

## FINAL REPORT

### PROJECT #10-1-06-1

Fire management and habitat quality for endangered bats in Kentucky's Mammoth Cave National Park during the swarming and staging periods: predator-prey interactions and habitat use of bats threatened by White-Nose Syndrome.

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## 1. Abstract

This project addressed the relationships of prescribed fire with habitat of insectivorous bats in central hardwood forests. We used Airborne Laser Scanning (ALS) to quantitatively describe forest structure at the understory, mid-story and canopy layers in forested habitats, largely upland oak-hardwoods, subjected to a chronosequence of prescribed fire treatments at Mammoth Cave National Park, Kentucky. We compared response of bat activity and bat diets with abundance of insect prey across a series of fire histories and varying canopy structures throughout Mammoth Cave National Park. We monitored seedlings of select plant species to assess patterns and changes in insect herbivory with changes in insect abundance and fire. Lastly, we monitored population trends and physiological condition of bats during staging and swarming as bats emerged and entered hibernation, respectively. The project has produced four published papers, with three other manuscripts either in review or nearly ready for submission to peer-reviewed journals. An additional two papers are also in preparation. The principle findings from this research are: 1) forest canopy structure, as described using ALS data, shows promise for predicting bat activity, especially for high-frequency echolocators; 2) molecular assays of bat diets using *COI* fragments indicate that traditional dietary approaches fail to capture the full breadth of the diet (i.e., prey species and size) of insectivorous bats that feed on moths; 3) smaller-sized bats achieve lower body mass index values during staging and swarming rendering them potentially more vulnerable to stress from white-nose syndrome than larger bat species, at least for populations of cave-hibernating bats in Kentucky; 4) high-frequency bats showed a varied response to changes in flight space and canopy structure with prescribed fire as compared to low-frequency echolocators; and, 5) prey availability for bats is highly dependent on season, with mode and degree of herbivory being variable in severity over the growing season.

## 2. Background and Purpose

### Prescribed Fire and Bats

Use of prescribed fire to manage forested environments across North America has expanded in recent decades in efforts to control outbreaks of severe wildfires and to restore forests to historical patterns of plant species composition, distribution, and abundance (Dickinson 2006, Boerner et al. 2008). Prescribed burning has been used in central hardwoods to restore and maintain oak-dominated ecosystems (Dickinson 2006, Yaussy et al. 2008), with federal land management agencies conducting prescribed fire treatments in oak forests throughout the distributional limits of several bats species vulnerable to white-nose syndrome (WNS), including the Indiana bat, *Myotis sodalis* (Dickinson et al. 2009). The widespread application of prescribed fire, the seasonal effects of when fires are ignited, and the impacts of prescribed fire on aboveground forest structure in habitats where insectivorous bats feed have received limited attention (Dickinson et al. 2009, Perry 2012). A better understanding of how these practices affect habitat structure and, thus, the suitability of forested ecosystems for bats, for both roosting and feeding, are needed to evaluate the extent to which this management practice is consistent with maintaining habitat of insectivorous bats that winter in caves and are vulnerable to WNS.

Although studies exist describing bat responses to specific silvicultural systems, including prescribed fire (Loeb and Waldrop 2008, Dickinson et al. 2009, Lacki et al. 2009), data are equivocal in assessing the appropriateness of these systems in providing suitable foraging habitats for bats (Hayes and Loeb 2007). Findings are confounded by differences in forested environments, geographic locations, and the assemblage of bat species present among study sites. Further, most existing studies are site-specific in their coverage, rendering extrapolation of findings to other locations potentially suspect (Miller et al. 2003, Kroll et al. 2012). Regardless, the perception exists that use of fire is a way of improving bat habitat, through creation of snags, developing more open stands preferred for foraging, and increasing the abundance and diversity of insect prey (USDA FS 2003, USFWS 2007). Prescribed fires can produce or consume snags, with the long-term effects on size and density of snags in eastern hardwood forests unknown (Dickinson et al. 2009). Short-term increases in insect prey with prescribed fire have been demonstrated in Kentucky forests, with shifts in foraging areas of northern long-eared bats (*Myotis septentrionalis*) toward burn units following fire treatments (Lacki et al. 2009). Long-term effects of prescribed fire on abundance of insect prey remain unclear.

Response of bats when feeding to differing degrees of clutter (i.e., obstructed flight space) and habitat structure varies among forested environments (Brigham et al. 1997, Patriquin and Barclay 2003, Titchenell et al. 2011). In general, bats can be categorized by call structures into high frequency and low frequency groupings (Britzke 2004, Britzke et al. 2011), with high frequency bats typically possessing body forms with low wing loadings (i.e., mass/ wing surface area) and, therefore, increased capability of flight in cluttered air space (Bogdanowicz et al. 1999, Swartz et al. 2003). In Kentucky, high frequency bats are species in the genus *Myotis* and *Perimyotis*, and include the endangered Indiana bat and the northern long-eared bat; the latter species has recently been proposed for listing as endangered (USFWS 2013). Conversely, low frequency bats often possess high wing loadings and are more suited to flight in open air space. This grouping in Kentucky includes the tree bats [red bat (*Lasiurus borealis*), hoary bat (*L. cinereus*), silver-haired bat (*Lasionycteris noctivagans*), evening bat (*Nycticeius humeralis*)], and the big-brown bat (*Eptesicus fuscus*).

Both high frequency and low frequency bats inhabit forested environments in eastern North America, but how they partition the available foraging space and insect prey remains poorly understood (Dodd et al. 2012). Numerous studies across eastern North America have examined activity and foraging behavior of bats under a range of forested conditions, with data equivocal on the response of bats to differing silvicultural treatments and across geographic regions (Humes et al. 1999, Menzel et al. 2002, Tibbels and Kurta 2003, Owen et al. 2004; Hayes and Loeb 2007). Use of growing-season burns as silvicultural treatments in oak-dominated forests has led to concern over increased risks to bats, especially from effects of smoke and heat while bats are roosting with young (Dickinson et al. 2010). Never-the-less, burning has the capability of altering forest structure in ways that hold promise for enhancing foraging habitat for insectivorous bats, but data are needed to assess the risks and benefits of burning to bats (Dickinson et al. 2009).

## White-nose Syndrome

Insectivorous bats are the primary predators of night flying insects and consequently are a significant component of healthy forested ecosystems (Lacki et al. 2007). The arrival of the fungus *Pseudogymnoascus destructans* (formerly *Geomyces destructans*) to cave systems in North America in 2006 (USFWS 2012), and the subsequent widespread mortality of cave-roosting bats caused by the debilitating effects of the WNS fungus (Frick et al. 2010), has created concern for the potential effects that a loss of bats will have on the function and health of forested ecosystems in eastern North America. Recent estimates of mortality of bats at hibernacula in eastern North America suggest as many as 6.7 million bats have died due to the fungus since 2007 (USFWS 2012). The fungus continues to expand in distribution and has now affected 9 species of bats over multiple states and Canadian provinces (Foley et al. 2011), and has been found as far west as Minnesota, Iowa, Missouri, Arkansas, and possibly Oklahoma (Figure 2.1). Most of these species largely maintain themselves in forested environments during the growing season where quality foraging habitats are essential for: building body mass for winter hibernation; maintaining and improving health, especially if recovering from WNS infection; and, most significantly, successfully rearing young. The eventual expansion of the fungus throughout much of the North American continent is predicted, as is the imminent loss of the endangered Indiana bat across much of its range (Thogmartin et al. 2013), and at least one species of bat, little brown bat (*M. lucifugus*), that was formerly viewed as common throughout its distribution (Frick et al. 2010).

Bats consume large amounts of insects, with Lepidoptera (i.e., moths) eaten by all bat species in eastern North America (Lacki et al. 2007). Many species of Lepidoptera are harmful pests in the larval stage to plants in forests and agro-ecosystems (Covell 2005, Lacki and Dodd 2011), and the loss of bats as predators and natural control agents of these pest species is a concern for sustaining healthy ecosystems. Two bat species that feed extensively on moths in eastern North America, the Indiana bat and the northern long-eared bat (Lacki et al. 2007), are affected by the *Pseudogymnoascus* fungus during hibernation (Foley et al. 2011), and the ecological consequences of the loss of these two bat species from forested environments remains unclear. While the precise economic impacts that bats as predators have on insect populations are uncertain, they are likely substantial and total in the billions of dollars per year across North America (Boyles et al. 2011). The ecosystem services that these night-flying predators provide are well described (Kunz et al. 2011); however, the role that insectivorous bats play in top-down regulation of pestiferous forest herbivores needs further study (Fenton 2012). We do know that North American insectivorous bats are core predators of Coleoptera and Lepidoptera (Lacki et al. 2007). These insect orders form two of the most common and conspicuous groups of forest herbivores in the world.

Given the paucity of data that exists regarding the impacts of prescribed fire on the spatiotemporal patterns of insect herbivores in oak-hardwood forests (Kim and Holt 2012), and how this prey occurrence may influence the use of habitats by bats (Dickinson et al. 2009), this study provided an opportunity to assess interactions between a common management tool (fire), effects of an emerging forest disease (WNS), and abundance of insect prey (pest species) on foraging patterns and habitat use of bats in oak-hardwood forests of North America.

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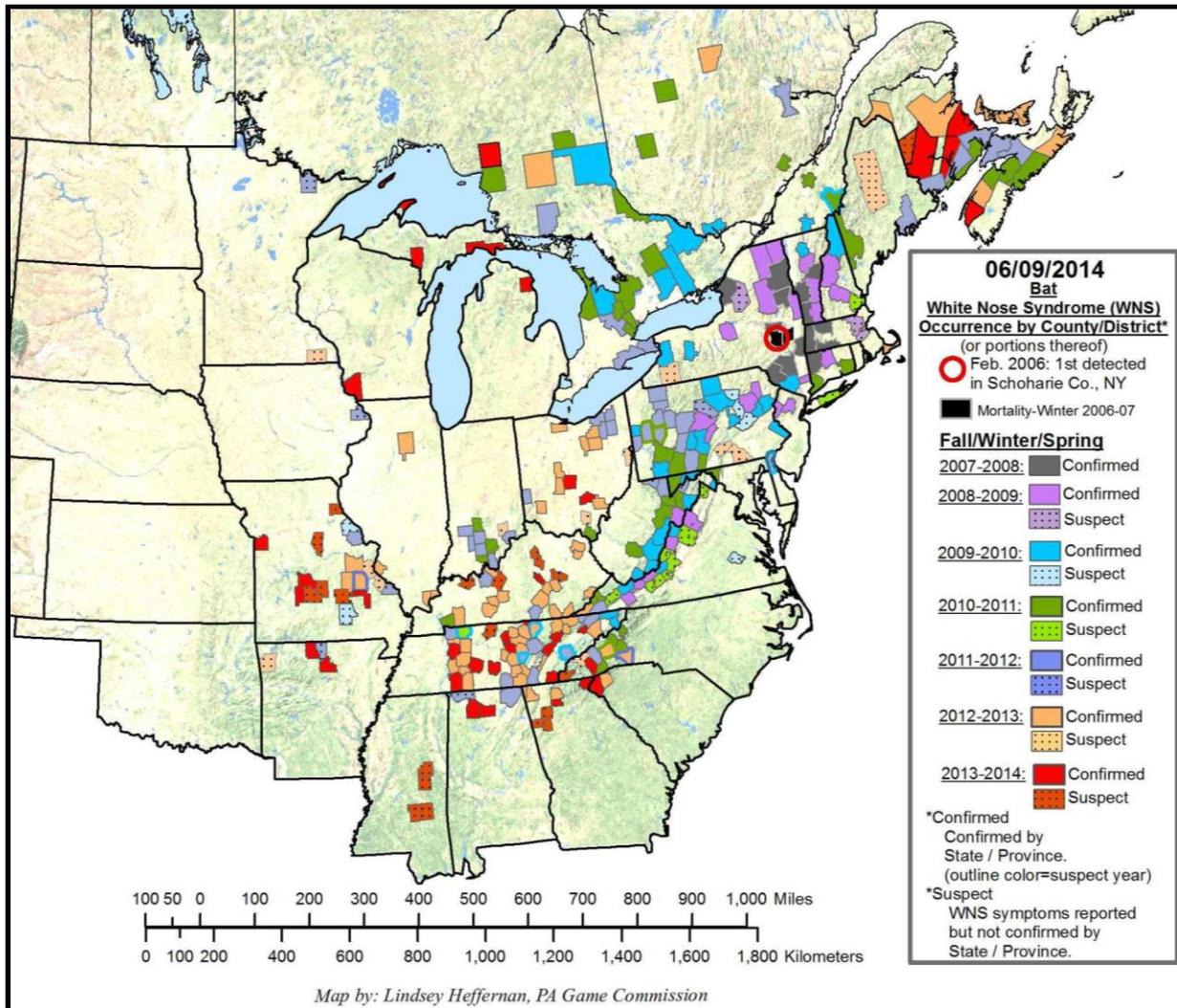


Figure 2.1. Chronological distribution of white-nose syndrome across eastern North America (Map by: L. Heffernan, Pennsylvania Game Commission, June 2014).

### 3. Study Description and Location

Mammoth Cave National Park (MCNP) is a 23,000-ha parcel, with an extensive cave system, history of fire management, and immediate proximity (80-160 km) to bat hibernacula infected with WNS in Indiana, Kentucky and Tennessee, prior to the start of this study. The fungus reached cave systems in Kentucky in 2011 (B. Hines, KDFWR, pers. comm.), and was discovered in MCNP during the winter of 2012 (R. Toomey, NPS, pers. comm.). Use of fire for

forest management has been ongoing at MCNP since 2002, with >25% of the park land base having been managed with prescribed fire under a proposed eight-year fire rotation. The prescribed fire program has created a forest mosaic with a range of conditions to which insect herbivores and bats can respond. Two caves at MCNP support over 1,000 hibernating bats in winter, including populations of the endangered Indiana bat and gray bat (*Myotis grisescens*). These caves are within 10 km (6 mi) of all burns implemented since 2002, with several existing burns encroaching within the 1-km (0.6 mi) buffer zone of one of these hibernacula. Thus, burned habitats are available to the populations of bats in these caves when they are foraging during the autumn pre-hibernation (swarming) and spring post-hibernation (staging) seasons. Our monitoring efforts included sampling in plots across all years of fire treatments since the fire program was initiated in 2002 and, since monitoring began in 2010, data have been collected in first-year prescribed burns, including pre- and post-burn measurements. Sampling efforts also span years prior to, commensurate with, and following arrival of the WNS fungus to MCNP.

The alarming decline in cave-hibernating bats in eastern North America suggests that research and management efforts targeting the ecology of these animals and their importance to forested ecosystems is of paramount importance. The *Pseudogymnoascus* fungus has been associated with mortalities in at least two endangered species, the Indiana bat and the gray bat (Foley et al. 2011), with several other species now under status review for possible listing as either threatened or endangered because of the large numbers of mortalities observed due to WNS since 2007 (M. Armstrong, USFWS, pers. comm.). The importance of field studies to identify preferred aboveground habitat conditions of these bat species are now sorely needed, and should be completed in advance of future population declines that may occur, as these declines could render obtaining and interpreting data even more difficult as bats become less abundant across eastern North American landscapes over time.

This project is an effort to begin the process of understanding how and whether prescribed fire can be used as a management tool for bats in oak-hardwood forests, especially species of bats vulnerable to WNS. Activities of this project were conducted under Kentucky state collecting permits (#SC1311182, #SC1311183) and a Federal collecting permit (#TE38522A-0). The federal permit covered capture and handling of Indiana bats and gray bats. All animal handling procedures used in this study were approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC No. 2010-0660).

#### **4. Key Findings**

Project Objective 1. Measure forest conditions associated with burned and unburned habitats in MCNP to quantify disturbance history, species composition, 3-D canopy density, solar exposure, and potential productivity as these relate to insect populations and levels of bat activity.

Airborne Laser Scanning data (ALS) was acquired during leaf-on conditions during October 2010 over the extent of Mammoth Cave National Park. Data were collected at a scan angle of  $\pm 13$  degrees with 100% overlap. Four returns per pulse were digitized with an average density of 8 returns per  $m^2$ . The raw ALS data was reduced using the Toolbox for LiDAR Data Filtering and Forest Studies (Tiffs). The resulting data products included a 1 x 1-m horizontal resolution digital elevation model, canopy height model, and object height model for the extent.

Data products based on a 25 x 25-m horizontal resolution were developed that included mean height, quadratic mean height, standard deviation of heights, percentile heights, skewness, and kurtosis. Canopy height profiles were also developed at 25 x 25-m horizontal resolution to describe the bulk density and clutter of the canopy at different levels; both the raw and reduced datasets have been provided to the Mammoth Cave International Center for Learning and the North Carolina State Geography Department for future research and management applications.

ALS data were valuable for exploring relationships between bats, their prey, and forest structure (Key Reports 10, 11, 13). We found that bat activity correlated with clutter throughout the canopy, and that predictive models can relate the activity of bats to reduced clutter within the understory strata (which is impacted by prescribed fire). Bat activity in general was greater in areas with less clutter. Insect prey exhibit varied, albeit weaker, responses to forest clutter, which we attribute to dependence on specific host materials.

Project Objectives 2 and 3. Monitor activity patterns of bats and their prey at MCNP across available habitats, including burned and unburned, during spring, summer and autumn.

Summarizing Key Report 12, activity of high-frequency bats tended to be lower in areas that had been burned at MCNP, but our data suggest that the time since burn may influence activity levels of both high and low-frequency echolocators. Activity of northern long-eared bats, in particular, was higher in areas that had never been burned versus areas that had been recently burned. Strong intra- and inter-seasonal differences were seen for bat activity, with dramatic, inconsistent variation observed for low-frequency echolocators. Differential responses to burning were observed for the primary prey of bats, with abundance of Coleoptera being heightened in recently-burned areas and abundance of Lepidoptera greater in unburned areas. Seasonally, prey abundance was generally lowest when bats were swarming versus earlier portions of the growing season.

Project Objective 4. Capture bats at MCNP to collect feces for use in morphological and DNA-based diet studies; evidence of WNS infection of individual bats will be recorded and quantified according the USFWS recommendations.

Molecular assessments of diet provide valuable resolution regarding prey selection by bats. Our findings in Key Report 14 suggest that North American *Corynorhinus* consume a wider range of sizes and species of Lepidoptera than previously reported in studies based solely on identification of culled prey wings beneath feeding roosts. Further, a comparison of our molecular assessment for the Rafinesque's big-eared bat versus data for the northern long-eared bat suggest separation of foraging niches for these co-occurring gleaning species.

Our harp-trapping efforts resulted in body condition assessments of well over 1,000 bats, thus establishing a benchmark for understanding the eminent changes in bat community structure and health at MCNP. Summarizing Key Report 15, we found that body mass index (BMI) varied among species, and that variation was strongly associated with the seasonal differences of staging and swarming. Smaller-sized bats achieved lower BMI values during staging and swarming rendering them potentially more vulnerable to stress from WNS than larger bat species.

Project Objective 5. Monitor changes in generalized forest herbivory and populations of herbivorous insects in MCNP during spring, summer and autumn, emphasizing those species that feed on forest vegetation and also occur in the diet of bats. Evaluate changes in bats, insects, and herbivory for evidence of “top down” control on forest productivity.

We found seasonal variation in insect herbivores at MCNP (Key Reports 12, 16). Varied feeding guilds impacted white oaks throughout the growing season. Chewing insects such as larval Lepidoptera were the primary defoliators found early in the growing season, and continued to be significant herbivores throughout the year. We found skeletonizing and stippling insects to be a greater source of herbivory later in the growing season.

## **5. Management Implications**

Project Objectives 1-3 (Described Prior in Section 4).

Describing forest canopy structure using ALS data shows promise for predicting bat activity, especially for high-frequency echolocators. While our results suggest that bats are not more active in burned areas at MCNP, we also found that bat activity is heightened in areas with reduced clutter. Given that repeated low-intensity burn entries may ultimately achieve a persistent reduction in understory clutter, management plans that integrate fire may ultimately promote structurally-preferred foraging habitat for bats. However, considering these data in tandem with assessments of prey availability, our study suggests that prescribed fire programs should maintain unburned parcels (or infrequently burned ) parcels of forest habitat in order to maintain an array of foraging conditions and prey types for the multi-species bat assemblages that are found in central hardwood forest ecosystems.

Project Objective 4 (Described Prior in Section 4).

Our data demonstrate that hibernating bats face a ‘squeeze-play’ during the periods of staging and swarming. Given a reduced and/or unreliable abundance of prey during these periods, managers should be mindful of prescribed fire activities that could reduce foraging efficiency of bats prior to and after hibernation (particularly given concerns of WNS). If afforded the opportunity, the implementation of burns later in the spring season could benefit bats facing critical foraging requirements immediately after hibernation.

Project Objective 5 (Described Prior in Section 4).

While no attributable impacts from herbivory were observed on seedling growth, our data demonstrate increased growth of oak seedlings in burned areas. The impacts of bats as a source of top-down predation pressure in the MCNP ecosystem remains to be seen. With declines of these predators expected due to WNS, continuing research (JFSP #14-1-05-22) is poised to evaluate shifts in herbivory in future years.

## 6. Relationship to Other Recent Findings and Ongoing Work

A body of work has emerged regarding the effects of prescribed fire on bats in the oak forests of eastern North America (Perry 2012). Recent studies have demonstrated potential benefits to bats that inhabit or use forests managed with prescribed fire, including creation of selected roosting and foraging habitats (Lacki et al. 2009, Dickinson et al. 2010, Johnson et al. 2010, Buchalski et al. 2013, Womack et al. 2013). Our data for bat activity across the landscape aligns more closely with Loeb and Waldrop (2008), who did not discern a difference in bat activity between burned and unburned habitats. Our study tempers the contemporary understanding of how foraging bats may respond to prescribed fire. While prescribed fire clearly serves the function of reducing clutter within forests used by foraging bats, our data suggest that bats at MCNP were not more active in burned habitats. Even so, the benefits gained through the creation of optimal roosting habitats by management with prescribed fire could confer an energetic advantage to bats during the maternity period. In terms of current JFSP research, our study offers a multi-species complement to other ongoing projects in the region investigating the effects of prescribed fire on the Indiana bat (JFSP #09-1-08-2) and northern long-eared bat (14-1-05-42).

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

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Womack, K.M., S.K. Amelon, and F.R. Thompson. 2013. Resource selection by Indiana bats during the maternity season. Journal of Wildlife Management 77: 707-715.

## 7. Future Work Needed

There is a critical need for comprehensive, continuous datasets that characterize the population dynamics and landscape-level distribution of endangered bat species and their prey prior to and concurrent with the arrival WNS. Given its history of prescribed fire, MCNP offers an ideal location to develop these data sets in the context of land management. Considering this, future field collections under JFSP #14-1-05-22 will allow for pre- and post-burn measurements, and will expand upon the design implemented in this study. JFSP funding will permit the development of a unique long-term data set ( $\geq 8$  years) that encompasses pre- and post-WNS conditions in cave hibernating bats and surrounding land parcels that span a gradient of time since fire ( $\geq 15$  years post-burn).

Specific project objectives for JFSP #14-1-05-22 include: 1) development of a post-WNS understanding of bat activity patterns at MCNP in relation to prescribed fire, 2) assessment of the developing impacts of WNS on bat body conditions, 3) evaluate potential changes in relative abundance and species diversity of insect herbivores in response to effects of WNS on cave-dwelling populations of bats, 4) evaluate growth and herbivory across oaks and their shade-tolerant competitors, and 5) develop an understanding of how prescribed fire may directly impact the productivity of foraging habitats for bats. This final objective for JFSP #14-1-05-22 will complement the current study by providing insight as to whether habitat structure or habitat “quality” in terms of prey availability is more fundamentally important to bats. In total, these data will provide an understanding of how prescribed fire may directly impact the productivity of foraging habitats for all insectivorous bats.

## 8. Deliverables Crosswalk Table

Proposed	Delivered	Status
Dataset	GIS maps of LiDAR-based forest structure	Complete.
Dataset	Relative abundance of insect prey	Complete.
Dataset	Multispecies bat foraging activity	Complete.
Scientific publications	Separate papers on 1) Fire effects on bat insect prey and 2) Bat foraging behavior	Ongoing. See Key Reports #12, 14, 15.
Synthesis publications	Synthesis of data on insect prey and foraging in a landscape context	Ongoing. See Key Reports #10-13, 16.
Website	Link at MCNP website	Server and web maintenance have not permitted NPS weblink. However, a description and partial deliverables of #10-1-06-1 are at <a href="http://brimsbg.org/">http://brimsbg.org/</a> , hosted in collaboration with MCNP.
Final workshop	MCNP workshop with partners, researchers, and fire managers	Hosted in conjunction with CAFMS on 30 Apr – 1 May 2014. A total of 95 individuals registered for workshop.

## 9. Comprehensive List of Deliverables to Date (as of June 2014)

### Final Report (This Document)

Lacki, M.J., L.E. Dodd, N.S. Skowronski, M.B. Dickinson, and L.K. Rieske. 2014. Fire management and habitat quality for endangered bats in Kentucky's Mammoth Cave National Park during the swarming and staging periods: predator-prey interactions and habitat use of bats threatened by White-Nose Syndrome. Final Project Report (JFSP Project Number 10-1-06-1). June 26, 2014. Lexington, KY. 104 p.

### Knowledge Transfer Workshops and Advisory Meetings

Discussing the relationships between fire management and the quality of habitat for bats: A workshop for scientists and land managers. Organized in conjunction with the Consortium of Appalachian Fire Managers and Scientists. 30 April – 1 May 2014, Mammoth Cave, KY. Presentations specific to JFSP Project #10-1-06-1 include:

Dodd, L.E., M.J. Lacki, N.S. Skowronski, M.B. Dickinson, and L.K. Rieske. Investigating the effects of fire & forest canopy conditions on the abundance & diversity of insects at Mammoth Cave National Park.

Dodd, L.E., M.J. Lacki, N.S. Skowronski, M.B. Dickinson, and L.K. Rieske. Modeling bat activity across the fire-managed landscape of Mammoth Cave Nat'l Park using remotely-sensed forest canopy data.

Dodd, L.E., and L.K. Rieske. Assessing the effects of prescribed fire on the herbivore load and growth of white oak seedlings.

M.J. Lacki, D.R. Cox, L.E. Dodd, and M.B. Dickinson. Response of northern long-eared bats (*Myotis septentrionalis*) to prescribed fires in eastern Kentucky forests.

M.J. Lacki, L.E. Dodd, R.S. Toomey, S.C. Thomas, Z. Couch, and B. Nichols. Body condition of bats during staging and swarming periods in Mammoth Cave National Park: Before and after Arrival of White-Nose Syndrome.

Dodd, L.E., M.J. Lacki, N.S. Skowronski, D.R. Cox, M.B. Dickinson, and L.K. Rieske-Kinney. Responses of *Myotis* bats & their prey to the effects of prescribed fire in the central hardwood forests of Kentucky. Prescribed Fire and Indiana Bats Workshop, Hosted by the USDA Forest Service, Indiana State University, and the Consortium of Appalachian Fire Managers and Scientists. 16-17 April 2013, Fontana Dam, NC.

### Other Professional Presentations and Invited Talks

Dodd, L.E., M.J. Lacki, N.S. Skowronski, M.B. Dickinson, and L.K. Rieske-Kinney. Predicting bat activity across Mammoth Cave National Park using LiDAR-derived predictors of forest

canopy conditions. Annual Meeting of the Kentucky Bat Working Group. 18 November 2013, Frankfort, KY.

Dodd, L.E., M.J. Lacki, and L.K. Rieske-Kinney. Exploring prey size and diet breadth of Rafinesque's big-eared bat, a lepidopteran specialist. Annual Meeting of the Entomological Society of America. 10-13 November 2013, Austin, TX.

Dodd, L.E., M.J. Lacki, and L.K. Rieske-Kinney. Assessing dietary specialization of Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) from a maternity colony at Mammoth Cave National Park. 2<sup>nd</sup> International Symposium on the Detection of Trophic Interactions. 13-16 May 2013, Lexington, KY.

Dodd, L.E., M.A. Floyd, and D.A. Etnier. Seasonal occurrence and habitat affiliations of Trichoptera at Mammoth Cave National Park. (Poster) Mammoth Cave National Park's 10<sup>th</sup> Research Symposium, 14-15 February 2013, Mammoth Cave, KY.

Dodd, L.E., N. S. Skowronski, M. B. Dickinson, M. J. Lacki, and L. K. Rieske. Using LiDAR to link forest canopy structure with bat activity and insect occurrence: preliminary findings. Mammoth Cave National Park's 10<sup>th</sup> Research Symposium, 14-15 February 2013, Mammoth Cave, KY.

Dodd, L.E., N.S. Skowronski, M.B. Dickinson, M.J. Lacki, and L.K. Rieske-Kinney. Using LiDAR to link forest canopy conditions with diversity patterns of Lepidoptera at Mammoth Cave National Park. 54<sup>th</sup> Annual Southern Forest Insect Work Conference. 24-27 July 2012, Charlottesville, VA.

Dodd, L.E., T.L. Culbertson, M.J. Lacki, and L.K. Rieske-Kinney. Investigating the relationships between bats, insects, and fire at Mammoth Cave National Park. National Parks Service's Junior Ranger Day. 28 April 2012, Mammoth Cave National Park, KY.

