North America (Barbour and Davis 1969). The objectives of my study were three-fold. First, I demonstrate a means of extracting and amplifying mitochondrial DNA of prey from fecal samples suitable for food habits analysis from bats captured in the field and, using web-based searches, identify prey taxa in the diet of these bats at the genus/species level. Species-level identifications may sometimes be made using morphological means either directly (e.g., the spotted cucumber beetle, *Diabrotica undecimpunctata*, heavily eaten by many species of bats) (Whitaker 2004) or

indirectly (e.g., the golden dung fly, *Scatophaga stercoraria*, eaten by both myotine and plecotine species in Europe) (Shiel *et al.* 1991). However, DNA-based techniques offer direct identification at a resolution of prey greater than that attainable with morphological identification (Brigham 2007), particularly for soft-bodied prey items such as Lepidoptera (J.O. Whitaker, Jr., pers. comm.). Given this, my second research objective was to assess consumption patterns of forest Lepidoptera by *M. septentrionalis* as related to prey abundance and selection. Though Lepidoptera are a widely and heavily consumed by bats in eastern North America (Lacki *et al.* 2007a), consumption patterns within the Lepidoptera are unknown for myotine species. Thus, my null hypothesis was that consumption across available Lepidoptera would be in proportion to abundance, i.e., no prey selection would occur. Lastly, by sequencing prey DNA from the same fecal samples which I evaluated concurrently using morphological dissection, I compared prey inferences generated using different web-based database searches and that of commonly-accepted microscopic analyses. Thus, for my third research objective, I investigated methodological bias of approaches to the analysis of food habits of this common forest-dwelling species.

Materials and Methods

Study Areas and Field Collection

Fecal samples of bats were collected regionally across the Central Appalachians of eastern North America (Appendix A), including the Daniel Boone National Forest, Kentucky (Lat. 38°2′ N, Long. 83°35′ W), the Raccoon Ecological Management Area, Ohio (Lat. 39°11′ N, Long. 82°22′ W), and the Royal Blue Wildlife Management Area, Tennessee (Lat. 39°11′ N, Long. 82°23.′ W). Field collections took place in a matrix of

upland forestland actively managed for timber production and used for scientific research.

Bats were captured throughout their active period across my study areas from March through September of 2007 and 2008 in monofilament nylon mist nets (2.6, 6, 9 m in length; 6.8 m^2, 15.6 m^2, 23.4 m^2 in area) (Avinet, Dryden, NY, USA) placed throughout the study areas over flyways formed by roads and road-ruts with pooled water, small streams, trails, and ridgelines (i.e., < 3 m above ground level). Individuals captured in this study thus had access to a broad range of heights over which to capture prey which I subsequently detected within their fecal pellets. Captured bats were handled in accordance with the University of Kentucky Institutional Animal Care and Use Committee (#01019A2006) and state and federal collection permits. Bats were held separately in single-use, disposable cotton bags (20 × 30.5 cm) (Avinet) for ca. 20 min to allow defecation. Fecal samples from each individual were then collected into 1.5 mL microcentrifuge tubes, placed on ice or in a mobile freezer (ca. 0°C) (MT17, Engel USA, Jupiter, FL, USA), and transferred to long-term freezer storage (−80°C) immediately upon return to the laboratory. At no time were fecal samples allowed to contact any surface other than the single-use cotton bag or the microcentrifuge tube.

Concurrent with mist net captures of bats, I also surveyed forest Lepidoptera across the same study areas to assess prey abundance during the growing seasons of 2007 and 2008. Because *M. septentrionalis* is known to depredate Lepidoptera via both aerial-hawking and gleaning (Faure *et al.* 1993; Caceres and Barclay 2000; Ratcliffe and Dawson 2003), I make no discernment of availability of prey either in flight or at rest. Further, the "true availability" of insect prey can never be known to bats; rather, I

assessed overall catch of Lepidoptera over the course of entire survey nights as a relative index of availability (Whitaker 1994). Surveys of prey abundance in each study area were ≤ 2.5 km from mist net locations. Lepidoptera were surveyed in upland deciduous forests across a continuum of silvicultural disturbance (i.e., unharvested uneven-aged forest and three increasing levels of harvest established at each study site during the dormant season of 2006-2007); covering slope positions known to be used as foraging habitat by *M. septentrionalis* in the Central Appalachians (Lacki *et al.* 2009).

Lepidoptera were trapped using 10 W blacklight traps (Universal Light Trap, Bioquip Products, Rancho Dominguez, CA, USA) suspended 2.5 m above ground (Burford, Lacki and Covell 1999; Dodd, Lacki and Rieske 2008). Though light traps are biased towards phototactic taxa of Lepidoptera, they are widely considered the standard technique for sampling lepidopteran assemblages (Southwood 1978; Summerville *et al.* 2001; Covell 2005; Dodd *et al.* 2008). Consequently, Lepidoptera that were diurnal, not phototactic, or that are only attracted to bait were not sampled in this study. As taxa with these characters are undersampled with blacklight traps, total numbers of lepidopteran assemblages are also underestimated and should be considered conservative estimates (Summerville *et al.* 2001). Fixed survey locations were established for blacklight traps across the disturbance gradient, and were chosen according to representation of the habitat under study, potential use by predator and prey (i.e., flyways and corridors), and accessibility. Traps were operated through the night and a cotton wad soaked in ethyl acetate was used to kill trapped insects. Lepidoptera were removed the following day, frozen, and returned to the laboratory for identification. Lepidoptera with wingspans ≥ 20 mm were identified using available keys (Holland 1903; Covell 2005) and reference

collections at the University of Kentucky. Lepidoptera with wingspans < 20 mm were not identified or enumerated due to damage to specimens from the blacklighting technique (Burford *et al.* 1999; Dodd *et al.* 2008).

Screening Fecal Samples

Fecal pellets of collected bats were dissected microscopically and prey remains identified to the most specific taxon possible (on the basis of key determination by Whitaker 1988). Individual fecal pellets were placed in a sterile pour boat (4.1 × 3.2 × 0.8 cm) (Fisher Scientific, Pittsburgh, PA, USA), diluted with 100% ethanol and teased apart using a disposable pestle (Fisher Scientific) for microscopic dissection. I estimated percent frequency of prey items in the diet among individual bats, and estimated the percent volume of prey items in pellets from each bat to the nearest five percent. Up to three pellets were dissected from each bat, and average values across pellets were used in determining percent volumes of prey in the diet (Lacki *et al.* 2007b). Individual fecal pellets were then preserved in ca. 1.5 mL 95% ethanol and placed in freezer storage (−80°C) until subsequent DNA-based analysis.

Molecular remains of prey are not homogenous within feces (Deagle *et al.* 2005). Considering the number of fecal pellets generally yielded by each bat, up to three fecal pellets from each individual bat were used for DNA-based analysis to increase the likelihood of accurate and reliable identification of all prey items consumed. The entire individual pellet that was used for morphological identification was then used for each individual DNA extraction. Prior to DNA extraction, each fecal sample was homogenized for ca. 1 min in 2.0 mL mortar-and-pestle microcentrifuge tubes, vortexed ca. 1 min, then centrifuged at 20,000 × *g* for 3 min, discarding the resulting supernatant. Following this

process, 1 mL TE buffer was added to samples which were then vortexed ca. 1 min, centrifuged at 20,000 × *g* for 3 min and the supernatant discarded. DNA was then extracted using a QIAamp DNA Stool Mini Kit (Qiagen Inc., Chatsworth, California, USA). Protocol was followed according to the manufacturer's instructions for the isolation of DNA from stool for pathogen detection carrying out lysis with the ASL buffer at 70°C, and using all applicable extra centrifugation steps.

PCR reactions (total volume = 50 µL) for nucleotide sequencing of C*OI* were carried out with C1-J-1859 with C1-N-2191 primers, resulting in a 333 base amplicon (Simon *et al.* 1994). The PCR cocktail contained 2 µL template DNA solution of unknown concentration, 1.25 U Qiagen HotStar Taq polymerase, Qiagen 0.2 mM dNTP, 0.25 mM of each primer, 1.5 mM 10× reaction buffer, and 1 mM $MgCl_2$. Cycling conditions were 15 min at 95°C, 50 cycles of 50 sec at 94°C, 45 sec at 45°C, 45 sec at 72°C, and a final elongation for 5 min at 72°C. Reaction success was then confirmed by electrophoresis of 10 µL of PCR product in 1.0 % agarose (Sigma-Aldrich Co., St. Louis, Missouri, USA) in 1× TAE (Promega Corp., Madison, Wisconsin, USA). Sequencing (University of Kentucky Advanced Genetic Technologies Center, Lexington, Kentucky, USA) was carried out for those reactions that yielded strong PCR bands of expected size, using BigDye terminator kits (v. 3.1) and the previously-mentioned primer set on an ABI3100 sequencer (Applied Biosystems, Foster City, California, USA). Reactions were sequenced bi-directionally to reduce the possibility of "chimeric sequences" consisting of multiple prey DNA fragments; overlapping forward and reverse sequences were edited and assembled using Vector NTI (v. 10.3, Invitrogen Corp., Carlsbad, California, USA). If strong, corresponding signals were not present in forward and reverse chromatographs,

such portions of sequences were marked as unidentifiable (or discarded if the bulk of a sequence was unknown). Thus, I generated a single sequence per fecal pellet.

Prey Identification and Comparison across Techniques

Prey identities were inferred using web-based searches to compare unknown DNA sequences with the Barcode of Life Data System (BOLD) and GenBank. Using BOLD, species-level identification of unknown sequences was carried out using methods previously outlined by Clare *et al.* (2009). I compared my sequences to reference sequences from arthropods present with species-level barcodes in BOLD (Ratnasingham and Hebert 2007) in November 2009. Matches of ≥ 99% similarity between my unknown sequence and a single species in the database were considered close enough to warrant species identification (Clare *et al.* 2009). Coarser taxonomic identifications of unknown sequences were made in the absence of species-level matches if there was a 100 % "probability of placement" within the broader phylogeny indexed by BOLD. Using GenBank, similarity of unknown sequences was considered using a basic local alignment and search tool (Altschul *et al.* 1990); the megablast variant was used with the default settings. Identity of prey was inferred by the closest match generated by this search; ranking was according to maximum similarity and maximum score parameters.

Order-level data were compared using a 2×3 contingency table with a χ^2 test of independence (Triola 1986) across the three methods of identification (morphological, GenBank, BOLD) using presence/absence counts across fecal pellets. Separate χ^2 tests of independence were conducted for each of the most frequently identified orders of prey (Coleoptera, Diptera, Hemiptera, and Lepidoptera). Calculated expected values were defined as: observations within category × sum of observations across categories / total

observations) (Triola 1986). Following a significant test value, individual variation of each identification method from the calculated expected value was assessed in terms of contribution (%) towards the overall χ^2 test score. Doing so allowed assessment of which identification technique(s) deviated the most from the null test hypothesis (i.e., observed = expected; Lacki *et al.* 1984), thus allowing assessment of differences in the frequency of occurrence of prey orders across identification procedures.

At a more specific resolution, I calculated the mean wingspan for all genera/species of Lepidoptera identified in fecal samples using BOLD. Prey inferences generated with BOLD were used to calculate wingspan values (as opposed to GenBank) due to the precedence for species-level prey inferences reported by Clare *et al.* (2009). Wingspan values were taken from Covell (2005) and the Bug Guide web-based database hosted by Iowa State University (www.bugguide.net). For taxa which wingspan values could not be determined (i.e., species/genus not indexed in the source), a wingspan value at a coarser level of taxonomic resolution was used (i.e., family-level). As a comparison with the data collected in this study for a myotine species, a similarly-calculated wingspan for prey of plecotine species was taken from Lacki and Dodd (*In Press*) and a mean wingspan was calculated for a lasiurine species from the lepidopteran species reported by Clare *et al.* (2009).

Results

A total of 139 fecal pellets from 62 bats showed evidence of consumption of seven insect orders, as well as Arachnida, using the morphological identification technique (Figure 4.1). Lepidoptera and Coleoptera constituted the greatest volume within fecal samples, means ±SE: 48.8 ± 2.5 % and 38.2 ± 1.8 %, respectively, and were

identified in all fecal pellets using morphological identification. Data gathered by morphological identification were then converted to presence/absence of prey orders per fecal pellet for comparison with DNA-based identification procedures (Figure 4.2).

I successfully extracted and amplified DNA from 123 fecal pellets from the total dataset (88% success); 120 pellets were sequenced (86% success). Web-based identification procedures using DNA sequences identified four prey orders with BOLD (n = 56) and five prey orders with GenBank (n = 120), respectively (Figure 4.2). Overwhelmingly, 93% of the pellets that could be identified using BOLD were identified as Lepidoptera. The majority of the pellets (86%) that could not be identified using BOLD were identified as non-lepidopteran using GenBank. With GenBank, 53% of all sequences were identified as Lepidoptera and other prey orders (e.g., Coleoptera, Diptera, Hemiptera) were identified more frequently than with BOLD.

Detection of the most commonly recorded orders of prey varied across identification procedures; each χ^2 test of independence conducted for each order of prey was significant ($P < 0.001$; Table 4.1). Individual χ^2 contributions to the overall test score ($\chi^2 = 236.8$) indicate morphological identification of Coleoptera varied most from expected values. Those for Diptera ($\chi^2 = 37.8$) indicated identification using BOLD, and the presence of Diptera using morphological identification, varied most from the expected value. Individual χ^2 contributions to the overall test score for Hemiptera ($\chi^2 = 56.1$) indicated presence of this prey order within fecal pellets contributed the most variation to the overall test score, with the exception of BOLD. Finally, individual χ^2 contributions to the overall test score for Lepidoptera ($\chi^2 = 93.3$) indicated that absence in

morphological identification and GenBank procedures varied most substantially from expected values.

At a finer resolution, BOLD allowed identification of 21 distinct species or genera (n = 29; Table 4.2). All sequences for which these inferences were generated were placed as Lepidoptera (Figure 4.3), with a dipteran exception (n = 2). The majority of Lepidoptera were Tortricidae (n = 13) and Noctuidae (n = 9). Other Lepidoptera identified included two each of Acrolophidae and Arctiidae, and one each of Coleophoridae, Epipyropidae, Gelechiliidae, Geometridae, Lasiocampidae, Saturniidae, and Tineidae. In total, 52% of these observations fell within the paraphyletic group of moths historically designated as microlepidoptera (Covell 2005). The mean (±SE) wingspan of all Lepidoptera identified using BOLD was 27.2 ± 3.6 mm, in contrast with 34.1 ± 1.6 mm calculated from Lepidoptera previously documented in the diet of the eastern red bat (*Lasiurus borealis* Müller) (identifications from Clare *et al.* 2009).

My survey of Lepidoptera served as an index of prey availability and yielded 20,256 moths, representing 23 families from 184 blacklight trap samples (Figure 4.3). Noctuidae were the most abundant, with 6,273 individuals captured. Other common families included the Geometridae (n = 3,800), Arctiidae (n = 3,334), Notodontidae (n = 2,291), Pyralidae (n = 1,553), Lasiocampidae (n = 765), Saturniidae (n = 724), Oecophoridae (n = 325), Limacodidae (n = 171), Tortricidae (n = 166), and Lymantriidae (n = 118). Families classified as ‘Uncommon’ (n < 100) included the Apatelodidae, Cossidae, Drepanidae, Epiplemidae, Megalopygidae, Mimallonidae, Pterophoridae, Sesiidae, Sphingidae, Yponomeutidae, and Zygaenidae (Figure 4.3).

Discussion

My study is the first to demonstrate the importance of microlepidoptera as a prey group of *M. septentrionalis* and reveals cryptic trophic linkages previously undocumented for *Myotis* species (Table 4.2). At a broader resolution, both DNA-based identification procedures that I employed indicated the majority of prey sequences belonged to Lepidoptera, corroborating my data from morphological identification, and providing evidence that my DNA-based results are congruent with previous studies of *M. septentrionalis* in my region (Griffith and Gates 1985; Lacki *et al.* 2009).

For my first research objective, my data demonstrate *M. septentrionalis* consume Lepidoptera that are smaller than those documented for either lasiurine or plecotine bats (Clare *et al.* 2009; Lacki and Dodd *In Press*). This may be a reflection of a feeder constrained to handling smaller prey due to its size (Alderidge and Rautenbach 1987; Caceres and Barclay 2000; Lacki *et al.* 2007a). Working in concert with this, the capacity for *M. septentrionalis* and other myotine species to echolocate across at a higher peak frequencies lends increased potential to better locate and capture smaller-sized prey (Fenton 1990; Lacki *et al.* 2007a). *M. septentrionalis* is a small myotine predator that, consequently, consumes smaller prey (27.2 ± 3.6 mm). In contrast, data from discarded wings of Lepidoptera suggest plecotine bats in the genus *Corynorhinus* consume taxa with a wingspan of 47 ± 1.3 mm (Lacki and Dodd *In Press*). However, assessment of such culled prey remnants only allows identification of prey from parts that are culled, thus smaller prey items which may be eaten in their entirety, or those with little chitin, may not be recorded (Lacki *et al.* 2007a). Even so, the data that do exist for plecotine species suggest that these lepidopteran specialists consume larger prey. Similarly,

lepidopterans consumed by *L.borealis*, a common lasiurine species, have wingspans of 34.1 ± 1.6 mm (Clare *et al.* 2009). Given my data, I hypothesize *M. septentrionalis* and likely other similar-sized myotine species that both hawk and glean prey (Ratcliffe and Dawson 2003; Whitaker 2004), occupy a niche of prey selection distinct from other taxonomic groups of insectivorous bats in North America.

In relation to my second research objective, my study helps further elucidate prey consumption by an insectivorous bat in the context of prey availability. Microlepidoptera are difficult to identify and enumerate in assessments of prey availability; consequently, identification efforts of Lepidoptera have focused on larger specimens (Burford *et al.* 1999; Dodd *et al.* 2008). The Lepidoptera consumed by *M. septentrionalis* in my study generally corresponded to the size-class of prey (i.e., wingspans ≥ 20 mm) identified in my assessment of prey abundance. However, some prey species did fall below this threshold [i.e., mean wingspans < 20 mm; *Blastobasis* sp., *Chionodes adamas* (Hodges), *Clepsis* spp., *Fulgoraecia exigua* (Edwards)]. Thus the importance of these smaller prey items to foraging bats, and consequently to food habits studies, should not be understated and should be considered in subsequent studies. As web-based DNA databases grow in taxonomic and regional representation, DNA-based prey identification procedures should become increasingly powerful. Furthermore, using web-based search tools to identify sequences from microlepidoptera and other taxa that are difficult to identify will allow ecologists to assess both prey availability and consumption of taxa that would otherwise require expert identification, thus allowing ecologists to further assess cryptic trophic linkages previously inaccessible.

My results illustrate differences in the sequence composition between the BOLD and GenBank databases (Fig ure 4.2). While it is possible the preponderance of sequence matches from smaller Lepidoptera in *M. septentrionalis* diet could be due to primer bias, I suggest this is unlikely given the use of my primer set in other studies amplifying DNA from a wide breadth of insect taxa (Simon *et al.* 1994; Harper *et al.* 2006; Jourdie *et al.* 2008). Further, the diet breadth as identified using GenBank, suggests DNA amplification across a broad cross-section of arthropod taxa. The total number of BOLD sequences across Lepidoptera (Table 4.3) generally corresponds with my prey abundance data. Despite their high frequency within fecal samples of *M. septentrionalis*, larger Tortricidae (≥ 20 mm) were not a major component of my blacklight trap catches, suggesting that frequent consumption of Tortricidae and other microlepidoptera is real. However, I do suggest that bias does exist for my DNA-based technique at a broader taxonomic resolution. If only considering data generated with BOLD, I would have reached the conclusion that *M. septentrionalis* is a highly-specialized predator of Lepidoptera consistent with observations for plecotine bats in eastern North America (>80% of diet; Lacki *et al.* 2007a). Given prey consumption data generated using GenBank and the morphological technique, as well as the results from other studies (Griffith and Gates 1985; Caceres and Barclay 2000; Brack and Whitaker 2001; Carter *et al.* 2003; Lee and McCracken 2004; Lacki *et al.* 2007a; Feldhammer *et al.* 2009; Lacki *et al.* 2009), this is likely not the case for *M. septentrionalis*. The distribution of C*OI* sequences in both databases offers a more likely explanation (Table 4.3). In the case of both GenBank and BOLD, Lepidoptera are the most amply represented prey order, though more so in BOLD. Therefore, it is logical that my lepidopteran sequence matches

were greater using BOLD versus GenBank. I suggest that BOLD allowed species-level inferences of Lepidoptera but precluded species-level inferences of other insect orders. Thus, I suggest that the species-level data I have generated is correct, albeit an incomplete view of the food habits of *M. septentrionalis*. Considering this, *a priori* knowledge of diet breadth may dictate which identification algorithm and database provides the optimal basis for analysis of a given predator species.

My results relating to my third research objective (comparing identification procedures) also provide an indication of the biases across techniques. Trends in the individual contributions of variation to overall χ^2 test scores suggested that variation exists among identification procedures' deviation from expected values (Table 4.1). A review of previous studies suggests morphological identification may over-represent hard-bodied prey (Lacki *et al.* 2007a); my data corroborate this. Notably, the insect taxa for which I can best achieve higher-resolution identifications (i.e., Lepidoptera), are the taxa that are precluded from higher-resolution identifications using traditional techniques (i.e., hard-bodied prey) (Whitaker 2004). Therefore, DNA-based prey identification techniques lend insight where it is most needed for bat food identification (J.O. Whitaker, Jr., pers. comm.). Even so, quantitative assessment of prey consumption is difficult with current DNA-based techniques (Harwood and Greenstone 2008). Until DNA-based methods evolve further (e.g., real-time PCR) (Harwood and Greenstone 2008), a union between DNA-based and morphological identification will best allow high resolution prey identification in conjunction with quantitative estimates of prey consumption.

Unlike recent work evaluating food habits of *L. borealis* that suggest a much broader diet breadth than previously reported (Clare *et al.* 2009), the sample units in my

study (individual fecal pellets) do provide a more narrower perspective of specific prey items by specific individual bats. Thus, my study is not indicative of strong differences in diet breadth between myotine and lasiurine bats; rather, the sample unit in my study is more conservative due to the single prey inferences generated per pellet. I have likely amplified the most common DNA products within fecal pellets and, thus, provide an indication of the most common items by volume within the diet of *M. septentrionalis*.

I also document predation by a common bat species on numerous Lepidoptera of importance as agricultural and forest pests (Table 4.2), many of which demonstrate outbreak behavior (Covell 2005). Tortricidae larvae are leaf rollers and tiers, and root, stem and fruit borers with broad economic importance (Covell 2005). The eastern tent caterpillar, *Malacasoma americanum* (Fabricius) (Lasiocampidae) is a serious defoliator of Rosaceous trees (Covell 2005), and sporadically impacts equine health (Webb *et al.* 2004). My study provides intriguing data that suggest that forest bats may play a role in depredating lepidopteran pest species; future research should further consider the role that forest bats may play in regulating these populations.

DNA-based assessments of foraging hold a number of implications for current ecological knowledge and natural resource management, as well as future research. My model predator, a common myotine forest-dwelling bat, selects prey across multiple taxonomic levels. My data reaffirms the importance of Lepidoptera as a key prey group. While the prey consumed by *M. septentrionalis* were not uncommon across the landscape, they were often not the most abundant recorded, and presumably not the most available. Further, these prey are smaller relative to those reported for other bat species that broadly exist at either end of a continuum of foraging behavior (i.e., gleaning and

aerial-hawking), suggesting that *M. septentrionalis* differentiates itself from sympatric insectivorous bats with the prey it selects (Arlettaz *et al.* 1997). Given the diversity of Lepidoptera consumed across bat species, conservation goals should promote land management and forest stewardship practices that contribute to a diverse prey base for these ecologically-sensitive predators. Finally, I have evaluated prey consumption with both innovative and traditional approaches; comparing these is a central consideration in the application of alternative methods. Integration of several techniques has allowed my study to consider prey consumption of various taxa at multiple levels of resolution. I hope these results contribute to further development and refinement of DNA-based techniques to evaluate cryptic trophic linkages, and for broader use in food web ecology.

Table 4.1. Contribution of percent variation to overall $\chi 2$ scores among methods of identification. Separate tests were conducted for each order of prey; critical $\chi^2_{\alpha = 0.001, 4\ DF} = 18.47$. Percentages in bold indicate values exceeding equitable variation.

Prey Order ($\chi 2$ Score)	Occurrence	Variation from Expected $\chi 2$ Score (%)		
		Morph. ID	GenBank	BOLD
Coleoptera (236.8)	Presence	**30.0**	11.5	10.5
	Absence	**27.7**	10.6	9.7
Diptera (37.82)	Presence	**27.4**	1.3	**38.5**
	Absence	13.4	0.6	**18.8**
Hemiptera (56.1)	Presence	**49.8**	**24.5**	11.2
	Absence	8.5	4.2	1.9
Lepidoptera (93.3)	Presence	6.9	12.0	1.3
	Absence	**27.3**	**47.5**	5.1

Table 4.2. List of insect prey species identified by comparing C*OI* sequences from the fecal samples of *Myotis septentrionalis* by comparison with BOLD. Nomenclature and authorities of Lepidoptera follow Covell (2005). Nomenclature and authority of Dipteran entry follows BOLD.

Order	Family	Taxon ID
Diptera	Tipulidae	*Tipula submaculata* Loew
Lepidoptera	Acrolophidae	*Acrolophus propinqua* (Wlsm.)
	Arctiidae	*Halysidota tessellaris* (Sm.)
	Coleophoridae	*Blastobasis* sp.
	Epipyropidae	*Fulgoraecia exigua* (Edw.)
	Gelechiliidae	*Chionodes adamas* (Hodges)
	Geometridae	*Hypagyrtis* sp. complex
		Macaria sp. complex
	Lasiocampidae	*Malacasoma americanum* (F.)
	Noctuidae	*Abagrotis alternata* (Grt.)
		Idia julia (B. and McD.)
		Noctua pronuba (L.)
	Saturniidae	*Antheraeopsis castanea* Jordan *
	Tineidae	*Isocorypha mediostriatella* (Clem.)
	Tortricidae	*Choristoneura fractivittana* (Clem.)
		Clepsis peritana (Clem.)
		Clepsis virescana (Clem.)

Table 4.2. (continued)

	Eucosma derelecta Heinrich
	Paralobesia liriodendrana (Kft.)
	Phaecasiophora confixana (Wlk.)
	Pseudexentera sp. complex

*Asiatic in origin; this identification is likely incorrect and reflects high sequence similarity between Saturniid species.

Table 4.3. Comparison of total C*OI* sequences from BOLD and GenBank databases (accessed February 2010). Search phrases for GenBank consisted of "<taxon of interest> AND cytochrome oxidase subunit I." For BOLD, all taxa are as indexed by BOLD, with the exception of “Pyralidae,” which is the sum of data indexed as Pyralidae and Crambidae.

Taxon	BOLD		GenBank
	Specimens	Species	Total Hits
Hemiptera	12,838	1,934	7,965
Coleoptera	14,727	3,246	18,471
Diptera	43,773	5,017	19,753
Lepidoptera	354,473	39,387	26,587
Notodontidae	15,311	866	97
Tortricidae	15,840	1,662	1,245
Arctiidae	17,067	1,991	882
Pyralidae	26,883	2,839	944
Geometridae	53,852	8,183	919
Noctuidae	65,801	6,778	980

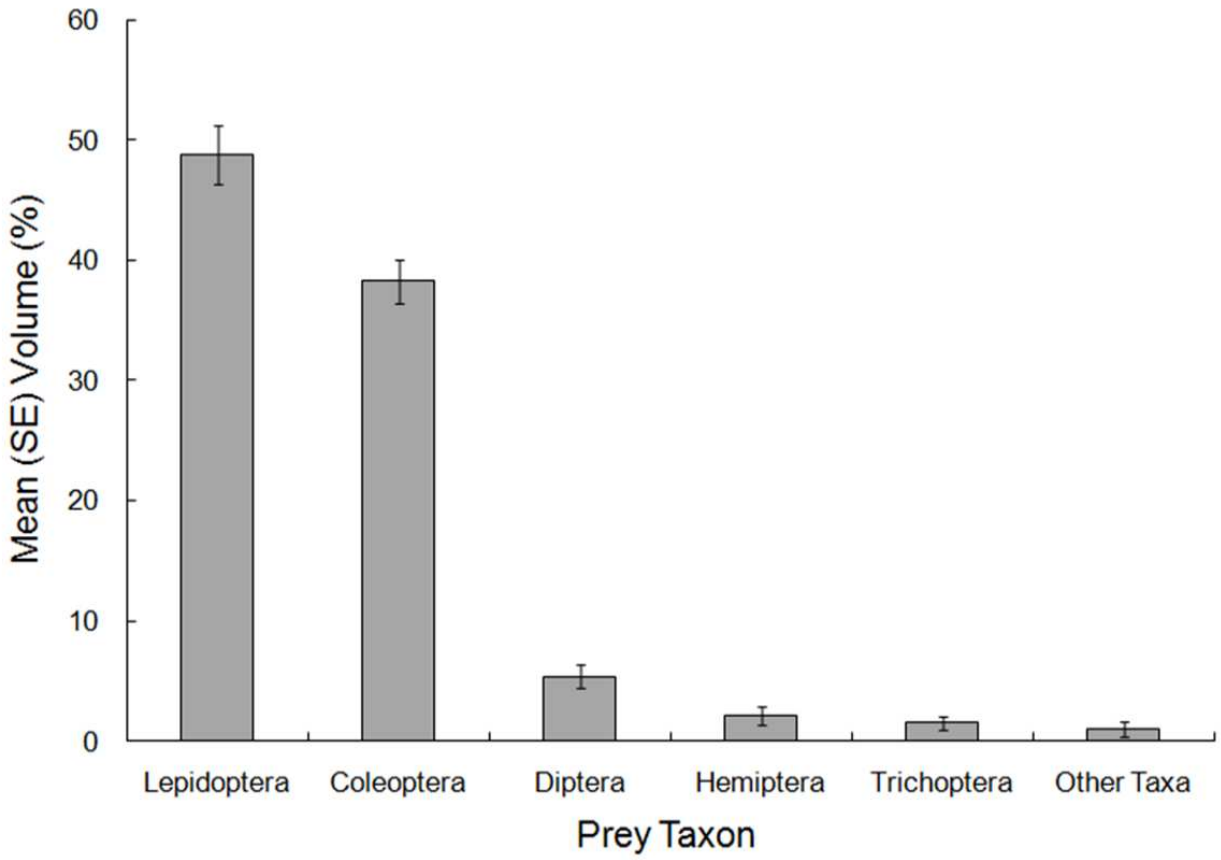


Figure 4.1. Prey volume identified in fecal samples of *Myotis septentrionalis* using morphological identification. "Other Taxa" include all taxa with mean volumes < 1%, including: Hymenoptera, Neuroptera, Arachnida.

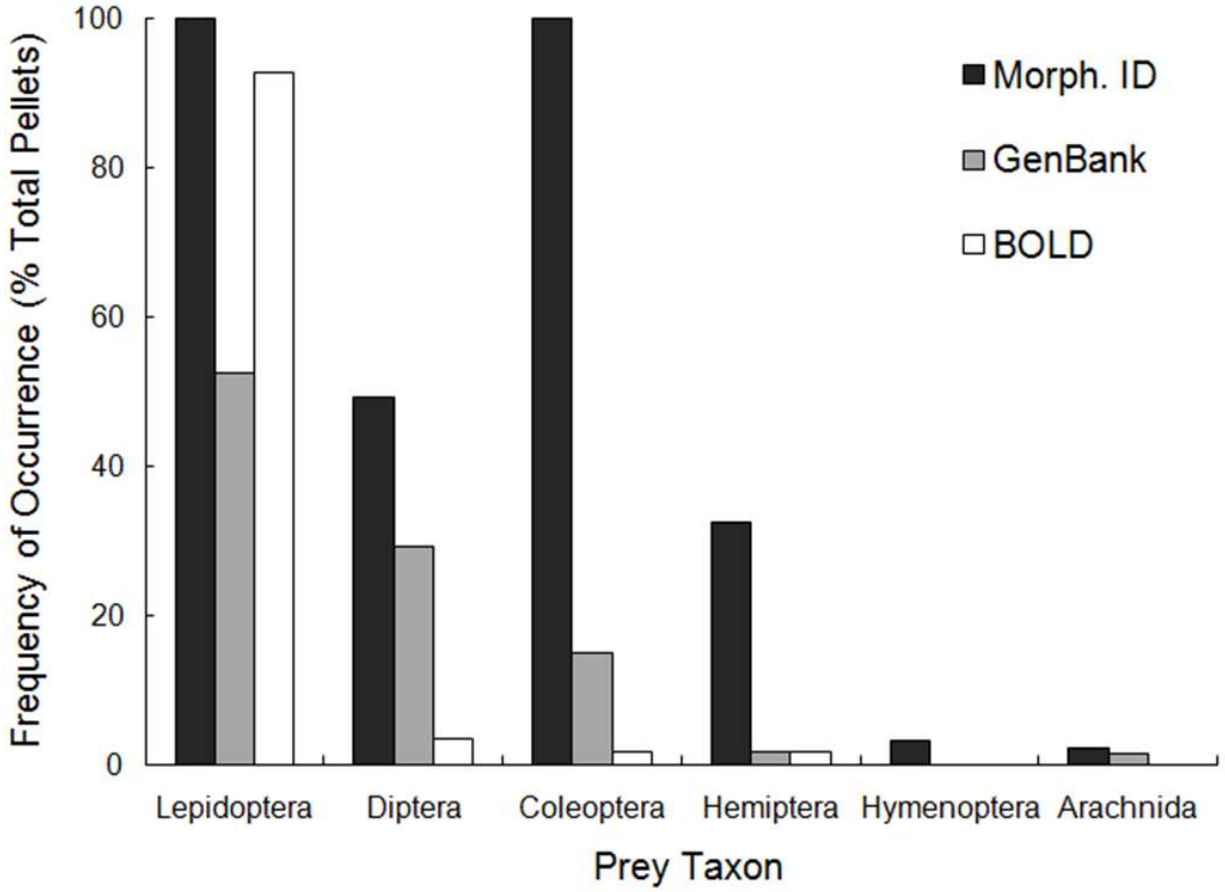


Figure 4.2. Frequency of occurrence of prey taxa in fecal pellets of *Myotis septentrionalis* across identification procedures.

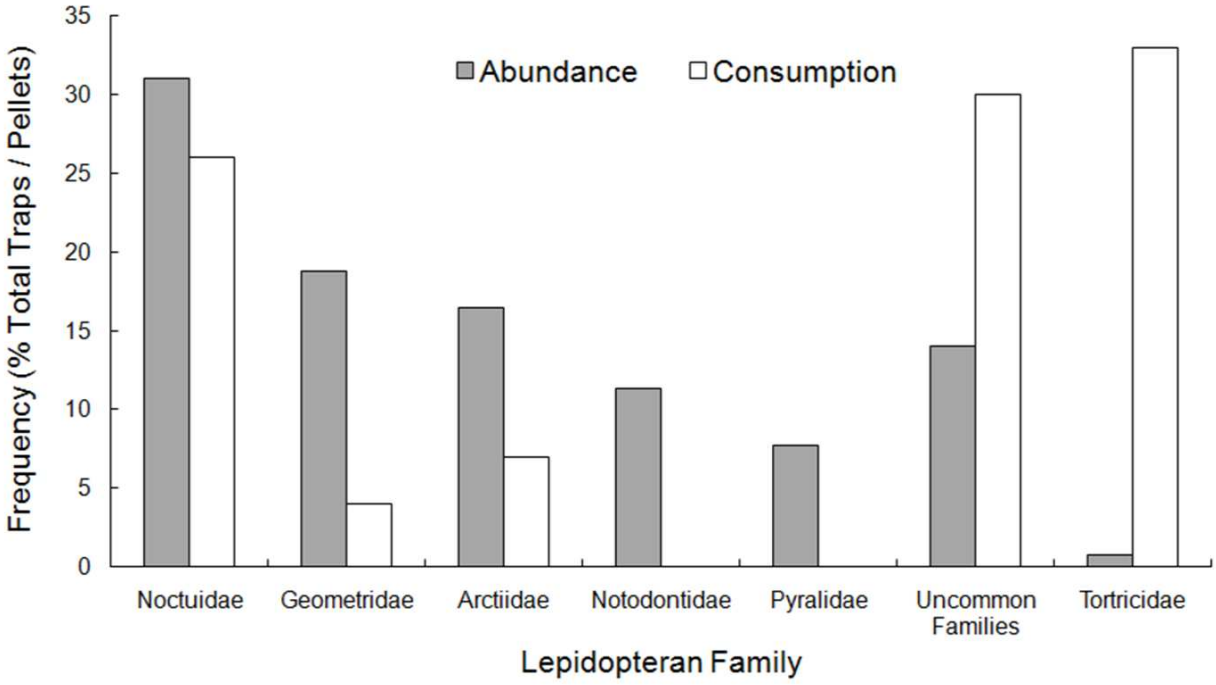


Figure 4.3. Lepidopteran prey abundance, as assessed by blacklight traps, compared with lepidopteran consumption, as assessed using BOLD. "Uncommon Families" include all families < 5% of total catch in blacklight traps, with the exception of the Torticidae (0.8%).