Dickinson, M.B., L.E. Dodd, N. Skowronski, M.J. Lacki, and L.K. Rieske-Kinney. Direct and habitat effects of fire on forest bats. Annual Meeting of the Kentucky Prescribed Fire Council. 13 September 2011. Greenville, KY.

Dodd, L.E., T.L. Culbertson, M.J. Lacki, and L.K. Rieske-Kinney. Investigating the relationships between bats, insects, and fire at Mammoth Cave National Park. 1<sup>st</sup> Annual International Bat Night. 27 August 2011, Mammoth Cave National Park, KY.

Dodd, L.E., M.J. Lacki, and L.K. Rieske-Kinney. First-year responses of forest bats and their arthropod prey to prescribed fire during the swarming period at Mammoth Cave National Park (Poster). Joint Meeting of the Northeastern Bat Working Group, Southeastern Bat Diversity Network, and Colloquium on the Conservation of Mammals in the Eastern United States. 23-25 February 2011, Louisville, KY.

### Publications In Print / In Press

Dodd, L.E., and L.F. Faust. 2014. Seasonal occurrence and habitat affiliations of Lampyridae at Mammoth Cave National Park, Kentucky. *Journal of the Kentucky Academy of Science*, In Press.

Dodd, L.E., M.A. Floyd, and D.A. Etnier. 2013. Seasonal occurrence and habitat affiliations of Trichoptera at Mammoth Cave National Park. *Proceedings of Mammoth Cave National Park's 10<sup>th</sup> Research Symposium* (ed Trimboli, S.R.). Publication available at: <http://brimsg.org/wp-content/uploads/2013/02/Symposium-Proceedings.pdf>  
Pp. 44-49 (of 207 p.).

Dodd, L.E., N. S. Skowronski, M. B. Dickinson, M. J. Lacki, and L. K. Rieske. 2013. Using LiDAR to link forest canopy structure with bat activity and insect occurrence: preliminary findings. *Proceedings of Mammoth Cave National Park's 10<sup>th</sup> Research Symposium* (ed Trimboli, S.R.). Publication available at: <http://brimsg.org/wp-content/uploads/2013/02/Symposium-Proceedings.pdf>  
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Dodd, L.E., and J.S. Johnson. 2012. Observations of potential spring mating behavior in the eastern pipistrelle (*Perimyotis subflavus*). *Bat Research News*, 53: 37-38.

### Publications Pending

Dodd, L.E., M.J. Lacki, J.S. Johnson, and L. K. Rieske. In Review. Prey size and dietary breadth in a gleaning bat, *Corynorhinus rafinesquii*. *Acta Chiropterologica*.

Dodd, L.E., M.J. Lacki, N.S. Skowronski, M.B. Dickinson, and L.K. Rieske. In Preparation. Multi-year impacts of prescribed fire on bats and their prey in a central hardwoods ecosystem. Target Journal: *Forest Ecology and Management*.

Dodd, L.E., L.K. Rieske. In Preparation. Multi-year impacts of fire on vigor and herbivory loads of white oak seedlings. Target: Undecided.

Dodd, L.E., N.S. Skowronski, M.B. Dickinson, M.J. Lacki, and L.K. Rieske. In Preparation. Predicting bat activity across eastern deciduous habitats using forest canopy conditions. Target Journal: *Remote Sensing of Environment*.

Lacki, M.J., L.E. Dodd, R.S. Toomey, S.C. Thomas, Z.L. Couch, and B.S. Nichols. In Preparation. Body condition of cave-hibernating bats during staging and swarming at Mammoth Cave National Park. Target Journal: *Journal of Fish and Wildlife Management*.

## **10. Key Report: Using LiDAR to Link Forest Canopy Structure with Bat Activity and Insect Occurrence.**

*Published in the Proceedings of Mammoth Cave National Park's 10<sup>th</sup> Research Symposium.*

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### Abstract

Bats are an imperiled, yet ecologically-important group of vertebrate predators. Our ongoing research focuses on testing hypotheses about the relationships between the effects of fire on canopy structure and insect prey availability, and how these factors relate to use of foraging space by bats during the pre- and post-hibernation periods at Mammoth Cave National Park (MCNP). LiDAR-derived data (October 2010) were intersected with spatially explicit sampling of bat and insect populations (2010-2011) in order to characterize relationships between canopy structure, insect abundance, and bat activity. A canonical correspondence analysis for bat data suggested that forest canopy structure has a strong relationship with bat activity, particularly for species that echolocate at higher frequencies. Less variation was accounted for in a canonical correspondence analysis of insect occurrence. Even so, this analysis still demonstrated that variation in forest canopy structure influences the insect community at MCNP, albeit in varied ways for specific orders of insects.

### Introduction

Remote sensing techniques such as light-detection and ranging (LiDAR) have expanded the scale and scope of ecological studies, allowing for more effective management of an expanding number of wildlife species (Vierling et al. 2008, Hudak et al. 2009). As bats are an imperiled and ecologically-important group of vertebrate predators, our study was initiated to relate the relative activity of these predators with the occurrence of their insect prey across the gradient of forest conditions found at Mammoth Cave National Park (MCNP). This ongoing project focuses on testing hypotheses about the relationships between the effects of fire on insect prey availability and canopy structure, and how these factors relate to use of foraging space by bats during the pre- and post-hibernation periods at MCNP. Aboveground habitat quality pre- and post-hibernation is critical because bats must go into hibernation with sufficient fat reserves and often leave hibernation in poor condition. A better understanding of the spatial and temporal patterns associated with bat foraging is important given the recent arrival of White-nose Syndrome (WNS) at MCNP.

## Methods

Mammoth Cave National Park encompasses 23,000 ha in Barren, Hart, and Edmonson counties on the edge of the Crawford-Mammoth Cave Uplands of the Interior Plateau of Kentucky (Woods et al. 2002). We developed three-dimensional canopy height models across the entirety of MCNP in October of 2010 using discrete-return scanning LiDAR ( $>4$  pulses /  $m^2$ ). We processed these data using “Toolbox for LiDAR data Filtering and Forest studies” software (Chen et al. 2007). The output from this processing included high resolution digital elevation models, canopy height models, as well as three-dimensional canopy height profiles (Skowronski et al. 2007). These canopy height profiles allowed assessment of the density of vegetation throughout the forest canopy (Figure 10.1).

LiDAR-derived data were intersected with spatially explicit sampling of bat and insect populations in order to characterize relationships between canopy structure, insect occurrence, and bat activity. We conducted surveys for bat activity and nocturnal insect occurrence from September 2010 through October 2011 using acoustic detectors and blacklight traps, respectively. These surveys took place across an array of upland and riverine habitats that covered a range of forest canopy heights. Transects were used for both techniques, which entailed multiple surveys points (all  $\geq 100$  m apart). We surveyed transects in tandem so that monitoring took place at a burned land parcel simultaneous with an unburned land parcel.

We assessed bat activity using the Anabat II system (Titley Electronics, Columbia, Missouri) powered by a 12 V gel-cell battery and housed in plastic containers to protect equipment from inclement weather (O’Ferrell 1998). Acoustic surveys spanned multiple (2-3) nights to account for nightly variation ( $n = 4$  acoustic detectors / transect). Despite standard placement and operation, the potential existed for microphone sensitivity to vary over time, as well as between units, so we regularly calibrated acoustic detectors using an ultrasonic insect repeller (Britzke 2004). Analysis of acoustic data collected between sunset and sunrise was carried out using Echoclass v.1.1, an automated software package for acoustic identification developed by the U.S. Army Engineer Research and Development Center and provided by the U.S. Fish and Wildlife Service (USFWS 2012). With this software, echolocation pulses are isolated into high frequency ( $> 34$  kHz) and low frequency ( $\leq 34$  kHz) categories (E. Britzke, U.S. Army Engineer Research and Development Center, pers. comm.). The resulting response variables we considered for bat activity were the numbers of echolocation files and pulses within the high and low-frequency categories, on a per night basis. The number of feeding buzzes isolated per night from echolocation data was considered as an additional response variable indicative of foraging activity by bats.

We assessed insect occurrence using 10-W blacklight traps (Universal Light Trap, Bioquip Products, Gardena, California). A single survey night for insects was conducted in the same land parcels as that for concurrent acoustic surveys ( $n = 4$  traps / transect). As per recommendations of Yela and Holyoak (1997) for sampling Lepidoptera, survey were conducted on nights with temperatures  $\geq 16^\circ$  C at sunset, no precipitation, and low wind speeds. We suspended blacklight traps 2.5-m aboveground prior to sunset and operated traps throughout the entire night. A dichlorvos-based ‘pest strip’ (ca.  $2 \times 6$ -cm) was placed within each blacklight trap to subdue specimens. Insects were identified using keys (Covell 2005, Triplehorn and Johnson

2005) and reference collections at the University of Kentucky. Insects  $\geq 10$  mm in length were identified to the lowest taxon practical. Response variables were numbers per night for the most abundant orders we recorded: Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera.

We used canonical correspondence analysis (CCA) to explore relationships between forest canopy structure and bats and insects separately. Variables describing density of vegetation throughout the forest canopy follow those developed by Lesak et al. (2011) and were based on a 15-m radius around each faunal survey point. These forest canopy variables describe the relative density of vegetation in the understory, midstory, and overstory (referred to as “canopy” in Lesak et al. 2011), and the relative proportions of these strata in relation to one another (i.e., ratios of midstory to overstory, understory to midstory, and understory to overstory). We generated a gap index for each faunal survey point; this variable was a proportional expression of the absence of vegetation  $>3$  m in height. This index thus considered the lack of taller vegetation (or “gap”) within a 15-m radius around each faunal sampling point. Data were analyzed in PC-ORD v.4.25 following standard ordination techniques (McCune and Grace 2002) using default settings; Monte Carlo tests of significance were run for 300 iterations. Relationships within and between faunal and LiDAR-derived data were explored using biplots.

## Results

Bat surveys were carried out over 114 nights during August-October of 2010 and April-October of 2011, yielding a total of 769 detector-nights. These data were collected prior to the detection of WNS at MCNP. The CCA of bat activity with forest canopy structure was significant (Table 10.1), and explained over 47% of the variation in acoustic data. High-frequency and low-frequency variables were broadly separated in multivariate space (Figure 10.2). A closer association was observed between the high frequency variables than between the low frequency variables. Variation in high frequency variables was more closely associated with variation of forest canopy variables than was variation in low frequency variables. The proportion of overstory, proportion of midstory, and gap index had the strongest relationships with bat activity. In contrast, the ratio of understory to overstory strata had the weakest relationship. High frequency bat activity was positively associated with an increased proportion of vegetation density in the overstory and midstory. Low frequency bat activity was less associated with forest canopy variables; however, low frequency pulses closely aligned with gap index, indicating a weak positive association between these variables. The incidence of feeding buzzes did not have a strong association with forest canopy variables.

Insect surveys were carried out over 41 nights concurrent with acoustic surveys, yielding a total of 205 trap-nights. The CCA of insect occurrence with forest canopy structure was significant (Table 10.1), and explained over 10% of the variation in the insect data. Abundance of various insect orders separated out in multivariate space (Figure 10.3). Abundance of Diptera and Hemiptera were closely associated with one another and separate from abundance of Coleoptera and abundance of Lepidoptera. The latter two orders were also separated from one another. Abundance of Hymenoptera was widely separated from other variables, and consequently had little weight on the analysis. The proportion of understory, proportion of overstory, and gap index had the strongest relationships with insect abundance, whereas the ratio of midstory to overstory strata had the weakest relationship. Abundance of Diptera and

Hemiptera were positively associated with an increased proportion of vegetation density in both the overstory and understory. Abundance of Coleoptera was distantly associated with gap index. Abundance of Lepidoptera was less associated with the first axis, but closely aligned with the ratio of understory to overstory strata.

## Discussion

These analyses are a first step towards elucidating the role that forest canopy structure plays in determining aboveground habitat use by bats at MCNP. Our data suggest that forest structure has a strong relationship with bat activity, particularly for species that echolocate at higher frequencies. This finding largely agrees with observations that show bats that echolocate at higher frequencies tend to be more capable of flight in “cluttered” habitats that possess an increased density of vegetation (Barclay and Brigham 1991, Swartz et al. 2003). Conversely, we found a reduced association between low frequency bat activity and forest canopy variables. This outcome is consistent with the use of open “uncluttered” foraging space by low-frequency echolocating bats in other habitats (Aldridge and Rautenbach 1987, Saunders and Barclay 1992), and with data that demonstrate North American bats which use low frequency echolocation also possess wing morphologies suited for flight in habitats with decreased clutter (Bogdanowicz et al. 1999, Lacki et al. 2007). The association we observed between low frequency bat activity and an increased gap index, while weak, further supports these patterns in habitat use.

While less variation was accounted for in the CCA of insect occurrence, those data still demonstrate that variation in forest canopy structure influences the insect community at MCNP. Multiple insect orders were positively related with an increased density of vegetation in the understory strata (Diptera, Hemiptera, and Lepidoptera). The associations between specific insect orders and canopy conditions are complex, however, given: 1) the ordination positions of forest canopy variables relating to the upper strata, and 2) the wide ecological and taxonomic diversity seen across these common insect orders. Regardless, affiliations between insect groups and specific strata in the forest canopy likely relates to varied abundance and utilization of host resources (Ober and Hayes 2008, Dodd et al. 2012). The orders of prey most consistently consumed by North American bats (Coleoptera, Diptera, and Lepidoptera; Lacki et al. 2007) separated from one another in our ordination. This suggests broad differences in forest canopy conditions where these insect orders are most common. Since the relative consumption of these orders of prey does vary across bat species, it will be important to determine in future analyses whether any affiliations between insects and cluttered foraging spaces may translate to increased availability of preferred prey for specific species groups of bats (i.e., those tending to use either high or low frequency echolocation).

Despite the link between cluttered forest canopies and high frequency bat activity, we did not see a strong association between feeding buzzes and any forest canopy variable. We offer several possible explanations. First, high-frequency bats may actively move through cluttered space, but may not feed extensively in these canopy conditions due to reduced foraging success (Bogdanowicz et al. 1999, Swartz et al. 2003). Second, some high-frequency bats (i.e., the northern myotis, *Myotis septentrionalis*), are capable of feeding in cluttered habitats by gleaning insects from the surface of vegetation, where feeding activity is based on insects located by passive listening and not echolocation (Faure et al. 1993, Ratcliffe and Dawson 2003). Third, the

feeding buzz variable considered in our analysis incorporated both high and low frequency echolocation pulses. Thus, potential relationships between forest canopy variables and a variable representing foraging success for bats that echolocate at either high or low frequencies may have been masked. Regardless, our findings indicate that forest canopy structure influences activity of bats. The extent to which feeding behavior of insectivorous bats is influenced by canopy structure, however, remains less clear. Based on our findings we postulate that canopy structure may be of less importance for feeding success of insectivorous bats than previously hypothesized (Hayes and Loeb 2007). Further studies are needed to confirm or refute this possibility.

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Table 10.1. Summary of canonical correspondence analyses relating both bat activity and insect occurrence to forest canopy variables for Mammoth Cave National Park.

Summary Statistic	Bat CCA	Insect CCA
Total Variance ("Inertia") of Response Variables	0.82	1.03
Eigenvalue for First Axis	0.390	0.108
Variance Explained by First Axis (%)	47.4	10.5
Monte-Carlo Test of Correlations in First Axis ( <i>P</i> -value)	0.001	0.05
Eigenvalue for Second Axis	0.002	0.022
Variance Explained by Second Axis (%)	0.3	2.1
Monte-Carlo Test of Correlations in Second Axis ( <i>P</i> -value)	0.10	0.61

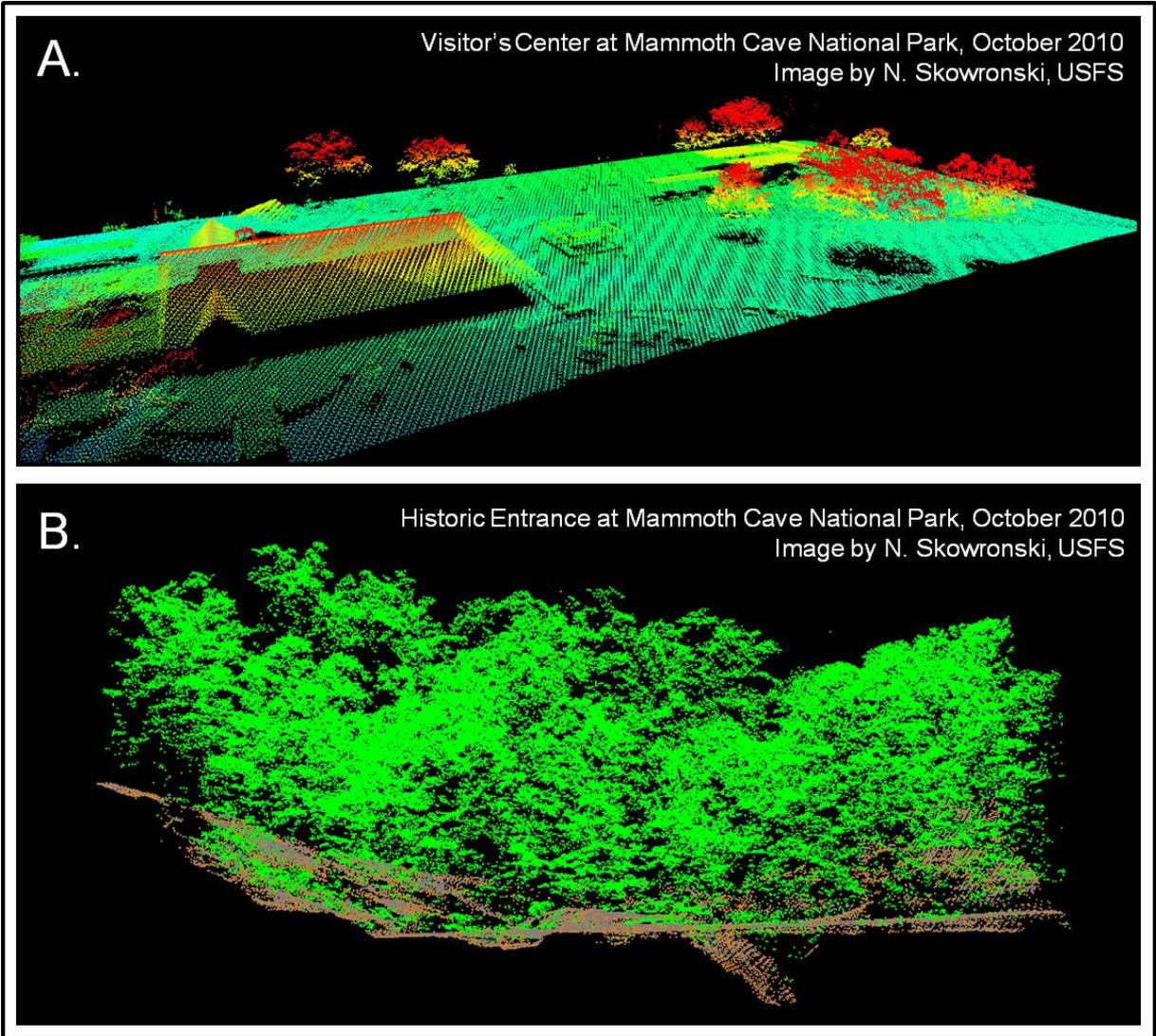


Figure 10.1. LiDAR-derived images demonstrating three-dimensional data derived for Mammoth Cave National Park.

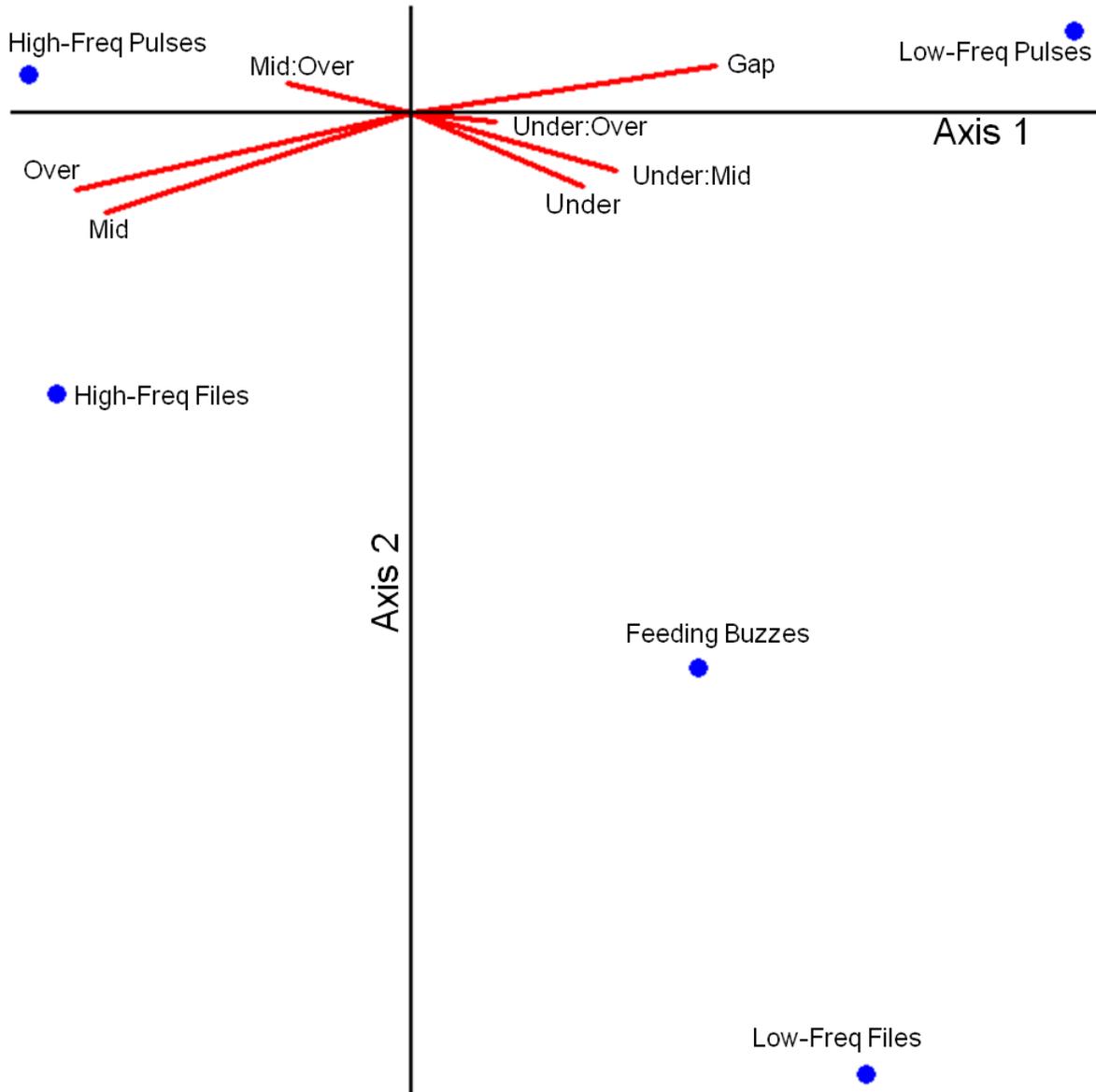


Figure 10.2. A biplot based on a canonical correspondence analysis of bat activity and forest canopy variables for Mammoth Cave National Park (using LC scores). The ordination shows the relative relationships between bat activity variables (circles) and forest canopy variables (vectors). Abbreviated forest canopy variables are: gap index (gap), relative proportion of midstory (mid), relative proportion of overstory (over), relative proportion of understory (under), ratio of relative proportion of midstory to relative proportion of overstory (mid:over), ratio of relative proportion of understory to relative proportion of midstory (under:mid), and ratio of the relative proportion of understory to relative proportion of overstory (under:over).

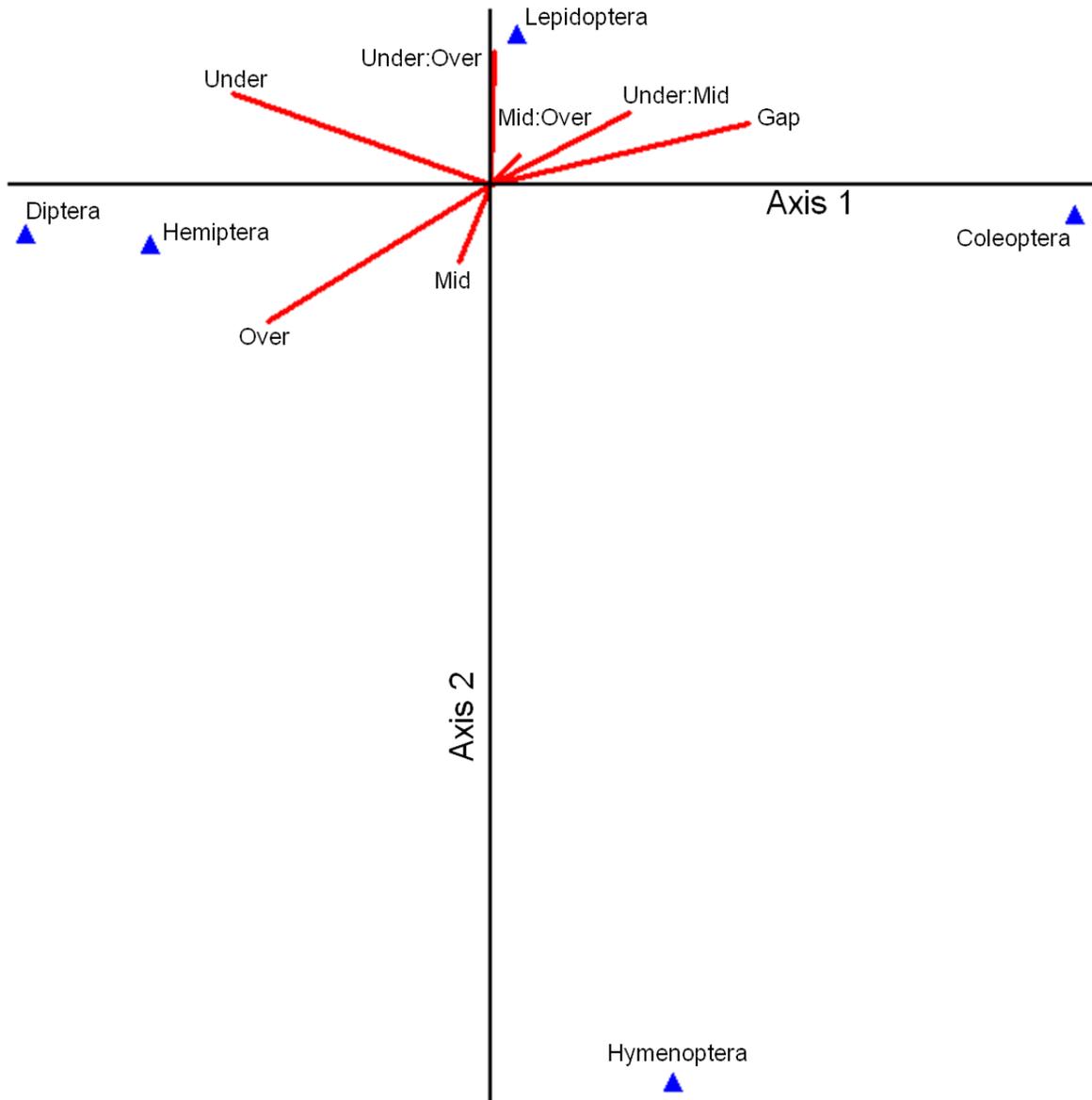


Figure 10.3. A biplot based on a canonical correspondence analysis of insect abundance and forest canopy variables for Mammoth Cave National Park (using LC scores). The ordination shows the relative relationships between insect abundance variables (triangles) and forest canopy variables (vectors). Abbreviated forest canopy variables are: gap index (gap), relative proportion of midstory (mid), relative proportion of overstory (over), relative proportion of understory (under), ratio of relative proportion of midstory to relative proportion of overstory (mid:over), ratio of relative proportion of understory to relative proportion of midstory (under:mid), and ratio of the relative proportion of understory to relative proportion of overstory (under:over).

## **11. Key Report: Modeling bat activity at Mammoth Cave National Park using ALS-derived descriptors of forest canopy conditions.**

### Introduction of Methods

Mammoth Cave National Park encompasses 23,000 ha in Barren, Hart, and Edmonson counties on the edge of the Crawford-Mammoth Cave Uplands of the Interior Plateau of Kentucky (Woods et al. 2002). Airborne Laser Scanning data (ALS) was collected across the entirety of MCNP in October of 2010 (>4 pulses / m<sup>2</sup>). We processed these data using “Toolbox for ALS data Filtering and Forest studies” software (Chen et al. 2007). The output from this processing estimated digital elevation models, canopy height models, variables derived from the pulse height distribution (e.g. mean height, max height, percentile heights, etc.), as well as three-dimensional canopy height profiles (CHP; Skowronski et al. 2007) at various resolutions continuously across the study extent. These ALS-derived data products were also estimated within individual polygons where it intersected with spatially explicit surveys of bat activity in order to characterize relationships with canopy structure.