CHAPTER FIVE: DEVELOPMENT OF A C*OI* LIBRARY OF FOREST LEPIDOPTERA AND IDENTIFICATION OF THE PREY OF *MYOTIS SEPTENTRIONALIS* USING TREE-BASED CLADISTIC ANALYSES

Introduction

As DNA-based and other molecular approaches increase in popularity among ecologists, the applications and means of interpreting data generated from these approaches continues to expand (Harwood and Greenstone 2008; San Mauro and Agorreta 2010). Regardless, for DNA-based approaches, a central component for inferring phylogeny is sequence similarity (San Mauro and Agorreta 2010). At a base level, the algorithms that are used in concert with web-based databases to identify a sequence of interest do so by comparing sequence similarity with those already existing within the database (e.g., BLAST) (Altschul *et al.* 1990).

I suggest an investigator can identify predator-prey trophic linkages on a local scale using simple tree-building techniques that are readily available and easily implemented by investigators with little expertise in cladistic and barcoding analyses. In doing so, limitations regarding DNA fragment length and sequence ambiguities within fragments that may skew or limit the efficacy of BLAST or other identification algorithms may be minimized (E. Chapman, pers. comm.). These are both issues encountered when working with prey sequences extracted from fecal samples (Deagle *et al.* 2005). Intuitively, such an approach would be best-suited in instances where the trophic linkages between a particular predator species and multiple prey species are either well-known by investigators or limited in number (e.g., a dietary specialist).

In addition to the prey inferences I generated in Chapter Three and Chapter Four, also assessed the suitability of applying tree-based phylogenetic approaches towards inferring prey identityof the same unknown DNA sequences amplified from fecal samples of the northern bat (*Myotis septentrionalis* Trouessart). This study presents exploratory analyses to determine the merit of comparing unknown DNA sequences to a discrete pool of known DNA sequences for identification purposes; this pool thus represents a bank of potential prey within a specific location versus the cosmopolitan pool of samples that are present in a web-based database (e.g., GenBank).

Materials and Methods

Study Areas and Field Collection

Fecal samples of bats were collected regionally across the Central Appalachians of eastern North America (Appendix A), including the Daniel Boone National Forest, Kentucky (Lat. 38°2′ N, Long. 83°35′ W), the Raccoon Ecological Management Area, Ohio (Lat. 39°11′ N, Long. 82°22′ W), and the Royal Blue Wildlife Management Area, Tennessee (Lat. 39°11′ N, Long. 82°23.′ W). Field collections took place in a matrix of upland forestland actively managed for timber production and used for scientific research.

Bats were captured throughout their active period across my study areas from March through September of 2007 and 2008 in monofilament nylon mist nets (2.6, 6, 9 m in length; 6.8 m^2, 15.6 m^2, 23.4 m^2 in area) (Avinet, Dryden, NY, USA) placed throughout the study areas over flyways formed by roads and road-ruts with pooled water, small streams, trails, and ridgelines. Captured bats were handled in accordance with the University of Kentucky Institutional Animal Care and Use Committee

(#01019A2006) and state and federal collection permits. Bats were held separately in single-use, disposable cotton bags (20 × 30.5 cm) (Avinet) for ca. 20 min to allow defecation. Fecal samples from each individual were then collected into 1.5 mL microcentrifuge tubes, placed on ice or in a mobile freezer (ca. 0°C) (MT17, Engel USA, Jupiter, FL, USA), and transferred to long-term freezer storage (−80°C) immediately upon return to the laboratory. At no time were fecal samples allowed to contact any surface other than the single-use cotton bag or the microcentrifuge tube.

Potential lepidopteran prey of *M. septentrionalis* were collected regionally in conjunction with the collection of fecal samples. Lepidoptera were collected from May to September, 2006 – 2008, using a light-weight cotton sheet (1.9 m x 1.0 m) stretched taut at ground level and illuminated with a 10 w blacklight and electrical harness (Universal Light Trap, Bioquip Products, Rancho Dominguez, CA, USA). Specimens attracted to the sheet were collected individually into sterile jars (7 mL, 30 mL) (Dynalab Corp.) in a manner to prevent contamination (i.e., jar placed over the specimen and not handled by the collector). Specimens were stored at ambient temperature for ca. 12 hours to allow clearance of gut contents and then transferred to long-term freezer storage (−80°C). Specimens were identified using available keys (Holland 1903; Covell 2005) and reference collections at the University of Kentucky.

Screening Fecal Samples

Fecal pellets of collected bats were dissected microscopically and prey remains identified to the most specific taxon possible (on the basis of key determination by Whitaker 1988). Individual fecal pellets were placed in a sterile pour boat (4.1 × 3.2 × 0.8 cm) (Fisher Scientific, Pittsburgh, PA, USA), diluted with 100% ethanol and teased apart

using a disposable pestle (Fisher Scientific) for microscopic dissection. I estimated percent frequency of prey items in the diet among individual bats, and estimated the percent volume of prey items in pellets from each bat to the nearest five percent. Up to three pellets were dissected from each bat, and average values across pellets were used in determining percent volumes of prey in the diet (Lacki *et al.* 2007b). Individual fecal pellets were then preserved in ca. 1.5 mL 95% ethanol and placed in freezer storage (−80°C) until subsequent DNA-based analysis.

Molecular remains of prey are not homogenous within feces (Deagle *et al.* 2005). Considering the number of fecal pellets generally yielded by each bat, up to three fecal pellets from each individual bat were used for DNA-based analysis to increase the likelihood of accurate and reliable identification of all prey items consumed. The entire individual pellet that was used for morphological identification was then used for each individual DNA extraction. Prior to DNA extraction, each fecal sample was homogenized for ca. 1 min in 2.0 mL mortar-and-pestle microcentrifuge tubes, vortexed ca. 1 min, then centrifuged at 20,000 × *g* for 3 min, discarding the resulting supernatant. Following this process, 1 mL TE buffer was added to samples which were then vortexed ca. 1 min, centrifuged at 20,000 × *g* for 3 min and the supernatant discarded. DNA was then extracted using a QIAamp DNA Stool Mini Kit (Qiagen Inc., Chatsworth, California, USA). Protocol was followed according to the manufacturer's instructions for the isolation of DNA from stool for pathogen detection carrying out lysis with the ASL buffer at 70°C, and using all applicable extra centrifugation steps.

PCR reactions (total volume = 50 μL) for nucleotide sequencing of C*OI* were carried out with C1-J-1859 with C1-N-2191 primers, resulting in a 333 base amplicon

(Simon *et al.* 1994). The PCR cocktail contained 2 μL template DNA solution of unknown concentration, 1.25 U Qiagen HotStar Taq polymerase, Qiagen 0.2 mM dNTP, 0.25 mM of each primer, 1.5 mM 10× reaction buffer, and 1 mM $MgCl_2$. Cycling conditions were 15 min at 95°C, 50 cycles of 50 sec at 94°C, 45 sec at 45°C, 45 sec at 72°C, and a final elongation for 5 min at 72°C. Reaction success was then confirmed by electrophoresis of 10 μL of PCR product in 1.0 % agarose (Sigma-Aldrich Co., St. Louis, Missouri, USA) in 1× TAE (Promega Corp., Madison, Wisconsin, USA). Sequencing (University of Kentucky Advanced Genetic Technologies Center, Lexington, Kentucky, USA) was carried out for those reactions that yielded strong PCR bands of expected size, using BigDye terminator kits (v. 3.1) and the previously-mentioned primer set on an ABI3100 sequencer (Applied Biosystems, Foster City, California, USA). Reactions were sequenced bi-directionally to reduce the possibility of "chimeric sequences" consisting of multiple prey DNA fragments; overlapping forward and reverse sequences were edited and assembled using Vector NTI (v. 10.3, Invitrogen Corp., Carlsbad, California, USA). If strong, corresponding signals were not present in forward and reverse chromatographs, such portions of sequences were marked as unidentifiable (or discarded if the bulk of a sequence was unknown). Thus, I generated a single sequence per fecal pellet.

Development of Sequence Library

A library of C*OI* sequences was compiled from lepidopteran samples. DNA was extracted using a QIAamp DNA Stool Mini Kit (Qiagen Inc., Chatsworth, California, USA). To prepare samples for DNA extraction, an entire leg of a vouchered individual was removed and partially homogenized for ca. 1 min in 2.0 ml mortar-and-pestle microcentrifuge tubes in 1.4 mL Buffer ASL solution. Protocol was followed according

to the manufacturer's instructions for the isolation of DNA from stool for pathogen detection carrying out lysis with the ASL buffer at 70°C, and using all applicable extra centrifugation steps.

PCR reactions (total volume = 50 μL) for nucleotide sequencing of lepidopteran C*OI* were carried out with C1-J-1751 with C1-J-2191 (Simon *et al.* 1994) and HCO1490 with HCO2198 (Folmer *et al.* 1994). The PCR cocktail contained 2 μL template DNA solution of unknown concentration, 1.25 U Qiagen HotStar Taq polymerase, Qiagen 0.2 mM dNTP, 0.25 mM of each primer, 1.5 mM 10× reaction buffer, and 1 mM $MgCl_2$. Cycling conditions were 15 min at 95°C, 50 cycles of 50 sec at 94°C, 45 sec at 45°C, 45 sec at 72°C, and a final elongation for 5 min at 72°C. Reaction success was then confirmed by electrophoresis of 10 μL of PCR product in 1.0 % agarose (Sigma-Aldrich Co., St. Louis, Missouri, USA) in 1× TAE (Promega Corp., Madison, Wisconsin, USA). Sequencing (University of Kentucky Advanced Genetic Technologies Center, Lexington, Kentucky, USA) was carried out for those reactions that yielded strong PCR bands of expected size, using BigDye terminator kits (v. 3.1) on an ABI3100 sequencer (Applied Biosystems, Foster City, California, USA). Overlapping sequences were edited and assembled using Vector NTI (v. 10.3, Invitrogen Corp., Carlsbad, California, USA).

Analyses for Prey Identification

To supplement the library of C*OI* sequences from Lepidoptera, sequences from additional insect taxa known to be preyed upon by *M. septentrionalis* (Griffin and Gates 1985; Whitaker 2004; Lacki *et al.* 2009) were secured from GenBank and incorporated into the framework I used in tree-building identification procedures. Prey identies were inferred using phylogenetic analyses; tree-building followed neighbor-joining (N-J) and

maximum likelihood (ML) approaches. In both cases, phylogenetic trees imbedded unknown prey sequences within the larger database of known sequences of potential prey taxa.

A N-J tree was generated using Geneious (v. 4.7.6; Saitou and Nei 1987) with the default settings for a Tamura-Nei genetic distance model. A best ML tree was generated using GARLI (v. 0.951; Zwickl 2006) using default settings except for the following: automatically terminate run 100,000 generations after last improved topology, lnL increase for significantly better topology = 0.0001 and score improvement threshold = 0.0005. In the case of both phylogenetic trees, prey identity was assigned to unknown sequences from fecal pellets at the ordinal taxonomic level by measuring the shortest genetic distance to the node belonging to an identified insect. Unknown sequences occurring on isolated nodes were recorded as ambiguities.

Results

A total of 153 individuals across 89 species of Lepidoptera were successfully sequenced and assembled into the library of potential prey (Table 5.1). Representation across taxa within this database was weighted such that more common taxa across study areas were represented more within the database. C*OI* sequences from 32 additional insect taxa were accessed on GenBank and assimilated into the pool of potential prey (Table 5.2).

Tree-building procedures identified 5 different taxonomic orders of prey, respectively (Figure 5.1). In both cases, the vast majority of unknown DNA sequences from fecal pellets were placed in closest genetic distance to lepidopteran sequences; 74.5 % for N-J and 67.1% for ML, respectively. In the case of the N-J tree, the most common

other taxonomic placements included Hymenoptera (13.8%) and Diptera (7.4%), whereas in the case of the ML tree, Coleoptera (10.9%) and Diptera (6.1%) followed behind Lepidoptera. Identification of unknown sequences as Hemiptera was absent with the N-J tree and limited with the ML tree (3.4%). Placement of unknown sequences outside the Insecta, in relation to Araneae, occurred with both identification procedures (1.4% for N-J tree and 2.5% for ML tree).

Discussion

These results are a novel application of a phylogenetic tool as a means of assessing prey barcodes in predator-prey relationships. The two tree-building approaches offer varied strengths and weaknesses. A N-J tree, while limited in application in modern phylogenetic study, offers a direct, efficient means of comparing sequences regardless of quality. Further, a N-J tree is more easily constructed by investigators not familiar with phylogenetic techniques. Even so, this approach holds limited application in the modern suite of techniques at the disposal of phylogenetic researchers (San Mauro and Agorreta 2010). As an alternative, ML trees bridge the gap between the complexity of web-based algorithms and the basic approach of a N-J tree. ML trees are a more statistically rigorous technique (E. Chapman, pers. comm.). Even so, the N-J tree was not parsimonious even at a course resolution. Hence, results from this study suggest that future efforts to implement tree-building approaches either 1) continue using the ML approach, or 2) consider a more robust pool of sequences of potential prey (that are parsimonious) if using the N-J approach.

The tree-based analyses presented in this study present a set of results that more closely correspond with the data generated using morphological identification in Chapter

Four (versus comparisons with web-based databases). While the pool of potential prey considered in this study is certainly skewed towards Lepidoptera, just as with the web-based databases considered in Chapter Four, it is intriguing that the results in this study more closesly match those generated using traditional approaches versus those using web-based databases. Regardless, the data from this study underscores the importance of Lepidoptera in the diet of *M. septentrionalis*; in the case of either tree, this insect group was identified in more than half of all sequences.

In total, data generated using tree-building approaches did not differ substantially from those data presented in Chapter Four. Even so, the methods and analyses presented in this study provide a useful resource for further studies that delineate trophic linkages using DNA-based approaches. The methods presented in this study may serve as a base for further application of these tree-building approaches. Specific to bats, future application should focus more on those bat species for which there is a better-defined pool of potential prey (e.g., *Corynorhinus* spp.) (Lacki and Dodd *In Press*).

Table 5.1 Forest Lepidoptera collected across Central Appalachia and integrated into a C*OI* sequence library. Nomenclature and authorities of Lepidoptera follow Covell (2005).

Lepidopteran Family	Library Entry
Arctiidae	*Cisseps fulvicollis* (Hbn.)
	Clemensia albata (Pack.)
	Crambidia pallida (Pack.)
	Halysidota tessellaris (J.E. Sm.)
	Hypoprepria fucosa (Hbn.)
	Hypoprepia miniata (Kby.)
	Pyrrharctia isabella (J.E. Sm.)
	Spilosoma congrua (Wlk.)
Drepanidae	*Drepana arcuata* (Wlk.)
Epiplemidae	*Calledapteryx dryopterata* (Grt.)
Geometridae	*Anacamptodes ephyraria* (Wlk.)
	Antepione thisoaria (Gn.)
	Campaea perlata (Gn.)
	Ecliptopera atricolorata (Grt. and Rob.)
	Epimecis hortaria (F.)
	Eubaphe mendica (Wlk.)
	Euchlaena amoenaria (Gn.)
	Euchlaena irraria (B. and McD.)
	Eulithis diversilineata (Hbn.)

Table 5.1. (continued)

	Heliomata cycladata (Grt. and Rob.)
	Hydrelia inornata (Hulst)
	Hydria prunivorata (Fgn.)
	Hypargyrtis unipunctata (Haw.)
	Iridopsis larvaria (Gn.)
	Itame pustularia (Gn.)
	Lambdina fervidaria (Hbn.)
	Metanema inatomaria (Gn.)
	Nemoria lixaria (Gn.)
	Pero hubneraria (Gn.)
	Plagodis alcoolaria (Gn.)
	Plagodis phlogosaria (Gn.)
	Probole amicaria (H.-S.)
	Prochoerodes transversata (Dru.)
	Semiothisa promiscuata (Fgn.)
	Xanthotype urticaria (Swett)
Lasiocampidae	*Malacosoma americanum* (F.)
	Malacosoma disstria (Hbn.)
Limacodidae	*Apoda biguttata* (Pack.)
	Apoda y-inversum (Pack.)
	Prolimacodes badia (Hbn.)
	Sibine stimulea (Clem.)

Table 5.1. (continued)

Lymantriidae	*Dasychira manto* (Stkr.)
	Dasychira obliquata (Grt. and Rob.)
	Orgyia definita (Pack.)
Noctuidae	*Abagrotis alternata* (Grt.)
	Acronicta americana (Harr.)
	Acronicta morula (Grt. and Rob.)
	Agriopodes fallax (H.-S.)
	Agrotis ipsilon (Hufn.)
	Baileya levitans (Sm.)
	Baileya ophthalmica (Gn.)
	Catocala ilia (Cram.)
	Catocala micronympha (Gn.) (?)
	Catocala obscura (Stkr.)
	Eudryas grata (F.)
	Euplexia benesimilis (McD.)
	Euparthenos nubilis (Hbn.)
	Idia aemula (Hbn.)
	Lithacodia carneola (Gn.)
	Panopoda carneicosta (Gn.)
	Panopoda rufimargo (Hbn.)
	Parallelia bistriaris (Hbn.)
	Pantograpta decoralis (Hbn.)

Table 5.1. (continued)

	Plathypena scabra (F.)
	Polygrammate hebraeicum (Hbn.)
	Renia discoloralis (Gn.)
	Renia fraternalis (Sm.) (?)
	Scolecocampa liburna (Gey.)
	Thioptera nigrofimbria (Gn.)
	Xestia dolosa (Franc.)
	Zale lunata (Dru.)
	Zanclognatha ochreipennis (Grt.)
Notodontidae	*Datana angusii* (Grt. and Rob.)
	Datana perspicua (Grt. and Rob.)
	Nadata gibbosa (J.E. Sm.)
Pyralidae	*Blepharomastix ranalis* (Gn.)
	Conchylodes ovulalis (Gn.)
	Crambus agitatellus (Clem.)
	Desmia funeralis (Hbn.)
	Euzophera ostricolorella (Hulst)
	Pantographa limata (Grt. and Rob.)
	Pyrausta niveicilialis (Grt.)
Saturniidae	*Automeris io* (F.)
	Dryocampa rubicunda (F.)

Table 5.1. (continued)

Sphingidae	*Darapsa myron* (Cram.)
	Laothoe juglandis (J.E. Sm.)
	Paonias myops (J.E. Sm.)
Tortricidae	*Choristoneura* sp.
Yponomeutidae	*Atteva punctella* (Cram.)

*Denotes species-level identification is questionable.

Table 5.2 Arthropods accessed from GenBank (November 2009) and integrated into a C*OI* sequence library.

Order	Family	Taxon Accessed	Accession Number
Araneae	Larinioidae	*Larinioides cornutus*	FJ525322
	Tetragnathidae	*Tetragnatha montana*	FJ899831
Coleoptera	Carabidae	*Harpalus herbivagus*	DQ059801
		Lebia grandis	DQ059806
		Poecilus chalcites	DQ059814
	Chrysomelidae	*Chrysomela lapponica*	EF656221
		Gonioctena pallida	FJ346979
	Curculionidae	*Curculio camelliae*	AB367611
		Curculio hilgendorfi	AB501119
		Naupactus cervinus	GQ406842
	Scarabaeidae	*Maladera holosericea*	DQ295297
		Pachysoma gariepinus	AY965138
Hemiptera	Cicadellidae	Deltocephalinae sp.	EU981889
		Euscelidius variegatus	EU981886
	Lygaeidae	*Laryngodus luteomaculatus*	FJ824823
		Lygaeus kalmii	GU013621
	Miridae	*Stenotus rubrovittatus*	AB518907
Diptera	Culicidae	*Aedes denderensis*	GQ165781
		Culex annulioris	GQ165780

Table 5.2. (continued)

	Tachinidae	*Lespesia aletiae*	EF181756
		Patelloa sp.	EF182280
	Tipulidae	*Tipula* sp.	EU005476
Ephemeroptera	Baetidae	*Baetis rhodani*	AM494632
	Ephemeridae	*Ephemera simulans*	GU013596
Hymenoptera	Formicidae	*Camponotus pennsylvanicus*	FJ943563
		Myopopone castanea	DQ353381
	Ichneumonidae	*Barycnemis gravipes*	FJ415046
		Tryphoninae sp.	FJ415063
Neuroptera	Chrysopidae	*Chrysoperla lucasina*	AB354065
	Hemerobiidae	*Hemerobius humulinus*	AB353938
Trichoptera	Hydropsychidae	*Ceratopsyche bronta*	GU013580
	Limnephilidae	*Limnephilus externus*	GU013619

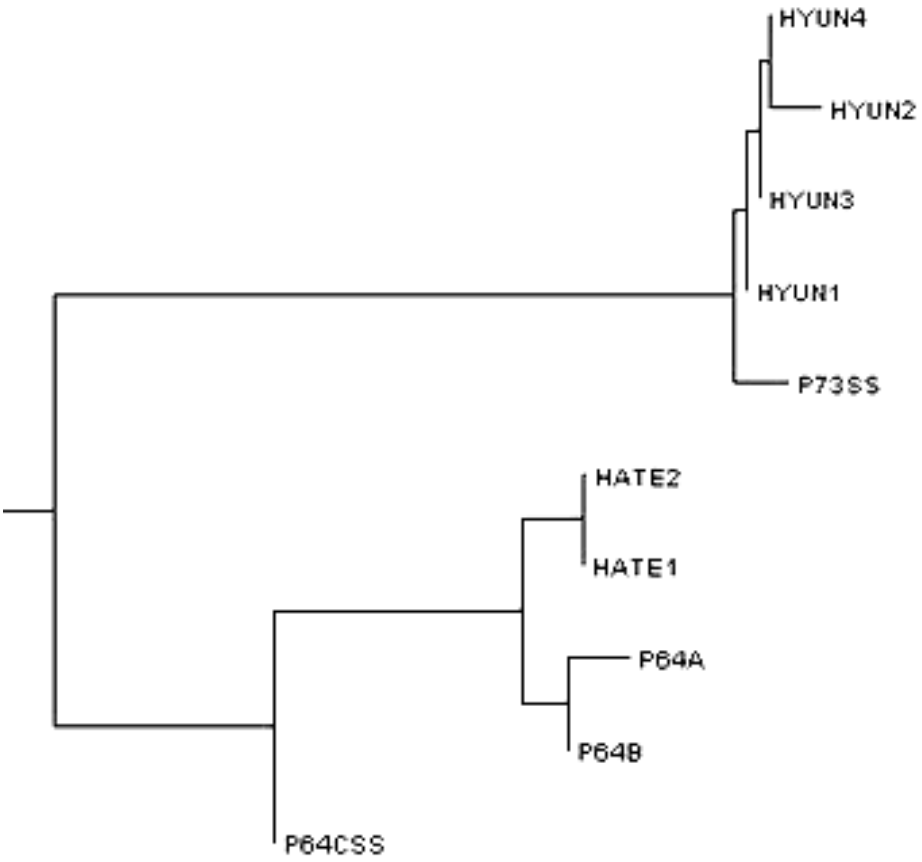


Figure 5.1. Representative portion of the neighbor-joining tree constructed using potential prey taxa and fecal samples of *Myotis septentrionalis* collected in Central Appalachia, 2007-2008. Branch length represents relative genetic distance. Whereas sequences from fecal samples #P64A, #P64B, and #P64SS (all collected from the same bat) are most similar to *Halysidota tessellaris* J.E. Sm. (Arctiidae), the sequence from fecal sample #P73SS is most similar to *Hypagyrtis unipunctata* Haw. (Geometridae). In the case of either subtree, fecal samples most closely match lepidopteran sequences and, hence, were identified as such.

APPENDICES

Appendices A-C provide methodological insight. Appendices D-G provide comprehensive analysis results not included in Chapter Two. Appendix H provides a checklist of Lepidoptera species identified in my research. Appendix I provides a behavioral observation ancillary to primary research objectives.

Appendix A: Description of study areas and land use history

The study area in Kentucky lies in the Cumberland District of the Daniel Boone National Forest at the juncture of Bath and Menifee counties (Lat. 38°2′ N, Long. 83°35′ W), which is part of the Western Allegheny Plateau (Level III Ecoregion) and includes portions of the Knobs-Lower Scioto Dissected Plateau and the Northern Forested Plateau Escarpment (Level IV Ecoregions) (Woods *et al.* 2002). Study plots most closely resemble the Knobs-Lower Scioto Dissected Plateau in character with rugged knobs, ridges, and foothills dominating the area. Local elevation ranges from 150 – 500 m, with topographic relief of 15-240 m (Woods *et al.* 2002). Non-calcareous upland areas are dominated by an oak (*Quercus* spp.) and hickory (*Carya* spp.) overstory, whereas calcareous areas are dominated by oak and ash (*Fraxinus* spp.); a mixed deciduous forest dominates the more mesic upland and cove areas (Woods *et al.* 2002). Prior to extirpation, the American chestnut (*Castanea dentata*) dominated xeric areas. Human land use has contributed to the land cover, yielding forests of varied composition. Timber harvest is common. Ridgelines and valleys may be forestland or farmland (Woods *et al.* 2002).

The study area in Ohio is located in Vinton County at the Vinton Furnace Experimental Forest (490 ha) and surrounding Raccoon Ecological Management area, which covers 6,500 ha (Lat. 39°11′ N, Long. 82°22′ W). As with the Kentucky site, this site lies on the Western Allegheny Plateau (Level III Ecoregion) but is a part of the Ohio/Kentucky Carboniferous Plateau (Level IV Ecoregion) (Woods *et al.* 1998) and is dissected by flat-bottomed valleys. Elevation varies from 150-370 m with relief of 60-150 m (Woods *et al.* 1998). Mixed oak forest dominates, though other habitats include

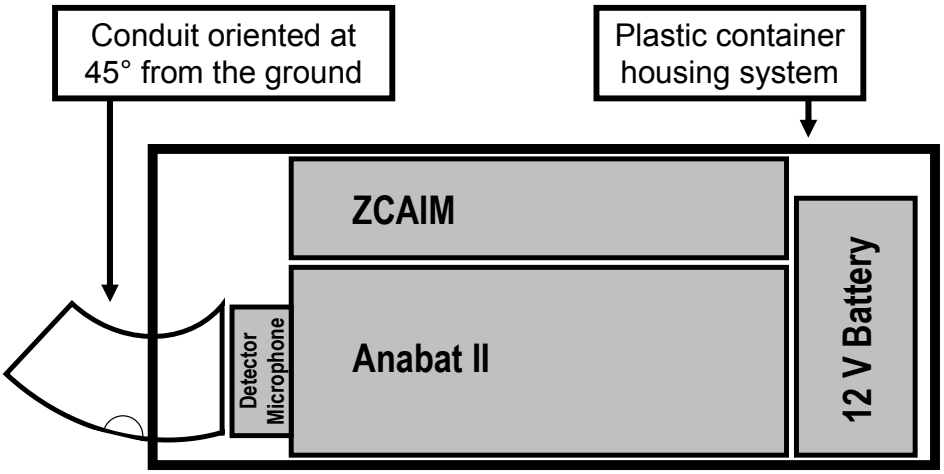
ravines with hemlock (*Tsuga* spp.) and hardwoods, as well as floodplain swamp areas with maple (*Acer* spp.) and ash (Woods *et al.* 1998). These forest types, in conjunction with livestock and farmland, form the bulk of the area's land cover. Coal mining and gas production are also common (Woods *et al.* 1998).

The Tennessee study site lies in the southern unit of the Royal Blue Wildlife Management Area (Campbell and Scott counties), which covers over 21,450 ha (Lat. 39°11′ N, Long. 82°23.′ W). It lies in the Central Appalachians (Level III Ecoregion) and is a part of the Cumberland Mountains (Level IV Ecoregion) (Griffith, Omernik and Azevedo 1998). Elevation varies from 370-1100 m with relief of 450-600 m (Griffith *et al.* 1998). The area is characterized by low mountains and narrow winding valleys. Vegetation varies with local physiography, but is a mixed mesophytic forest that includes maple, buckeye (*Aesculus* spp.), beech (*Betula* spp.), tulip poplar (*Liriodendron tulipifera*), and oak (Griffith *et al.* 1998). The area has been extensively mined and the timber harvested (Griffith *et al.* 1998).

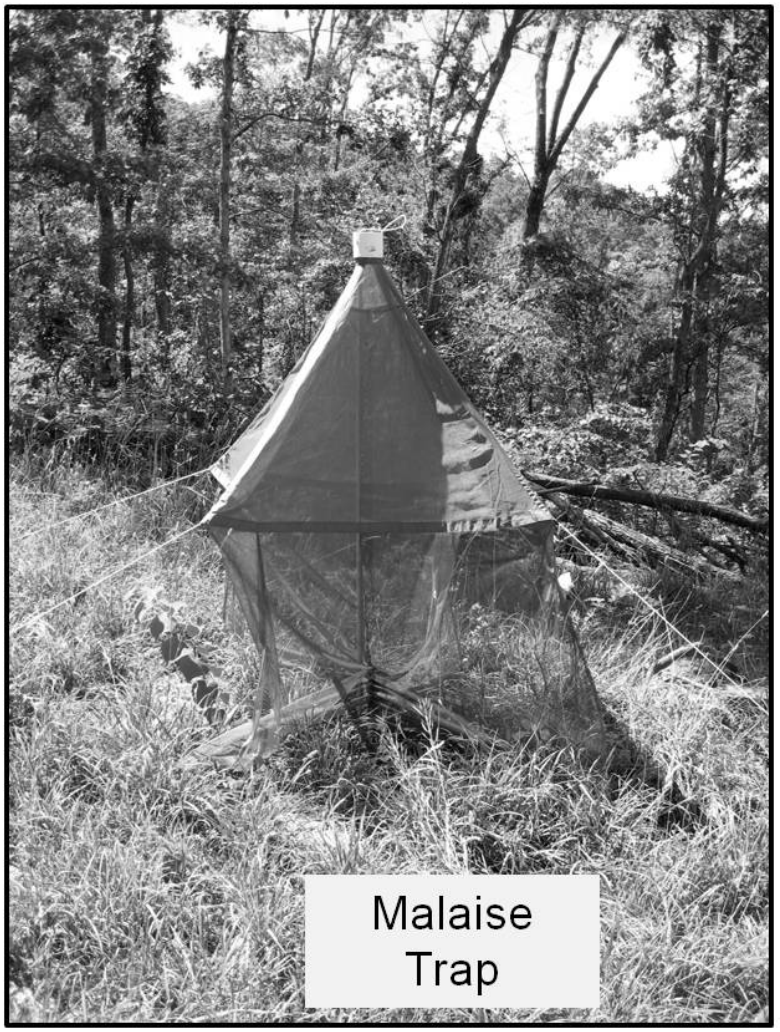
The study area in West Virginia (Wyoming County) lies within commercial timberland owned by Wagner Forestry Company (Lat. 37°30′ N, Long. 81°36′ W). It is located in the Central Appalachians (Level III Ecoregion) and is part of the Dissected Appalachian Plateau (Level IV Ecoregion) (Woods *et al.* 1999). The plateau is dominated by narrow ridgetops with steep slopes leading to deep coves (Woods *et al.* 1999). Ridge crests range in elevation from 366-1097 m and are 107-168 m above narrow valleys (Woods *et al.* 1999). Vegetation varies with local physiography, but mesophytic forests dominate. Oaks dominate upper slopes; beech, yellow poplar, and sugar maple variously dominate middle and lower northern and eastern slopes, whereas mixed oaks dominate

middle and lower southern and western slopes. Prior to extirpation, the American chestnut dominated xeric areas. A mixed deciduous forest or a hemlock and magnolia (*Magnolia* spp.) component are found in coves and bottomlands (Woods *et al.* 2002). Towns and small-scale livestock farms are found in wider valleys, and commercial forestland is common (Woods *et al.* 1999). Coal mining and gas and oil production, in conjunction with logging, have degraded stream quality (Woods *et al.* 2002).

Appendix B: Diagram and photograph of passive-monitoring acoustic detection system for bats (based on O'Ferrell 1998)



Appendix C: Photographs of trapping methods used for surveying nocturnal aerial insects



Appendix D: Canonical correspondence analysis of the relationship between bat activity and vegetation attributes in Central Appalachia, 2007-2008. The overall analysis and both ordination axes were significant ($P < 0.05$).

Variable ($\lambda_{14, 622} = 4.86$)	First Axis ($F_{14, 622} = 4.86$)		Second Axis ($F_{6, 312} = 4.71$)	
	Standardized Coefficients	Correlation of Datasets	Standardized Coefficients	Correlation of Datasets
Basal Area	-0.7482	0.0666	0.1887	0.2597
Canopy Tree Diameter	-0.318	-0.1761	0.0768	0.0613
Canopy Tree Richness	1.1241	0.1084	0.6285	0.2607
Sapling Cover	-0.1664	0.1044	0.4455	0.0766
Sapling Density	-0.1342	0.1285	-0.0131	-0.0246
Sapling Richness	0.5922	0.2388	-0.4517	-0.0778
Shrub Cover	0.4724	0.2179	-0.0189	-0.0502
Lasiurine Pulses	-0.1635	-0.2146	-1.1604	-0.2225
Myotine Pulses	-0.905	-0.3345	0.7446	0.0402

Appendix E: Canonical correspondence analysis of the relationship between Lepidoptera and vegetation attributes in Central Appalachia, 2007-2008. The overall analysis and two ordination axes were significant ($P < 0.05$).

Variable	First Axis		Second Axis	
($\lambda_{70, 951} = 1.79$)	($F_{70, 951} = 1.79$)		($F_{54, 836} = 1.55$)	
	Standardized Coefficients	Correlation of Datasets	Standardized Coefficients	Correlation of Datasets
Basal Area	-0.9352	-0.0997	-1.4859	0.0088
Canopy Tree Diameter	0.4297	0.1462	-0.083	-0.0928
Canopy Tree Richness	0.678	-0.06	1.5346	0.0798
Sapling Cover	0.3778	0.1584	0.1019	0.2605
Sapling Density	-0.8353	-0.0337	0.8258	0.3641
Sapling Richness	0.7765	0.2474	-0.17	0.2211
Shrub Cover	0.3246	0.1957	0.0499	0.0861
Lepidopteran Abundance				
Arctiidae	0.7237	0.2769	0.8532	0.0482
Geometridae	0.4673	0.1559	-0.3079	0.1505
Noctuidae	0.7051	0.2645	1.0878	0.162
Notodontidae	-0.4679	0.2226	-0.9391	-0.0959
Pyralidae	-0.1298	0.1756	-0.3928	0.0646

Apendix E. (continued)

Lepidopteran Richness				
Arctiidae	-0.2743	0.1881	-0.8472	-0.0167
Geometridae	-1.1626	0.0141	0.6589	0.168
Noctuidae	-0.0593	0.2021	0.0005	0.1337
Notodontidae	0.8839	0.2512	-0.3575	-0.0509
Pyralidae	-0.1367	0.1159	0.361	0.0892

Appendix F: Canonical correspondence analysis of the relationship between Coleoptera and vegetation attributes in Central Appalachia, 2007-2008. The overall analysis and first ordination axis was significant ($P < 0.05$).

Variable ($\lambda_{14, 354} = 2.53$)	First Axis ($F_{14, 354} = 2.53$)	
	Standardized Coefficients	Correlation of Datasets
Basal Area	-2.4655	0.036
Canopy Tree Diameter	0.6757	0.2728
Canopy Tree Richness	2.7349	0.078
Sapling Cover	-0.5275	-0.13
Sapling Density	0.3611	-0.085
Sapling Richness	-0.254	-0.1729
Shrub Cover	0.0865	-0.0145
Coleopteran Abundance	1.7407	0.3231
Coleopteran Diversity (H')	-1.0677	0.1623

Appendix G: Canonical correspondence analysis of the relationship between Diptera and vegetation attributes in Central Appalachia, 2007-2008. The overall analysis and first ordination axis was significant ($P < 0.05$).

Variable ($\lambda_{14, 350} = 2.66$)	First Axis ($F_{14, 350} = 2.66$)	
	Standardized Coefficients	Correlation of Datasets
Basal Area	0.3248	-0.0987
Canopy Tree Diameter	0.4185	0.1615
Canopy Tree Richness	-1.0052	-0.2508
Sapling Cover	0.3039	0.0716
Sapling Density	-0.0096	0.0359
Sapling Richness	0.0186	0.0703
Shrub Cover	0.3384	0.132
Dipteran Abundance	0.9134	0.3193
Dipteran Diversity (H')	0.4376	0.1449

Appendix H: Species checklist of forest Lepidoptera captured across a gradient of silvicultural disturbance in Central Appalachia, 2007-2008. Nomenclature and authorities follow Covell (2005).