We conducted surveys for bat activity from August 2010 through October 2011 using acoustic detectors deployed along a series of transects across MCNP (Figure 11.1, Figure 11.2). For each survey location, we recorded location and altitude using digital topographic maps and Global Positioning Systems (GPS). Locations were recorded at an accuracy  $\pm 10$  m. Surveys took place across an array of upland and riparian habitats that covered a range of forest canopy heights; transects were surveyed in tandem so that monitoring took place at a burned land parcel simultaneous with an unburned land parcel (or riparian corridor). These survey locations were selected using a stratified random approach, wherein land parcels were identified based on burn history. Once selected, the specific positions of survey transects were randomly selected and then surveyed in a random order. Repeated sampling occurred without replacement across the complement of land parcels in both staging and swarming periods. A subsample of long-term monitoring locations was also monitored during the maternity season.

Bat activity was assessed using the Anabat II system (Titley Electronics, Australia) powered by a 12 V gel-cell battery and housed in plastic containers to protect equipment from inclement weather (O’Ferrell 1998). Housed detection systems were mounted upon 1.5-m camera tripods for surveys, allowing position above re-growth of understory vegetation while sampling within the forest canopy. Each acoustic survey spanned multiple nights ( $\geq 3$ ) to account for nightly variation and equipment failure. Despite standard placement and operation, the potential exists for microphone sensitivity to vary over time, as well as between units, so the sensitivity of detection systems was calibrated annually using an ultrasonic sound emitter (Britzke 2004). Analysis of acoustic data collected between sunset and sunrise was carried out using Echoclass v.1.1, an automated software package for acoustic identification developed by the U.S. Army Engineer Research and Development Center and provided by the U.S. Fish and Wildlife Service (USFWS 2012). With this software, echolocation pulses are isolated into high frequency ( $> 34$  kHz) and low frequency ( $\leq 34$  kHz) categories (E. Britzke, U.S. Army Engineer Research and Development Center, pers. comm.). Echolocation data were then visually inspected for quality control using Analook software (v. 4.9). The resulting response variables considered for bat activity were the numbers of echolocation pulses isolated into the high and low-frequency

categories, on a per night basis. We also considered the echolocation pulses identified as belonging to the Indiana bat (*Myotis sodalis*) as an additional species-specific response variable (following default settings of Echoclass v. 1.1).

We then derived a suite of forest canopy descriptors for our acoustic survey points using the ALS data set. This suite of variables incorporated descriptors based on the absolute measurements of ALS hits at 10 m increments throughout the forest canopy, as well as measurements for total canopy height and canopy gap (Figure 11.3). This suite also incorporates predictive variables developed by Lesak et al. (2011), which scales the incidence of ALS hits throughout the forest canopy by collapsing ALS data into 10 proportionate bins scaled to the height of the canopy. All descriptors were based on a 15 m radius centered on an acoustic survey point. These descriptive variables included:

- total density (sum of all ALS-derived CHP from the ground to the top of the canopy)
- gap index (percent of open air space >3 m in height without vegetative structure)
- canopy height (height of canopy at the 90<sup>th</sup> percentile of ALS hits aboveground)
- understory density (sum of ALS-derived CHP from the ground to 10-m aboveground)
- midstory density (sum of ALS-derived CHP from 10 to 20-m aboveground)
- overstory density (sum of ALS-derived CHP from 20 to 30-m aboveground)
- legacy density (sum of ALS-derived CHP > 30-m aboveground)
- $P_{\text{Understory}}$  (percent of ALS-derived CHP in the bottom 2 bins of scaled data)
- $P_{\text{Midstory}}$  (percent of ALS-derived CHP in intermediate 3<sup>rd</sup> through 6<sup>th</sup> bins of scaled data)
- $P_{\text{Canopy}}$  (percent of ALS-derived CHP in the upper 7<sup>th</sup> through 10<sup>th</sup> bins of scaled data)
- $R_{\text{Understory:Midstory}}$  (ratio of  $P_{\text{Understory}}$  to  $P_{\text{Midstory}}$ )
- $R_{\text{Understory:Canopy}}$  (ratio of  $P_{\text{Understory}}$  to  $P_{\text{Canopy}}$ )
- $R_{\text{Midstory:Canopy}}$  (ratio of  $P_{\text{Midstory}}$  to  $P_{\text{Canopy}}$ )
- $R_{\text{Total:Understory}}$  (ratio of total density to understory density)

We used multiple linear regression in conjunction with Akaike’s Information Criterion (AIC) model rankings (Burnham and Anderson 2002) to identify the most parsimonious models for predicting activity of high-frequency echolocators, low-frequency echolocators, and the Indiana bat. We derived 4 *a priori* canopy structure models to be evaluated for each bat response variable. These models corresponded to specific portions of the forest canopy (understory, midstory, and overstory), as well as a model describing the entirety of clutter (hereafter, “total clutter”). Component predictor variables for the models are as follows:

- total clutter: total returns, gap index, canopy height
- overstory: overstory density, legacy tree density,  $P_{\text{Canopy}}$
- midstory: midstory density,  $P_{\text{Midstory}}$ ,  $R_{\text{Understory:Midstory}}$ ,  $R_{\text{Midstory:Canopy}}$
- understory: understory density,  $R_{\text{Total:Understory}}$ ,  $P_{\text{Understory}}$ ,  $R_{\text{Understory:Canopy}}$

We used AIC scores relative to the smallest AIC value ( $\Delta\text{AIC}$ ) and Akaike weights ( $w_i$ ) to assess the suitability of habitat models (Burnham and Anderson 2002, Arnold 2010). For models with strong support, we identified significant parameter estimates ( $P < 0.05$ ) to elucidate which canopy descriptors within a model best described the variation observed for a bat response variable.

## Discussion of Results

A total of 39 acoustic surveys were conducted from 2010-2011, with effort covering 125 nights. This resulted in 836 detector-nights from 109 survey locations used for model development. From this, 35,872 echolocation files were recorded. A total of 63% of the 369,253 pulses were classified as high-frequency, and the remainder as low-frequency. Of these recordings, 790 files were identified as belonging to the Indiana bat.

For high-frequency activity, both understory and midstory models were significant ( $F_{4,836} = 13.1$ ,  $P = 0.001$ , and  $F_{4,836} = 9.8$ ,  $P = 0.001$ , respectively). Of these, little support was seen for the midstory model versus the understory model (Table 11.1). For this understory model, parameter estimates for understory density,  $P_{\text{Understory}}$ ,  $R_{\text{Understory:Canopy}}$  were significant (Table 11.2). This model suggests that while high-frequency echolocators are found in habitats with less clutter in the understory strata, some level of structure in this understory versus the overstory strata is also relevant (i.e., an uncluttered mixed-age stand condition).

For low frequency activity, total clutter and understory models were significant ( $F_{3,836} = 6.5$ ,  $P = 0.001$ , and  $F_{4,836} = 4.1$ ,  $P = 0.003$ , respectively). Of these, the understory model received limited support versus the total clutter model (Table 11.1). Parameter estimates for the total clutter model suggest that low-frequency echolocators are more active in areas with a greater gap index (i.e., more open air space) (Table 11.2). Considering the less-supported understory model, parameter estimates for  $R_{\text{Total:Understory}}$  and  $P_{\text{Understory}}$  suggest increased activity at areas with proportionately lower measurements of understory clutter.

For activity of Indiana bats, models were significant for total clutter ( $F_{3,836} = 4.1$ ,  $P = 0.01$ ), understory ( $F_{4,836} = 9.2$ ,  $P = 0.001$ ), midstory ( $F_{4,836} = 5.9$ ,  $P = 0.001$ ), and overstory ( $F_{3,836} = 6.0$ ,  $P = 0.001$ ). Considering AIC rankings, however, only the understory model received support. For this understory model, parameter estimates for  $P_{\text{Understory}}$  and  $R_{\text{Understory:Canopy}}$  were significant (Table 11.2). Similar to the most parsimonious model for high-frequency echolocators, the strength of these parameter estimates suggests that Indiana bats were more active in areas with proportionately less clutter in the understory strata.

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<http://www.fws.gov/midwest/Endangered/mammals/inba/inbasummersurveyguidance.html>

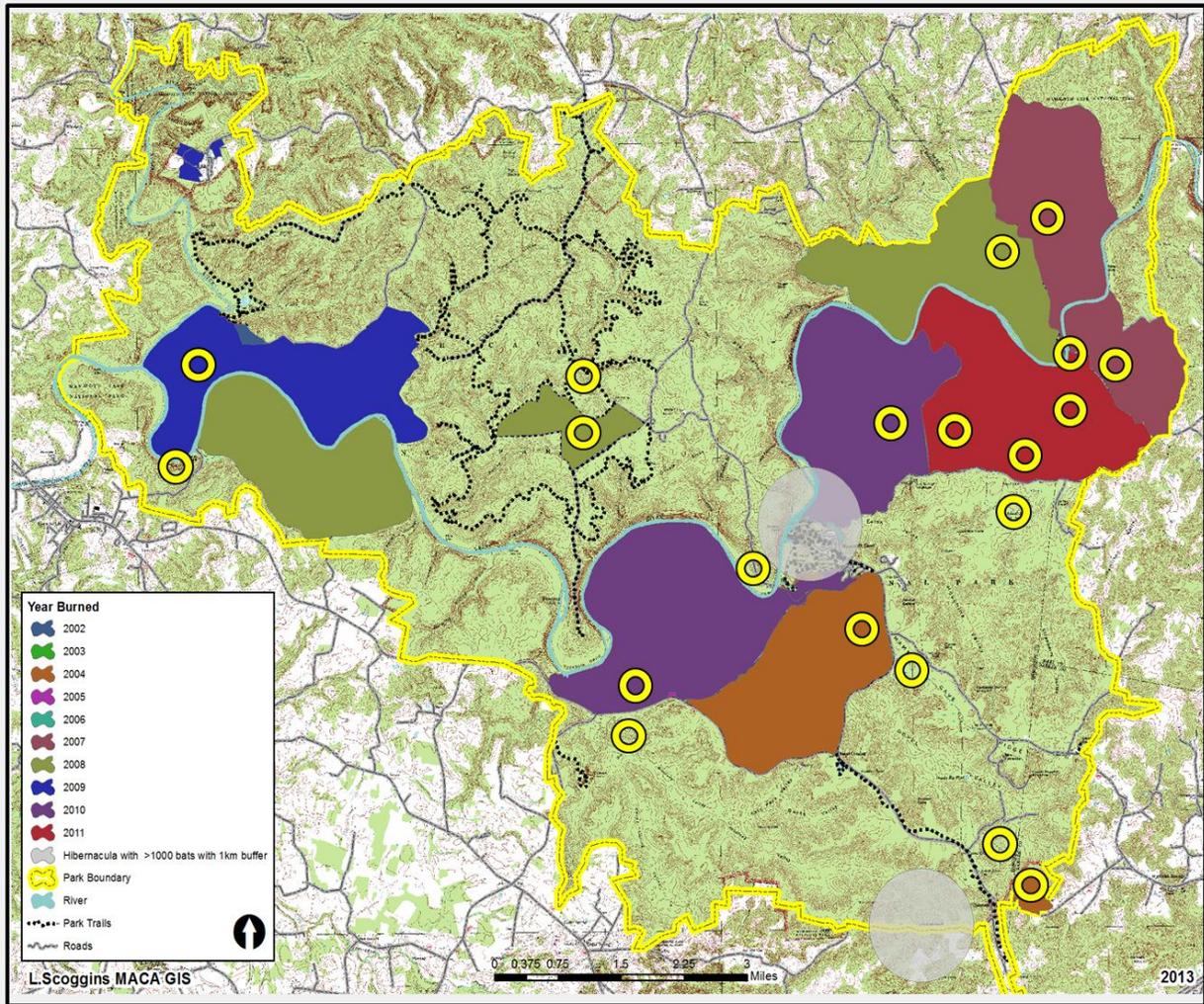
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Table 11.1. Akaike's Information Criterion scores (AIC), difference in AIC values ( $\Delta$ AIC), Akaike weights ( $w_i$ ), and number of parameters ( $K$ ) for linear regression models of bat activity by ALS-derived descriptors of vegetation throughout the forest canopy at Mammoth Cave National Park, USA, from 2010-2011. Models with an asterisk were significant ( $P \leq 0.05$ ).

Response Variable	Model	AIC	$\Delta$ AIC	$w_i$	$K$
High-Frequency Pulses ( $n = 836$ )	Understory*	10944.3	0.0	0.99	6
	Midstory*	10957.1	12.8	< 0.01	6
	Total Clutter	10988.2	43.9	< 0.01	5
	Overstory	10992.6	48.3	< 0.01	5
Low-Frequency Pulses ( $n = 836$ )	Total Clutter*	12476.36	0	0.93	5
	Understory*	12481.47	5.1	0.07	6
	Overstory	12490.56	14.2	< 0.01	5
	Midstory	12492.59	16.2	< 0.01	6
<i>M. sodalis</i> Pulses ( $n = 836$ )	Understory*	7525.05	0.0	0.99	6
	Midstory*	7537.94	12.9	< 0.01	6
	Overstory*	7541.34	16.3	< 0.01	5
	Total Clutter	7546.81	22	< 0.01	5

**Table 11.2.** Parameter estimates ( $\beta$ ) and standard errors (SE) for ALS-derived descriptors of the forest canopy used for models of bat activity at Mammoth Cave National Park, USA, from 2010-2011. Parameter estimates indicated by an asterisk were significant within a model ( $P \leq 0.05$ ).

Model	Canopy Descriptor	Parameter Estimate ( $\beta$ ) $\pm$ SE		
		High-Frequency Pulses	Low-Frequency Pulses	<i>M. sodalis</i> Pulses
Total Clutter	Total Density	-44.2 $\pm$ 40.1	39.4 $\pm$ 97.6	2.7 $\pm$ 5.1
	Gap Index	300.6 $\pm$ 448.7	3846.4 $\pm$ 1092.6*	95.2 $\pm$ 57.3
	Canopy Height	4.6 $\pm$ 6.0	4.5 $\pm$ 14.6	2.4 $\pm$ 0.8*
Overstory	Overstory Density	-42.3 $\pm$ 56.4	-28.4 $\pm$ 138.2	-1.8 $\pm$ 7.2
	Legacy Tree Density	226.8 $\pm$ 294.9	-402.3 $\pm$ 722.5	140.4 $\pm$ 37.4*
	P <sub>Canopy</sub>	76.8 $\pm$ 152.0	-713.3 $\pm$ 372.4*	-20.4 $\pm$ 19.3
Midstory	Midstory Density	67.1 $\pm$ 46.9	-199.7 $\pm$ 117.6	-2.7 $\pm$ 6.1
	P <sub>Midstory</sub>	-1653.3 $\pm$ 346.7*	-434.2 $\pm$ 868.5	-72.7 $\pm$ 44.9
	R <sub>Understory:Midstory</sub>	-22.9 $\pm$ 9.9*	-7.7 $\pm$ 24.9	-1.4 $\pm$ 1.3
	R <sub>Midstory:Canopy</sub>	365.2 $\pm$ 59.5*	129.8 $\pm$ 149.0	30.0 $\pm$ 7.7*
Understory	Understory Density	-254.3 $\pm$ 85.5*	-598.7 $\pm$ 214.5*	-19.2 $\pm$ 11.1
	R <sub>Total:Understory</sub>	291.4 $\pm$ 378.3	1875.4 $\pm$ 948.7*	-2.5 $\pm$ 48.9
	P <sub>Understory</sub>	-1635.6 $\pm$ 344.5*	130.6 $\pm$ 864.0	-172.2 $\pm$ 44.6*
	R <sub>Understory:Canopy</sub>	408.3 $\pm$ 65.0*	93.0 $\pm$ 162.9	46.5 $\pm$ 8.4*



**Figure 11.1.** Map of MCNP showing most recent prescribed fires across burn units. Generalized locations of transects used for surveying bats and insects are denoted with ring symbols. Map courtesy of Lillian Scoggins, Mammoth Cave National Park, US National Park Service.



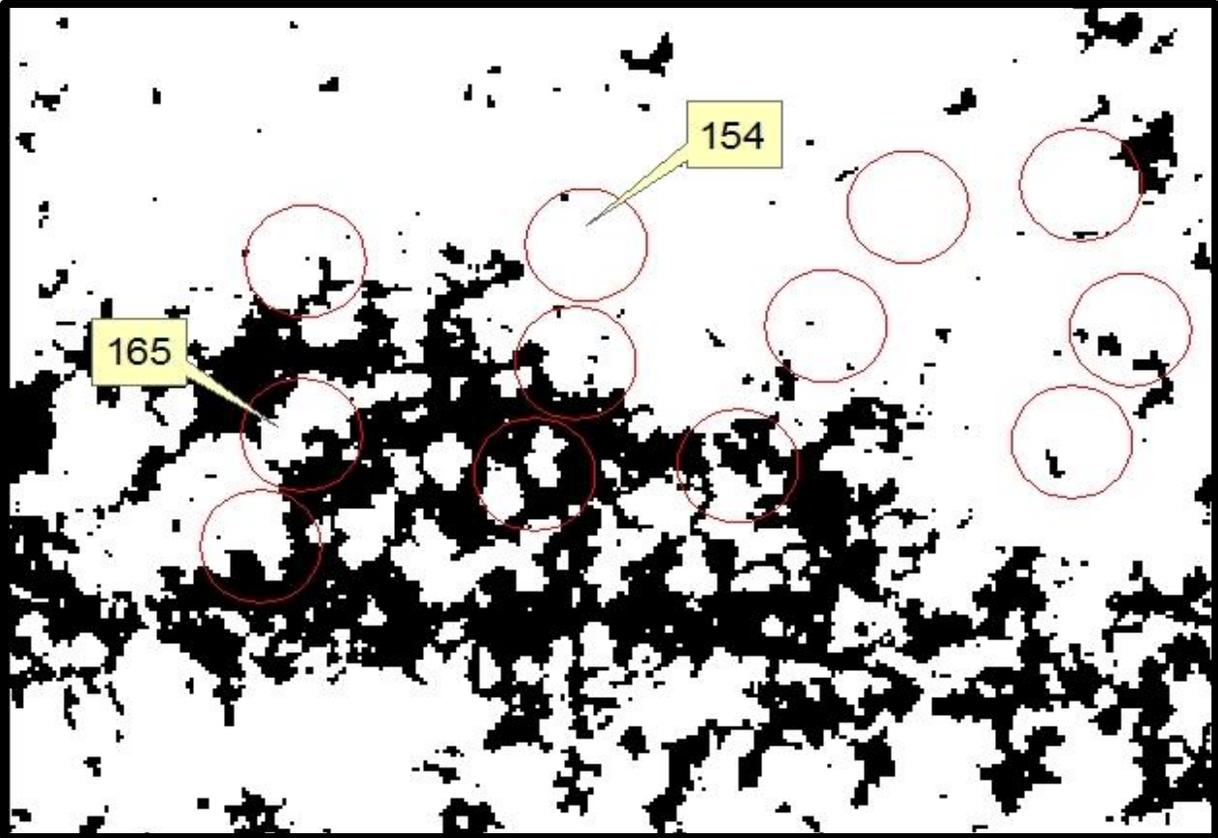


Figure. 11.3. Visualization of canopy gaps along a transect used for faunal surveys. Red circles denote the area of measurement centered on survey points. White pixels denote presence of canopy vegetation (> 3 m in height), and black pixels denote a lack of canopy vegetation as measured using ALS. Our gap index is the ratio of black pixels to white pixels.

## 12. Key Report: Impacts of prescribed fire on bat and insect populations at Mammoth Cave National Park.

### Summary of Fire History and Methods

Use of fire for forest management at Mammoth Cave National Park (MCNP) has been ongoing since 2002, with two prescribed fires occurring in the spring that our project began in 2010 (Figure 12.1). By the end of data collection, over 25% of the park land base has been managed with prescribed fire, and multiple burn units had repeated burn entries (Figure 12.2). This prescribed fire program created a forest mosaic with a range of conditions to which bats can respond, and allowed us to test and assess the impacts of time since fire and frequency of fire on both bats and their prey. We used existing GIS data bases to identify burned habitats and key landscape elements at MCNP, namely location of hibernacula and accessible portions of the riparian corridor of the Green River.

We conducted surveys for bat activity and nocturnal insect occurrence from August 2010 through October 2012 using acoustic detectors and insect traps deployed in tandem along a series of transects across MCNP (Figure 12.1, Figure 12.3). Surveys took place across an array of upland and riparian habitats that covered a range of forest canopy heights; transects were surveyed in tandem so that monitoring took place at a burned land parcel simultaneous with an unburned land parcel (or riparian corridor). These survey locations were selected using a stratified random approach, wherein land parcels were identified based on burn history. Once selected, the specific positions of survey transects were randomly selected and then surveyed in a random order. Repeated sampling occurred without replacement across the complement of land parcels in both staging and swarming periods. A subsample of long-term monitoring locations was also monitored during the maternity season.

Bat activity was assessed using the Anabat II system (Titley Electronics, Australia) powered by a 12 V gel-cell battery and housed in plastic containers to protect equipment from inclement weather (O'Ferrell 1998). Housed detection systems were mounted upon 1.5-m camera tripods for surveys, allowing position above re-growth of understory vegetation while sampling within the forest canopy. Each acoustic survey spanned multiple nights ( $\geq 3$ ) to account for nightly variation and equipment failure. Despite standard placement and operation, the potential exists for microphone sensitivity to vary over time, as well as between units, so the sensitivity of detection systems was calibrated annually using an ultrasonic sound emitter (Britzke 2004). Analysis of acoustic data collected between sunset and sunrise was carried out using Echoclass v.1.1, an automated software package for acoustic identification developed by the U.S. Army Engineer Research and Development Center and provided by the U.S. Fish and Wildlife Service (USFWS 2012). With this software, echolocation pulses are isolated into high frequency ( $> 34$  kHz) and low frequency ( $\leq 34$  kHz) categories (E. Britzke, U.S. Army Engineer Research and Development Center, pers. comm.). Echolocation data were then visually inspected for quality control using Analook software (v. 4.9). The resulting response variables considered for bat activity were the numbers of echolocation pulses isolated into the high and low-frequency categories, on a per night basis. We also considered the echolocation pulses identified as belonging to the Indiana bat (*Myotis sodalis*) and northern long-eared bat (*M. septentrionalis*) as additional response variables (following default settings of Echoclass v. 1.1).

To minimize potential bias introduced by any single approach, we used two techniques to assess prey occurrence (Kunz, 1988). Insect surveys were conducted on nights with temperatures  $\geq 16^{\circ}\text{C}$  at sunset, no precipitation, and low wind speeds (Yela and Holyoak 1997). These surveys occurred on a single night in each sampling interval, concurrent with acoustic surveys for bats. Nocturnal phototactic insects were surveyed using a 10-W blacklight trap (Bioquip, Rancho Dominguez, California, USA) suspended at 2.5 m (Dodd et al. 2012). We suspended blacklight traps 2.5-m aboveground prior to sunset and operated them throughout the entire night. A dichlorvos-based ‘pest strip’ (ca.  $2 \times 6$  cm) was placed within each blacklight trap to subdue specimens. Additionally, malaise traps (Bioquip, Rancho Dominguez, California, USA) were placed at ground level to survey non-phototactic insects. Collection jars containing a similar ‘pest strip’ were affixed to the traps at dusk to capture only nocturnal insects. Insects were removed the following day and stored in 70% ethanol.

Insects were identified using available keys (Covell, 2005, Triplehorn and Johnson 2005) and reference collections at the University of Kentucky. For blacklight traps, insects  $\geq 10$  mm in length were identified to the lowest taxon practical; Lepidoptera were identified to species and other insects to the family level. Our classification of noctuid Lepidoptera follows that of LaFontaine and Schmidt (2010). Smaller insects ( $< 10$  mm) captured in light traps were identified to order. All insects captured in malaise traps were identified to the lowest taxon practical (generally family level). For the purposes of this report, we report the abundance of Coleoptera and Lepidoptera recorded using blacklight traps and the abundance of Diptera as recorded using malaise traps.

The effects and interactions of time since fire and seasonality were assessed for bat and insect response variables using Analysis of Variance (ANOVA). A total of three categories were considered when defining time since fire: unburned, recently burned ( $\leq 3$  growing seasons since burn), and burned in the past ( $> 4$  growing seasons since burn). This classification accounted for the duration of our study (i.e., all burns occurring during the window of our study were “recently burned”) and allowed consideration of the changing effects of fire over time. The temporal effect in our analysis followed the seasonal activity of resident and migratory bats at MCNP, including staging (April-May), maternity (June-July), and swarming periods (August-October). Wide variation across land units was observed early on in our study. Thus, we chose to conduct discrete analyses for each year. This avoided use of confounding and un-interpretable interactions between site and time since fire, and allowed assessment of a more resolved seasonal effect during swarming in 2010. Further, the impact of fire frequency (never burned, burned once, burned twice) was assessed in conjunction with seasonality. Lastly, we assessed variation in bat activity between riparian areas versus similarly unburned upland habitats, with a focus towards understanding species-level differences in habitat use between Indiana bats and northern long-eared bats. In all cases, response variables were tested for homogeneity of variance using Variance Ratio  $F_{\text{MAX}}$  tests, with ANOVAs conducted on log-transformed values when variances were heterogeneous (Sokal and Rohlf, 1969). We used Tukey’s Honestly Significant Difference means separation procedures to evaluate effects when models were significant (Zar 1999).

## Discussion of Results

A total of 60 acoustic surveys were conducted from 2010-2012, with efforts covering 170 nights and resulting in 1,092 detector/nights. From these surveys, 46,656 echolocation files were recorded. Of the 462,837 echolocation pulses recorded, 65% were classified as high-frequency, and the remainder as low-frequency. Of these recordings, a total of 5,842 files were identified as belonging to the northern long-eared bat and 872 files belonging to the Indiana bat.

Considering the effects of time since burn (Figure 12.4) and seasonality (Figure 12.5), models were significant for high frequency activity in 2010 ( $F_{8,378} = 9.1, P = 0.001$ ), 2011 ( $F_{7,431} = 6.3, P = 0.001$ ), and 2012 ( $F_{7,274} = 7.9, P = 0.001$ ). For 2010, high frequency activity was higher in August versus later in swarming and, considering the significant interaction, high frequency activity was greater in unburned habitats. For 2011, high frequency activity was greater in unburned habitats, with no temporal variation evident. For 2012, high frequency activity dropped during swarming and, considering the significant interaction, activity was greater in unburned habitats and those habitats that had been burned in the past. While the model for low frequency activity was not significant in 2010 ( $P > 0.05$ ), models were significant for 2011 ( $F_{7,431} = 3.9, P = 0.001$ ) and 2012 ( $F_{7,274} = 2.8, P = 0.001$ ). Low frequency activity was greater in the maternity season versus staging or swarming in 2011 and 2012 (Figure 12.5). The effect of time since burn was only apparent for 2011 (Figure 12.4); more low frequency activity was observed in areas that had not been burned during the maternity season. Models were significant for the northern long-eared bat in 2010 ( $F_{8,378} = 3.6, P = 0.001$ ), 2011 ( $F_{7,431} = 11.9, P = 0.001$ ), and 2012 ( $F_{7,274} = 7.9, P = 0.001$ ). Activity of the northern long-eared bat was greater in unburned habitats versus burned habitats in 2010 and 2011, and areas that were unburned or had been burned in the past were greater than recently burned areas in 2012 (Figure 12.6). Northern long-eared activity was higher in August versus later swarming months in 2010 (Figure 12.7). While no seasonal difference was detected in 2011, activity of northern long-eared bats was lower in during swarming in 2012 (Figure 12.7). Models for activity of the Indiana bat were not significant in 2010 or 2012, ( $P > 0.05$ ), but were significant in 2011 ( $F_{7,431} = 3.5, P = 0.001$ ). No seasonal effect was detected (Figure 12.7), but activity of Indiana bats was greater in unburned habitats versus burned habitats in 2011 (Figure 12.6).

Models for fire frequency were significant for high and low frequency activity ( $F_{8,1023} = 6.7, P = 0.001$ , and  $F_{8,1023} = 13.4, P = 0.001$ , respectively). Models for fire frequency were also significant for the Indiana bat ( $F_{8,1023} = 2.1, P = 0.001$ ) and northern long-eared bat ( $F_{8,1023} = 10.4, P = 0.001$ ). In all cases, greater activity was observed in unburned areas versus burned areas (Figure 12.7).

While no differences were detected between riparian and upland habitats for high frequency activity ( $P > 0.05$ ), the model for low frequency activity was significant ( $F_{3,501} = 8.1, P = 0.001$ ). However, only season was significant in this test and no difference was detected in habitat type. Models for burn frequency were significant for both the Indiana bat ( $F_{3,501} = 18.2, P = 0.001$ ) and northern long-eared bat ( $F_{3,501} = 2.8, P = 0.04$ ), with inverse trends apparent for these species with activity greater for northern long-eared bats in upland habitats and activity of Indiana bats higher in riparian habitats (Figure 12.8). There was no seasonal effect in either species-specific model.

A total of 53 nights was surveyed from 2010-2012 with both blacklight traps and malaise traps. This resulted in 337 and 294 trap/nights for blacklight traps and malaise traps, respectively. A total of 185,714 insects was captured using blacklight traps, and 22,001 insects were captured in malaise traps. The primary orders captured, Coleoptera, Diptera and Lepidoptera, accounted for 72% of the insects captured in blacklight traps. Diptera was the principal order captured in malaise traps, accounting for 63% of the total catch.