Taxon	Number of Individuals Captured			
	Undisturbed	Single Tree	Shelterwood	Seed Tree
Apatelodidae				
Apatelodes torrefacta (J.E. Sm.)	7	2	2	3
Olceclostera angelica (Grt.)	5	4	3	5
Arctiidae				
Apantesis sp.	3	1	4	15
Apantesis phalerata (Harr.)		1		3
Apantesis vittata (F.)	3	5	4	4
Cisseps fulvicollis (Hbn.)				13
Cisthene sp.	1			
Cisthene plumbea (Stretch)	7	17	2	6
Cisthene packardii (Grt.)		1		
Clemensia albata (Pack.)	270	109	64	43
Crambidia sp.	1	4	51	1
Crambidia cephalica (Grt. & Rob.)	11	29	21	1
Crambidia lithosioides (Dyar)		2		
Crambidia pallida (Pack.)	46	79	32	14
Ctenucha virginica (Esper)	3		2	7
Cycnia sp.	3	4	3	2
Cycnia inopinatus (Hy. Edw.)		1		
Cycnia oregonensis (Stretch)		1	2	2
Cycnia tenera (Hbn.)	4	2	4	21
Ecpantheria scribonia (Stoll)	11	17	35	24
Estigmene acrea (Dru.)				1
Euchaetes egle (Dru.)	6	5	3	5
Grammia sp.	3	16	2	6
Grammia anna (Grt.)	8	4	12	5
Grammia figurata (Dru.)	2	12	30	57
Grammia parthenice intermedia (Stretch)				3

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Grammia phyllira (Dru.)			1	
Grammia virgo (L.)				1
Halysidota tessellaris (J.E. Sm.)	615	291	288	197
Haploa sp.	4	4	2	8
Haploa clymene (Brown)	7	17	38	39
Haploa contigua (Wlk.)	18	1		
Haploa lecontei (Guer.)	15	3	3	2
Holomelina sp.	9	8	5	10
Holomelina opella (Grt.)	2		4	
Hyphantria cunea (Dru.)	100	113	91	88
Hypoprepria fucosa (Hbn.)	409	192	228	54
Hypoprepia miniata (Kby.)	7	3	1	1
Lophocampa sp.		25		
Lophocampa caryae (Harr.)	99	74	70	86
Lycomopha pholus (Dru.)	1			
Pygarctia sp.	15			2
Pyrrharctia isabella (J.E. Sm.)	9	6	11	17
Spilosoma sp.	36	49	57	38
Spilosoma congrua (Wlk.)	63	80	34	32
Spilosoma latipennis (Stretch)	1	3		
Spilosoma virginica (F.)	8	11	11	17
Cossidae				
Prionoxystus macmurtrei (Guér.)	1	1	1	
Prionoxystus robiniae (Pack)	1	3	5	1
Drepanidae				
Drepana arcuata (Wlk.)	8	4	3	2
Oreta rosea (Wlk.)	10	4	4	2
Epiplemidae				
Calledapteryx dryopterata (Grt.)	6	2	1	
Geometridae				
Anacamptodes sp.	2	2	1	6
Anacamptodes defectaria (Gn.)	4	2	1	15

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Anacamptodes ephyraria (Wlk.)	9	2	9	3
Anacamptodes vellivolata (Hulst)			1	
Anagoga occiduaria (Wlk.)		1	1	
Anavitrinella pampinaria (Gn.)			1	
Antepione thisoaria (Gn.)	2	2	6	12
Anticlea multiferata (Wlk.)	1			
Besma sp.	13	1	2	
Besma endropiaria (Grt. & Rob.)	17	7	5	1
Besma quercivoraria (Gn.)	15	14	9	9
Biston betulaira cognataria (Gn.)	12	4	8	31
Cabera sp.	1			
Cabera erythemaria (Gn.)	21	5	6	5
Cabera variolaria (Gn.)			2	1
Calothysanis amaturaria (Wlk.)			1	1
Campaea perlata (Gn.)	30	4	6	3
Caripeta divisata (Wlk.)	1	3	1	
Chlorochlamys chloroleucaria (Gn.)	3			
Cladara atroliturata (Wlk.)	4		3	
Cyclophora packardi (Prout)	7		1	1
Cyclophora pendulinaria (Gn.)	2	3		
Dyspteris abortivaria (H.-S.)	1			1
Ecliptopera atricolorata (Grt. & Rob.)	9	9	4	7
Ectropis crepuscularia (D. & S.)	3	5	2	2
Ennomos magnaria (Gn.)				1
Ennomos subsignaria (Hbn.)	2		14	
Epimecis hortaria (F.)	2	4	5	1
Epirrhoe alternata (Müller)	2			
Eubaphe mendica (Wlk.)	9	7	7	2
Euchlaena sp.	12	1	1	11
Euchlaena amoenaria (Gn.)	44	28	25	19
Euchlaena irraria (B. & McD.)	8	4	2	5
Euchlaena johnsonaria (Fitch)				1
Euchlaena obtusaria (Hbn.)			1	1
Euchlaena serrata (Dru.)				1
Euchlaena pectinaria (D. & S.)	1	5	5	3
Euchlaena tigrinaria (Gn.)	1		2	

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Eufidonia notataria (Wlk.)	1		1	
Eugonobapta nivosaria (Gn.)	14	5	3	1
Eulithis sp.		3		1
Eulithis diversilineata (Hbn.)	9	3	5	
Euphyia unangulata intermediata (Gn.)	10	1	5	8
Eupithecia sp.	2	5		5
Eupithecia herefordaria (C. & S.)	1	3	1	1
Eupithecia miserulata (Grt.)	23	10	17	13
Eusarca confusaria (Hbn.)	1			2
Eutrapela clemataria (J.E. Sm.)	4			
Glena cribrataria (Gn.)	8		5	1
Glenoides texanaria (Hulst)	3			
Haematopis grataria (F.)	1			1
Heliomata cycladata (Grt. & Rob.)	25	12	22	29
Heterophleps refusaria (Wlk.)	2		6	1
Heterophleps triguttaria (H.-S.)	6	2	2	3
Horisme intestinata (Gn.)	6	2	5	6
Hydrelia albifera (Wlk.)			1	
Hydrelia inornata (Hulst)	51	36	60	38
Hydria prunivorata (Fgn.)	1		2	
Hydriomena sp.	33	1	11	9
Hydriomena divisaria (Wlk.)	1			
Hydriomena pluviata meridianata (McD.)		1	1	
Hypagyrtis sp.	1			1
Hypagyrtis brendae (R.L. Heitzman)		1		
Hypagyrtis esther (Barnes)		2		
Hypargyrtis unipunctata (Haw.)	17	32	5	8
Hypomecis umbrosaria (Hbn.)	1	1		
Idaea demissaria (Hbn.)			1	
Idaea obfusaria (Wlk.)	3	4	1	
Iridopsis larvaria (Gn.)	77	32	55	26
Itame sp.			2	
Itame coortaria (Hulst)	1			
Itame pustularia (Gn.)	172	40	62	17
Lambdina sp.	198	115	70	67
Lambdina fervidaria (Hbn.)		1	38	2

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Lambdina fervidaria athasaria (Wlk.)	21	24	22	18
Lambdina fiscellaria (Gn.)	2			
Lambdina pellucidaria (Grt. & Rob.)	3	2	1	
Lobocleta ossularia (Gey.)	1			
Lobocleta plemyraria (Gn.)				1
Lomographa glomeraria (Grt.)	2		1	1
Lomographa vestaliata (Gn.)	15	10	10	19
Lytrosis unitaria (H.-S.)	6		2	
Melanolophia sp.			4	
Melanolophia canadaria crama (Rindge)		4	15	2
Melanolophia signataria (Wlk.)				2
Metanema inatomaria (Gn.)	1			
Metarranthis sp.	2	1		1
Metarranthis angularia (B. & McD.)	2	1	1	
Metarranthis hypochraria (H.-S.)	13	3	5	2
Metarranthis indeclinata (Wlk.)	2			
*Metarranthis obfirmari*a (Hbn.)	1			
Nacophora quernaria (J.E. Sm.)	26	1	1	4
Nematocampa limbata (Haw.)	4			
Nemoria sp.	1	3		
Nemoria lixaria (Gn.)	15	7	10	7
Nemoria rubrifrontaria (Pack.)	6	3	1	3
Orthonama centrostrigaria (Woll.)	9	2	3	1
Orthonama obstipata (F.)	1		1	4
Pero sp.	51	35	81	8
Pero honestaria (Wlk.)	6	1	1	3
*Plagodi*s sp.	19	10	1	3
Plagodis alcoolaria (Gn.)	32	38	4	4
Plagodis fervidaria (H.-S.)	30	3	17	7
Plagodis kuetzingi (Grt.)	7	10	3	1
Plagodis phlogosaria (Gn.)	10	1	4	7
Plagodis serinaria (H.-S.)	9	10	9	3
Pleuroprucha insulsaria (Gn.)	2		4	2
Probole sp.	21	3	7	10
Probole amicaria (H.-S.)	67	18	21	12
Probole nyssaria (Gn.)	3	18	4	

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Prochoerodes transversata (Dru.)	4	3	1	2
Protitame virginalis (Hulst)			2	
Protoboarmia porcelaria (Gn.)	31	10	23	7
Scopula sp.	1			1
Scopula inductata (Gn.)			2	
Scopula limboundata (Haw.)	2	8	5	6
Semiothisa sp.	53	30	30	8
Semiothisa aemulataria (Wlk.)	2			
Semiothisa bisignata (Wlk.)	7	1		
Semiothisa continuata (Wlk.)		2		
Semiothisa fissinotata (Wlk.)	1			1
Semiothisa granitata (Gn.)			1	
Semiothisa gnophosaria (Gn.)	9	1	4	3
Semiothisa minorata (Pack.)		1		
Semiothisa multilineata (Pack.)	1			
Semiothisa ocellinata (Gn.)	11	9	12	7
Semiothisa promiscuata (Fgn.)	18	20	12	5
Semiothisa pustularia (Gn.)	3			
Semiothisa quadronotaria (H.-S.)	38	11	5	8
Semiothisa signaria (Hbn.)	2			
Semiothisa signaria dispuncta (Wlk.)		1		
Semiothisa transitaria (Wlk.)	35			
Sicya macularia (Harr.)	1			
Synchlora aerata (F.)	1	1	2	1
Tetracis sp.	1			
Tetracis cachexiata (Gn.)	9	5	15	20
Tetracis crocallata (Gn.)	7	2	1	2
Trichodezia albovittata (Gn.)	4		3	
Xanthorhoe sp.			1	1
Xanthorhoe labradorensis (Pack.)	1	2		
Xanthorhoe lacustrata (Gn.)				1
Xanthotype sp.				1
Xanthotype urticaria (Swett)	1	1	4	7
Lasiocampidae				
Artace cribraria (Ljungh)		1		1

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Malacosoma sp.	6	2	7	
Malacosoma americanum (F.)	243	249	79	41
Malacosoma disstria (Hbn.)	26	24	13	10
Pyllodesma americana (Harr.)	3			
Limacodidae				
Adoneta spinuloides (H.-S.)	1			
Apoda biguttata (Pack.)	1	8		1
Apoda y-inversum (Pack.)	8	7	12	10
Euclea delphinii (Bdv.)	16	19	19	5
Isa textula (H.-S.)	1	2		
Lithacodes fasciola (H.-S.)	6	10	4	1
Natada nasoni (Grt.)	6	2	1	1
Packardia geminata (Pack.)	5	1		
Parasa sp.		2	2	
Parasa indetermina (Bdv.)			1	1
Parasa chloris (H.-S.)	15	5	9	6
Prolimacodes badia (Hbn.)	4	2	3	
Tortricidia sp.		1		
Tortricidia flexuosa (Grt.)	21	30	22	11
Tortricidia testacea (Pack.)	14	12	1	2
Lymantriidae				
Dasychira sp.	23	20	23	4
Dasychira basiflava (Pack.)	10	2	5	
Dasychira basiflava (Pack.)	3			
Dasychira obliquata (Grt. & Rob.)	2	10	4	1
Dasychira manto (Stkr.)	1			1
Dasychira vagans (B. & McD.)	2	3		1
Orgyia sp.	5	4	1	9
Orgyia antiqua (L.)		1		
Orgyia definita (Pack.)	4	3	4	1
Orgyia leucostigma (J.E. Sm.)		2		
Megalopygidae				
Lagoa crispata (Pack.)	6	11	11	5

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Lagoa pyxidifera (J.E. Sm.)		1	1	
Megalopyge opercularis (J.E. Sm.)	2	2	3	2
Norape ovina (Sepp)	13	2	5	7
Mimallonidae				
Cicinnus melsheimeri (Harr.)	4			1
Lacosoma chiridota (Grt.)				1
Noctuidae				
Abagrotis alternata (Grt.)	25	32	16	8
Achatia distincta (Hbn.)	1			
Acontia aprica (Hbn.)				4
Acronicta sp.	407	264	221	146
Acronicta americana (Harr.)	6	5	5	8
Acronicta afflicta (Grt.)	7	6	6	3
Acronicta exilis (Grt.)		1		
Acronicta fragilis (Gn.)	1	3		
Acronicta haesitata (Grt.)	39	25	32	6
Acronicta impleta (Wlk.)	6	1	5	8
Acronicta inclara (Sm.)	55	12	19	3
Acronicta innotata (Gn.)	1			
Acronicta laetifica (Sm.)				1
Acronicta lithospila (Grt.)	2	1	1	1
Acronicta lobeliae (Gn.)	4	5	4	1
Acronicta morula (Grt. & Rob.)	1			
Acronicta ovata (Grt.)	17	1	2	
Acronicta pruni (Harr.)	4			1
Acronicta retardata (Wlk.)	4	1	1	
Acronicta spinigera (Gn.)	3		3	
Acronicta vinnula (Grt.)		1		
Agriopodes fallax (H.-S.)	7	4	1	1
Agriopodes teratophora (H.-S.)	2			1
Agrotis sp.		1		
Agrotis ipsilon (Hufn.)	4		4	10
Allotria elonympha (Hbn.)	49	19	16	14
Amolita fessa (Grt.)				3

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Amphipoea americana (Speyer)		1		5
Amphipyra pyramidoides (Gn.)	1	1		2
Anagrapha falcifera (Kby.)		1	2	1
Anaplectoides pressus (Grt.)	3			
Anicla infecta (Ochs.)				1
Anorthodes tarda (Gn.)	211	153	164	92
Apamea finitima (Gn.)	4	6	7	1
Argyrogramma basigera (Wlk.)	1	1	1	1
Arugisa latiorella (Wlk.)		4	2	1
Autographa biloba (Steph.)			1	
Autographa precationis (Gn.)	1			
Baileya sp.	48	19	14	13
Baileya australis (Grt.)	16	12	9	3
Baileya levitans (Sm.)	48	9	11	2
Baileya ophthalmica (Gn.)	61	26	26	13
Balsa sp.	4	1	3	4
Balsa labecula (Grt.)	10	6	2	2
Balsa malana (Grt.)	1	4		
Balsa tristrigella (Wlk.)			1	
Basilodes pepita (Gn.)			4	3
Bleptina caradrinalis (Gn.)	14	9	8	31
Bomolocha sp.	20	11	9	5
Bomolocha abalienalis (Wlk.)				1
Bomolocha baltimoralis (Gn.)	4	6	5	3
Bomolocha bijugalis (Wlk.)	8	2		
Bomolocha deceptalis (Wlk.)		2	3	4
Bomolocha edictalis (Wlk.)	5		2	
Bomolocha madefactalis (Gn.)			1	
Bomolocha manalis (Wlk.)	15	1		
Bomolocha palparia (Wlk.)	1			
Caenurgia sp.	1	1	5	13
Caenurgia chlorophy (Hbn.)				1
Caenurgina crassiuscula (Haw.)	1		1	
Caenurgina erechtea (Cram.)		9	8	29
Callopistria cordata (Ljungh)	1		1	
Callopistria mollissima (Gn.)	25	10	18	11

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Calyptra canadensis (Bethune)		1	1	2
Catocala sp.	19	20	28	55
Catocala amica (Hbn.)	2	7	5	1
Catocala connubialis (Gn.)	1			
Catocala dejecta (Stkr.)	4	9		
Catocala flebilis (Grt.)	1			
Catocala ilia (Cram.)	2	1	2	3
Catocala nebulosa (Edw.)	2		2	
Catocala obscura (Stkr.)	5			
Catocala palaeogama (Gn.)	11	13	15	
Catocala residua (Grt.)	1	3		
Catocala retecta (Grt.)	3	2		1
Catocala subnata (Grt.)	3			
Catocala ulalume (Stkr.)	1		1	
Catocala vidua (J.E. Sm.)	2		1	
Celiptera frustulum (Gn.)				1
Cerastis tenebrifera (Wlk.)				1
Cerma cerintha (Tr.)	13	11	4	7
Charadra deridens (Gn.)	1	1	2	9
Chrysanympha formosa (Grt.)			1	
Chytolita morbidalis (Gn.)	1		2	
Chytonix palliatricula (Gn.)	5	32	10	2
Celiptera frustulum (Gn.)	1			
Crambodes talidiformis (Gn.)				9
Crocigrapha normani (Grt.)	13			7
Cosmia calami (Harv.)	6	1	3	6
Discestra trifolii (Hufn.)			1	1
Dypterygia rozmani (Berio)	1			4
Dysgonia smithii (Gn.)	2			
Egira alternans (Wlk.)		2		
Elaphria sp.			2	1
Elaphria festivoides (Gn.)	1	2		
Elaphria grata (Hbn.)	6	4	2	30
Elaphria versicolor (Grt.)	6	6	2	3
Eosphoropteryx thyatyroides (Gn.)	2	1	1	
Euagrotis lubricans (Gn.)				1

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Euclidia cuspidea (Hbn.)		1	3	1
Eudryas sp.				4
Eudryas grata (F.)	31	32	41	19
Euparthenos nubilis (Hbn.)	10	5	13	12
Euplexia benesimilis (McD.)	5		8	2
Eutelia pulcherrima (Grt.)	2		1	
Eutolype grandis (Sm.)		1		
Euxoa sp.	5		3	
Euxoa messoria (Harr.)	2			
Euxoa tessellata (Harr.)		1	1	1
Faronta diffusa (Wlk.)			1	1
Feltia sp.		3	1	13
Feltia jaculifera (Gn.)			1	1
Feltia subgothica (Haw.)		1		
Galgula partita (Gn.)		1	3	15
Gluphisia septentrionis (Wlk.)			1	
Harrisimemna trisignata (Wlk.)	1			
Heliothis sp.				3
Heliothis turbatus (Wlk.)	1			
Heliothis zea (Boddie)				3
Homohadena badistriga (Grt.)	1			
Hyperstrotia pervertens (B. & McD.)	3	2		
Hyppa xylinoides (Gn.)	1		1	
Hypsoropha hormos (Hbn.)	1		1	
Idia sp.	74	17	120	15
Idia aemula (Hbn.)	51	20	18	22
Idia americalis (Gn.)	34	13	24	23
Idia lubricalis (Gey.)				5
Idia scobialis (Grt.)	15	9	8	21
Isogona tenuis (Grt.)			1	
Lacanobia grandis (Gn.)		2		
Lacinipolia sp.			1	6
Lacinipolia implicata (McD.)	2	2		4
Lacinipolia lorea (Gn.)			3	4
Lacinipolia olivacea (Morr.)		2		
Lacinipolia renigera (Steph.)		1	8	3

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Lascoria ambigualis (Wlk.)	2	1		1
Ledaea perditalis (Wlk.)	1			
Lesmone detrahens (Wlk.)				1
Leucania sp.	2	3	7	14
Leucania inermis (Fbs.)	3	5		
Leucania multilinea (Wlk.)			4	
Leucania scirpicola (Gn.)	1	9	13	3
Leuconycta diphteroides (Gn.)		1	1	
Lithacodia sp.		3		3
Lithacodia carneola (Gn.)	5	12	12	22
Lithacodia muscosula (Gn.)	3	1		1
Lithacodia synochitis (Grt. & Rob.)	1	4	1	
Macrochilo absorptalis (Wlk.)	4			1
Magusa orbifera (Wlk.)	1	1		
Marathyssa sp.		1	2	2
Marathyssa inficita (Wlk.)	1			1
Melanchra adjuncta (Gn.)	1	5	2	
Meganola minuscula (Zell.)	4			1
Metalectra sp.	11	17		
Metalectra discalis (Grt.)	1			
Metalectra quadrisignata (Wlk.)		1		
Metalectra richardsi (Brower)	6		12	
Metalectra tantillus (Grt.)			11	5
Metarranthis hypochraria (H.-S.)		1		
Mocis texana (Morr.)	1			
Morrisonia sp.	2		12	1
Morrisonia confusa (Hbn.)	32	11	9	11
Morrisonia evicta (Grt.)	1			
Nedra ramosula (Gn.)		4	1	
Noctua pronuba (L.)	1		1	2
Nola triquetrana (Fitch)	1		1	
Ochropleura plecta (L.)		1		3
Ogdoconta cinereola (Gn.)	3		1	4
Oligia illocata (Wlk.)	6			
Orthodes sp.	15	2	4	
Orthodes crenulata (Btlr.)	3	12	15	5

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Orthodes cynica (Gn.)	14	7	8	6
Orthosia sp.	2			
Ozarba aeria (Grt.)			1	1
Paectes sp.	2		1	
Paectes abrostoloides (Gn.)			3	1
Paectes oculatrix (Gn.)	4		2	
Paectes pygmaea (Hbn.)	3		2	1
Palthis sp.	6	9	5	10
Palthis angulalis (Hbn.)	2	1	1	2
Palthis asopialis (Gn.)		6	3	7
Pangrapta decoralis (Hbn.)	24	15	16	13
Panopoda sp.	4	2	1	2
Panopoda carneicosta (Gn.)	9	4	5	3
Panopoda repanda (Wlk.)	1			
Panopoda rufimargo (Hbn.)	9	9	6	2
Papaipema sp.	1			1
Papaipema arctivorens (Hamp.)	1			
Papaipema rigida (Grt.)	1			
Parallelia bistriaris (Hbn.)	10	2	6	
Peridroma saucia (Hbn.)			1	
Perigea xanthioides (Gn.)	15	2	10	44
Phalaenophana pyramusalis (Wlk.)	2	2	1	1
Phalaenostola larentioides (Grt.)	6			
Phlogophora periculosa (Gn.)				2
Phosphila miselioides (Gn.)	3		2	
Plathypena scabra (F.)	6	2	1	3
Platysenta sp.	5	1		
Platysenta vecors (Gn.)			2	3
Platysenta videns (Gn.)	1			1
Polia sp.	1	1	1	1
Polia latex (Gn.)	4	2	1	4
Polygrammate hebraeicum (Hbn.)	153	106	126	57
Protolampra brunneicollis (Grt.)	1	1	1	1
Pseudaletia unipuncta (Haw.)	10	2	2	10
Pseudeva purpurigera (Wlk.)			1	2
Pseudorthodes vecors (Gn.)				1

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Pyreferra hesperidago (Gn.)	1			
Pyrrhia umbra (Hufn.)		1	1	
Rachiplusia ou (Gn.)			1	
Raphia frater (Grt.)			1	1
Renia sp.	3	7	5	
Renia discoloralis (Gn.)	3	9	9	6
Renia sobrialis (Wlk.)		1		1
Rivula propinqualis (Gn.)	1	1		1
Shinia sp.	1			1
Schinia florida (Gn.)				1
Schinia rivulosa (Gn.)		3	2	4
Schinia trifascia (Hbn.)	1		2	
Scolecocampa liburna (Gey.)				1
Spargaloma sexpunctata (Grt.)	1			
Spaelotis clandestina (Harr.)	1			
Spodoptera dolichos (F.)		1		
Spodoptera ornithogalli (Gn.)		2	1	4
Spragueia sp.				1
Spragueia leo (Gn.)	1		1	
Stiriodes obtusa (H.-S.)	1			1
Synedoida grandirena (Haw.)		1		1
Syngrapha rectangula (Kby.)				1
Tarachidia sp.		1		
Tarachidia candefacta (Hbn.)	3			2
Tarachidia erastrioides (Gn.)	2	2	1	4
Tetanolita sp.	17	62	18	12
Tetanolita mynesalis (Wlk.)	7	60	6	3
Thioptera nigrofimbria (Gn.)	9	9	24	16
Tricholita signata (Wlk.)			2	3
Trichordestra legitima (Grt.)	3			1
Ulolonche culea (Gn.)			3	5
Xestia sp.		1		
Xestia dolosa (Franc.)	1	3	2	4
Xestia smithii (Snell.)	1	4		4
Zale sp.	6	2	4	3
Zale calycanthata (J.E. Sm.)	1			

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Zale galbanata (Morr.)	2			
Zale horrida (Hbn.)	1	1		
Zale lunata (Dru.)	2	4		1
Zale lunifera (Hbn.)	9	3	3	1
Zale minerea (Gn.)		1		
Zale unilineata (Grt.)	3		1	2
Zanclognatha sp.	70	30	26	19
Zanclognatha cruralis (Gn.)	4		3	1
Zanclognatha laevigata (Grt.)		1	1	
Zanclognatha lituralis (Hbn.)	2	23		
Zanclognatha ochreipennis (Grt.)	2			2
Zanclognatha obscuripennis (Grt.)	1	2	4	
Notodontidae				
Cerura scitiscripta (Wlk.)				1
Clostera albosigma (Fitch)	2	1		
Clostera inclusa (Hbn.)				1
Dasylophia anguina (J.E. Sm.)	3	4	2	
Dasylophia thyatiroides (Wlk.)	2	1	4	
Datana sp.	36	37	24	21
Datana angusii (Grt. & Rob.)	11	1	2	4
Datana contracta (Wlk.)	2	2	1	4
Datana drexelii (Hy. Edw.)		6	2	3
Datana integerrima (Grt. & Rob.)			2	2
Datana ministra (Drury)	2	3		2
Datana perspicua (Grt. & Rob.)		3	3	7
Ellida caniplaga (Wlk.)	24	2	17	6
Furcula borealis (Guer.)	2	1		2
Furcula cinerea (Wlk.)			2	2
Gluphisia septentrionis (Wlk.)	5	4	3	2
Heterocampa sp.	52	17	11	4
Heterocampa biumbrata (Wlk.)	4			
Heterocampa biundata (Wlk.)	4	1	9	4
Heterocampa guttivitta (Wlk.)	3	2	2	
Heterocampa obliqua (Pack.)	13	4	4	3
Heterocampa subrotata (Harv.)	8			

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Heterocampa umbrata (Wlk.)	50	27	23	7
Hyperaeschra georgica (H.-S.)	67	39	13	10
Lochmaeus sp.	44	16	18	17
Lochmaeus bilineata (Pack.)	4			4
Lochmaeus manteo (Doubleday)	31	15	23	2
Macrurocampa marthesia (Cram.)	6	9	6	1
Misogada unicolor (Pack.)	1			
Nadata gibbosa (J.E. Sm.)	86	69	47	42
Nirice bidentata (Wlk.)	1	2	1	
Oligocentria sp.				1
Oligocentria lignicolor (Wlk.)	8	6	6	4
Oligocentria semirufescens (Wlk.)	4	2	2	2
Peridea sp.	28	55	33	18
Peridea angulosa (J.E. Sm.)	13	15	9	15
Peridea basitriens (Wlk.)	37	18	7	21
Peridea ferruginea (Pack.)	5	5	5	7
Pheosia rimosa (Pack.)		2		1
Schizura sp.	9	1	6	6
Schizura apicalis (Grt. & Rob.)		2		
Schizura concinna (J.E. Sm.)			2	
Schizura ipomoeae (Doubleday)	10	3	3	2
Schizura unicornis (J.E. Sm.)		1	1	
Symmerista albifrons (J.E. Sm.)	55	16	22	12
Oecophoridae				
Agonopterix robiniella (Pack.)	6	8	5	3
Antaeotricha sp.	19	10	9	8
Antaeotricha leucillana (Zell.)	11	2	1	1
Antaeotricha schlaegeri (Zell.)	15	19	12	6
Ethmia zelleriella (Cham.)	5	12	15	3
Machimia tentoriferella (Clem.)		1		
Psilocorsis sp.	40	11	11	3
Psilocorsis reflexella (Clem.)	45	31	30	12
Pyralidae				
Achyra rantalis (Gn.)	1		2	5

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Aglossa cuprina (Zell.)	35	24	10	18
Agriphila vulgivagella (Clem.)		2	7	
Blepharomastix ranalis (Gn.)	15	12	5	14
Compacta capitalis (Grt.)	3			
Conchylodes ovulalis (Gn.)	1		1	
Crambus sp.	13	57	24	83
Crambus agitatellus (Clem.)	33	16	37	15
Crocidophora tuberculalis (Led.)		1		
Desmia funeralis (Hbn.)	34	24	37	24
Desmia maculalis (Westwood)		1		
Diacme elealis (Wlk.)	4	1	4	2
Epipagis huronalis (Gn.)	1			1
Epipaschia superatalis (Clem.)	9		3	1
Euzophera ostricolorella (Hulst)	25	7	5	8
Evergestis unimacula (Grt. & Rob.)	1		1	1
Galasa nigrinodis (Zell.)		1		
Helvibotys helvialis (Wlk.)	5	5	2	6
Herculia sp.	2	4		5
Herculia infimbrialis (Dyar)	1	5	4	2
Herculia olinalis (Gn.)	45	11	10	7
Herpetogramma thestealis (Wlk.)	4			
Ostrinia nubilalis (Hbn.)	7	6	4	5
Munroessa gyralis (Hulst)	1			
Nomophila nearctica (Mun.)			1	5
Pediasia trisecta (Wlk.)		1	10	1
Palpita magniferalis (Wlk.)	63	38	12	10
Pantographa limata (Grt. & Rob.)	83	34	31	11
Parapoynx obscuralis (Grt.)			1	2
Pilocrocis ramentalis (Led.)			1	
Plodia interpunctella (Hbn.)	5	1		
Polygrammodes flavidalis (Gn.)	7		4	2
Pyrausta bicoloralis (Gn.)	3			
Pyrausta niveicilialis (Grt.)	2		1	
Tetralopha asperatella (Clem.)	39	19	19	7
Udea rubigalis (Gn.)	7	14	9	49
Urola nivalis (Dru.)		2	8	1

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Sesiidae				
Synanthedon acerni (Clem.)	4	5	5	14
Synanthedon exitiosa (Say)	1			
Saturniidae				
Actias luna (L.)	44	28	45	23
Anisota stigma (F.)	21	31	17	15
Anisota virginiensis (Dru.)				1
Antheraea polyphemus (Cram.)	13	8	10	17
Automeris io (F.)	5	14	3	4
Callosamia sp.	1		2	
Callosamia angulifera (Wlk.)	3	5	3	2
Callosamia promethea (Dru.)			1	
Citheronia regalis (F.)	13	16	2	2
Dryocampa rubicunda (F.)	120	71	56	70
Eacles imperialis (Dru.)	54	46	50	33
Hyalophora cecropia (L.)	1			
Sphingidae				
Ceratomia hageni (Grt.)				1
Ceratomia undulosa (Wlk.)	5	3	3	4
Darapsa myron (Cram.)	3	1	2	1
Deidamia inscripta (Harr.)			6	1
Deidamia inscripta (Harr.)		1		
Laothoe juglandis (J.E. Sm.)	6		3	
Lapara coniferarum (J.E. Sm.)			2	
Paonias sp.				1
Paonias astylus (Dru.)		1		1
Paonias exaecatus (J.E. Sm.)	14	13	20	11
Paonias myops (J.E. Sm.)	5	1	2	3
Sphinx sp.	1			
Tortricidae				
Amorbia humerosana (Clem.)	1			
Archips argyrospila (Wlk.)	1			

Appendix H. (continued)

Taxon	Undisturbed	Singletree	Shelterwood	Seedtree
Archips fervidana (Clem.)			2	1
Argyrotaenia sp.	10			1
Argyrotaenia alisellana (Rob.)	10	7	8	
Argyrotaenia mariana (Fern.)	8	5		1
Argyrotaenia quercifoliana (Fitch)	3			
Argyrotaenia velutinana (Wlk.)	2	1		1
Choristoneura sp.	13	23	14	8
Choristoneura parallela (Rob.)			3	1
Choristoneura pinus (Freeman)		1	2	
Choristoneura rosaceana (Harr.)	5		1	2
Clepsis melaleucana (Wlk.)	5			1
Ecdytolopha insiticiana (Zell.)	1			1
Melissopus latiferreanus (Wlsm.)	1			
Pandemis limitata (Rob.)		1	1	
Sparganothis reticulatana (Clem.)				1
Sparganothis sulfureana (Clem.)		1		
Syndemis afflictana (Wlk.)		1		
Yponomeutidae				
Atteva punctella (Cram.)	9	22	29	37
Yponomeuta multipunctella (Clem.)	1		1	
Zygaenidae				
Harrisina americana (Guér)	4		3	2
Pyromorpha dimidiata (H.-S.)	16	5	3	1

Appendix I: Observations of mating behavior in *Lasiurus borealis*

On 13 September 2007, I observed mating of the eastern red bat (*Lasiurus borealis* Müller) while conducting a mist-netting survey at a closed-canopy stream in the Cumberland District of the Daniel Boone National Forest, Bath County, Kentucky (Appendix A). The sky was clear, with fair weather and a temperature at sunset of 19 °C. I captured one male northern bat (*Myotis septentrionalis* Trouessart) and four *L. borealis*. All *L. borealis* were males; two individuals possessed descended testes and two did not. Bats captured on this night were more agitated than normally encountered when being handled. After collecting data on sex and reproductive condition, I released the bats ca. 7 m from the netting area.

A pair of bats was observed ca. 2 h after sunset (2030 h EDT), flying in a looping pattern (ca. 2 m in diameter), with one individual following the other. These bats were making vocalizations detectable by both the human ear and an ultrasonic detector (Anabat II, Titley Electronics, Australia). Less than a minute later, the bats landed on the stream bank and began copulating within 3 m of myself. The bank consisted of gravel lightly littered with deciduous foliage, which may have provided a cryptic location for terrestrial activity. After the bats landed, I observed their behavior intermittently (ca. every 1 min) using the low-light setting of a headlamp.

Copulation consisted of a series of 2–3 min bursts of activity followed by 3–5 min of rest. During bursts of mating activity, the mounted individual, presumably a female, appeared motionless. The top bat, presumably a male, clasped the female at the torso, and made readily discernable thrusts. During a period of inactivity, I approached to 1 m of the mating bats. This allowed positive identification as an eastern red bat based on body size

and coloration, which are unique to bats in this region (Barbour and Davis 1969). When approached, the copulating bats remained motionless. Mating activity recommenced once a photograph was taken to verify my observations and after I retreated (Figure I.1). Despite the vocalizations heard while the bats were in flight, no audible or ultrasonic sounds were detected while the bats were on the ground. After ca. 15 min of copulation, activity ceased, but the mating pair remained joined and stationary for an additional 15 min. The two bats eventually took flight in separate directions.

In other regions, mating by *L. borealis* typically occurred in late summer and autumn (Cryan and Brown 2007; Shump and Shump 1982), and the timing of my observation in eastern Kentucky was similar. However, most previous descriptions of mating in *L. borealis* noted that coupling occurred in flight (Cryan and Brown 2007), whereas I witnessed apparent pre-copulatory behavior in the air and independent landing on the ground. While capturing *L. borealis*, Saugey *et al.* (1989) observed multiple males entering mist nets within a few centimeters of a female and suggested that males were pursuing females for breeding; my observations support their interpretation. In a later paper, Saugey *et al.* (1998) noted a male *L. borealis* entering a mist net and initiating copulation with a female that was already caught in the net, indicating as in my observation, that coupling may not always occur in flight. Thus, I suggest that observations of mating may not be the consequence of aerial accidents on the part of the copulating bats, as suggested by Glass (1966).

Further, given the skewed number of male *L. borealis* captured and the activity of free-flying bats that I observed, I offer two comments. First, my observations indicate that *L. borealis* invests a considerable amount of time when mating. This invokes an

obvious risk of predation, but I found it surprising that the bats remained coupled even after copulation appeared to have ended. I suggest that this delay may serve to prevent the female from immediately copulating with another male. To my knowledge, the existing literature gives little indication as to the degree of promiscuity in *L. borealis* or other lasiurine bats. Even so, I suggest that simply reducing the potential for a female to mate multiple times in a given night may play a role in sperm competition, which has been suggested across multiple bat taxa (Wilkinson and McCracken 2003). Additionally, though the mating pair vocalized prior to copulation, they were silent during the copulation event. We suggest that the lack of vocalizations while on the ground may not only serve as a means of avoiding predation, but may also potentially serve as a measure to prevent intrusion by another male. Disturbance of mating by extra-pair males has been documented in *Saccopteryx bilineata*, a harem-keeping species (Tannenbaum 1975), although there is no evidence for such a social structure in *L. borealis.* I suggest that it is logical for a mating pair, already investing time and risking predation, to employ cryptic behavior to avoid disruption by other individuals seeking partners with which to mate.

Figure I.1. A copulating pair of *Lasiurus borealis*.

REFERENCES

Agosta, S.J., Morton, D and Kuhn, K.M. (2003) Feeding ecology of the bat *Eptesicus fuscus*: 'preferred' prey abundance as one factor influencing prey selection and diet breadth. *Journal of Zoology*, **260,** 169-177.

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

Allgood, D.W., Miller, D.W. and Kalcounis-Rueppell, M.C. (2009). Influence of intensive pine management on dipteran community structure in coastal North Carolina. *Environmental Entomology,* **38,** 657-666.

Altenbach, J.S. and Best, T.L. (1999) Bats of the United States. Arkansas Game and Fish Commission. 64 pp.

Altschul, S., Gish, F., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology,* **215,** 403-410.

Arlettaz, R., Perrin, N. and Hausser, J. (1997) Trophic resource partitioning and competition between two sibling bat species *Myotis myotis* and *Myotis blythii*. *Journal of AnimalEcology,* **66,** 897-911.

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

Barbour, R.W. and Davis, W.H. (1969) *Bats of America*. University Press of Kentucky, Lexington, Kentucky.

Barclay, R.M.R. (1991) Population structure of temperate zone insectivorous bats in relation to foraging behavior and energy demand. *Journal of Animal Ecology,* **60,** 165-178.