Considering the effects of time since burn and seasonality on Coleoptera, models were significant for 2010 ( $F_{7,71} = 14.0$ ,  $P = 0.001$ ), 2011 ( $F_{7,131} = 6.4$ ,  $P = 0.001$ ), and 2012 ( $F_{7,106} = 3.3$ ,  $P = 0.001$ ), with no interaction between main effects. Generally, Coleoptera were more abundant in areas that had been recently burned versus areas burned in the past (Figure 12.10). Seasonally, Coleoptera decreased over the swarming period of 2010, and were less abundant during swarming versus earlier portions of the growing season in 2011 and 2012 (Figure 12.11). For Diptera, models were significant in 2010 ( $F_{7,79} = 6.8$ ,  $P = 0.001$ ) and 2011 ( $F_{5,114} = 10.5$ ,  $P = 0.001$ ), but not 2012 ( $P > 0.05$ ). Time since burn had no effect on abundance of Diptera (Figure 12.10). While this group was less abundant at the end of swarming in 2010, Diptera were more abundant during swarming versus staging in 2011 (Figure 12.11). No seasonal difference was detected for Diptera in 2011. Considering Lepidoptera, models were significant for 2010 ( $F_{7,71} = 7.5$ ,  $P = 0.001$ ), 2011 ( $F_{7,131} = 10.8$ ,  $P = 0.001$ ), and 2012 ( $F_{7,106} = 4.3$ ,  $P = 0.001$ ). Time since burn did not significantly impact abundance of Lepidoptera in 2010 or 2012, but this group was more abundant in areas that had never been burned versus areas burned in the past (Figure 12.10). Abundance of Lepidoptera was lowest at the end of swarming in 2010, and lower numbers of this group were captured in swarming than during the maternity period (Figure 12.11). Models for fire frequency were significant for Coleoptera, Diptera, and Lepidoptera ( $F_{8,328} = 8.1$ ,  $P = 0.001$ ,  $F_{8,328} = 6.1$ ,  $P = 0.001$ , and  $F_{8,328} = 5.3$ ,  $P = 0.001$ , respectively). In all cases, seasonality was a significant effect (Figure 12.11). Only for Lepidoptera did fire frequency impact prey abundance (Figure 12.12).

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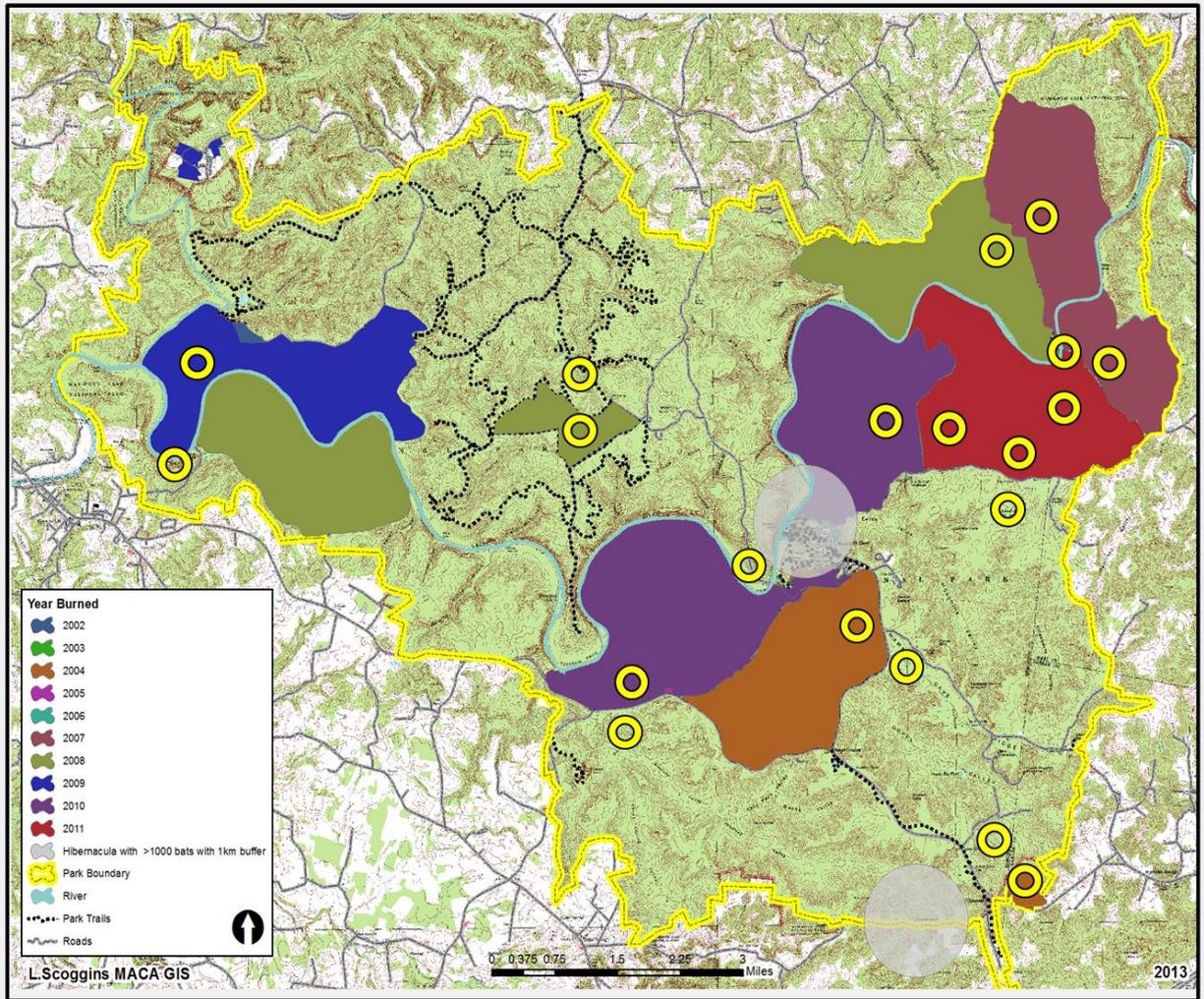
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**Figure 12.1.** Map of MCNP showing most recent prescribed fires across burn units. Generalized locations of transects used for surveying bats and insects are denoted with ring symbols. Map courtesy of Lillian Scoggins, Mammoth Cave National Park, US National Park Service.

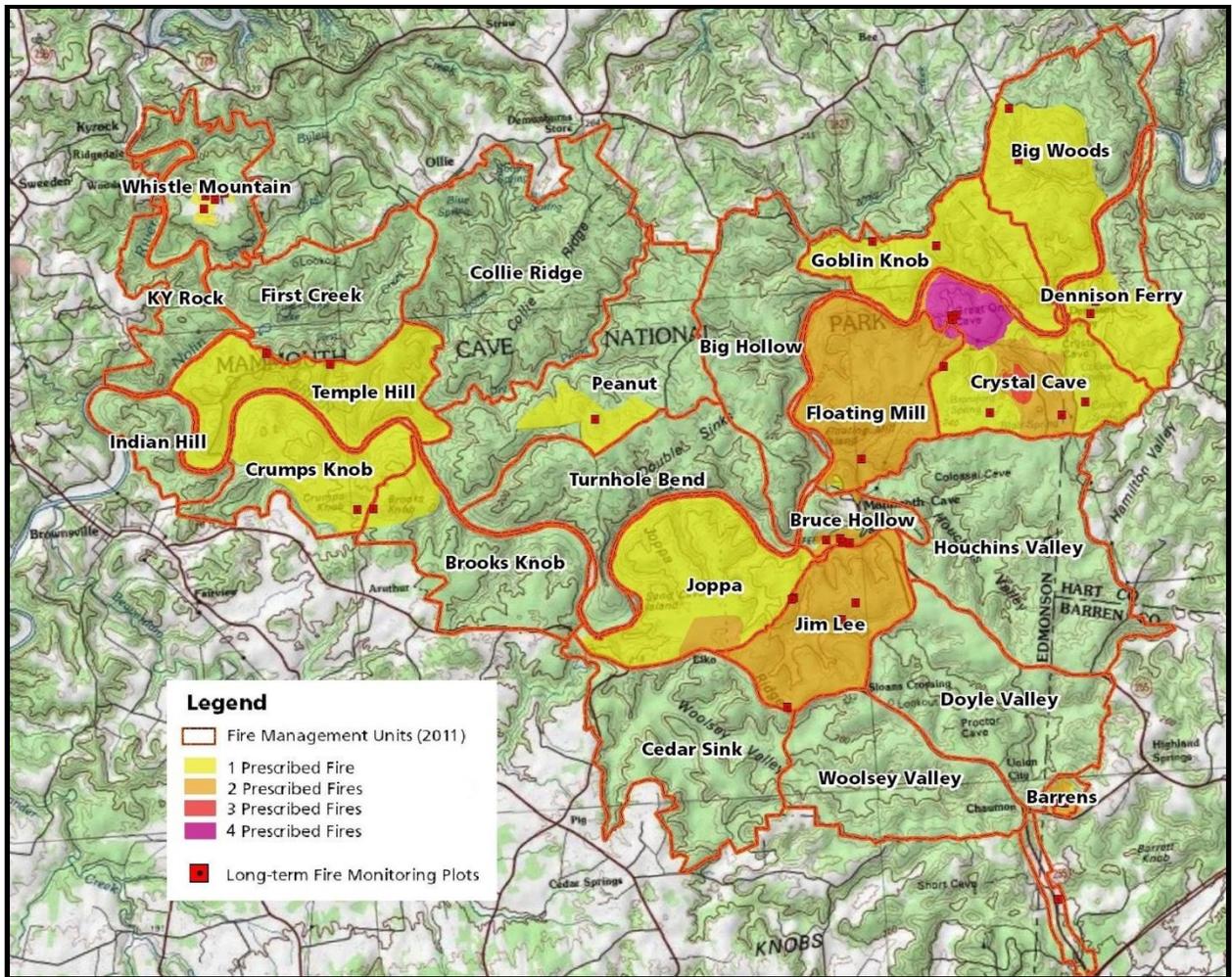
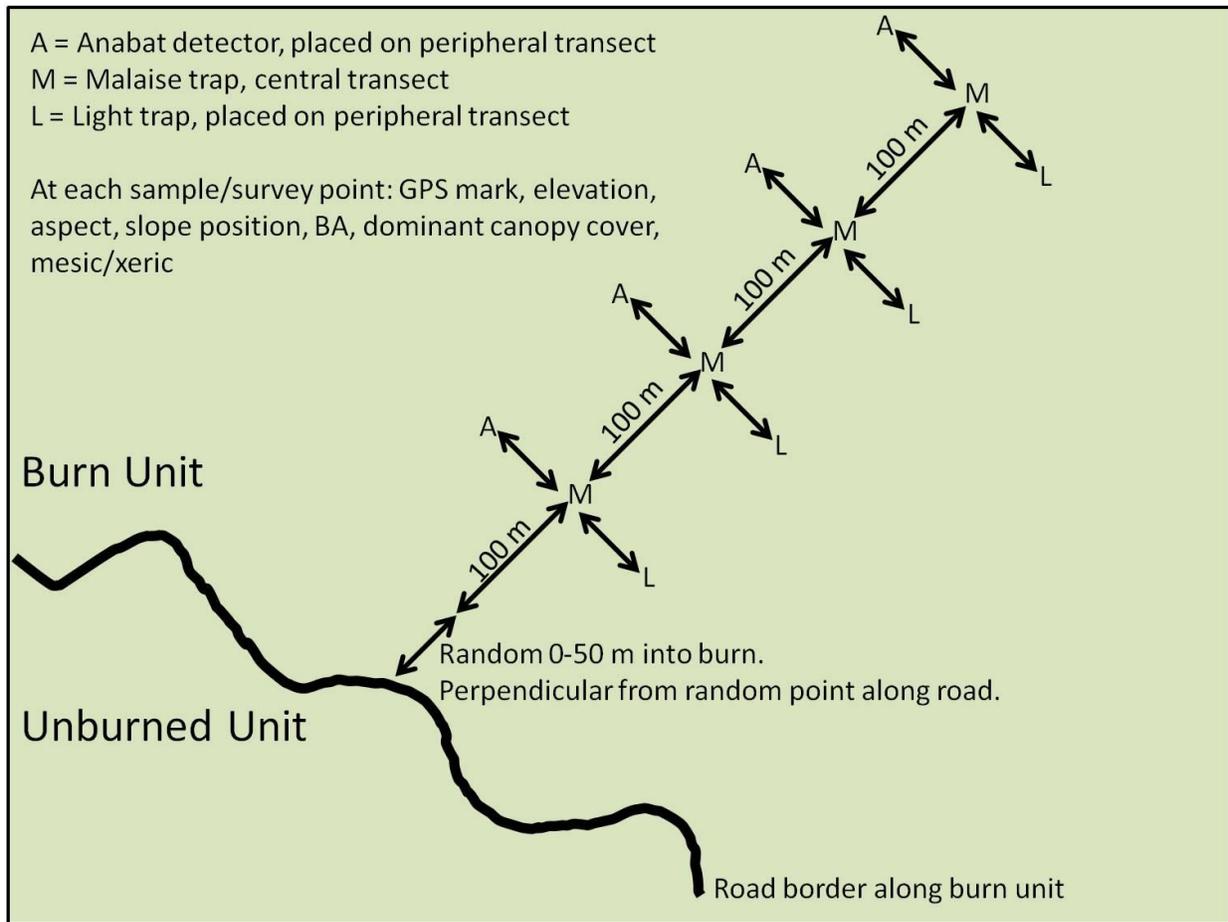
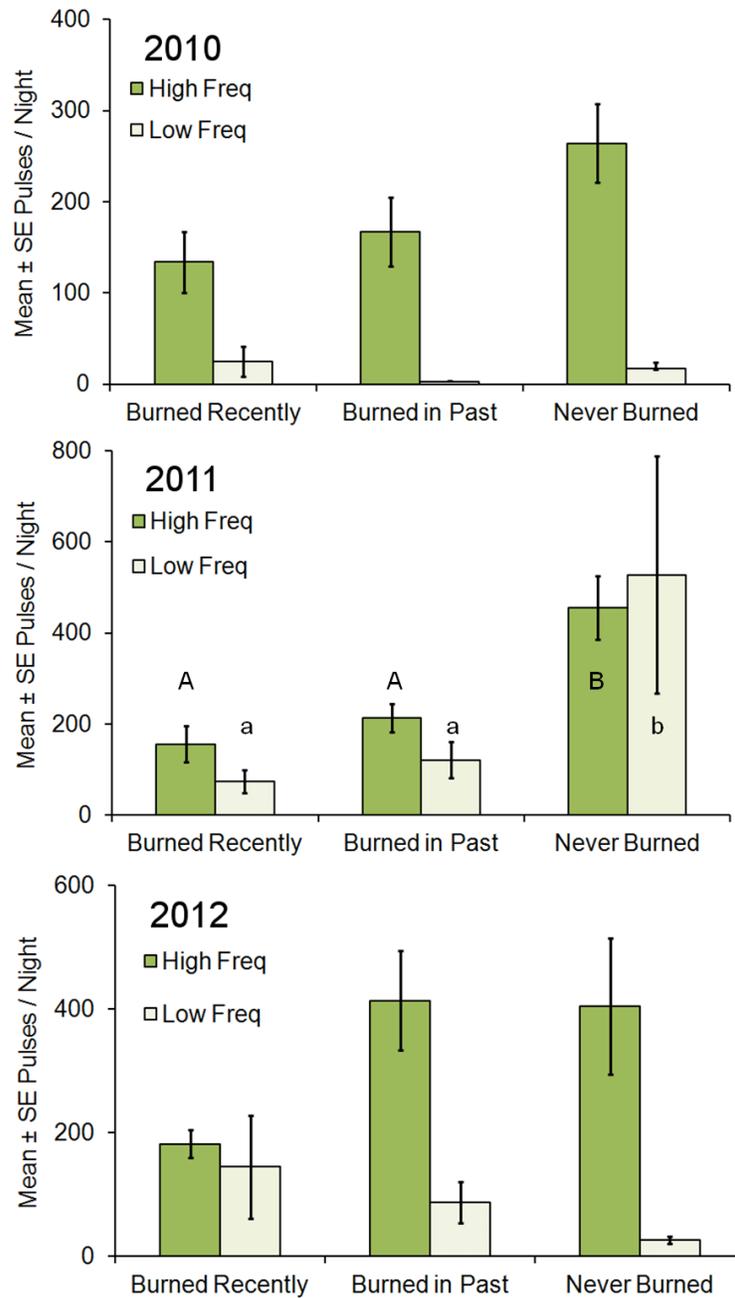


Figure 12.2. Map of MCNP showing frequency of prescribed fires across burn units at the time of project's conclusion. Map courtesy of J. Burton, Barrens to Bayous Fire Monitoring Network, US National Park Service.



**Figure 12.3.** Generalized layout of transects used to survey bat activity and insect occurrence. A pair of these transects were deployed during each survey period.



**Figure 12.4.** The effect of burning on bat activity. Different letters within a data series depict a significant means separation for fire as a main effect ( $P \leq 0.05$ ). The interaction of time since burn and season was significant for high frequency activity in 2010 and 2012.

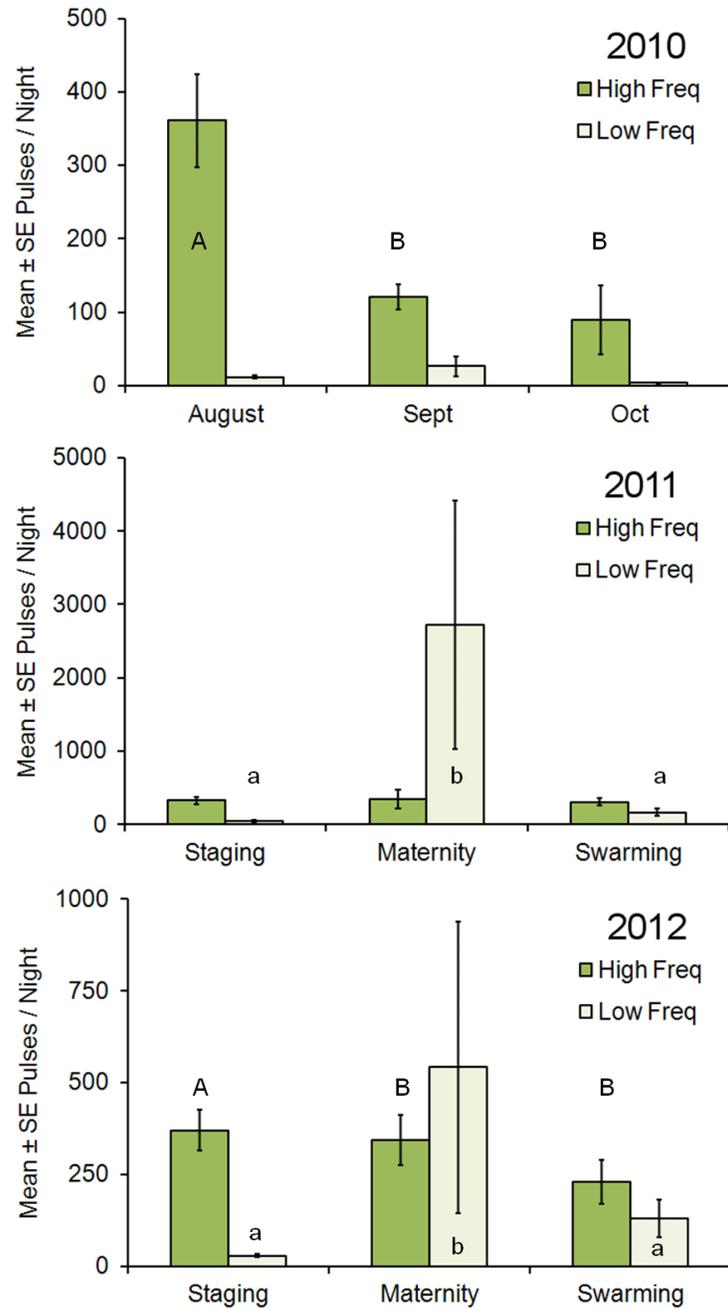
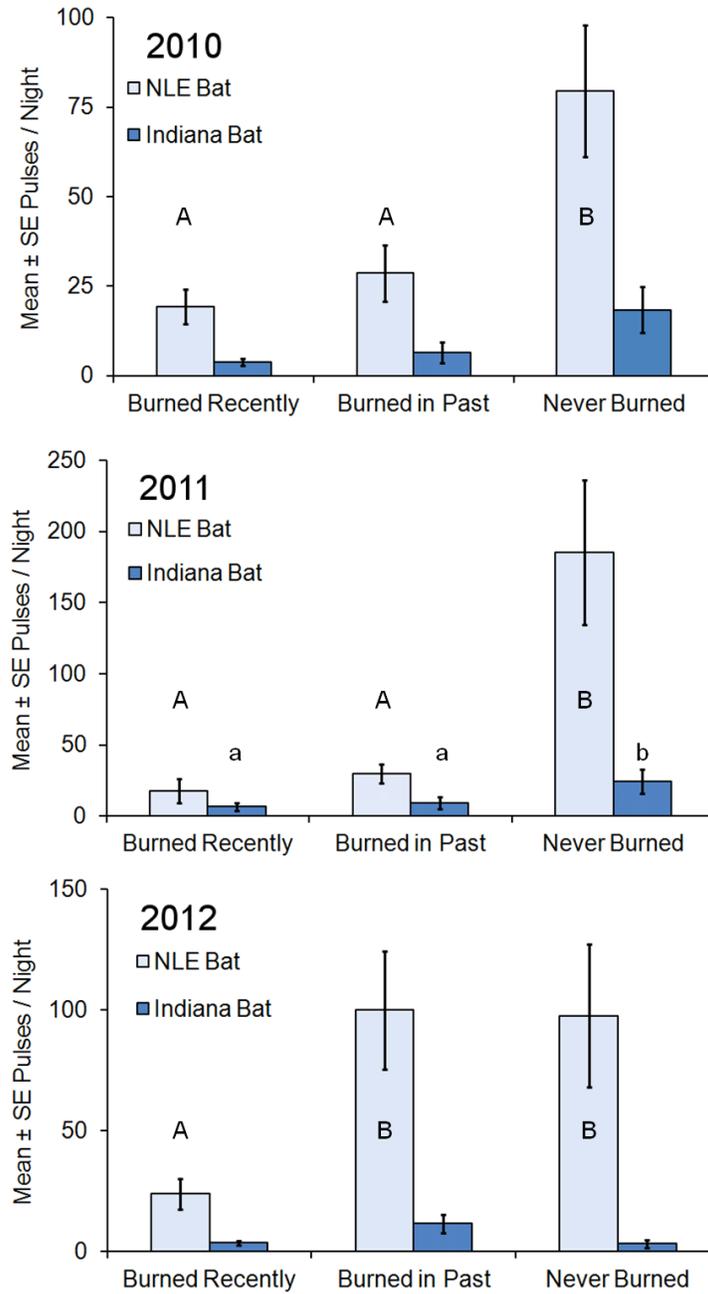
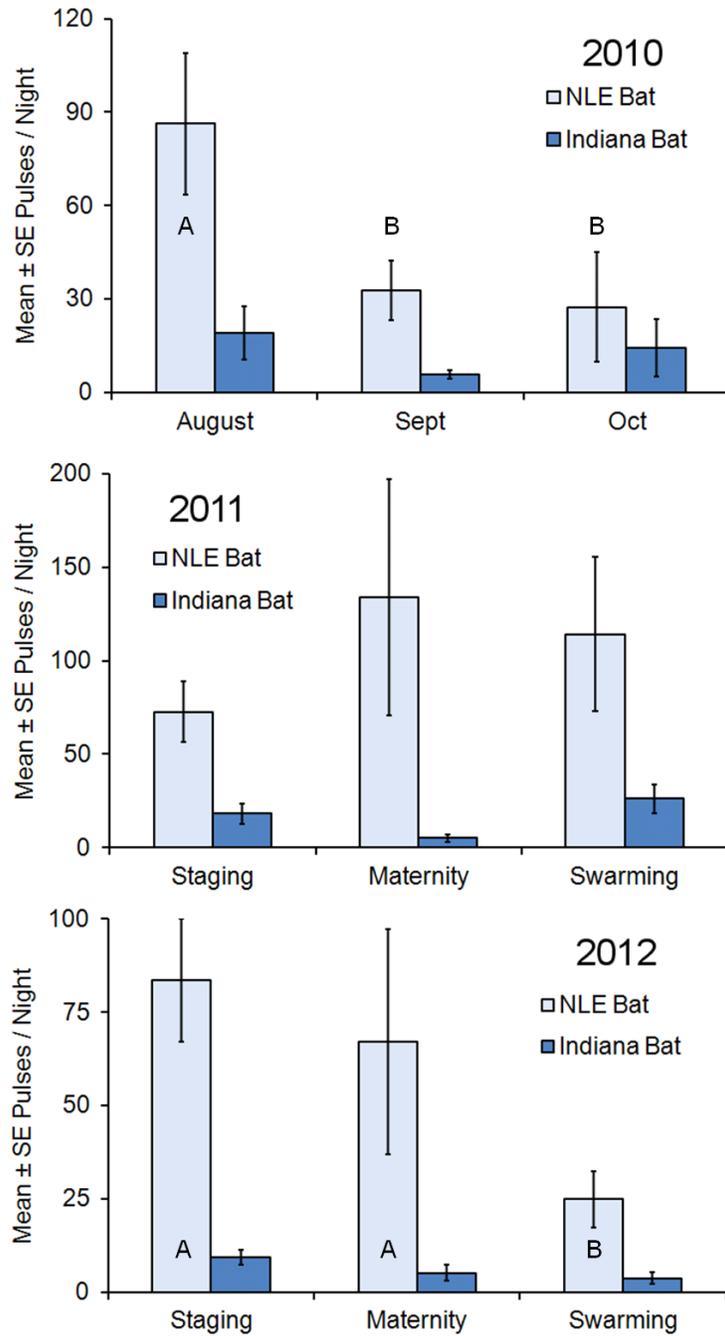


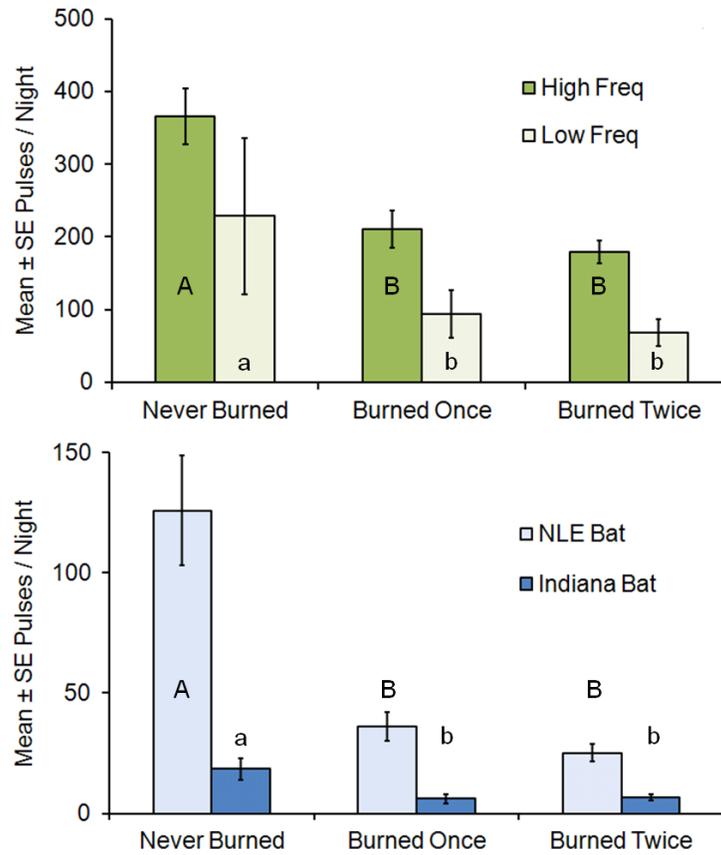
Figure 12.5. Seasonal trends in bat activity. Different letters within a data series depict a significant means separation for season as a main effect ( $P \leq 0.05$ ).



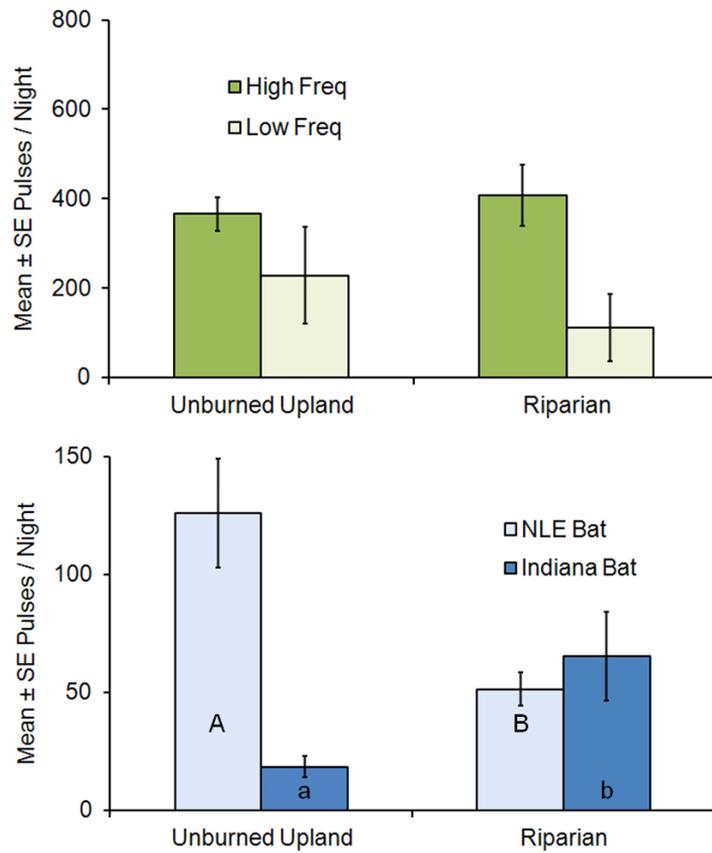
**Figure 12.6.** The effect of burning on activity of the Indiana bat and northern long-eared bat. Different letters within a data series depict a significant means separation for fire as a main effect ( $P \leq 0.05$ ).



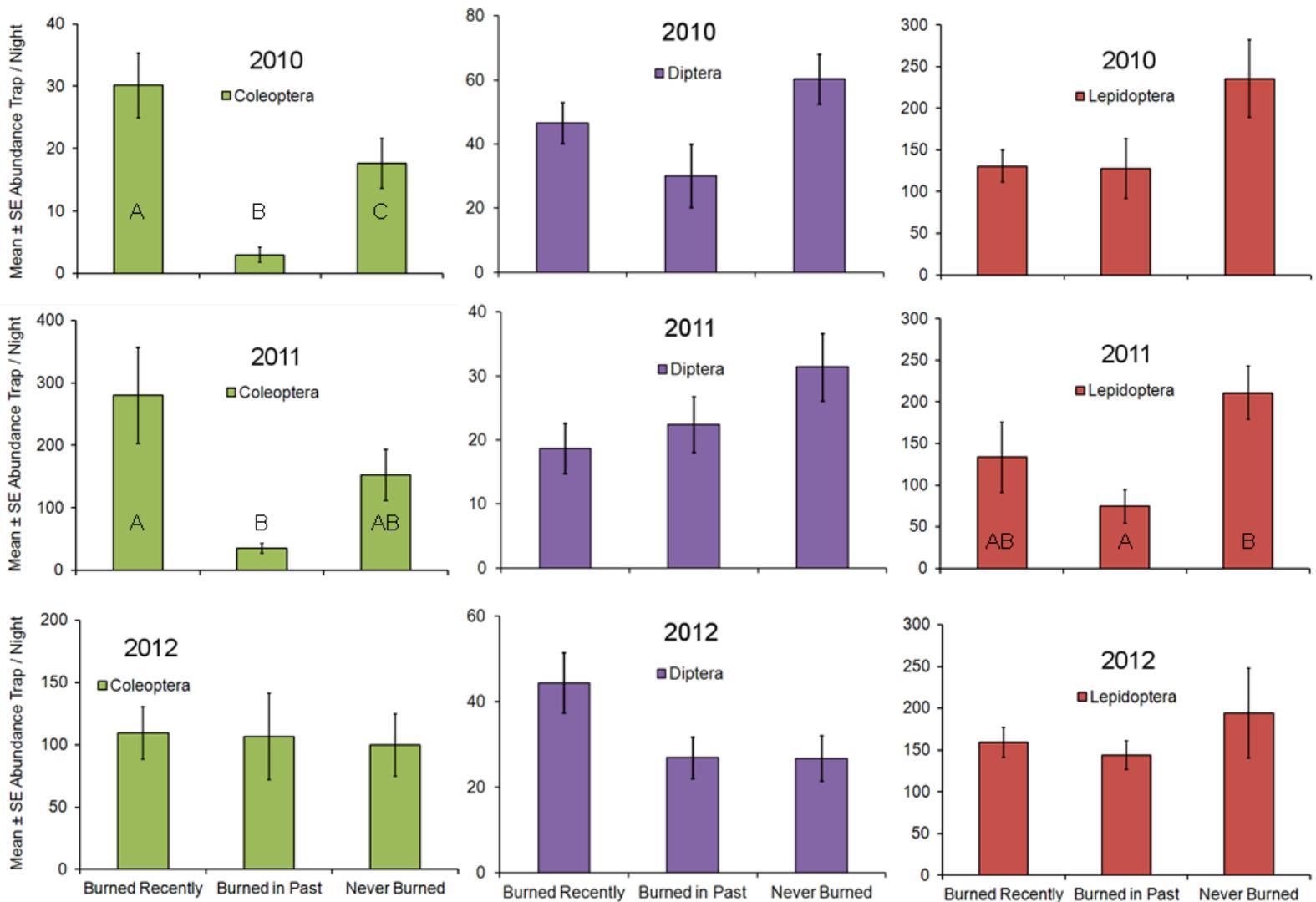
**Figure 12.7.** Seasonal trends in the activity of the Indiana bat and northern long-eared bat. Different letters within a data series depict a significant means separation for fire as a main effect ( $P \leq 0.05$ ).



**Figure 12.8.** Trends in bat activity in relation to burn frequency across habitats. Different letters within a data series depict a significant means separation for fire as a main effect ( $P \leq 0.05$ ).



**Figure 12.9.** Variation in bat activity between upland and riparian habitats. Different letters within a data series depict a significant means separation for fire as a main effect ( $P \leq 0.05$ ).



**Figure 12.10.** Trends in the abundance of the primary prey of bats in relation to burn frequency across habitats. Abundance of Coleoptera and Lepidoptera was measured using blacklight traps and abundance of Diptera was measured with malaise traps. Different letters within a data series depict a significant means separation for fire as a main effect ( $P \leq 0.05$ ).

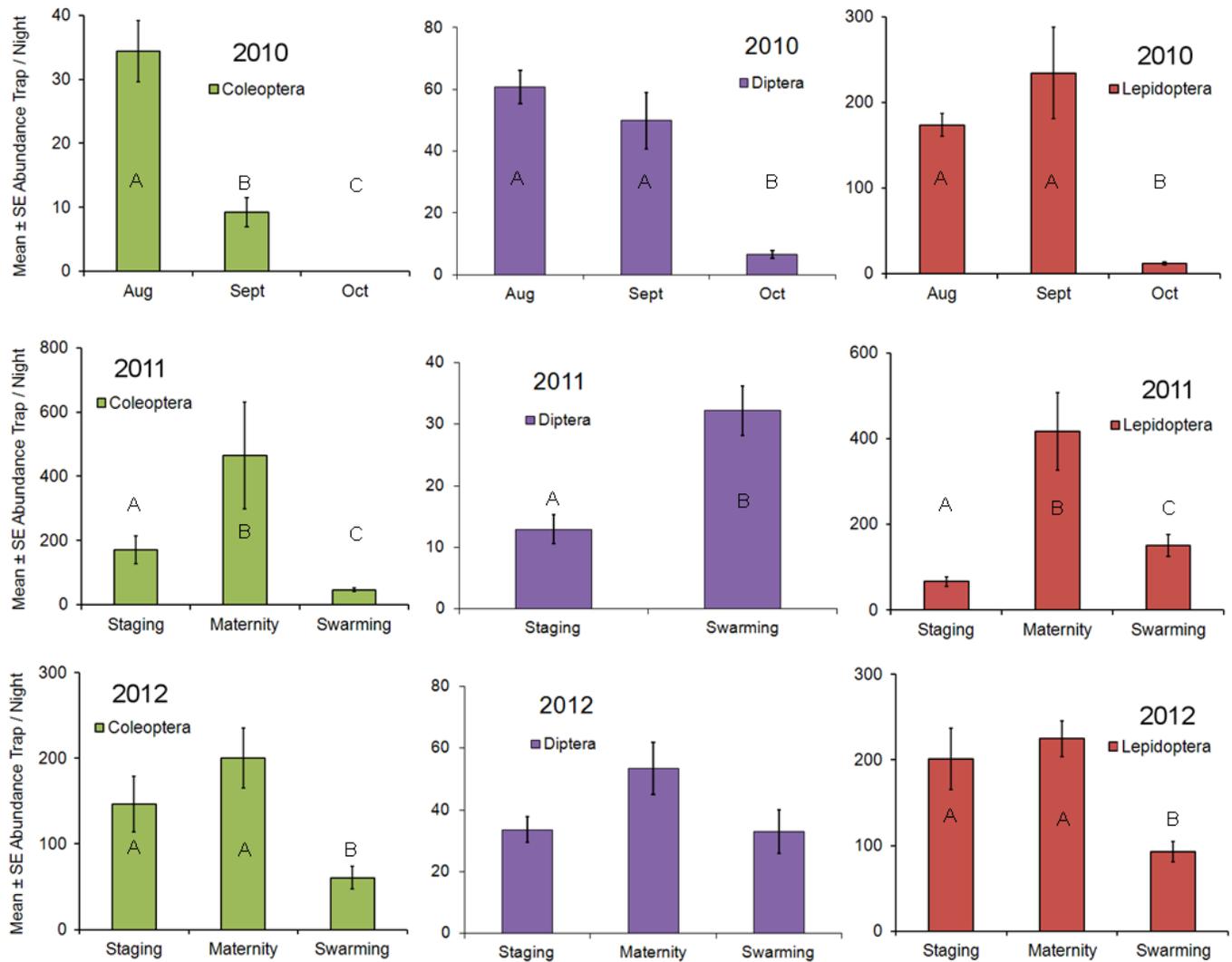
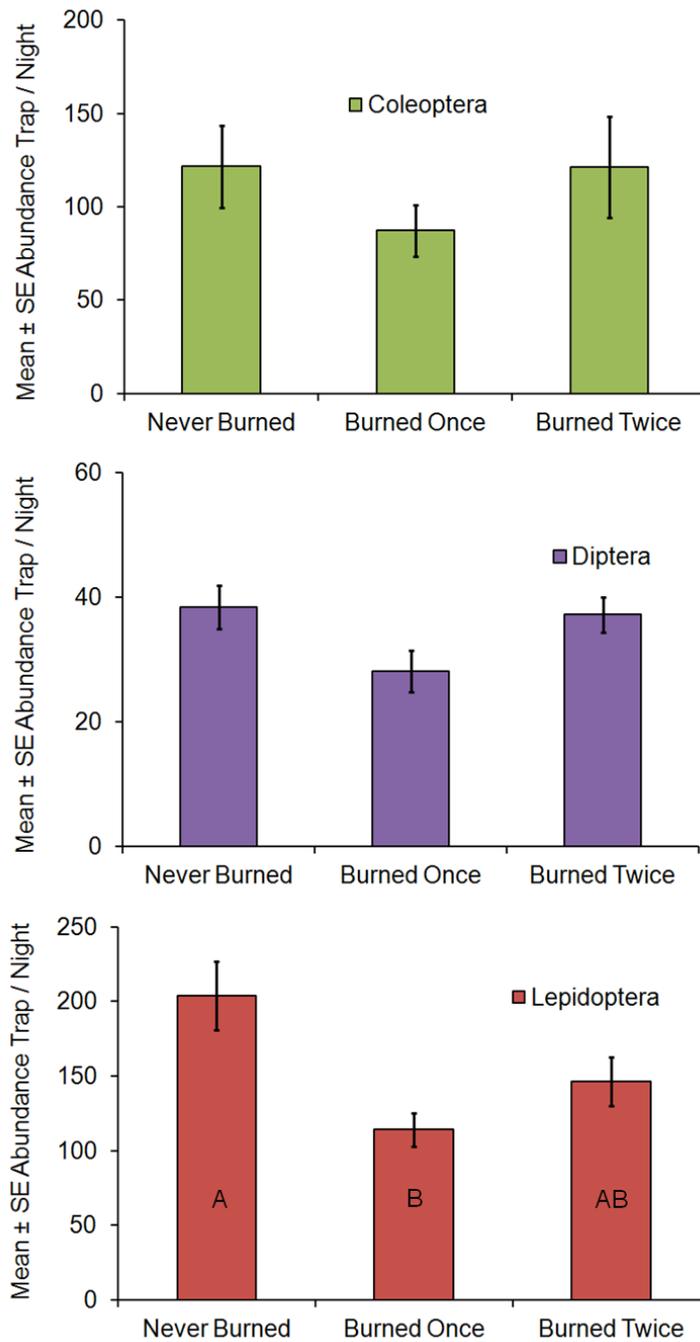


Figure 12.11. Seasonal trends in the abundance of the primary prey of bats. Abundance of Coleoptera and Lepidoptera was measured using blacklight traps and abundance of Diptera was measured with malaise traps. Different letters within a data series depict a significant means separation for fire as a main effect ( $P \leq 0.05$ ).



**Figure 12.12.** Trends in the abundance of the prey of bats in relation to burn frequency across habitats. Abundance of Coleoptera and Lepidoptera was measured using blacklight traps and abundance of Diptera was measured with malaise traps. Different letters within a data series depict a significant means separation for fire as a main effect ( $P \leq 0.05$ ).

### **13. Key Report: Assessing the impact of burn severity on forest canopy conditions using ALS-derived descriptors.**

#### Summary of Methods

Mammoth Cave National Park (MCNP) has diligently developed and archived geospatial burn severity products from many of the major fires (prescribed or wildfire) over the past decade. These products were developed by calculating the difference in normalized burn ratio (dNBR; Key and Benson 2005) using multi-temporal Landsat scenes from the Monitoring Trends in Burn Severity website ([www.MTBS.gov](http://www.MTBS.gov)) within the extent of digital fire perimeters. Park personnel collected Composite Burn Index (CBI; Key and Benson 2005) following each fire event to train the dNBR imagery. The resulting data products are 30 x 30-m resolution, categorical, estimates of burn severity for each burned unit. Using these data, we evaluated relationships between fire severity and forest canopy characteristics by comparing forest habitat structure, derived from Airborne Laser Scanning (ALS) data, with degree of fire intensity from prescribed fires at MCNP.

We extracted CBI values for our field sampling plots for the 9 burns at MCNP. In tandem with this, ALS data was collected for the same field sampling plots in October of 2010 (>4 pulses / m<sup>2</sup>). We processed these data using “Toolbox for ALS data Filtering and Forest studies” software (Chen et al. 2007). The output from this processing estimated digital elevation models, canopy height models, variables derived from the pulse height distribution (e.g. mean height, max height, percentile heights, etc.), as well as three-dimensional canopy height profiles (CHP; Skowronski et al. 2007) at various resolutions continuously across the study extent. These ALS-derived data products were also estimated within individual polygons where it intersected with spatially explicit surveys.

Using this ALS data, we derived a suite of habitat variables for model-building with CBI values. This suite of variables incorporated descriptors based on the absolute measurements of ALS hits at 10 m increments throughout the forest canopy, as well as measurements for total canopy height and canopy gap (Figure 11.3). This suite also incorporates predictive variables developed by Lesak et al. (2011), which scales the incidence of ALS hits throughout the forest canopy by collapsing ALS data into 10 proportionate bins scaled to the height of the canopy. All descriptors were based on a 15 m radius centered on an acoustic survey point. These descriptive variables included:

- total density (sum of all ALS-derived CHP from the ground to the top of the canopy)
- gap index (percent of open air space >3 m in height without vegetative structure)
- canopy height (height of canopy at the 90<sup>th</sup> percentile of ALS hits aboveground)
- understory density (sum of ALS-derived CHP from the ground to 10-m aboveground)
- midstory density (sum of ALS-derived CHP from 10 to 20-m aboveground)
- overstory density (sum of ALS-derived CHP from 20 to 30-m aboveground)
- legacy density (sum of ALS-derived CHP > 30-m aboveground)
- P<sub>Understory</sub> (percent of ALS-derived CHP in the bottom 2 bins of scaled data)
- P<sub>Midstory</sub> (percent of ALS-derived CHP in intermediate 3<sup>rd</sup> through 6<sup>th</sup> bins of scaled data)
- P<sub>Canopy</sub> (percent of ALS-derived CHP in the upper 7<sup>th</sup> through 10<sup>th</sup> bins of scaled data)

- $R_{\text{Understory:Midstory}}$  (ratio of  $P_{\text{Understory}}$  to  $P_{\text{Midstory}}$ )
- $R_{\text{Understory:Canopy}}$  (ratio of  $P_{\text{Understory}}$  to  $P_{\text{Canopy}}$ )
- $R_{\text{Midstory:Canopy}}$  (ratio of  $P_{\text{Midstory}}$  to  $P_{\text{Canopy}}$ )
- $R_{\text{Total:Understory}}$  (ratio of total density to understory density)

We used linear discriminant analysis and canonical discriminant analysis to examine associations of habitat characteristics with degree of fire severity (SAS Institute Inc., Cary, NC). Habitat variables were initially screened for their potential to differentiate among burn severity classes using one-way ANOVAs (PROC GLM), with a  $P \leq 0.15$  used for retaining variables for further analysis. Remaining habitat variables were then evaluated for multicollinearity effects using multiple regression (PROC REG) with tolerance (TOL) and variance inflation factor (VIF) metrics (Green 1978). Variables with the lowest TOL and highest VIF values were selectively removed from the model and the model rerun until all remaining variables possessed a  $TOL > 0.1$  and  $VIF < 10$ . Habitat variables retained were then evaluated for their ability to separate among burn severity scores (0, 1, and 2) using linear discriminant analysis (PROC DISCRIM). Homogeneity of covariance matrices was tested following Morrison (1976), with within covariance matrices used for subsequent analyses if the Chi-square test outcome was significant. Because 3 classes of fire severity were evaluated, the discriminant model generated two canonical axes (one less than the number of groupings). Model effectiveness was verified by testing classification outcomes using Cohen's kappa (Titus et al. 1984). Cohen's kappa assesses the percent improvement over chance in classification outcomes of individual observations among classes.

Equality of group centroids (i.e., burn severity classes) was tested for both canonical axes using Likelihood Ratios ( $LR$ ) from canonical discriminant analysis (PROC CANDISC). Mahalanobis  $D^2$  scores were generated for each pair of classes and tested for significance using Wilk's Lambda. Importance of and directionality of individual habitat variables in separating among burn severity classes was assessed with total standardized canonical coefficients. Coefficient scores exceeding 0.4, either positive or negative, were required for use in interpretation of model outcomes. Relationships of habitat variables with degree of burn severity were evaluated using means and standard deviations by burn severity classes. The relationship of burn severity with topographic position was also assessed using one-way ANOVAs, with burn severity evaluated by elevation (m), slope (%), and aspect ( $^\circ$ ), respectively. Unless otherwise indicated, all statistical tests were evaluated for significance at  $P \leq 0.05$ .

## Discussion of Results

Initial screening for importance of habitat characteristics found  $P_{\text{Canopy}}$  ( $F = 0.91$ ,  $P = 0.4049$ ) and  $R_{\text{Understory:Canopy}}$  ( $F = 1.08$ ,  $P = 0.3425$ ) to possess limited variation in explaining degree of burn severity, so these variables were removed from further consideration. Tests for multicollinearity demonstrated highest levels for TOL (0.01) and VIF (99.0) with total density included in the regression model. Removal of total density resulted in satisfactory TOL and VIF scores for all remaining habitat characteristics with  $P_{\text{Understory}}$  possessing the lowest TOL (0.126) and highest VIF (7.95) scores. The test of homogeneity of covariance matrices was significant ( $\chi^2 = 950$ , d.f. = 110,  $P < 0.0001$ ), so the within covariance matrices were used in creating discriminant functions. Both canonical axis 1 [ $LR = 0.518$  (SE+ 0.042),  $F_{20, 582} = 8.57$ ,  $P <$

0.0001] and canonical axis 2 [ $LR = 0.429$  ( $SE = 0.047$ ),  $F_{9, 292} = 7.31$ ,  $P < 0.0001$ ] were significant at separating group centroids; however, examination of class means on canonical axes for burn severity groupings demonstrated different orderings of burn severity classes between axes (Table 13.1), with Mahalanobis  $D^2$  values (Wilk's Lambda = 0.5967,  $P < 0.0001$ ) demonstrating greatest separation between classes 1 and 2 ( $D^2 = 5.2$ ), and the least separation between classes 0 and 1 ( $D^2 = 1.28$ ). Model verification using Cohen's kappa demonstrated a classification outcome significantly better than chance ( $K = 0.356$ ,  $P < 0.001$ ), with 65.3% of observations placed into the correct burn severity grouping.

Habitat characteristics important to class separation on canonical axis 1 included midstory density, overstory density, canopy height, gap index, and  $P_{\text{Understory}}$  (Table 13.1). Gap index was associated with increasing burn severity, with increasing midstory density and overstory density associated with unburned habitat (Table 13.2). Habitat characteristics important to class separation on canonical axis 2 included midstory density, canopy height, gap index,  $P_{\text{Understory}}$ ,  $P_{\text{Midstory}}$ , and  $R_{\text{Understory:Midstory}}$  (Table 13.1); however, the inconsistent ordering of burn severity classes on this axis confounded interpretation of variable importance. For example, canopy height and  $P_{\text{Understory}}$  were important to both canonical axes but demonstrated inconsistent relationships with burn severity classification between axes.

As with canonical axis 1, midstory density was directly associated with unburned habitat and gap index directly associated with moderately burned habitat on canonical axis 2 (Tables 13.1 and 13.2).  $P_{\text{Midstory}}$  was directly related to lightly burned habitat relative to unburned and moderately burned habitats (Table 13.2). Burn severity differed by aspect ( $F_{2, 255} = 3.54$ ,  $P = 0.0304$ ) but not elevation ( $F_{2, 300} = 2.31$ ,  $P = 0.101$ ), with increasing burn severity on south to southwest-facing slopes than on southeast-facing slopes (Table 13.3). Effect of slope was moderate ( $F_{2, 161} = 2.94$ ,  $P = 0.0557$ ), with burn severity increasing slightly with steeper slopes.

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Table 13.1. Total standardized canonical coefficients of habitat characteristics, and class means for burn severity groupings, by canonical axes from linear discriminant analysis of burn severity from prescribed fires at Mammoth Cave National Park, Kentucky, 2010. Analyses based on  $n = 303$  observations.

	Canonical axis 1	Canonical axis 2
Habitat characteristic		
Understory density	-0.179	0.172
Midstory density	0.464	0.886
Overstory density	0.506	0.087
Legacy tree density	0.392	0.029
Canopy height	-0.513	0.789
Gap index	-1.190	0.570
$P_{\text{Understory}}$	0.671	0.930
$P_{\text{Midstory}}$	0.164	-0.673
$R_{\text{Understory:Midstory}}$	0.184	-0.771
$R_{\text{Understory:Canopy}}$	0.296	0.034
Severity class		
Unburned (0)	0.256	0.258
Lightly burned (1)	-0.059	-0.829
Moderately burned (2)	-2.001	0.356

Table 13.2. Means (SD) for habitat characteristics by burn severity classes from prescribed fires at Mammoth Cave National Park, Kentucky, 2010.

Habitat characteristic	Burn severity		
	Unburned ( <i>n</i> = 205)	Lightly burned ( <i>n</i> = 74)	Moderately burned ( <i>n</i> = 24)
Midstory density	1.19(0.58)	1.1(0.55)	0.57(0.43)
Overstory density	0.69(0.46)	0.55(0.54)	0.48(0.37)
Canopy height	25.4(4.58)	23.5(4.8)	24.1(2.25)
Gap index	0.03(0.08)	0.03(0.07)	0.19(0.19)
P <sub>Understory</sub>	0.2(0.14)	0.15(0.13)	0.3(0.15)
P <sub>Midstory</sub>	0.3(0.14)	0.34(0.17)	0.16(0.1)
R <sub>Understory:Midstory</sub>	0.96(1.2)	1.16(3.76)	2.94(3.22)

Table 13.3. Variables describing topographic position [mean (SD)] by burn severity class for Mammoth Cave National Park, Kentucky, 2010.

		Burn severity		
	<i>n</i>	Unburned	Lightly burned	Moderately burned
Slope	117	6.58(5.33)	9.03(5.31)	7.17(3.76)
Aspect	172	163(121)	209(107)	180(91.1)
Elevation	205	232(35.8)	240(23.7)	242(13.9)

## 14. Key Report: Prey size and dietary niche of a gleaning bat, *Corynorhinus rafinesquii*

*In review with Acta Chiropterologica.*

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### Abstract

Bats in the genus *Corynorhinus* possess a suite of morphological characters that permit them to effectively use both gleaning and aerial-hawking foraging strategies to capture Lepidoptera. Consequently, they occupy a specialized feeding niche within North American bat assemblages and are of particular interest for dietary studies. We collected fecal pellets from a colony of Rafinesque's big-eared bat (*C. rafinesquii*) at Mammoth Cave National Park (USA) from August – October 2011, and extracted and amplified Cytochrome c oxidase subunit I fragments of prey from these fecal pellets. We used the Barcode of Life Database to identify prey, and evaluated the size of prey species based on published values. The mean wingspan of prey recorded was smaller than average values reported for *C. rafinesquii* using traditional methods ( $P \leq 0.01$ ), suggesting that surveys of culled insect parts beneath roosting sites may lead to biased estimates of the size and breadth of prey species eaten by gleaning bats. Mean wingspan of lepidopteran prey consumed by *C. rafinesquii* was larger ( $P \leq 0.01$ ) than values reported for a smaller, sympatric gleaner in eastern North America (*Myotis septentrionalis*), but was similar in size to prey of an Old World species of big-eared bat (*Plecotus macrobullaris*). Further, comparisons of our diet data with abundance of prey suggest common macrolepidopteran taxa are consistently consumed by *C. rafinesquii*. Our findings reaffirm that *C. rafinesquii* is a lepidopteran specialist and suggest that North American *Corynorhinus* consume a wider range of sizes and species of Lepidoptera than previously reported in studies based solely on identification of culled prey wings beneath feeding roosts.

### Introduction

Bats in the genus *Corynorhinus* (Vespertilionidae) are among the best examples of dietary specialization found within diverse assemblages of insectivorous bats. Members of this genus and other vespertilionid species within the plecotine tribe possess adaptations that permit them to glean prey from surfaces in structurally complex environments (Lacki et al. 2007). The diets of these bats are well studied and, in contrast to many insectivorous bat species, use of traditional identification methods has permitted high resolution identification of prey (Lacki and Dodd 2011). This is largely due to the behavioral habit of these bats at capturing prey and returning to a roost for feeding. While at feeding roosts, *Corynorhinus* typically consumes the soft portions of prey and discard the remnant wings. These remnants provide diagnostic characteristics that permit the identification of prey to the species level. Even so, the high-resolution dietary patterns generated for these bats are not without limitations or potential bias. For example, while data collected indicate that both New and Old World plecotine bats consume

larger-sized Lepidoptera (Lacki and Dodd 2011, Alberdi et al. 2012), it remains unclear whether historical methods result in a complete picture of the dietary breadth of these bats (i.e., dissection and morphological identification of gut contents or fecal pellets). Some have postulated that *Corynorhinus* also captures smaller prey which are consumed in flight or in their entirety at the feeding roost (Sample and Whitmore 1993, Burford and Lacki 1998). The application of alternative DNA-based methods can be used to investigate hypotheses regarding the feeding behavior (e.g., gleaning versus aerial hawking, location of prey consumption) of these bats and the possibility of bias of using established methods of dietary analysis.

To date there has been no molecular investigation of the diet of any *Corynorhinus*. Regardless, there is an emerging body of data for Old World plecotine species that provide a foundation for comparison with North American species. Razgour et al. (2011) investigated overlap in the dietary niches of *Plecotus auritus* (brown long-eared bat) and *Plecotus austriacus* (gray long-eared bat) and found they ate many of the same prey species, leading the authors to infer little dietary differentiation between these two species of bats. Even so, the authors concluded that differences in the host-plant affinities of prey might result in variation in occurrence of prey and, subsequently, in the spatiotemporal separation of foraging activity between these sympatric species. More recently, Alberdi et al. (2012) documented the diet of *Plecotus macrobullaris* (mountain long-eared bat) using molecular techniques. Their findings indicate this rare bat consumes lepidopteran prey similar in size to those documented at feeding roosts of *Corynorhinus* in eastern North America (Lacki and Dodd 2011). Because of the need for a refined understanding of the prey consumed by these specialist insectivores, we sought to delineate, using molecular methods, the diet of Rafinesque's big-eared bat (*C. rafinesquii*), the most wide-ranging *Corynorhinus* in eastern North America (Bat Conservation International and the Southeastern Bat Diversity Network 2013).