Barclay, R.M.R. and Brigham, R.M. (1991) Prey detection, dietary niche breadth, and body size in bats – why are aerial insectivorous bats so small? *American Naturalist,* **137,** 693-703.

Beachy, T.A. (2007) Cerulean warbler (*Dendroica cerulean*) breeding ecology and habitat selection, initial response to forest management, and association with anthropogenic disturbance in the Cumberland Mountains of Tennessee. M.S. Thesis, University of Tennessee, Knoxville, Tennessee.

Blehert, D.S., Hicks, A.C., Behr, M., *et al.* (2009) Bat white-nose syndrome: an emerging fungal pathogen? *Science,* **323,** 227-227.

Borer, D.J. and White, R.E. (1970) A field guide to insects: America north of Mexico. Houghton Mifflin Co., Boston, MA. 404 pp.

Boshoff, A.F., Palmer, N.G. and Avery, G. (1990) Regional variation in the diet of martial eagles in the Cape Province, South Africa. *South African Journal of Wildlife Research,* **20,** 57-68.

Brack, V. and Whitaker, J.O. (2001) Foods of the northern long-eared myotis, *Myotis septentrionalis*, from Missouri and Indiana, with notes on foraging *Acta Chiropterologica,* **3,** 203-210.

Brigham, R.M. (2007) Bats in forests: what we know and what we need to learn. In *Bats in forests: conservation and management*, (eds Lacki, M.J., Hayes, J.P. and Kurta, A.), John Hopkins University Press, Baltimore, Maryland, pp 1-17.

Britzke, E.R. (2003) Use of ultrasonic detectors for acoustic identification and study of bat ecology in the eastern United States. Ph.D Dissertation, Tennessee Technical University, Cookeville, Tennessee.

Britzke, E.R., Murray, K.L., Hadley, B.M. and Robbins, L.W. (1999) Measuring bat activitywith the Anabat II system. *Bat Research News,* **40,** 1-3.

Britzke, E.R., Murray, K.L., Heywood, J.S. and Robbins, L.W. (2004) Acoustic identification. In. The Indiana bat: biology and management of an endangered species (eds Kurta, A. and Kennedy, J.). Bat Conservation International, Austin, TX. pp 221-225.

Brooks, R.T. (2009) Habitat-associated and temporal patterns of bat activity in a diverse forest landscape of southern New England, USA. *Biodiversity and Conservation,* **18,** 529-545.

Brooks, R.T. and Ford, W.M. (2005) Bat activity in a forest landscape of central Massachusetts. *Northeastern Naturalist,* **12,** 447-462.

Bulluck, L.P and Buehler, D.A. (2008) Factors influencing golden-winged warbler (*Vermivora chrysoptera*) nest-site selection and nest survival in the Cumberland Mountains of Tennessee. *Auk,* **125,** 551-559.

Burford, L.S., Lacki, M.J. and Covell, C.V. (1999) Occurrence of moths among habitats in a mixed-mesophytic forest: implications for management of forest bats. *Forest Science,* **45,** 323-332.

Butterfield, J., Luff, M.L., Baines, M. and Eyre, M.D. (1995) Carabid beetle communities as indicators of conservation potential in upland forests. *Forest Ecology and Management,* **79,** 63-77.

Caceres, M.C. and Barclay, R.M.R. (2000) *Myotis septentrionalis*. *Mammalian Species Account,* **634,** 1- 4.

Carter, T.C., Menzel, M.A., Owen, S.F., Edwards, J.W., *et al.* (2003) Food habits of seven species of bats in the Allegheny Plateau and Ridge and Valley of West Viriginia. *Northeastern Naturalist,* **10,** 83-88.

Carter, T.C., Menzel, M.A., Chapman, B.R. and Miller, K.V. (2004) Partitioning of food resources by syntopic eastern red bat (*Lasiurus borealis*), Seminole (*L. seminolus*) and evening (*Nycticeius humeralis*) bats. *American Midland Naturalist,* **151,** 186-191.

Carter, G.G., Coen, C.E., Stenzler, L.M. and Lovette, J.J. (2006) Avian host DNA isolated from the feces of white-winged vampire bats (*Diaemus youngi*). *Acta Chiropterologica,* **8,** 255-258.

Charnov, E.L. (1976) Optimal foraging, the marginal value theorem. *Theoretical Population Biology,* **9,** 129-136.

Clare, E.L., Fraser, E.E., Braid, H.E., Fenton, M.B. and Hebert, P.D.N. (2009) Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. *Molecular Ecology,* **18,** 2532-2542.

Cleveland, C.J., Betke, M., Federico, P., *et al.* (2006) Economic value of the pest control service provided by Brazilian free-tailed bats in south-central Texas. *Frontiers in Ecology and the Environment,* **4,** 238-243.

Constanz, G. (2000) Grassroots-based watershed conservation in Central Appalachia. *Mountain Research and Development,* **20,** 122-125.

Covell, C.V. (2005) *A field guide to moths of Eastern North America: Special Publication Number 12,* Virginia Museum of Natural History, Martinsville, Virginia.

Cryan, P.M. and Brown, A.C. (2007) Migration of bats past a remote island offers clues toward the problem of bat fatalities at wind turbines. *Biological Conservation,* **139,** 1–11.

Cryan, P.M. and Barclay, R.M.R. (2009) Causes of bat fatalities at wind power turbines: hypotheses and predictions. *Journal of Mammalogy,* **90,** 1330-1340.

Deans, A.M., Malcolm, J.R., Smith, S.M and Bellocq, M.I. (2004) Edge effects and the responses of aerial insect assemblages to structural-retention harvesting in Canadian boreal peatland forests. *Forest Ecology and Management,* **204,** 249-266.

Deagle, B.E., Jarman, S.N., Pemberton, D. and Gales, J.N. (2005) Genetic screening for prey in the gut contents from a giant squid (*Architeuthis* sp.). *Journal of Heredity,* **96,** 417-423.

Deagle, B.E., Gales, N.J., Evans, K., *et al.* (2007) Studying seabird diet through genetic analysis of faeces: a case study on Macaroni penguins (*Eudyptes chrysolophus*). *PLoS One,* **2,** e831.

Deagle, B.E., Kirkwood, R. and Jarman, S.N. (2009) Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology,* **18,** 2022-2038.

Dodd, L.E. (2006) Diet and prey abundance of the Ozark big-eared bat in Arkansas. M.S. Thesis, University of Kentucky, Lexington, KY. 252 pp.

Dodd, L.E., Lacki, M.J. and Rieske, L.K. (2008) Variation in moth occurrence and the implications for foraging habitat of the Ozark big-eared bat. *Forest Ecology and Management,* **255,** 3866-3872.

Duffy, C.D., Wilson, R.P. and Wilson, M.P. (1987) Spatial and temporal patterns of diet in the cape cormorant off southern Africa. *The Condor,* **89,** 830-834.

Erickson, J. L. and West, S.D. (1996) Managed forests in the Western Cascades: the effects of seral stage on bat habitat use patterns. In. *Bats and forests symposium* (eds Barclay, R.M.R. and Brigham, R.M). Research Branch, British Columbia Ministry of Forests, Victoria, British Colombia, Working Paper 23/1996, pp 215-227.

Faure, P.A., Fullard, J.H and Dawson, J.W. (1993) The gleaning attacks of the northern long-eared bat, *Myotis septentrionalis,* are relatively inaudible to moths. *Journal of Experimental Biology,* **178,** 173-189.

Feldhake, C.M. and Schumann, C.M. (2005) Tree establishment for a temperate agro forest in Central Appalachia, USA. *Agroforestry System,* **65,** 187-195

Feldhammer, G.A., Carter, T.C. and Whitaker, J.O. (2009) Prey consumed by eight species of insectivorous bats from southern Illinois. *American Midland Naturalist,* **162,** 43-51.

Feldman, R., Whitaker, J.O., Jr. and Yom-Tov, Y. (2000) Dietary composition and habitat use in an insectivorous bat community in Israel. *Acta Chiropterologica,* **2,** 15-22.

Fenton, M.B. and Bell, G.P. (1979) Echolocation and feeding behaviour in four species of *Myotis* (Chiroptera). *Canadian Journal of Zoology,* **57,** 1271-1277.

Fenton, M.B. (2003) Science and the conservation of bats: where to next? *Wildlife Society Bulletin,* **31,** 6-15.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* **3,** 294-297.

Freeman, P.W. (1981) Correspondence of food habits and morphology in insectivorous bats. Journal of Mammalogy, **62,** 166-173.

Gargas, A., Trest, M.T., Christensen, M., Volk, T.J. and Blehert, D.S. (2009) *Geomyces destructans* sp nov associated with bat white-nose syndrome. *Mycotaxon,* **108,** 147-154.

Glass, B.P. (1966) Some notes on reproduction in the red bat, *Lasiurus borealis*. *Proceedings of the Oklahoma Academy of Science,* **46,** 40–41.

Gragson, T.L. and Bolstad, P.V. (2006) Land use legacies and the future of Southern Appalachia. *Society and Natural Resources,* **19,** 175-190

Greenstone, M.H. (2006) Molecular methods for assessing insect parasitism. *Bulletin of Entomological Research,* **96,** 1-13.

Grendal, S.D. (1996) Habitat use by bats in fragmented forests. In *Bats and forests symposium,* (eds Barclay, R.M.R. and Brigham, R.M.), BC Ministry of Forests, Victoria, British Columbia, pp 260-272.

Grendal, S.D. and Brigham, R.M. (1998) Short-term effects of small-scale habitat disturbance on activity by insectivorous bats. *Journal of Wildlife Management,* **62,** 996-1003.

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

Griffith, L.A. and Gates, J.E. (1985) Food habits of cave-dwelling bats in the central Appalachians. *Journal of Mammalogy*, **66,** 451-460.

Griffith, G.E., Omernik, J.M. and Azevedo, S.H. (1998) Ecoregions of Tennessee (color poster with map, descriptive text, summary tables, and photographs). Reston, VA., U.S. Geological Survey (map scale 1:940,000).

Guldin, J.M., Emmingham, W.H., Carter, S.A. and Saugey, D.A. (2007) Silvicultural practices and management of habitats for bats. In. *Bats in forests: conservation and management,* (eds Lacki, M.J., Hayes, J.P. and Kurta, A.), John Hopkins University Press, Baltimore,Maryland, pp 177-205.

Haddad, N. M., Tilman, D., Haarstad, J., Ritchie, M. and Knops, J.M.H. (2001) Contrasting effects of plant richness and composition on insect communities: a field experiment. *The American Naturalist,* **158**, 17-35.

Hamer, K.C. and Hill, J.K. (2000) Scale-dependent effects of habitat disturbance on species richness in tropical forests. *Conservation Biology* **14,** 1435-1440.

Hammond, P.C. and Miller, J.C. (1998) Comparison of the biodiversity of Lepidoptera within three forested ecosystems. *Annals of the Entomological Society of America*, **91,** 323-328.

Harper, G.L., Sheppard, S.K., Harwood, J.D., *et al.* (2006) Evaluation of temperature gradient gel electrophoresis for the analysis of prey DNA within the guts of invertebrate predators. *Bulletin of Entomological Research,* **96,** 295-304.

Harwood, J.D., Desneux, N., Yoo, H.J.S., *et al.* (2007) Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. *Molecular Ecology,* **16,** 4390-4400.

Harwood, J.D. and Greenstone, M.H. (2008) Molecular diagnosis of natural enemy-host interactions. In. Recent advances in insect physiology, toxicology and molecular biology (ed Liu, N.). Research Signpost, Kerala, India. Pp. 41-57.Harvey, M.J.,

Hayes, J.P. (1997) Temporal variation in activity of bats and the design of echolocation studies. *Journal of Mammalogy,* **78,** 514-524.

Hayes, J.P. (2000) Assumptions and practical considerations in the design and interpretation of echolocation-monitoring studies. *Journal of Mammology,* **78,** 514-524.

Hayes, J.P. and Steidl, R.J. (1997) Statistical power analysis and amphibian population trends. *Conservation Biology,* **11,** 273-275.

Hill, J.K., Hamer, K.C., Lace, L.A. and Banham, W.M.T. (1995) Effects of selective logging on tropical forest butterflies in Buru, Indonesia. *Journal of Applied Ecology,* **32,** 754-760.

Hogberg, L.K., Patriquin, K.J. and Barclay, R.M.R. (2002) Use by bats of patches of residual trees in logged areas of the boreal forest. *American Midland Naturalist,* **148,** 282-288.

Holland, W.J. (1903) The moth book. Doubleday, Page and Company, New York City, NY. 479 pp.

Holling, C.S. (1961) Principles of insect predation. *Annual Review of Entomology,* **6,** 163-182.

Homyack, J.A. and Haas, C.A. (2009) Long-term effects of experimental forest harvesting on abundance and reproductive demography of terrestrial salamanders. *Biological Conservation,* **142,** 110-121.

Houston, R.D., Boonman, A.M., and Jones, G. (2004) Do echolocation signal parameters restrict bats' choice of prey? In. *Echolocation in bats and dolphins,* (eds Thomas, J.A., Moss, C.F. and Vater, M.), University of Chicago Press, Chicago, IL, pp 339-345.

Hughes, J.B., Daily, G.C. and Ehrlich, P.R. (2000) Conservation of insect diversity: a habitat approach. Conservation Biology, **14,** *1788-1797*

Humes, M. L., Hayes, J.P. and Collopy, M.W. (1999) Bat activity in thinned, unthinned, and old-growth forests in western Oregon. *Journal of Wildlife Management,* **63**, 553-561.

Jackson, R.R. (1977) Prey of jumping spider *Phidippus johnsoni* (Araneae: Salticidae). *Journal of Arachnology,* **5,** 145-149.

James, F.C. and Shugart, H.H. Jr. (1970) A quantitative method of habitat description. *Audubon Field Notes,* **24,** 727–736.

Jenness, J. (2005) Random point generator (randpts.avx) extension for ARCVIEW 3.x, v. 1.3 [Online.] Available at www.jennessent.com/arcview/random_points.htm.

Jones, G. and Rydell, J. (2003) Attack and defense: interactions between echolocating bats and their insect prey. In. *Bat Ecology,* (eds Kunz, T.H. and Fenton, M.B.), University of Chicago Press, Chicago, Illinois, pp 301-345.

Jones, E.B.D. III, Helfman, G.S., Harper, J.O. and Bolstad, P.V. (1999) The effects of riparian deforestation on fish assemblages in southern Appalachian streams. *Conservation Biology,* **13,** 1454-1465.

Jourdie, V., Alvarez, N. and Turlings, T.C.J. (2008) Identification of seven species of hymenopteran parasitoids of *Spodoptera frugiperda*, using polymerase chain reaction and restriction enzyme digestion. *Agricultural and Forest Entomology,* **10,** 129-136.

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

Kalka, M.B., Smith, A.R. and Kalko, E.K.V. (2008) Bats limit arthropods and herbivory in a tropical forest. *Science,* **320,** 71.

Karr, J.R. and James, F.C. (1975) Eco-morphological configurations and convergent evolution of species and communities. In. *Ecology and evolution of communities* (eds Cody, M.L. and Diamond, J.M.). Harvard University Press, Cambridge, MA.

Kitching, R.L., Orr, A.G., Thalib, L., Mitchell, H., Hopkins, M.S. and Graham, A.W. (2000) Moth assemblages as indicators of environmental quality in remnants of upland Australian rainforest. *Journal of Applied Ecology,* **37,** 284-297.

Koivula, M., Kukkonen, J. and Niemala, J. (2002) Boreal carabid-beetle (Coleoptera, Carabidae) assemblages along the clear-cut originated succession gradient. *Biodiversity and Conservation,* **11,** 1269-1288.

Krebs, C.J. (2000) *Ecological methodology.* 2nd edition, Addison-Wesley Longman, Menlo Park, CA.

Kunz, T.H. (1988) Methods of assessing the availability of prey to insectivorous bats. In. *Ecological and behavioral methods for the study of bats*. (ed Kunz, T.H.) Smithsonian Institution Press, Washington, D.C. pp 191-210.

Kunz, T.H., Arnett, E.B., Erickson, W.P., *et al.* (2007) Ecological impacts of wind energy development on bats: questions, research needs, and hypotheses. *Frontiers in Ecology and the Environment,* **5,** 315-324.

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

Lacki, M.J., Gregory, M.J. and Williams, P.K. (1984) Activity of *Tamias striatus* in response to supplemented food. *Journal of Mammalogy,* **65,** 521-524.

Lacki, M.J., Amelon, S.K. and Baker, M.D. (2007a) Foraging ecology of forest bats. In. *Bats in forests: conservation and management,* (eds Lacki, M.J., Hayes, J.P. and Kurta, A.), John Hopkins University Press, Baltimore, Maryland, pp 83-128.

Lacki, M.J., Johnson, J.S., Dodd, L.E. and Baker, M.D. (2007b) Prey consumption of insectivorous bats in coniferous forests of north-central Idaho. *Northwest Science,* **81,** 199-205.

Lacki, M.J., Cox, D.C., Dodd, L.E. and Dickinson, M.B. (2009) Response of northern bats (*Myotis septentrionalis*) to prescribed fires in eastern Kentucky forests. *Journal of Mammalogy,* **90,** 1165-1175.

Lacki, M.J. and Dodd, L.E. (*In Press*) Diet and foraging behavior of *Corynorhinus* bats in eastern North America. *Proceedings of the Symposium on the Conservation and Management of Big-Eared Bats in the Eastern United States,* (eds Loeb, S.C.,

Lacki, M.J. and Miller, D.A.), USDAForest Service Southeastern Experimental Station, General Technical Report, pp. XX-XX.

Larson, D.J. and Hayes, J.P. (2000). Variability in sensitivity of Anabat II detectors and a method of calibration. *Acta Chiropterologica,* **2,** 209-214.

Lattin, J., Carroll, D. and Green, P. (2003) Analyzing multivariate data. Duxbury Press. 556 pp.

Law, B. and Chidel, M. (2002) Tracks and riparian zones facilitate the use of Australian regrowth forest by insectivorous bats. *Journal of Applied Ecology,* **39,** 605-617.

Lee, Y.F. and McCracken, G.F. (2004) Flight activity and food habits of three species of *Myotis* bats (Chiroptera: Vespertilionidae) in sympatry. *Zoological Studies,* **43,** 589-597.

Lenski, R.E. (1982) The impact of forest cutting on the diversity of ground beetles (Coleoptera: Carabidae) in the southern Appalachians. *Ecological Entomology,* **7,** 385-390.

Leslie, D.M. Jr., and Clark, B.S. (2002) Feeding habits of the endangered Ozark big-eared bat (*Corynorhinus townsendii ingens*) relative to prey abundance. *Acta Chiropterologica,* **4**, 173-182.

Lundgren, J.G., Nichols, S., Prischmann, D.A. and Ellsbury, M.M. (2009) Seasonal and diel activity patterns generalist predators associated with *Diabrotica virgifera* immature (Coleoptera: Chrysomelidae). *Biocontrol Science and Technology,* **19,** 327-333.

Magurran, A.E. (1988) Ecological diversity and its measurement. Princeton University Press, Princeton, NJ.

Marques, E.S.D.A., Price, P.W. and Cobb, N.S. (2000) Resource abundance and insect herbivore diversity on woody fabaceous desert plants. *Environmental Entomology,* **29,** 696-703.

McCracken, G.F., Brown, V.A., Etheridge, M. and Westbrook, J.K. (2005) The use of fecal DNA to verify and quantify the consumption of agricultural pests. Proceedings of the 35th North American Symposium on Bat Research. In. *Bat Research News*, **46,** 195-196.

Menzel, J.M., Menzel, M.A., McCracken, G.F. and Chapman, B.R. (2000) Notes on bat activity above the forest canopy in the eastern United States. *Georgia Journal of Science,* **58,** 212–216.

Menzel, M.A., Carter, T.C., Menzel, J.M., Ford, W.M. and Chapman, B.R. (2002) Effects of group selection silviculture in bottomland hardwoods on the spatial activity patterns of bats. *Forest Ecology and Management,* **162,** 209-218.

Miller, D.A., Arnett, E.B. and Lacki, M.J. (2003) Habitat management for forest-roosting bats of North America: a critical review of habitat studies. *Wildlife Society Bulletin,* **31,** 30-44.

Milne, D.J. (2002) Key to the bat calls of the top end of the Northern Territory. Australian Parks and Wildlife Commission of the Northern Report, General Technical Report No. 71.

Morris, A.D., Miller, D.A. and Kalcounis-Rueppell, M.C. (2010) Use of forest edges by bats in a managed pine forest landscape. *Journal of Wildlife Management,* **74,** 26-34.

Muirhead-Thomson, R.C. 1991. Trap responses of flying insects. Academic Press, London. 287pp.

Niemela, J., Langor, D.W. and Spence, J.R. (1993) Effects of clear-cut harvesting on boreal ground beetle assemblages (Coleoptera: Carabidae) in western Canada. *Conservation Biology,* **7,** 551-561.

Noether, G.E. (1990) Introduction to statistics: the nonparametric way. Springer – Verlag, Düsseldorf, Germany.

Norberg, U.M. and Raynor, J.M.V. (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.,* **316,** 335-427.

O'Shea, T.J. and Bogan, M.A. (2003) Introduction. In. *Monitoring Trends in Bat Populations of the United States and Territories: Problems and Prospects* (eds O'Shea, T.J. and Bogan, M.A.), U.S. Geological Survey, Information and Technology Report, pp 69-77.

Ober, H.K. and Hayes, J.P. (2008) Influence of vegetation on bat use of riparian areas at multiple spatial scales. *Journal of Wildlife Management,* **72**, 396-404.

Ober, H.K. and Hayes, J.P. (2010) Determinants of nocturnal Lepidopteran diversity an community structure in a conifer-dominated forest. *Biodiversity and Conservation,* **19,** 761-774.

Okland, B. (1996) Unlogged forests: important sites for preserving the diversity of mycetophilids (Diptera: Sciaroidea). *Biological Conservation,* **76,** 297-310.

Okland, B., Gotmark, F. and Norden, B. (2008) Oak woodland restoration: testing the effects on biodiversity on mycetophilids in southern Sweden. *Biodiversity and Conservation,* **17,** 2599-2616.

Owen, S.F., Menzel, S.F., Ford, W.M., Chapman, B.R., Miller, K.V., Edwards, J.W. and Wood, P.B. (2003) Home-range size and habitat used by the northern myotis (*Myotis septentrionalis*). *American Midland Naturalist,* **150,** 352–359.

Owen, S.F., Menzel, M.A., Edwards, J.W., Ford, W.M., Menzel, J.M., Chapman, B.R., Wood P.B. and Miller, K.V. (2004) Bat activity in harvested and intact forest stands in the Allegheny Mountains. *Northern Journal of Applied Forestry,* **21,** 154-159.

Panzer, R. and Schwartz, M.W. (1998) Effectiveness of a vegetation-based approach to insect conservation. *Conservation Biology,* **12,** 693-702.

Parsons, S. (2001) Identification of New Zealand bats (*Chalinolobus tuberculatus* and *Mystacina tuberculata*) in flight from analysis of echolocation calls by artificial neural networks. *Journal of Zoology,* **253,** 447-457.

Patterson, P.D., Willig, M.R. and Stevens, R.D. (2003) Trophic strategies, niche partitioning, and patterns of ecological organization. In. *Bat Ecology,* (eds Kunz, T.H. and Fenton, M.B.), University of Chicago Press, Chicago, Illinois, pp 536-579.

Patriquin, K.J. and Barclay, R.M.R. (2003) Foraging by bats in cleared, thinned and unharvested boreal forest. *Journal of Applied Ecology,* **40,** 646–657.

Pierson, E.D. (1998) Tall trees, deep holes and scarred landscapes: conservation biology of North American bats. In. *Bat Biology and Conservation,* (eds Kunz, T.H. and Racey, P.A.), Smithsonion Institution Press, Washington DC, pp 309-325.

Racey, P.A. and Entwhistle, A.C. (2003) Conservation ecology of bats. In. *Bat Ecology,* (eds Kunz, T.H. and Fenton, M.B.), University of Chicago Press, Chicago, Illinois, pp 680-743.

Rakotoarivelo, A.A., Ranaivoson, N., Ramiliijaona, O.R., Kofoky, A.F., *et al*. (2007) Seasonal food habits of five sympatric microchiropterans in western Madagascar. *Journal of Mammalogy,* **88,** 959-966.

Ratcliffe J.M. and Dawson, J.W. (2003) Behavioral 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.

Ratnasingham, S. and Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes,* **7,** 355-264.

Read, D.S., Sheppard, S.K., Bruford, M.W., Glen, D.M. and Symondson, W.O.C. (2006) Molecular detection of predation by soil micro-arthropods on nematodes. *Molecular Ecology,* **15,** 1963-1972.

Reddy, E. and Fenton, M.B. (2003) Exploiting vulnerable prey: moths and red bats (*Lasiurus borealis*: Vespertilionidae). *Canadian Journal of Zoology,* **81,** 1553 1560.

Ries, L., Fletcher, R.J. Jr., Battin, J. and Sisk, T.D. (2004) Ecological responses to habitat edges: mechanisms, models, and variability explained. *Annual Review of Ecology and Systematics,* **35,** 491-522.

Rieske, L.K. and Buss, L.J. (2001) Influence of site on diversity and abundance of ground and litter-dwelling Coleoptera in Appahachian oak-hickory forests. *Environmental Entomology,* **30,** 484-494.

Rings, R. W. E., Metzler, E.H., Arnold, F.J. and Harris, D.H. (1992) The owlet moths of Ohio (Lepidoptera: Noctuidae). *Ohio Biological Survey Bulletin New Series,* **9,** 1-219.

Rosenheim, J.A. (1998) Higher-order predators and the regulation of insect herbivore populations. *Annual Review of Entomology,* **43,** 421-447.

Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution,* **4,** 406-425.

San Mauro, D. and Agorreta, A. (2010) Molecular systematics: a synthesis of the common methods and the state of knowledge. *Cellular and Molecular Biology Letters,* **15,** 311-341.

Saugey, D.A., Heath, D.R. and Heidt, G.A. (1989) The bats of the Ouachita Mountains. *Proceedings of the Arkansas Academy of Science,* **43,** 71-77.

Saugey, D.A., Heath, D.R. and Heidt, G.A. (1998) Notes on the natural history of *Lasiurus borealis* in Arkansas. *Journal of the Arkansas Academy of Science,* **52,** 92–98.

Samways, M.J. (2007) Insect conservation: a synthetic management approach. *Annual Review of Entomology,* **52,** 465-487.

Scott, S.J., McLaren, G., Jones, G. and Harris, S. (2010) The impact of riparian habitat on the foraging and activity of pipistrelle bats (*Pipistrellus* spp.). *Journal of Zoology,* **280,** 371-378.

Sheppard, S.K. and Harwood, J.D. (2005) Advances in molecular ecology: tracking trophic links through predator-prey food-webs. *Functional Ecology,* **19,** 751-762.

Shiel, C.B., McAney, C.M. and Fairley, J.S. (1991) Analysis of the diet of Natterer's bat *Myotis nattereri* and the common long-eared bat *Plecotus auritus* in the West of Ireland. *Journal of Zoology*, **223,** 299-305

Shoemaker, L.G. (1994) Diet preference of the endangered Virginia big-eared bat, *Plecotus townsendii virginianus*, in the Daniel Boone National Forest, Kentucky. M.S. Thesis, University of Kentucky, Lexington, KY. 139 pp.

Shump, K.A. and Shump, A.U. (1982) Lasiurus borealis. *Mammalian Species,* **183,** 1–6.

Simon, C., Frati, F., Beckenbach, A., *et al.* (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America,* **87,** 651-701.

Simmons, N.B. and Conway, T.M. (2003) Evolution of ecological diversity in bats. In. *Bat Ecology,* (eds Kunz, T.H. and Fenton, M.B.), University of Chicago Press, Chicago, Illinois, pp 493-535.

Sokal, R.R. and Rohlf, F.J. (1969) Biometry: the principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco, California.

Storm, J.J. and Whitaker, J.O. (2008) Prey selection of big brown bats (*Eptesicus fuscus*) during an emergence of 17-year cicadas (*Magicicada* spp.). American Midland Naturalist, **160,** 350-357.

Strong, D. R., Lawton, J.J.H. and Southwood, T.R.E. (1984) Insects on plants: community patterns and mechanisms. Harvard University Press, Cambridge, Massachussetts.

Summerville, K.S., Boulware, M.J., Veech, J.A. and Crist, T.O. (2003) Spatial variation in species diversity and composition of forest lepidoptera in eastern deciduous forests of North America. *Conservation Biology,* **17,** 1045-1057.

Summerville, K.S., and Crist, T.O. (2002) Effects of timber harvest on forest lepidoptera: community, guild, and species responses. *Ecological Applications,* **12,** 820-835.

Summerville, K.S., and Crist, T.O. (2003) Determinants of lepidopteran community composition and species diversity in eastern deciduous forests: roles of season, eco-region, and patch size. *Oikos,* **100,** 134-148.

Summerville, K.S. and Crist, T.O. (2008) Structure and conservation of lepidopteran communities in managed forests of northeastern North America: a review. *Canadian Entomologist,* **140,** 475-494.

Summerville, K.S., Metzler, E.H. and Crist, T.O. (2001) Diversity of lepidoptera in Ohio forests at local and regional scales: How heterogeneous is the fauna? *Annals of the Entomological Society of America*, **94,** 583-591.

Summerville, K.S., Ritter, L.M. and Crist, T.O. (2004) Forest moth taxa as indicators of lepidopteran richness and habitat disturbance: a preliminary assessment. *Biological Conservation,* **116,** 9-18.

Symondson, W.O.C. (2002) Molecular identification of prey in predator diets. *Molecular Ecology,* **11,** 627-641.

Taki, H., Inoue, T., Tanaka, H., *et al.* (2010) Responses of community structure, diversity, and abundance of understory plants and insect assemblages to thinning in plantations. *Forest Ecology and Management,* **259,** 607-613,

Tallamy, D.W. and Shropshire, K.J. (2009) Ranking lepidopteran use of native versus introduced plants. *Conservation Biology,* **23,** 941-947.

Tannenbaum, R. (1975) Reproductive strategies in the White-Lined Bat. Ph.D. Dissertation, Cornell University, Ithaca, NY.

Thomas, A.W. 2001. Moth Diversity in a Northeastern North American Red Spruce Forest: I. Baseline Study. Information Report, M-X-210E, Canadian Forest Service, Canada. 42 pp.

Thomas, D.L. and Taylor, E.J. (1990) Study designs and tests for comparing resource use and availability. *Journal of Wildlife Management,* **54,** 322-330.

Thomas, D.L. and Taylor, E.J. (2006) Study designs and tests for comparing resource use and availability II. *Journal of Wildlife Management***, 70,** 324-336.

Thomas, A.W., and G.M. Thomas. 1994. Sampling strategies for estimating moth species diversity using a light trap in a northeastern softwood forest. *Journal of the Lepidopterists' Society,* **48,** 85-105.

Tibbels, A.E. and Kurta, A. (2003) Bat activity is low in thinned and unthinned stands of red pine. *Canadian Journal of Forest Research,* **33,** 2436-2442.

Triola, M.F. (1986) *Elementary Statistics,* Third Edition, Benjamin/Cummings Publishing, Menlo Park, California.

Triplehorn, C.A., and Johnson, N.F. (2005) Borror and Delong's introduction to the study of insects. Seventh edition. Thomson Brooks/Cole, Belmont, CA. 864 pp.

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

Walsh, A.L. and Harris, S. (1996) Foraging habitat preferences of Vespertilionid bats in Britain. *Journal of Applied Ecology,* **133,** 508–518.

Waters, D.A. (2003) Bats and moths: what is there left to learn? *Physiological Entomology,* **28,** 237-250.

Webb, B.A., Barney, W.E., Dahlman, D.L., *et al.* 2004. Eastern tent caterpillars (*Malacosoma americanum*) cause mare reproductive loss syndrome. *Journal of Insect Physiology,* **50,** 185-193.

Weber, D.C. and Lundgren, J.G. (2009) Assessing the trophic ecology of the Coccinellidae: their roles as predators and prey. *Biological Control,* **51,** 199-214.

Weller, T.J., Seidman, V.M. and Zebel, C.J. (1998) Assessment of foraging activity using Anabat II: a cautionary note. *Bat Research News,* **39,** 61-65.

Werner, S.M. and Raffa, K.F. (2000) Effects of forest management practices on the diversity of ground-occurring beetles in mixed northern hardwood forests of the Great Lakes region. *Forest Ecology and Management,* **139,** 135-155.

Whitaker, J.O. Jr. (1972) Food habits of bats from Indiana. *Canadian Journal of Zoology*, **50,** 877-883.

Whitaker, J.O. Jr. (1988) Food habits analysis of insectivorous bats. In. *Ecological and behavioral methods for the study of bats,* (ed Kunz, T.H.), Smithsonian Institution Press, Washington, DC, pp 171-189.

Whitaker, J.O. Jr. (1994) Food availability and opportunistic versus selective feeding in insectivorous bats. *Bat Research News,* **35,** 75-77.

Whitaker, J.O. Jr. (2004) Prey selection in a temperate zone insectivorous bat community. *Journal of Mammalogy,* **85,** 460-469.

Whitaker, J.O. Jr., Maser, C. and Cross, S.P. (1981) Food habits of eastern Oregon bats, based on stomach and scat analyses. *Northwest Science,* **55,** 281-292.

Wickramasinghe, L.P., Harris, S., Jones, G. and Jennings, N.V. (2004) Abundance and species richness of nocturnal insects on organic and conventional farms: effects of agricultural intensification on bat foraging. *Conservation Biology,* **18,** 1283-1292.

Wilkinson, G.S. and McCracken, G.F. (2003) Bats and balls: sexual selection and sperm competition in the Chiroptera. In. *Bat Ecology* (eds Kunz, T.H. and Fenton, M.B.), University of Chicago Press, Chicago, IL.

Williams-Guillen, K., Perfecta, I. and Vandermeer, J. (2008) Bats limit insects in a neotropical agroforestry system, *Science,* **320,** 70

Wolf, J.M., Battaglia, L., Carter, T.C., Rodman, L.B., Britzke, E.R. and Feldhamer, G.A. (2009) Effects of tornado disturbance on bat communities in southern Illinois. *Northeastern Naturalist,* **16,** 553-562.

Woods, A.J., Omernik, J.M, Brockman, *et al.* (1998) Ecoregions of Indiana and Ohio. (color poster with map, descriptive text, summary tables, and photographs). Reston, VA., U.S. Geological Survey(map scale 1:1,500,000).

Woods, A. J., Omernik, J.M. and Brown, D.D. (1999) Level III and IV ecoregions of Delaware, Maryland, Pennsylvania, Virginia, and West Virginia. Corvallis, OR, U.S. Environmental Protection Agency.

Woods, A.J., Omernik, J.M., Martin, W.H., *et al.* (2002) Ecoregions of Kentucky (color poster with map, descriptive text, summary tables, and photographs). Reston, VA., U.S. Geological Survey (map scale 1:1,000,000).

Work, T.T., Jacobs, J.M., Spence, J.R. and Volney, W.J. (2010) High levels of green-tree retention are required to preserve ground beetle diversity in boreal mixed-wood forests. *Ecological Applications,* **20,** 741-751.

Zar, J. H. (1999) Biostatistical Analysis, Fourth Edition. Prentice Hall, Upper Saddle River, NJ. 663 pp.

Zimmerman, G.S. and Glanz, W.E. (2000) Habitat use by bats in eastern Maine. *Journal of Wildlife Management,* **64**, 1032-1040.

Zwickl, D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Dissertation (The University of Texas at Austin).

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Grants in Aid of Research, Sigma Xi, 2007.

University Scholarship, Arkansas Tech University, 2000-2004.

Biology Student of the Year, ATU Beta Beta Beta Biological Society, 2004.

Wildlife Student of the Year, ATU Fisheries and Wildlife Society, 2003.

Professional Publications

Lacki, M.J., and L.E. Dodd. In Press. Diet and foraging behavior of *Corynorhinus* bats in eastern North America. Proceedings of the Symposium on the Conservation and Management of Big-Eared Bats in the Eastern United States, (eds Loeb S.C., M. J. Lacki, and D.A. Miller), USDA Forest Service Southeastern Experimental Station, General Technical Report, pp. XX-XX.

Lacki, M. J., D. C. Cox, L. E. Dodd, and M. B. Dickinson. 2009. Response of northern bats (*Myotis septentrionalis*) to prescribed fires in eastern Kentucky forests. *Journal of Mammalogy,* **90,** 1165-1175.

Dodd, L. E., M. J. Lacki, and L. K. Rieske. 2008. Variation in moth occurrence and the implications for foraging habitat of the Ozark big-eared bat. *Forest Ecology and Management,* **255,** 3866-3872.

Dodd, L. E., and J. K. Adkins. 2007. Observations of mating behavior in the eastern red bat (*Lasiurus borealis*). *Bat Research News,* **48,** 155-156.

Dodd, L. E., and M. J. Lacki. 2007. Prey consumed by *Corynorhinus townsendii ingens* in the Ozark Mountain region. *Acta Chiropterologica,* **9,** 451–461.

Lacki, M. J., J. S. Johnson, L. E. Dodd, and M. D. Baker. 2007. Prey consumption of insectivorous bats in coniferous forests of north-central Idaho. *Northwest Science,* **81,** 199-205.

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