We determined whether the size of Lepidoptera identified using a DNA-based method differed from the size of known records of prey of *C. rafinesquii* and its congeners (Lacki and Dodd 2011), and hypothesized that past assessments for *C. rafinesquii* are likely incomplete, under-representing the range in size of prey consumed by this predator. Secondly, we compared the size of Lepidoptera consumed by *C. rafinesquii* in our study to sizes of prey reported for two other species of bats, *Lasiurus borealis* (eastern red bat) and *Myotis septentrionalis* (northern long-eared bat), for which there is species-level knowledge of their respective lepidopteran prey (Clare et al. 2009, Dodd et al. 2012a). These species are sympatric across much of their distributions in eastern North America (Jones 1977, Shump and Shump 1982, Caceres and Barclay 2000). An established framework for prey selection of insectivorous bats predicts that larger bats eat larger prey relative to smaller bats, due to ease in prey handling and inherent differences in detection of prey resulting from echolocation characteristics (Barclay and Brigham 1991, Bogdanowicz et al. 1998). Following this paradigm, we hypothesized that *C. rafinesquii* should consume larger Lepidoptera than reported for *M. septentrionalis* based on analyses using similar DNA-based methods, as *M. septentrionalis* is smaller in size than *C. rafinesquii* (Barbour and Davis 1969). *Myotis septentrionalis* is known to capture prey by both gleaning and aerial hawking (Faure et al. 1993). In contrast, *Lasiurus borealis* captures prey primarily by aerial-hawking (Acharya and Fenton 1992), yet is similar in size to *C. rafinesquii* (Barbour and Davis 1969). Aside from a difference in prey detection through echolocation call strategies or foraging behavior (i.e., aerial-hawking versus gleaning) we hypothesized similar sizes of Lepidoptera

would be consumed by *C. rafinesquii* and *L. borealis*. Lastly, we evaluated the lepidopteran prey consumed by *C. rafinesquii* in the context of lepidopteran prey abundance. Assuming *C. rafinesquii* does not select prey on the basis of size, we hypothesized that the consumption patterns of this predator should broadly follow the abundance patterns of Lepidoptera in habitats where these bats forage.

## Materials and Methods

Field work occurred at Mammoth Cave National Park (MCNP; 37.2072° N, 86.1319° W). This park encompasses 23,000 ha in Barren, Hart, and Edmonson counties and is positioned at the edge of the Crawford-Mammoth Cave Uplands of the Interior Plateau of Kentucky, USA (Woods et al. 2002). The area is primarily forested and is dissected by numerous small drainages that create a topographically diverse landscape. Forest cover consists of oak-hickory (*Quercus* and *Carya* spp.) and western mixed mesophytic forests (Braun 1950). During summer, *C. rafinesquii* roosts in hollow trees, sandstone outcrops, caves, and abandoned human-made structures (Johnson et al. 2012). Numerous caves occur at MCNP, and this location possesses one of the largest known winter concentrations of *C. rafinesquii* (Bayless et al. 2011).

We collected fecal pellets beneath a colony of *C. rafinesquii* during late summer 2011. This roost location was situated in the rafters of an equipment barn. Bats were found at this location throughout the maternity season of 2011 and were known to use this location as a maternity roost in past years (R. Toomey, U.S. National Park Service, pers. comm.). For a single sampling interval, the roost location was entered at night after nearly all bats had left to forage (ca. 1 – 1.5 hrs after sunset). At this time, a plastic tarpaulin (2.7 m × 3.7 m) was placed on the wooden floor of the loft of the barn. Fecal pellets were captured on the tarpaulin throughout the following day while bats were roosting. The roosting colony was checked during this time to ensure the roosting individuals were the target species, which is easily distinguished from other bats in the study area due to its conspicuous ears (Barbour and Davis 1969). We estimate that colony size varied between 20-100 individuals; no species other than *C. rafinesquii* was observed at the roost during our study. The tarpaulin was removed the following night after bats had left the roost, and up to 30 fecal pellets were collected arbitrarily from the tarpaulin and individually transferred to 1.5 ml microcentrifuge tubes filled with 100% ethanol. Fecal pellets were placed in long-term freezer storage (–80°C) upon return to the laboratory (within 3 days). At no time were fecal pellets allowed to contact any surface other than the plastic tarpaulin or the microcentrifuge tube. Ten fecal pellets were randomly selected from each sampling interval for subsequent dietary analysis. Sampling was conducted over biweekly intervals from August – October 2011, yielding a total of 60 fecal pellets. All methods used for this portion of the study were in accordance with the Institutional Animal Care and Use Committees of the University of Kentucky (IACUC No. A3336-01) and U.S. National Park Service (IACUC No. 2011-30), and followed the guidelines of the American Society of Mammalogists (Sikes et al. 2011) and requirements of state and federal collection permits.

We conducted surveys of nocturnal insects concurrent with collection of fecal pellets. *C. rafinesquii* is a habitat generalist (relative to other *Corynorhinus*). This species is known to forage within and along the edges of forest canopies, as well as within open field habitats (Lacki and Dodd 2011). As such, our surveys took place across an array of upland and riverine habitats

that covered the range of forest canopy heights at MCNP. Light traps are widely used to survey lepidopteran assemblages in forested environments (Burford et al. 1999, Covell 2005, Dodd et al. 2008). We placed 10-W blacklight traps (Universal Light Trap, Bioquip Products, Gardena, California) at multiple sites each survey night ( $n \geq 7$  traps / night). A blacklight trap was always placed in the immediate vicinity of the roost location (ca. 150 m away) to ensure sampling always occurred within the home range of bats at the roost location. In addition to this fixed survey location, survey transects were systematically chosen (without replacement) from land parcels definable as distinct prescribed burn units (Dodd et al. 2013). Blacklight traps were positioned at the micro-scale along these transects by the random assignment of the transect start position and bearing. All blacklight traps were  $\geq 100$  m apart; traps were spaced far enough apart to ensure no interference between traps (Muirhead-Thomson, 1991). Based on recommendations in Yela and Holyoak (1997), surveys were conducted on nights with temperatures  $\geq 16^\circ$  C at sunset, no precipitation, and low wind speeds. We suspended blacklight traps 2.5-m aboveground prior to sunset and operated them throughout the entire night. This deployment method ensured traps were visible within forest canopies, as well as near ground level, where *C. rafinesquii* is known to forage (Lacki and Dodd 2011). A dichlorvos-based ‘pest strip’ (ca.  $2 \times 6$ -cm) was placed within each blacklight trap to subdue specimens. Trap contents were collected the following morning and all Lepidoptera were enumerated. Specimens with wingspans  $\geq 20$  mm were identified to family level using Covell (2005) and reference collections at the University of Kentucky. Specimens with wingspans  $< 20$  mm were simply classified as microlepidoptera and not identified to family level. Discrimination of micro- versus macrolepidopteran families with wingspans  $\geq 20$  mm followed Covell (2005). Our classification of noctuids followed revisions of LaFontaine and Schmidt (2010).

Prey remains within fecal pellets were identified both morphologically and molecularly. Pellets were dissected microscopically and prey remains identified to the most specific taxon possible (Whitaker 1988). Individual fecal pellets were placed in a sterile pour boat ( $4.1 \times 3.2 \times 0.8$  cm; Fisher Scientific, Pittsburgh, Pennsylvania), diluted with 100% ethanol and teased apart using a disposable pestle (Fisher Scientific) for microscopic dissection. We estimated percent frequency of prey items at the ordinal level in each pellet to the nearest 5%. Individual fecal pellets were then preserved in ca. 1.5 ml of 95% ethanol and placed in freezer storage ( $-80^\circ$ C) for subsequent DNA-based analysis. The entire pellets used for morphological identification were used for each individual DNA extraction. Prior to DNA extraction, each fecal pellet was homogenized for ca. 1 min in 2.0 ml mortar-and-pestle microcentrifuge tubes, vortexed ca. 1 min, and then centrifuged at  $20,000 \times g$  for 3 min, discarding the resulting supernatant. Afterwards, 1 ml of water was added to samples which were then vortexed ca. 1 min, centrifuged at  $20,000 \times g$  for 3 min, and the supernatant discarded. DNA was then extracted using a QIAamp DNA Stool Mini Kit (Qiagen Inc., Chatsworth, California). Protocol followed the manufacturer instructions for the isolation of DNA from stool for pathogen detection, carrying out lysis with manufacturer’s premixed ASL buffer solution at  $70^\circ$ C and using all applicable extra centrifugation steps.

Polymerase chain reactions (PCR) (total volume = 50 uL) for nucleotide sequencing of the Cytochrome c oxidase subunit I gene 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 and cycling conditions followed Dodd et al. (2012a). Reaction success was confirmed as in Dodd et al.

(2012a) and sequencing of PCR products (University of Kentucky Advanced Genetic Technologies Center, Lexington, Kentucky) 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). Reactions were sequenced bi-directionally to reduce the possibility of chimeric sequences consisting of DNA fragments from multiple prey items (Dodd et al. 2012a); overlapping forward and reverse sequences were edited and assembled using Geneious (v. 6.0.3, Biomatters Ltd., Auckland, New Zealand). To reduce the possibility of chimeric sequences, all chromatograms were inspected for double peaks and potential sequencing error. If strong, corresponding signals were not present in forward and reverse chromatographs, such portions of sequences were marked as unidentifiable or discarded if the majority of a sequence was unknown. We generated a single sequence per fecal pellet (Dodd et al. 2012a).

Prey identities were inferred using web-based searches to compare unknown DNA sequences with the Barcode of Life Data System (BOLD) in December, 2012. Species-level identification of unknown sequences using BOLD was carried out following the methods of Clare et al. (2009). Matches of  $\geq 99\%$  similarity between our unknown sequence and a single species in the database were considered sufficiently close 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 (and  $\geq 98\%$  similarity) (Clare et al. 2011).

We calculated a mean  $\pm$  SE wingspan for all genera or species of Lepidoptera identified in fecal pellets using BOLD. Wingspan values are the standard measurement for the size of Lepidoptera in eastern North America (Covell 2005). We took the midpoints of the wingspan ranges provided by Covell (2005) and the Bug Guide database hosted by Iowa State University in 2013 ([www.bugguide.net](http://www.bugguide.net)). These midpoint values were then used to generate an unweighted mean  $\pm$  SE of the wingspan of prey, with each taxon being included once in the calculation. We then compared the mean wingspan of prey identified in this study with the mean wingspan of lepidopteran prey previously reported in the literature (Lacki and Dodd 2011) using a Wilcoxon rank-sum test (SAS Institute 2002). We then compared the mean wingspan of prey calculated in this study with the mean wingspans for Lepidoptera consumed by *L. borealis* and *M. septentrionalis* (Clare et al. 2009, Dodd et al. 2012a) using a Kruskal-Wallis test followed by a nonparametric means separation based on the Dunn–Nemenyi procedure (Elliot and Hynan 2011). Lastly, we assessed patterns of lepidopteran consumption at the family level with the relative occurrence of these prey families, as indexed across the landscape of MCNP.

## Results

We were able to extract and amplify DNA from 54 pellets (90% success). Multiple identifications ( $n = 7$ ) came back with a closest-similarity match to the DNA for *C. rafinesquii*. Beyond these non-target amplifications, nearly all sequences (98%) for which high resolution matches (genus/species) could be made were identified as Lepidoptera (Fig. 14.1), except for a single dipteran outcome (Chironomidae: *Chironomus*). Of the 22 lepidopteran species identified, 19 were new dietary records for *C. rafinesquii* (Table 14.1). Sequences complete with genus/species identities will be archived on GenBank upon peer-reviewed publication.

Morphological dissections showed Lepidoptera formed  $71.2 \pm 1.74$  % (mean  $\pm$  SE) of the volume of pellet.

The size of Lepidoptera eaten by *C. rafinesquii* in our study differed from that previously reported in the literature for the species, and also differed from the size of prey consumed by other bat species. The average wingspan of prey detected using DNA-based methods was smaller than that measured by collection of culled prey remnants below feeding roosts ( $35.8 \pm 1.5$  mm versus  $51.2 \pm 2.4$  mm) ( $\chi^2 = 21.5$ , *d.f.* = 1,  $P \leq 0.01$ , Fig. 14.2). Comparison of prey consumed by *C. rafinesquii* versus sympatric bat species demonstrated size of Lepidoptera eaten by *C. rafinesquii* overlapped with published values for prey eaten by *L. borealis*, ( $34.1 \pm 1.6$  mm) but were larger than prey eaten by *M. septentrionalis* ( $27.2 \pm 3.6$  mm) ( $\chi^2 = 9.9$ , *d.f.* = 2,  $P \leq 0.01$ , Fig. 14.3).

Of the 6,084 Lepidoptera that were captured on the same nights that fecal pellets were collected (6 nights, *n* = 48 trap-nights), most specimens (66%) possessed wingspans <20 mm. The most abundant families identified, however, were the Erebidae, Geometridae, Noctuidae, and Notodontidae (Fig. 14.1). While these same macrolepidopteran families were most heavily consumed by *C. rafinesquii*, reduced consumption was documented for microlepidoptera as well as the largest representatives in the macrolepidoptera (i.e., Saturniidae and Sphingidae).

## Discussion

This study demonstrates the ecological and methodological merit for expanded DNA-based dietary assessments of specialist insectivores. As per our expectations, our study provided evidence that *C. rafinesquii* consumes, on average, smaller Lepidoptera than previously documented using traditional methods (Lacki and Dodd 2011). Further, our study expands the breadth of known prey species for *C. rafinesquii* by more than 66% (Lacki and Dodd 2011). Given the similarities in size, foraging behaviors, and dietary preferences across *Corynorhinus* in North America, it is likely that bats in this genus consume a number of Lepidoptera previously unrecorded using traditional methods. These bats presumably consume the smaller prey in their entirety while in flight (Burford and Lacki 1998).

In an assemblage context, these data illuminate the variation inherent in patterns of consumption of Lepidoptera across the diversity of insectivorous bats in North America. There are two primary ensembles of bats in eastern North America that use gleaning as a foraging strategy, the species of *Corynorhinus* and *Myotis* (Lacki et al. 2007). Our data indicate a wider range of *C. rafinesquii* prey sizes than previously reported in the literature and suggest niche separation, in terms of the sizes and taxa of prey exploited, between these species of bats. Consistent with our hypothesis, *C. rafinesquii* in our study consumed Lepidoptera on average 33% larger than species documented for *M. septentrionalis* by Dodd et al. (2012a) ( $35.8 \pm 1.5$  mm versus  $27.2 \pm 3.6$  mm, respectively). Further, 97% of the Lepidoptera consumed by *C. rafinesquii* in this study were macrolepidoptera, whereas over half of the prey previously reported for *M. septentrionalis* using the same primers and PCR conditions were microlepidoptera (Dodd et al. 2012a). The mean  $\pm$  SE distance between MCNP and sites studied by Dodd et al (2012a) is only  $333 \pm 74$  km. We suggest the variation in prey size and identity is likely attributable to differences in morphology and echolocation call characteristics of the two

predator species. *C. rafinesquii* can be up to 75% heavier and more than 15% longer than *M. septentrionalis* (Jones 1977, Caceres and Barclay 2000). The larger size of *C. rafinesquii* likely aids in the capture and handling of larger Lepidoptera, which are thought to be energetically profitable prey items (Razgour et al. 2011). In addition, the propensity for plecotine species, including *C. rafinesquii*, to echolocate at lower amplitudes and lower frequencies (Bayless et al. 2011, Lacki and Dodd 2011) could contribute to their ability to detect and handle larger lepidopteran species than *M. septentrionalis* (Barclay and Brigham 1991). We suggest that although *Corynorhinus* and some *Myotis* bats may use similar gleaning strategies, the diets of these bats likely vary from one another across much of their distributions in North America. Conversely, we did not observe differences in the size of lepidopteran prey eaten by *C. rafinesquii* versus prey eaten by *L. borealis* (Clare et al. 2009). Given similarities in size between *C. rafinesquii* and *L. borealis*, it follows expectations that both predator species would capture larger-sized prey than *M. septentrionalis* (Barclay and Brigham 1991). Even so, *L. borealis* is a generalist species that consumes a variety of insect orders across its distribution (Carter et al. 2003, Clare et al. 2009, Feldhammer et al. 2009). As such, while the prey sizes considered in this study did not differ, the diets of these bats still fundamentally vary as a consequence of differences in habitat use and the reliance of *L. borealis* on aerial-hawking as a foraging strategy.

Despite variation in patterns of prey consumption for *C. rafinesquii* and sympatric bat species, the macrolepidopteran prey selected by *C. rafinesquii* aligned with observed patterns of abundance for the most common macrolepidopteran families in the study area, as well as across the region (Dodd et al. 2012b, 2013). Families frequently consumed by *C. rafinesquii* included Erebiidae, Geometridae, Noctuidae, and Notodontidae. Additionally, there was a noted absence of larger macrolepidoptera (i.e., Saturniidae and Sphingidae) in the diet of *C. rafinesquii* compared with previous reports in the literature (Lacki and Dodd 2011). Because the primers and PCR conditions used in this study have previously demonstrated amplification of these targets, in both bat fecal pellets (Dodd et al. 2012a) and from prey tissue (Dodd 2010), there is strong evidence that neither Saturniidae nor Sphingidae were consistently eaten by the colony of *C. rafinesquii* in our study. Even so, we concede that the timing of our study (i.e., during post-lactation when young were volant) does not preclude the possibility of seasonal variance between the prey consumed in our study and data previously reported for *C. rafinesquii* using traditional techniques (Lacki and Dodd 2011).

In a global context, our study builds on an expanding knowledge of prey consumption patterns of plecotine bats. Prey species consumed by *C. rafinesquii* in this study align with the size of prey reported for *P. macrobullaris* ( $37.6 \pm 1.5$  mm) (Alberdi et al. 2012). Additionally, the family-level and broader classifications of prey taxa are similar to that of *P. macrobullaris*, as well as *P. auritus* and *P. austriacus* (Razgour et al. 2011, Alberdi et al. 2012). DNA-delineated patterns in consumption for these bat species demonstrate a general absence of microlepidopteran prey; thus, it would appear that multiple *Corynorhinus* and *Plecotus* species are selecting larger-sized Lepidoptera across their distributions. Data from this and other DNA-based investigations of the food habits of predatory bats continue to offer an opportunity to test hypotheses relevant to behavior, foraging, and phylogeny.

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Table 14.1. Prey identified for *Corynorhinus rafinesquii* using a molecular technique. Asterisks denote novel prey documented in the diet of this bat species.

Order	Family	Genera / Species
Diptera	Chironomidae	<i>Chironomus</i> sp*
Lepidoptera	Crambidae	<i>Herpetogramma thestealis</i> *
	Erebidae	<i>Hypena scabra</i> *
		<i>Palthis angulalis</i> *
		<i>Spilosoma virginica</i> *
		<i>Zale lunata</i> *
		<i>Paectes abrostoloides</i> *
	Euteliidae	<i>Capusa senilis</i>
	Geometridae	<i>Melanolophia canadaria</i> *
		<i>Nemoria catachloa</i>
		<i>Agrotis gladiaria</i> *
	Noctuidae	<i>Amphipyra pyramidoides</i> *
		<i>Anicla infecta</i> *
		<i>Athetis tarda</i> *
		<i>Feltia subterranea</i> *
		<i>Mythimna unipuncta</i> *
<i>Nephelodes minians</i> *		
<i>Phlogophora periculosa</i> *		
<i>Spodoptera frugiperda</i> *		
<i>Spodoptera ornithogalli</i> *		
<i>Xestia maculata</i>		
Notodontidae	<i>Locmaeus manteo</i> *	

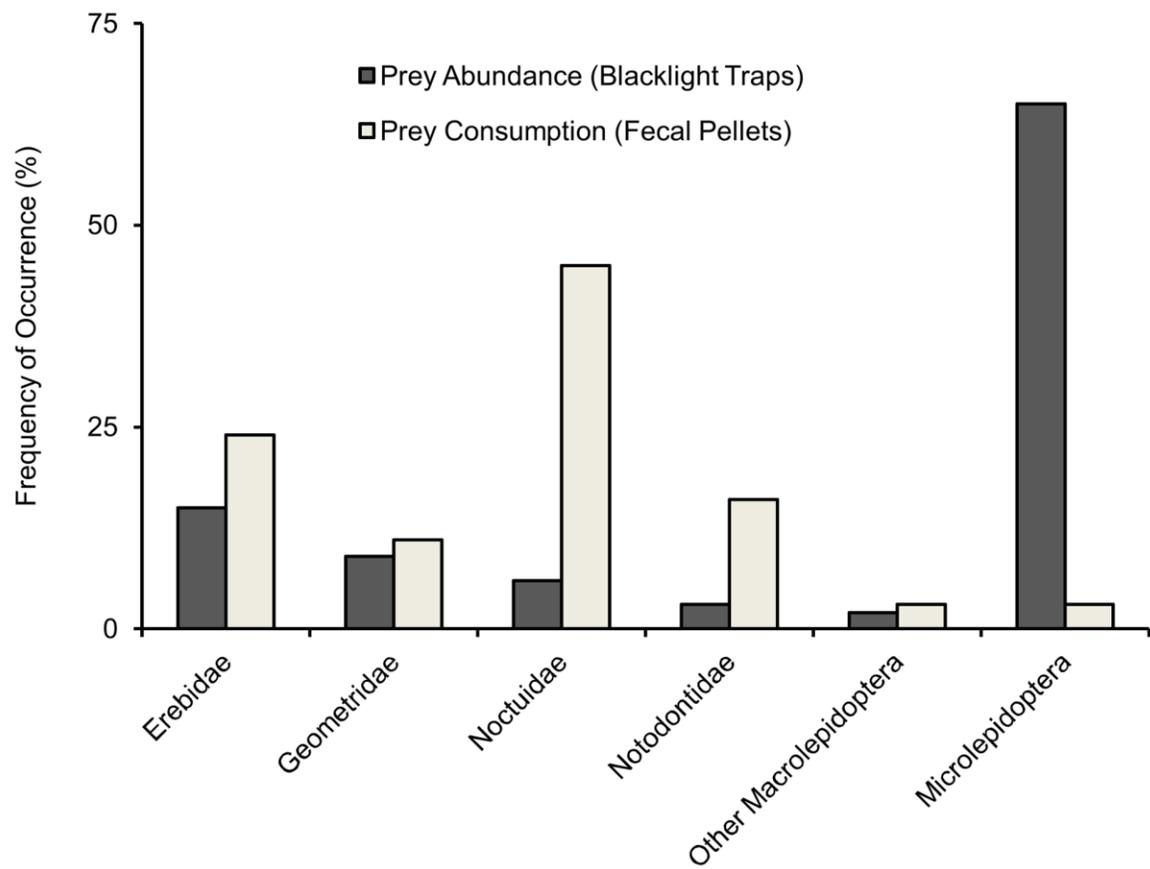


Fig. 14.1. Relative consumption and abundance patterns of Lepidoptera eaten by *Corynorhinus rafinesquii* at Mammoth Cave National Park, Kentucky, USA.

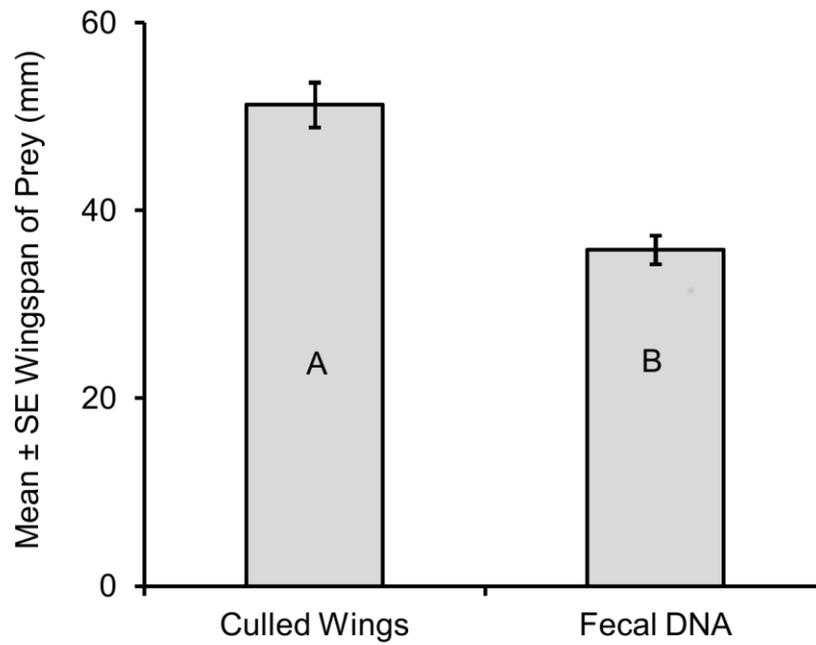


Fig. 14.2. Comparison of lepidopteran prey size of *Corynorhinus rafinesquii* as estimated using culled wings collected from feeding roosts versus a DNA-based assessment of fecal pellets. Estimates of prey size were significantly different at  $P \leq 0.01$  (indicated by differing letters between histograms). Bibliographic source for culled-wing data is given in the main text.

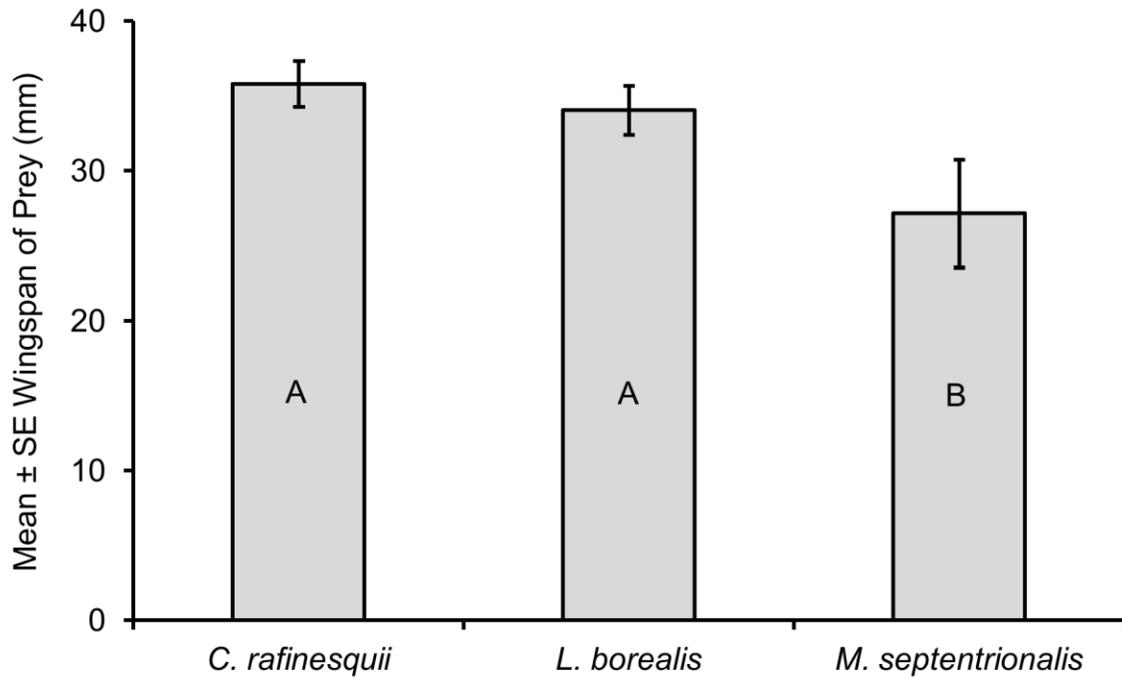


Fig. 14.3. Comparison of lepidopteran prey size of *Corynorhinus rafinesquii* and co-occurring bat species as estimated using DNA-based assessments of fecal pellets. Mean separations were significant between *C. rafinesquii* and *Myotis septentrionalis* ( $P \leq 0.01$ ), as well as between *Lasiurus borealis* and *M. septentrionalis* ( $P = 0.02$ ), but not between *C. rafinesquii* and *L. borealis* ( $P = 0.08$ ). Significant differences are represented by differing letters among histogram bars. Bibliographic sources for data corresponding to *L. borealis* and *M. septentrionalis* are given in the main text.

## 15. Key Report: Body condition of cave-hibernating bats during staging and swarming at Mammoth Cave National Park.

*In preparation for the Journal of Fish and Wildlife Management.*

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### Abstract

The rapid colonization of the *Pseudogymnoascus destructans* fungus across cave systems in eastern North America, and the associated disease effects leading to extensive bat mortalities (white-nose syndrome; WNS), necessitates studies of cave-hibernating bat populations that remain unaffected, but occur within sufficient proximity of the leading edge of the fungal distribution as to expect infection within a relatively short time frame. Studies of the physiological ecology of cave-hibernating bats during the staging and swarming periods are few, thus, our understanding of patterns in body condition of bats associated with emergence from and entry into hibernation is incomplete. We sampled bats at the entrance to a cave in Mammoth Cave National Park (MCNP), Kentucky, USA, during staging and swarming from 2011 to 2014, ahead of and concurrent with the arrival of the WNS fungus, and evaluated seasonal and annual changes in body condition of the assemblage of bats hibernating in the cave. We captured 1,112 bats of 8 species. Sex ratios of populations of all species were male-biased. Capture success was substantially reduced in spring 2014 one winter after the WNS fungus was detected in the cave. Body mass index (BMI) varied among species, with the largest bat, big brown bat (*Eptesicus fuscus*), demonstrating the highest mean BMI values and the smallest bats, tri-colored bat (*Perimyotis subflavus*) and eastern small-footed bat (*Myotis leibii*), having the lowest BMI values. Some temporal variation in BMI, seasonal, annual or sampling interval, was observed in all bat species evaluated except the Indiana bat (*Myotis sodalis*) and eastern small-footed bat. Most species demonstrated highest mean BMI values in late swarming compared to other sampling intervals, with tri-colored bats showing the greatest increase in BMI (53.8%) prior to entry into hibernation. Males and females of most species demonstrated a trend toward higher mean body mass (BM) and mean BMI values in swarming than staging, with differences significant for tri-colored bats and northern long-eared bats. These data indicate that significant temporal and intra-specific variation in body condition of cave-hibernating bat species exists, with patterns in variation strongly associated with the seasonal differences of staging and swarming. This variation in BMI across species is likely to have implications for the relative vulnerability of bat species to WNS infection, both in MCNP and elsewhere across the distribution of the *Pseudogymnoascus* fungus.

## Introduction

Bats in the northern temperate zone face energetic stressors in winter months due to cold ambient temperatures that lead to reduced availability of insect prey and higher metabolic costs of thermoregulation, forcing species to choose between migration in autumn to warmer climates (Fleming and Eby 2003; Cryan and Veilleux 2007) or moving shorter distances at the completion of the breeding season to roosting sites nearby and entering periods of deep torpor or hibernation (Speakman and Thomas 2003). Winter hibernation is preceded and followed by swarming and staging, two periods in the annual cycle of many temperate zone bats where breeding occurs and where bats must acquire sufficient fat reserves to either enter or emerge from hibernation. Swarming is associated with rapid flight, chasing behavior and vocalizations of large numbers of bats in and out of the entrances to caves (Fenton 1969; Thomas et al. 1979; Furmankiewicz et al. 2013). Most typically associated with *Myotis* bats, swarming is known to occur in seven genera of Microchiropteran bats (Parsons et al. 2003). This behavior begins in late summer, peaks in mid-autumn and ceases in mid-October to early November when bats enter hibernation (Šuba et al. 2008; Vintulis and Šuba 2010). Swarming is hypothesized to serve several functions including finding mates (Parsons et al. 2003; Rivers et al. 2005), introducing volant young to hibernation sites (Humphrey and Cope 1976; Veith et al. 2004), serving as rendezvous sites in migration (Fenton 1969), and assessing conditions inside hibernacula (Davis and Hitchcock 1965; Furmankiewicz and Gorniak 2002). Sex ratios of bats in swarming populations are usually male-biased (Piksa 2008; Šuba et al. 2008; Vintulis and Šuba 2010), although exceptions have been reported for populations of *Myotis myotis* (Pocora et al. 2012). Swarming behavior can facilitate gene flow, especially among relatively isolated summer colonies of bats (Kerth et al. 2003; Veith et al. 2004; Rivers et al. 2006), with male-biased hybridization also occurring among species in some instances suggesting at least some randomness in mating during swarming with high evolutionary potential for speciation and biodiversity (Bogdanowicz et al. 2012).

Less is known about the spring staging or transient (Neubaum et al. 2014) period. Females are believed to awaken and emerge from hibernacula earlier in spring than males (Cope and Humphrey 1977; Little and Brack 2001), with some males remaining at hibernacula well into the summer season (Hall 1962; Whitaker and Brack 2001). Both male- and female-biased sex ratios have been reported for populations of bats during spring staging (Little and Brack 2001). Mating is believed to occur throughout hibernation and into the spring staging season, but to a lesser extent than observed during autumn swarming (Thomas et al. 1979; Wai-Ping and Fenton 1988; Watt and Fenton 1995). The relative importance to successful reproduction of copulations in autumn versus spring is still unclear (Burland et al. 2001; Senior et al. 2005). Regardless, during both the swarming and staging periods temperate zone bats are faced with building fat reserves to survive either an extended hibernation period or to support pregnancy and the gestation of young (Speakman and Thomas 2003). Estimates suggest bats need to add up to 2.3 grams of fat to sustain hibernation periods that can last up to 9 months at more northern latitudes (Kunz et al. 1998). In swarming, the accumulation of fat reserves for hibernation is slowed by the energy expended in mating behaviors and flight (Piksa 2008; Šuba et al. 2011) with evidence suggesting males lower in body condition often spend more time in swarming activities than presumably healthier, males in better condition (Lowe 2012). Temporal patterns in fat accumulation for bats in the staging period are less well known.

The arrival of the fungus *Pseudogymnoascus destructans* (formerly *Geomyces destructans*) to cave systems in North America in 2006 (USFWS 2012a), and the subsequent widespread mortality of cave-roosting bats caused by the debilitating effects of the fungus (Frick et al. 2010), now known as white-nose syndrome (WNS), has created concern for the long-term conservation of cave-hibernating bats in eastern North America. Mortality numbers of bats at hibernacula in eastern North America likely exceed the estimated 6.7 million deaths reported since 2007 (USFWS 2012a) and, despite efforts to ensure that humans do not spread the fungus among cave systems (USFWS 2012b, Shelley et al. 2013), the fungus continues to expand in distribution and has now affected 9 species of bats over multiple states and Canadian provinces (Foley et al. 2011).

For bats that successfully reach the spring staging period, WNS can cause significant tissue damage to wing membranes (Reichard and Kunz 2009), with degree of recovery from injuries following the staging period correlated with body mass index (BMI); on average individuals with higher BMI in late spring and early summer were shown to have less overall wing damage (Fuller et al. 2011). Thus, replenishment of fat stores during spring staging is likely important to survival and reproduction of bats emerging from hibernation, especially individuals impacted by WNS. In this study, we monitored BMI and body mass of bats during autumn swarming and spring staging from 2011 to 2014 at a cave entrance in Mammoth Cave National Park, Kentucky, USA, in advance and commensurate with the arrival of WNS to the Park. We hypothesized that BMI would vary among species and across seasons, and that peaks in fat stores should be evident through increases in BMI with peaks in late swarming and late staging, when energy reserves are most needed for hibernation and gestation, respectively.

## Methods

Mammoth Cave National Park (MCNP) encloses 23,000 ha in Barren, Hart, and Edmonson counties (37.2072°N, 86.1319°W) on the edge of the Crawford-Mammoth Cave Uplands of the Interior Plateau of central Kentucky (Woods et al. 2002). The Park is largely forested and supports primarily oak-hickory (*Quercus* - *Carya* spp.) and western mixed mesophytic forests (Braun 1950). Management of forests at MCNP includes use of prescribed fire to restore historic native plant communities, with fires set from 2004 through 2010. Roughly 33% of the Park land surface has been burned 1 or more times.

Erosion of limestone and sandstone bedrock by numerous drainage systems has produced a topographically diverse landscape containing hundreds of small caves, including the longest known cave system in the world. Bats were captured at the entrance to Colossal Cave, a priority 2 hibernaculum for the Indiana bat (*Myotis sodalis*). Colossal Cave was a portion of the Mammoth Cave system receiving commercial use in the late 1800s, with the entrance used for capturing bats being an artificial access, created to allow entry into the cave by tour groups. Evidence reflecting prior commercial use remains, with entry inside now prevented by an angle iron gate and signage. White-nose syndrome was first confirmed in the Park in January 2013 on a northern long-eared bat (*Myotis septentrionalis*), and was discovered in Colossal Cave later that winter.

We captured bats at Colossal Cave using a harp trap placed at the cave entrance, with the surrounding flight space enclosed with netting to ensure that exiting bats passed through the trap wires. The trap was set for 6 nights in each of 2011, 2012, and 2013, and for 4 nights in spring 2014. During the first three years of sampling, we conducted three trapping nights during the spring staging period from 5 April to 12 May, and during the autumn swarming period from 21 August to 25 September. Trap nights in spring 2014 began on 4 April and, due to significantly lower captures, continued until 20 May. Trap nights each season were approximately 10 days apart, depending on weather. On each sampling night, the trap was operated from dusk till approx. 11:00 pm, or until 50-60 bats were captured.

All WNS protocols established by the USFWS for operating, disinfecting and cleaning trapping equipment, and for the handling of bats were followed to prevent the spread of fungal spores among bats and humans (USFWS 2012b). Bats were placed into separate disinfected cloth bags and then identified to species; weighed (g) using a digital scale; aged based on fusion of the finger joints (adult/juvenile) (Brunet-Rossinni and Wilkinson 2009); measured for forearm length (mm) with digital calipers; sexed; assessed for reproductive condition by examining for development of the epididymes (males) or the presence of a fetus, swollen vulva or swollen teats (females) (Racey 2009); assessed for wing damage (scale: 0-3; Reichard and Kunz 2009); scanned with ultraviolet light to check for fungal spores; fitted with a 2.7 or 2.4 mm identification arm band (*Myotis* species only; bands supplied by Kentucky Department of Fish and Wildlife Resources, Frankfort, KY); examined for external parasites; and then released.

Due to the decline in bat captures at the cave entrance in spring 2014 (Figure 15.1), analyses were based solely on data collected from 2011 through 2013 and, thus, largely reflect the physiological state of bats prior to impacts from WNS. We calculated sex ratios as males/female for all species captured. We generated body condition indices (BMI) as body mass (g) · forearm length (mm)<sup>-1</sup> (Pearce et al. 2008). We compared BMI among species using a one-way ANOVA with an LSD means comparison procedure. Two species, Rafinesque's big-eared bat (*Corynorhinus rafinesquii*) and eastern red bat (*Lasiurus borealis*) were excluded from analysis due to insufficient sample size. We extended this analysis by using linear regression to evaluate the relationship of mean BMI of bat species with mean body mass (BM) for each species.

For species with sufficient sample size ( $n > 50$  bats), we assessed temporal variation in BMI by modeling the effects of sampling interval (early, mid- and late staging; and, early, mid- and late swarming), year (2011, 2012 and 2013), and sampling interval\*year interaction with a multi-way ANOVA, with effect sizes evaluated using Type III Sums of Squares due to unbalanced sample sizes across cells (Pendleton et al. 1986). For the two most commonly captured species ( $n > 400$  bats), the tri-colored bat (*Perimyotis subflavus*) and northern long-eared bat, we evaluated BMI and BM by staging and swarming using the effects of sex (male and female), season (staging and swarming), and year (2011, 2012 and 2013) in multi-way ANOVA models resulting in seven effects each: year, season, year\*season interaction, sex, sex\*year interaction, sex\*season interaction, and sex\*year\*season interaction. Type III Sums of Squares were used to evaluate effect sizes. We generated mean values for BM and BMI by sex and season for all *Myotis* species and the tri-colored bat, with data pooled across the three years of sampling to ensure sufficient sample sizes for evaluating patterns between the staging and

swarming periods. All statistical procedures were run in SAS (SAS Inc. 2002). For all statistical analyses an effect was interpreted as significant when  $P < 0.05$ .

## Results

We captured 1,112 bats of eight species in harp trap samples from 2011 to 2014. Among the species captured included two eastern red bats which were captured attempting to enter the cave and not exiting the hibernaculum. Of the remaining seven species, tri-colored bats and northern long-eared bats were most abundant representing, collectively, 84.8% of the total captures (Table 15.1). All species captured demonstrated male-biased sex ratios, except for eastern red bats, suggesting that Colossal Cave may serve as a bachelor hibernaculum site. Juvenile bats represented 4.2% of the bats captured, and were only recorded during autumn swarming. Capture numbers were steady across the first 3 years of sampling, averaging 59 bats per capture interval; however, numbers of bats captured declined precipitously in spring 2014 (Figure 15.1), suggesting impacts due to the arrival of the WNS fungus in winter 2013. Only a single northern long-eared bat was recorded in 2014 samples despite this bat being an abundant species in previous capture sessions, and entry into the cave by NPS park personnel found no mass mortalities suggesting most bat species, northern long-eared bats in particular, had abandoned the hibernaculum in response to WNS infection.

Bats demonstrating a wing damage index score (WDI) of 1 represented 2.9% (2011), 16.4% (2012), 13.1% (2013), and 13.3% (2014) of individuals sampled, with only two bats exhibiting sufficient damage to assign a WDI = 2 across all sampling intervals. No bat received a WDI = 3. Evidence of fungal spores using UV light scans demonstrated 2.9% of bats with a positive scan in 2011; however, individuals sent off for testing were not found positive for the fungus. No bat in 2012 showed a positive UV light scan, but in 2013 and 2014, 23.4% and 51.1% of the bats scanned respectively, demonstrated evidence of fungal spores.

BMI differed among species ( $F = 24.8$ ,  $P < 0.0001$ ; Figure 15.2), with big brown bats (*Eptesicus fuscus*) possessing significantly higher values than all other species ( $P < 0.05$ ); little brown bats possessing the highest values of all *Myotis* species; and, the two smallest species, tri-colored bat and eastern small-footed bat (*Myotis leibii*), having significantly smaller mean BMI values than all other species ( $P < 0.05$ ). Linear regression analysis was in agreement, demonstrating a strong relationship of mean BMI with mean BM (adjusted  $R^2 = 0.945$ ;  $\beta_{\text{mass}} = 0.026$ ,  $t = 137$ ,  $P < 0.0001$ ; Figure 15.3), indicating that mass accumulation, or loss, in staging and swarming was directly proportional to skeletal dimensions across this assemblage of bat species.

Tri-colored bat ( $F = 24.41$ ,  $P < 0.0001$ ) and little brown bat (*Myotis lucifugus*;  $F = 2.88$ ,  $P = 0.0021$ ) demonstrated significant temporal variation in BMI. BMI in tri-colored bats varied significantly due to sampling interval ( $F = 15.83$ ,  $P < 0.0001$ ) but not year ( $F = 2.07$ ,  $P = 0.1276$ ), with mean BMI being highest in late swarming and lowest in early staging, when bats first begin emerging from hibernation (Table 15.2). For little brown bats, BMI varied significantly by year ( $F = 4.96$ ,  $P = 0.0103$ ) and sampling interval ( $F = 4.5$ ,  $P = 0.0016$ ), with the interaction not significant ( $F = 1.26$ ,  $P = 0.2802$ ). Mean BMI in little brown bats was lowest in

early swarming and increased to maximum levels in late swarming (Table 15.2); this increase in BMI was largely attributable to males and not females (Table 15.3).

BMI in northern long-eared bats differed by sex ( $F = 14.87, P < 0.0001$ ) and season ( $F = 60.72, P < 0.0001$ ), but not year ( $F = 2.47, P = 0.0855$ ). The interaction of sex and season was also significant ( $F = 27.76, P < 0.0001$ ), with females being lower in BMI in staging than in swarming (Table 15.3). For tri-colored bats, BMI was significant by season ( $F = 68.16, P < 0.0001$ ) and year ( $F = 6.04, P = 0.0026$ ), but not sex ( $F = 2.97, P = 0.0854$ ). Both male and female tri-colored bats had higher mean BMI values in swarming than in staging (Table 15.3). The interaction of season and year was not significant ( $F = 2.38, P = 0.0935$ ).

Variation in BM of northern long-eared bats was more confounded, as all effects and their interaction terms were significant ( $P \leq 0.03$ ; grand model:  $F = 9.1, P < 0.0001$ ), rendering patterns in BM change difficult to interpret. Patterns in BM for tri-colored bats were more clear, as effects of sex ( $F = 22.58, P < 0.0001$ ), season ( $F = 28.91, P < 0.0001$ ), and year ( $F = 5.35, P = 0.005$ ) were all significant, with no significant interactions ( $P \geq 0.06$ ). Both male and female tri-colored bats were heavier in BM during swarming than during staging (Table 15.3).

## Discussion

Male-biased sex ratios predominate in populations of bats at swarming caves during autumn (Piksa 2008; Šuba et al. 2008; Vintulis and Šuba 2010). Bats at Colossal Cave demonstrated the same pattern for all species that hibernated in the cave. Because swarming behavior was not observed at the entrance to the cave on any night of sampling across the three years of survey, and juvenile bats were uncommon in samples of captured bats at the cave, we suggest Colossal Cave serves primarily as a bachelor hibernation site. Levels of wing damage due to WNS did not change over the time intervals sampled, but the frequency of bats possessing fungal spores increased markedly during the final year of sampling, concurrent with noticeable declines in bat captures (Figure 15.1). The disappearance of northern long-eared bats from the cave following arrival of the WNS fungus is similar to patterns observed for populations of this species in Arkansas upon exposure to the WNS fungus (B. Sasse, Arkansas Game and Fish Commission, pers. commun.).

BMI varied significantly among species and was highly correlated with body mass (Figures 15.2 and 15.3), consistent with results for bats swarming at caves in Latvia (Šuba et al. 2011). Larger bats, such as big brown bat, possessing higher BMI values entering hibernation may have a selective advantage in responding to WNS by carrying greater fat reserves to sustain them through hibernation, regardless of degree of infection. Conversely, the smallest bats we examined, eastern small-footed bat and tri-colored bat, commonly had mean BMI values ( $\leq 0.16$ ), well below those reported for other species during swarming or staging (Fuller et al. 2011; Šuba et al. 2011; Lowe 2012), rendering these two bat species vulnerable to accelerated losses in body mass due to WNS infection. Tri-colored bats exhibited the greatest fluctuation (53.8%) in mean BMI from early staging to late swarming, suggesting a hibernation strategy that already leads to significant weight loss, even without WNS infection. This percent increase was larger than percent increases reported for body mass in little brown bats (32.9% males, 29.6% females) upon entering hibernation (Kunz et al. 1998). Mean BMI values of little brown bats (males =

0.18, females = 0.17) recorded in our study during swarming were smaller than those reported for the species in Nova Scotia (0.22; Lowe 2012) and two New England states ( $> 0.2$  for all groupings with  $WDI \leq 2$ ; Fuller et al. 2011), suggesting little brown bats at the southern end of the species distribution enter hibernation with lowered fat reserves relative to populations hibernating further north. These patterns allude to two possible scenarios for little brown bats hibernating in southern North American cave systems: i) lowered fat reserves may lead to high mortality rates upon WNS infection due to exhaustion of insufficient fat stores before spring emergence (Reeder et al. 2012), or ii) lowered fat reserves are indicative of bats that annually experience shorter hibernation periods, relative to bats hibernating further north in latitude, and are more likely to withstand impacts of WNS infection due to a reduced length of exposure time to the fungus each winter (Johnson et al. 2012).

Seasonal variation in BMI was observed in tri-colored bat, little brown bat and northern long-eared bat, with highest mean BMI values recorded in late swarming, as observed for other populations of bats during swarming (Šuba et al. 2011). Studies of male little brown bats in Nova Scotia, however, found BMI values to increase in mid-swarming only to decline just prior to entry of bats into hibernation (Lowe 2012). Mean BM and mean BMI values differed between staging and swarming for both male and female tri-colored bats, with lower values in staging likely due to limited fat reserves remaining on bats upon emergence from hibernation (Speakman and Thomas 2003). Female northern long-eared bats also demonstrated higher mean BMI values in swarming than in staging. BMI can vary with time of capture, especially in late summer and early swarming, but appears to stabilize thereafter (Šuba et al. 2011). We suggest this source of error was minimal in our analysis because bats were captured exiting the cave, i.e., prior to feeding, and the majority of captures came from the first 1 to 1 ½ hours post-sunset.

Although bats exhibit fidelity to swarming sites (Glover and Altringham 2008), movement of bats in autumn among swarming sites is well documented for many species in northern latitudes, with distances ranging from 5 to 21 km in Latvia (Šuba et al. 2008), 0.6 to 1.5 km in northern England (Rivers et al. 2006), and up to 31.5 km in Poland (Furmankiewicz 2008). In North America, patterns of movement among hibernacula for little brown bats among years can reach 500 km, with a median distance of 315 km (Norquay et al. 2013). Further, size of catchment areas associated with specific hibernacula and swarming sites can be large (Rivers et al. 2006; Glover and Altringham 2008), and varies geographically for Indiana bats in North America with larger catchment areas for hibernacula at more southerly latitudes (Britzke et al. 2012). Recent evidence indicates that migration of females across landscapes is believed to be a mechanism for bat-to-bat transmission of the WNS fungus for at least little brown bats (Miller-Butterworth et al. 2014). We suggest that movement of bats during swarming is likely contributing to the spread of the WNS fungus among eastern North American populations of bats because swarming provides an environment whereby fungal spores can be spread among individuals due to the extended intra-specific behavioral interactions that occur. What remains less clear is how the physiological condition of bats affects swarming behavior and the tendency for movement of individuals among cave sites. We postulate that bats in better physiological condition (i.e., higher BMI values) should be more capable of long-distance movements during swarming, however, these bats are also less likely to be affected by the WNS fungus and carrying loads of fungal spores (Fuller et al. 2011). Evidence for male little brown bats during swarming in Nova Scotia shows that individuals in better body condition return less frequently to

sites to engage in mating behavior than bats in poor physiological condition (Lowe 2012), demonstrating that the interplay of body condition, mating success and bat behavior in swarming is complex and in need of further study if we are to fully comprehend the impact of WNS on cave-hibernating populations of North American bats.

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Table 15.1. Totals and sex ratios by species of bats captured at Colossal Cave, Mammoth Cave National Park, Kentucky, 2011 to 2014.

Species	Number captured	Sex ratio (males:female)
<i>Perimyotis subflavus</i>	524	2.56:1.0
<i>Myotis septentrionalis</i>	419	3.66:1.0
<i>Myotis lucifugus</i>	75	4.36:1.0
<i>Myotis sodalis</i>	66	5.6:1.0
<i>Myotis leibii</i>	17	4.67:1.0
<i>Eptesicus fuscus</i>	4	3.0:1.0
<i>Corynorhinus rafinesquii</i>	6	males only
<i>Lasiurus borealis</i>	2	1.0:1.0

Table 15.2. Mean (SD) body mass index values (BMI, g/mm) by sampling interval for species of bats captured at Colossal Cave, Mammoth Cave National Park, Kentucky, 2011 to 2013.

Species	Sampling interval					
	Early staging	Mid-staging	Late staging	Early swarming	Mid-swarming	Late swarming
<i>Perimyotis subflavus</i>	0.13(0.01)	0.14(0.02)	0.14(0.02)	0.14(0.01)	0.15(0.03)	0.2(0.03)
<i>Myotis septentrionalis</i>	0.16(0.11)	0.16(0.01)	0.16(0.02)	0.15(0.01)	0.16(0.02)	0.18(0.14)
<i>Myotis lucifugus</i>	0.17(0.02)	0.18(0.02)	0.15(0.02)	0.17(0.01)	0.18(0.02)	0.19(0.02)
<i>Myotis sodalis</i>	0.17(0.01)	0.17(0.02)	0.15(0.01)	0.17(0.01)	0.17(0.01)	0.18(0.02)
<i>Myotis leibii</i>	0.14(0.01)	0.13(0.01)	0.12(-)	-	0.12(0.01)	0.14(0.02)

**Table 15.3.** Mean (SD) body mass (BM) and body mass index values (BMI) by sex and season (staging vs. swarming) for five species of bats captured at Colossal Cave, Mammoth Cave National Park, Kentucky, 2011 to 2013.

Species/metric	Staging		Swarming	
	Males	Females	Males	Females
<i>Perimyotis subflavus</i>				
BM (g)	4.37(0.92)	5.0(0.52)	5.15(0.88)	5.71(1.42)
BMI (g/mm)	0.13(0.02)	0.14(0.01)	0.15(0.03)	0.16(0.04)
<i>Myotis septentrionalis</i>				
BM (g)	5.55(0.55)	5.35(0.54)	5.75(0.82)	6.23(1.0)
BMI (g/mm)	0.16(0.02)	0.15(0.02)	0.16(0.02)	0.17(0.03)
<i>Myotis lucifugus</i>				
BM (g)	6.18(0.92)	6.53(0.4)	6.93(0.73)	6.44(0.32)
BMI (g/mm)	0.17(0.02)	0.18(0.01)	0.18(0.02)	0.17(0.01)
<i>Myotis sodalis</i>				
BM (g)	5.88(0.12)	6.84(0.39)	6.67(0.52)	7.73(1.77)
BMI (g/mm)	0.15(0.01)	0.17(0.01)	0.17(0.01)	0.2(0.04)
<i>Myotis leibii</i>				
BM (g)	4.0(0.28)	3.75(0.16)	4.18(0.53)	3.71(-)
BMI (g/mm)	0.13(0.01)	0.13(0.01)	0.14(0.02)	0.12(-)

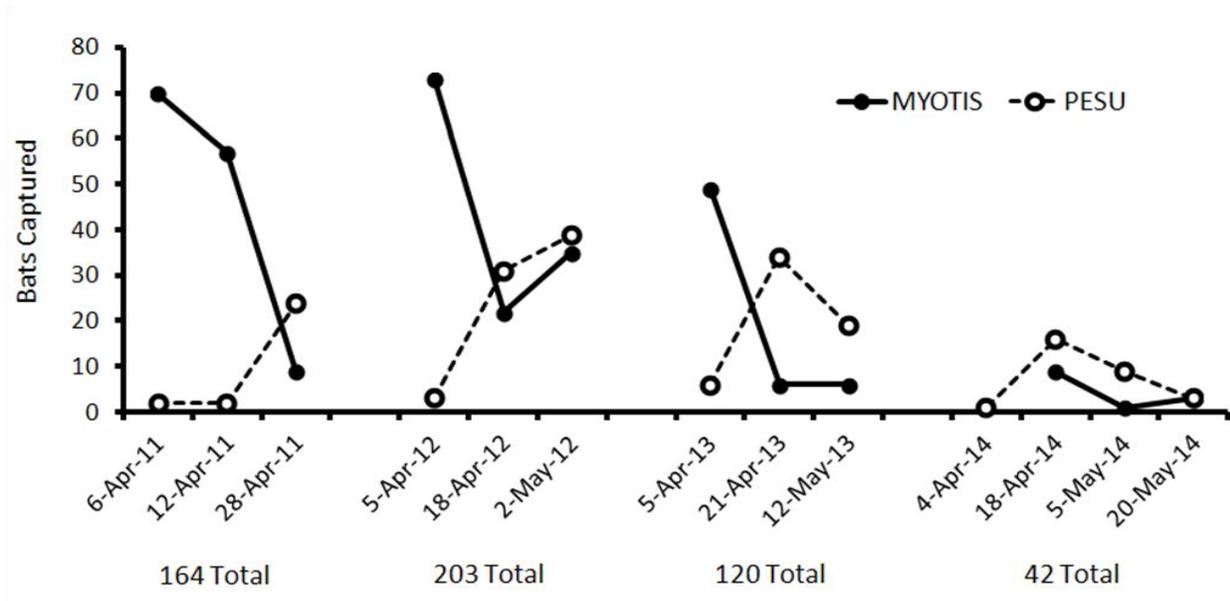


Figure 15.1. Total captures for *Myotis* bats and tri-colored bats (*Perimyotis subflavus*) at Colossal Cave, Mammoth Cave National Park, Kentucky, during harp trapping in spring emergence, 2011 to 2014.

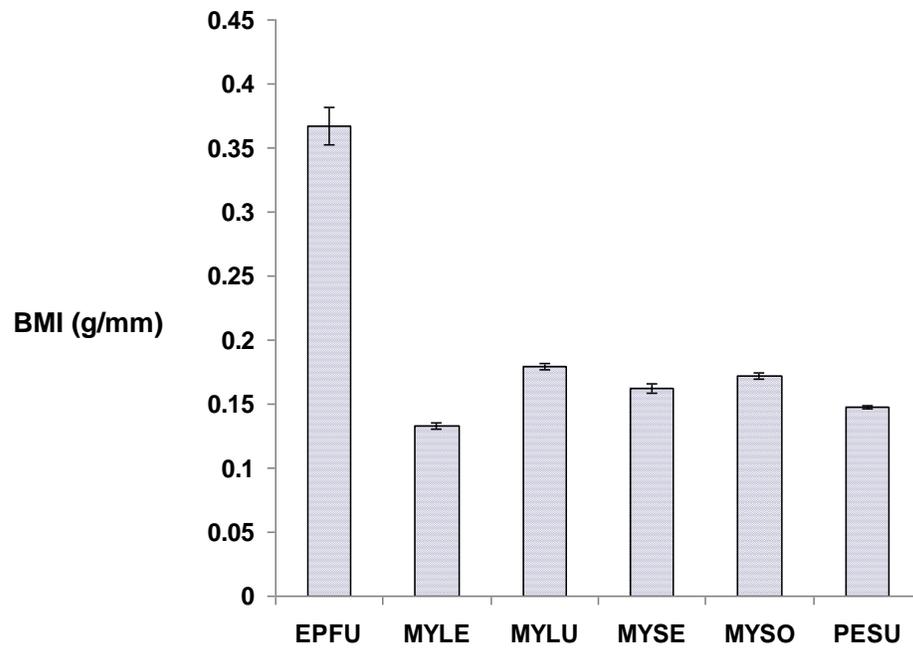


Figure 15.2. Mean (SE) body mass index values (g/mm) by species for bats captured at Colossal Cave, Mammoth Cave National Park, Kentucky, 2011 to 2013.

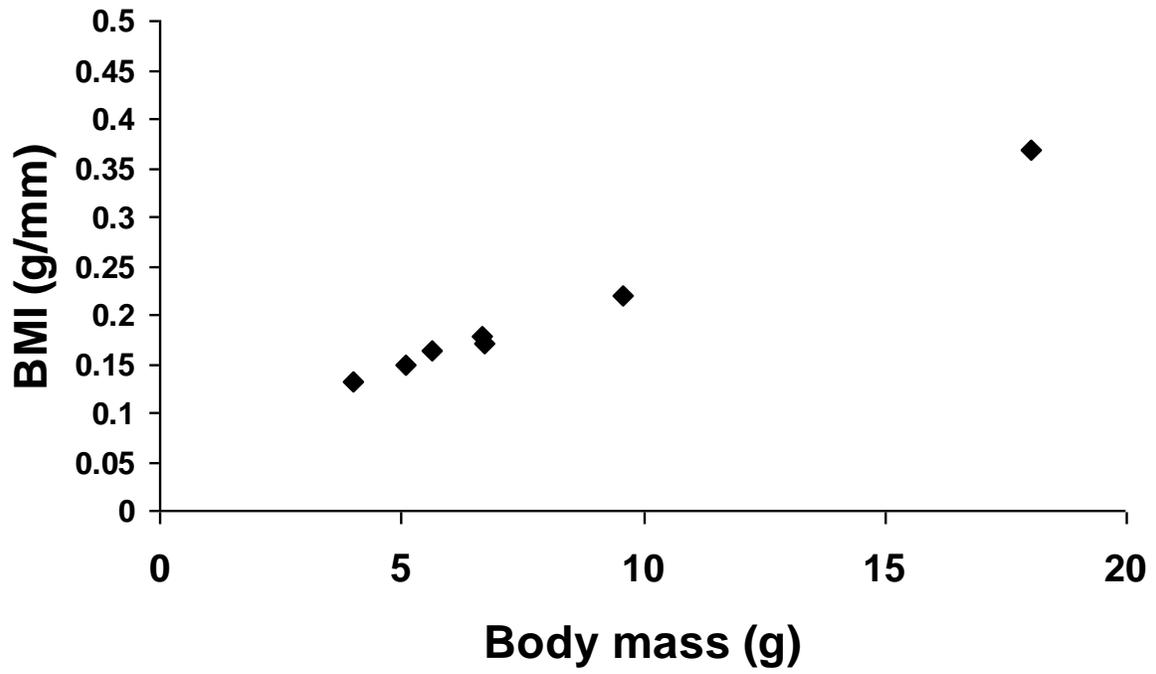


Figure 15.3. Relationship of mean body mass index (g/mm) with mean body mass for species of bats captured at Colossal Cave, Mammoth Cave National Park, Kentucky, 2011 to 2013.

## 16. Key Report: Seasonality of insects at Mammoth Cave and impacts of herbivory.

### Burn History and Summary of Methods

Use of fire for forest management at Mammoth Cave National Park (MCNP) has been ongoing since 2002, with two prescribed fires occurring in the spring that our project began in 2010. A third prescribed burn was implemented in spring of 2012. This fire program created a mosaic of recently burned areas immediately adjacent to unburned parcels of land. In addition to our assessments of insect activity across MCNP (see Key Report 12), these recent fires allowed us to assess the growth and herbivory loads on white oaks (*Quercus alba*) regenerating in burned areas. We chose white oak as the focal species for our herbivory assessments, as oaks are important to a wide diversity of wildlife in the central hardwoods and, further, white oaks are particularly one of the heaviest-masting species in this keystone group of trees (Greenberg 2000, McShea et al. 2007). Specific to insects, oaks are a major host plant for a wide array of Lepidoptera (Covell 2005), and Lepidoptera are the most consistently and heavily consumed prey of bats in eastern North America (Lacki et al. 2007). Considering this, we selected 20 white oak seedlings for each plot to receive manipulations of herbivory to investigate the interactions between insect feeding and prescribed fire, as well as investigate the role that fire could play in promoting oak seedling growth. We defined seedlings as small white oaks, appearing less than several years old from aboveground investigation and averaging 25 cm in height and 3.4 mm in diameter.

Plots were scouted prior to bud break in 2011 and 2012 to identify candidate seedlings. In 2011, plots were established in the two areas burned most recently (the previous year), and in adjacent unburned areas. This yielded a total of four plots (two burned, two unburned) that were assessed throughout the 2011 growing season. The seedlings in these plots were assessed again the following year, and an additional plot was established in the area newly burned in 2012. This resulted in assessments of seedling growth and herbivory loads over a chronosequence of up to two years post-burn with spatial and annual replication incorporated into our study design (Figure 16.1). To investigate general trends in insect abundance, we paired these data with insect abundance measurements from blacklight trap and malaise trap surveys occurring in 2011-2012 (see Key Report 12 for detailed methods).

Seedling manipulations followed Adams (2000). Seedlings were designated for manipulation of arthropod and vertebrate herbivory using a combination of insecticides and wire cages. This resulted in 5 seedlings per plot randomly receiving the following treatments: 1) exclusion of arthropod and vertebrate herbivory, 2) only exclusion of arthropod herbivory, 3) only exclusion of vertebrate herbivory, and 4) no herbivory exclusion. Seedlings receiving an exclusion of arthropod herbivory were treated with a pyrethroid (bifenthrin, Ortho Max, Marysville, OH, USA) at the label rate at 14-day intervals. Seedlings receiving an exclusion of vertebrate herbivory were enclosed in a wire tomato cages (35 cm height x 30.5 cm diameter) enclosed with 2.5 x 3.5 cm-chicken wire imbedded in the ground and surrounded at the base by 15 cm-metal flashing (Figure 16.2).

Seedling assessments followed Adams and Rieske (2001). In 2011 we made observations on herbivory levels every 14 days beginning 6 April and progressing throughout September.

During this time we assessed seedling growth monthly (i.e., on every other visit to assess herbivory). In 2012, we made observations of both herbivory and seedling growth on a monthly basis beginning 12 April and progressing through September. Herbivory was visually assessed for the entire seedling and incorporated defoliator feeding guilds (Figure 16.3) which included: leaf tissue removal (from chewing herbivory), skeletonization (removal of leaf cuticle to leave a network of veins), and stippling (disruption of leaf cuticle). Herbivory loads were then expressed as percent leaf area per seedling. Assessments of seedling growth included total height and stem diameter. Seedling height was measured from a predetermined basal stem mark to the tip of the leading branch. Stem diameter was the average of two basal measurements at a predetermined point near the root collar, taken in 2011 and 2012. These parameters were summarized in terms of absolute growth rates (AGR) over the course of the study, and also scaled to relative growth rates (RGR).

Annual and seasonal trends for insect abundance were assessed using a two-way Analysis of Variance (ANOVA) for the total insects captured on a per night basis using blacklight traps and malaise traps (PROC GLM using SAS 9.1). As herbivory assessments were taken over multiple instances on the same seedlings, we used repeated-measures ANOVAs (PROC GLM using SAS 9.1) to investigate the impact of prescribed fire on insect herbivory. We did not use insecticide-treated seedlings for this analysis. Data were kept separate across years to avoid a confounding interaction. We report the effect of the repeated measure using a multivariate score (Wilks  $\lambda$ ). If an interacting effect between fire and time was detected, we report Mauchley's Test of Sphericity ( $\chi^2$ -approximated) and report both multivariate and univariate results. Univariate  $F$ -scores are adjusted by Greenhouse-Geisser correction term. We tested for differences in seedling growth rates (AGR and RGR) due to prescribed fire and insect herbivory. We did this using ANOVAs with burning and herbivory (insecticide-treated and no insecticide) as main effects in our models. Finally, we investigated differences in seedling growth measurements across a chronosequence of years since burning using ANOVAs with time since burn as the main effect. Across all analyses, response variables were tested for homogeneity of variance using Variance Ratio  $F_{\text{MAX}}$  tests, with ANOVAs conducted on log-transformed values when variances were heterogeneous (Sokal and Rohlf, 1969). We used Tukey's Honestly Significant Difference test to evaluate effects when models were significant and a means separation procedure was merited (Zar 1999).

## Discussion of Results

The abundance of nocturnal insects varied between years and across growing seasons (Figure 16.4). In the case of data collected in blacklight traps ( $F_{5,264} = 12.0$ ,  $P = 0.001$ ), fewer insects were captured in 2011 versus 2012, but the abundance of insects captured in the maternity season of 2011 drove a seasonal difference in our analysis. Similarly for malaise traps ( $F_{3,205} = 20.1$ ,  $P = 0.001$ , fewer insects were captured in 2011 versus 2012. With only staging and swarming considered, fewer insects were captured during the staging period versus when bats were swarming.

Varied seasonal and fire responses were observed for herbivores of white oak seedlings (Figure 16.5). For insects that totally removed leaf tissue, herbivory peaked relatively early both years and, in 2012, gradually decreased in the latter portions of the growing season. The

repeated-measures effect of survey time was significant for this mode of herbivory in both 2011 and 2012 ( $\lambda_{11,21} = 4.5$ ,  $P = 0.001$ , and  $\lambda_{5,34} = 7.5$ ,  $P = 0.001$ , respectively), but neither burning nor a seasonal interaction with burning was significant ( $P > 0.05$ ). Notably greater levels of skeletonization were observed during the latter portions of both 2011 and 2012. The repeated-measures effect of survey time was significant for this mode of herbivory in 2011 and 2012 ( $\lambda_{11,21} = 10.1$ ,  $P = 0.001$ , and  $\lambda_{5,34} = 8.3$ ,  $P = 0.001$ , respectively), but neither burning nor a seasonal interaction with burning was significant ( $P > 0.05$ ). Similarly, greater levels of stippling were observed during the latter portions of both 2011 and 2012. The repeated-measures effect of survey time was significant for this mode of herbivory in 2011 and 2012 ( $\lambda_{11,21} = 5.7$ ,  $P = 0.001$ , and  $\lambda_{5,34} = 17.8$ ,  $P = 0.001$ , respectively). A significant interaction between burning and the repeated-measure was observed in 2011 (Mauchley's  $\chi_{65} = 238.7$ ,  $P = 0.001$ ; Wilk's  $\lambda_{11,21} = 2.7$ ,  $P = 0.001$ ; Adjusted  $F_{4.9,150.4} = 8.2$ ,  $P = 0.001$ ). A similar interaction was observed in 2012 (Mauchley's  $\chi_{14} = 186.1$ ,  $P = 0.001$ ; Wilk's  $\lambda_{5,34} = 1.6$ ,  $P = 0.20$ ; Adjusted  $F_{2.9,108.3} = 4.3$ ,  $P = 0.01$ ). In both years, stippling levels increased in unburned areas versus burned areas as the growing season progressed.

The growth of white oak seedlings was greater in burned areas versus unburned areas (Figure 16.6). Models for AGR, including height and diameter, were significant ( $F_{3,77} = 7.6$ ,  $P = 0.001$ , and  $F_{3,77} = 13.4$ ,  $P = 0.001$ , respectively). Models for RGR, including height and diameter, were also significant ( $F_{3,77} = 7.8$ ,  $P = 0.001$ , and  $F_{3,77} = 15.3$ ,  $P = 0.001$ , respectively). In all cases, seedling growth rates were greater in burned areas versus unburned areas and no effect was observed regarding insect herbivory.

The growth of white oak seedlings varied over years since burn (Figure 16.7). Models for AGR, including height and diameter, were significant ( $F_{3,175} = 18.7$ ,  $P = 0.001$ , and  $F_{3,175} = 16.1$ ,  $P = 0.001$ , respectively). Models for RGR, including height and diameter, were also significant ( $F_{3,175} = 22.4$ ,  $P = 0.001$ , and  $F_{3,175} = 23.6$ ,  $P = 0.001$ , respectively). In the case of height, seedling growth peaked in the year following a burn. In the case of diameter, seedling growth peaked in the year following a burn, but burning in general promoted seedling growth.

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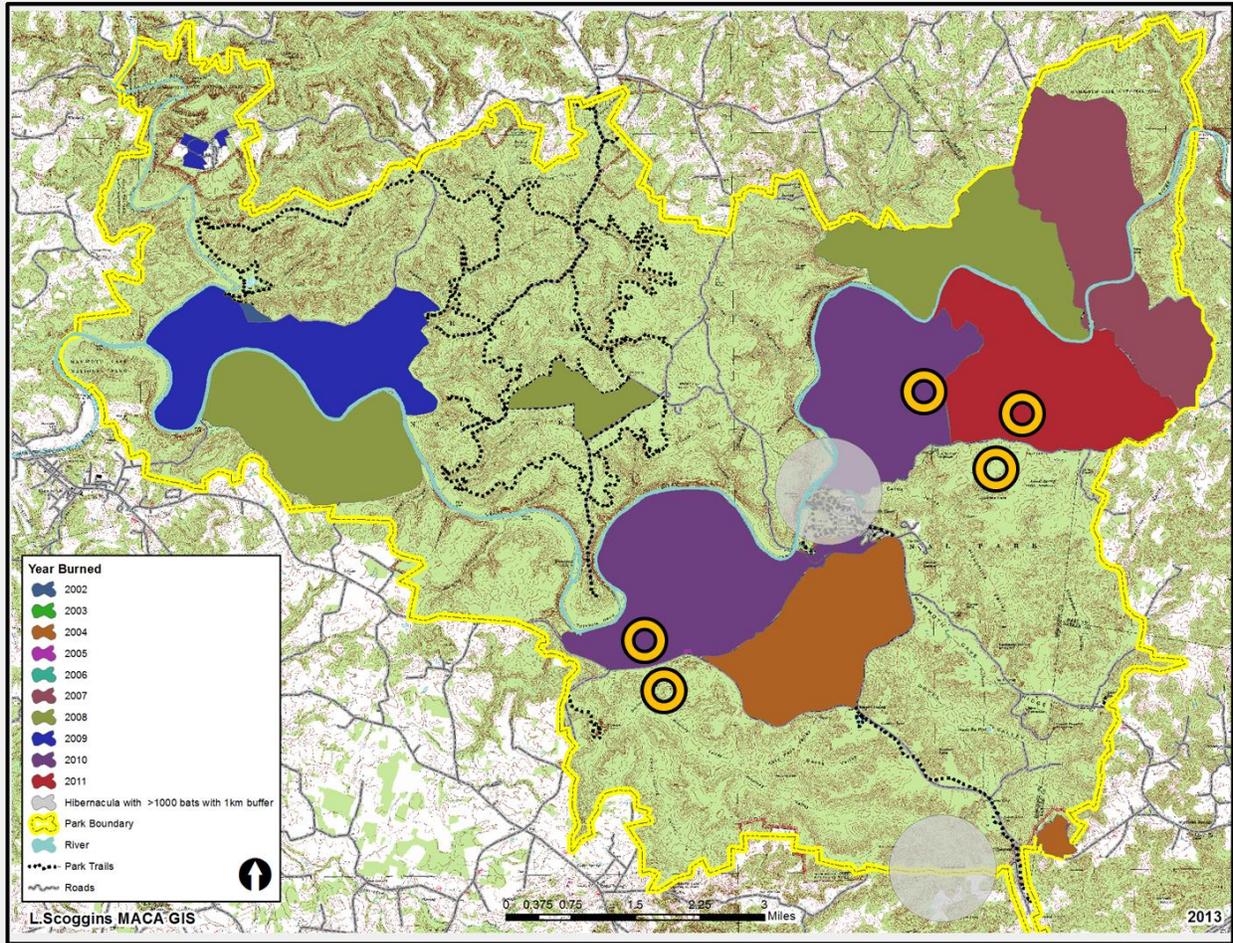


Figure 16.1. Map of MCNP showing most recent prescribed fires across burn units. Generalized locations of seedling plots used for assessments of herbivory are denoted with ring symbols. Map courtesy of Lillian Scoggins, Mammoth Cave National Park, US National Park Service.



Figure 16.2. Cage system used to exclude vertebrate herbivory of white oak seedlings: A) image of entire cage system, and B) close-up image detailing metal flashing used to deter small mammal access.

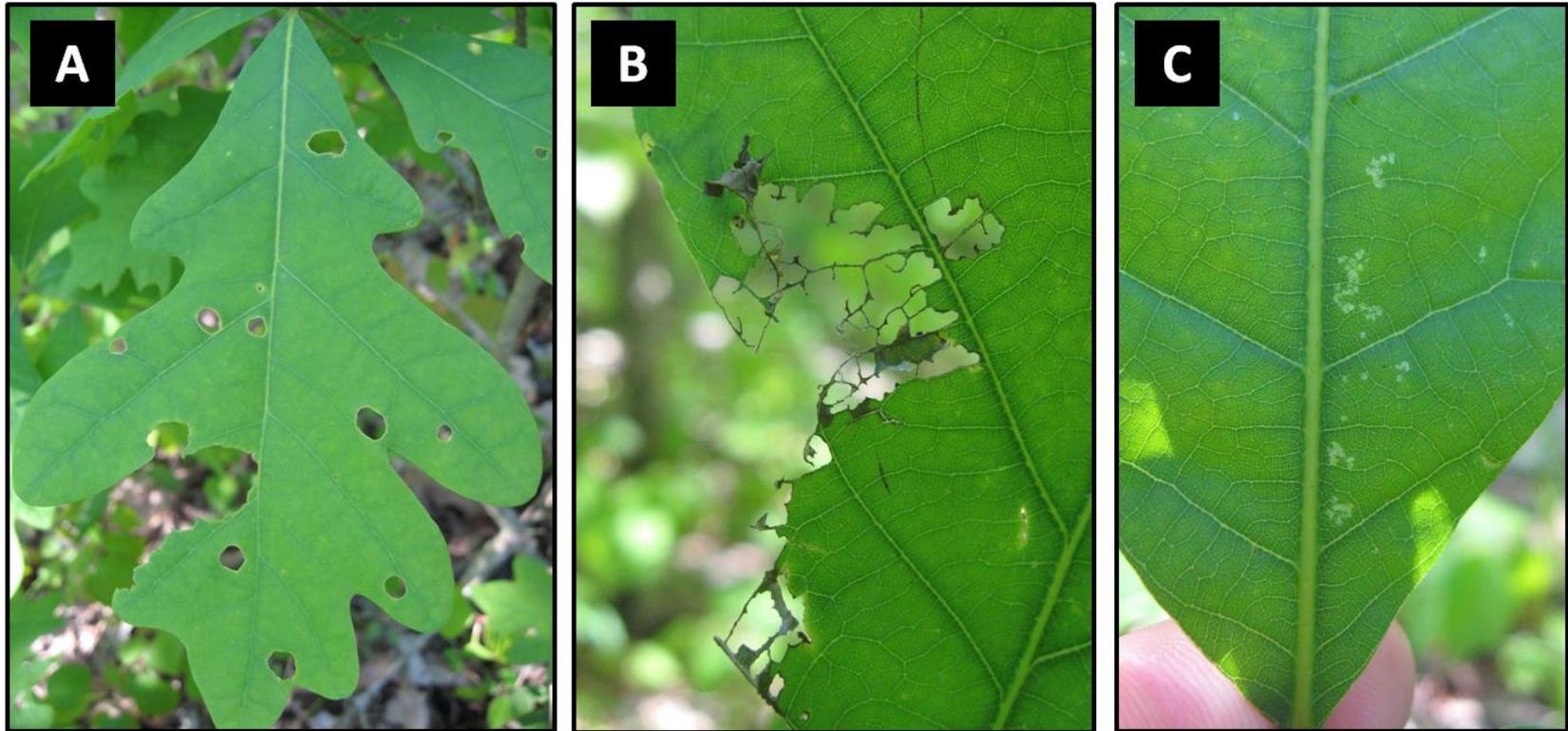


Figure 16.3. Images of the modes of herbivory assessed for white oak seedling in burned and unburned areas at Mammoth Cave National Park: A) tissue removal, B) skeletonization, and C) stippling. Images taken by T. Culbertson.

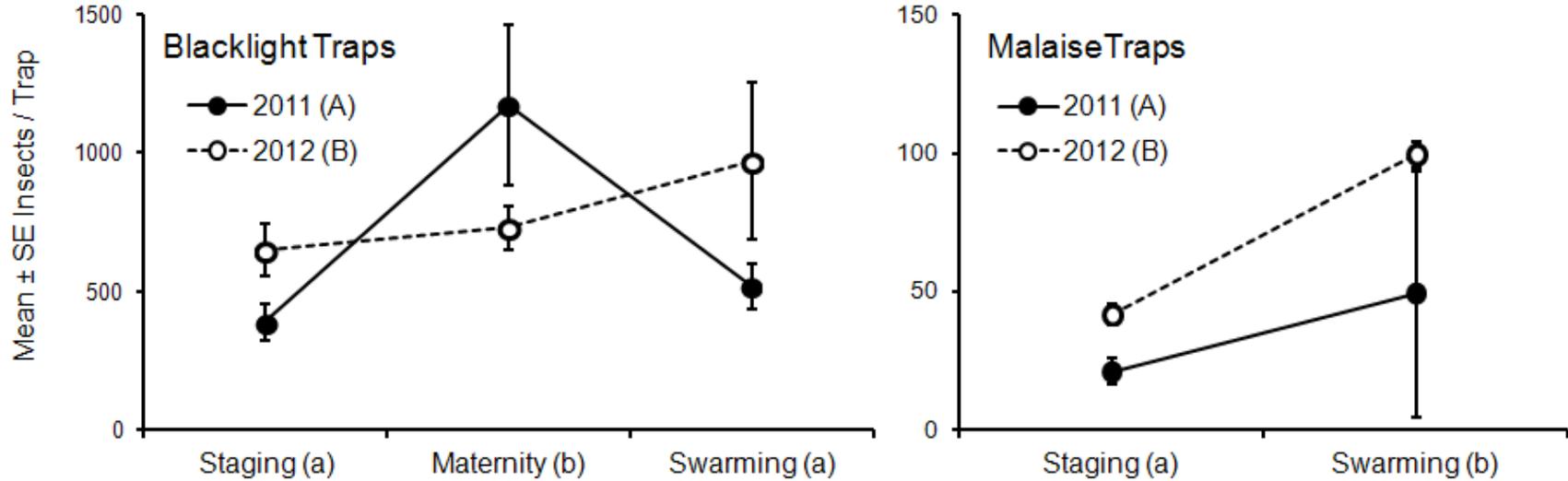
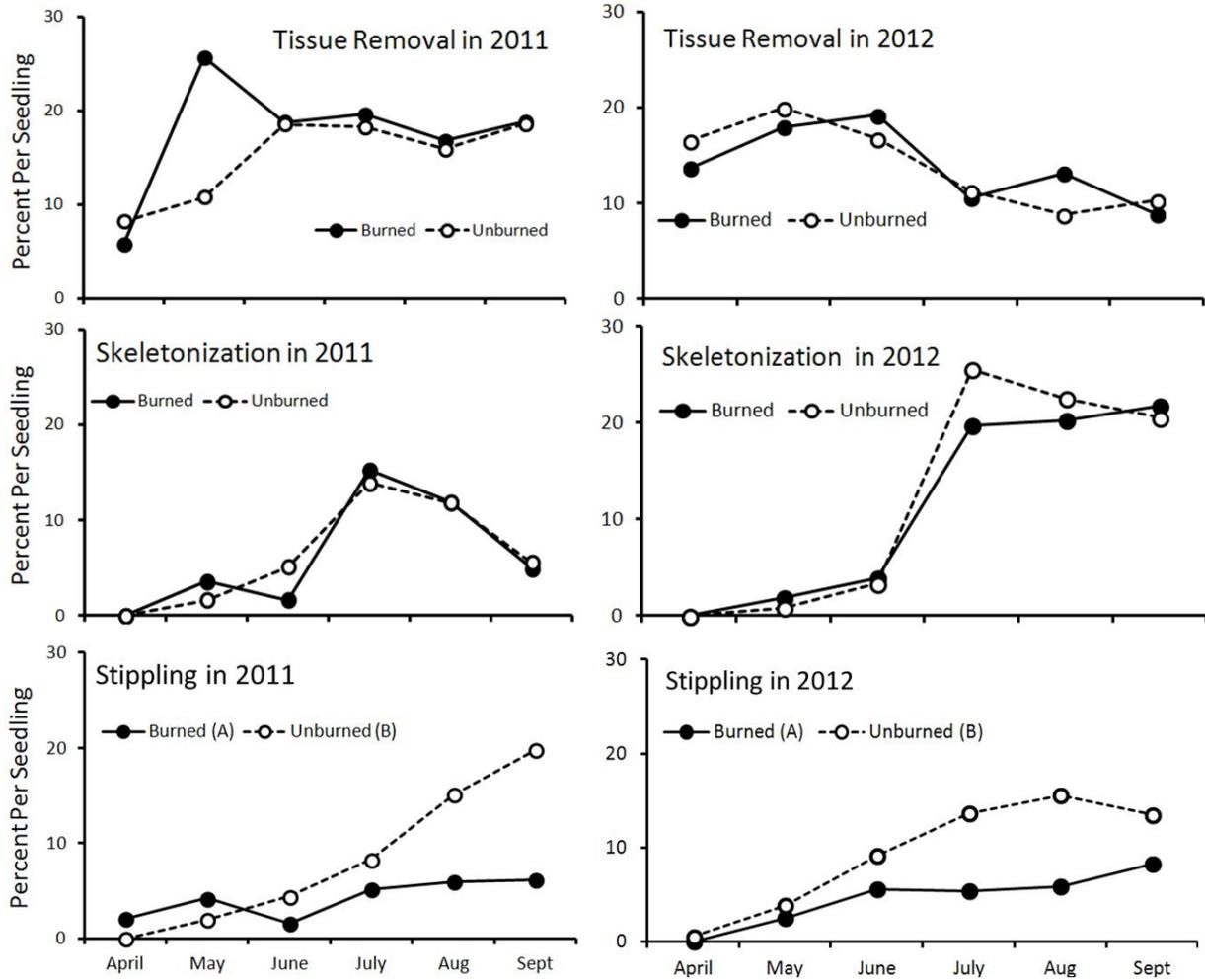
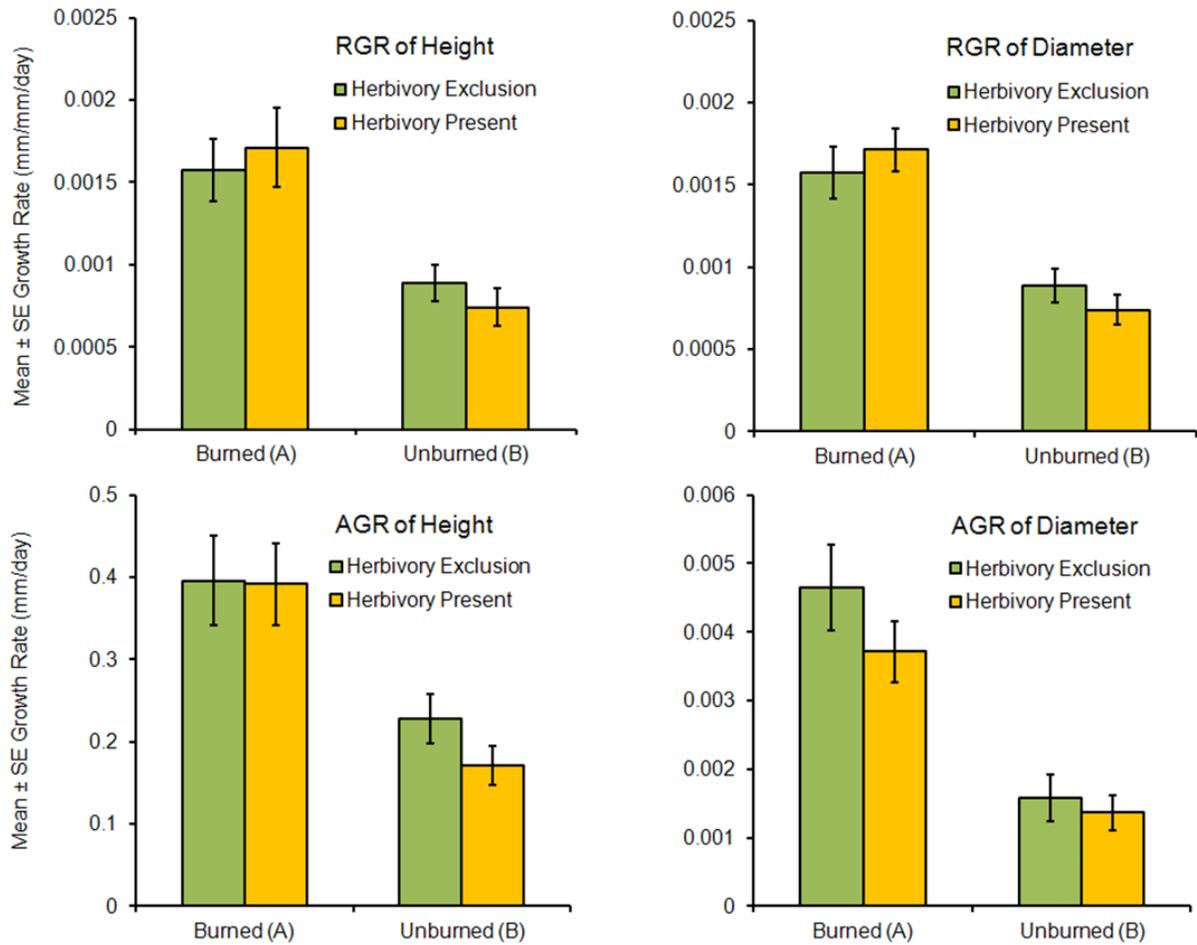


Figure 16.4. Variation in the abundance of nocturnal insects between years and across seasons at Mammoth Cave National Park. Different letters within a data series depict a significant means separation for year or season as a main effect ( $P \leq 0.05$ ). The interaction of these main effects were significant for data from both blacklight traps and malaise traps ( $P \leq 0.05$ ).



**Figure 16.5.** Seasonal variation of mean herbivory levels on white oak seedlings in burned and unburned areas at Mammoth Cave National Park. Seasonal variation within a year was observed across all modes of herbivory ( $P < 0.05$ ). Different letters within data series depict a significant means separation for fire as a main effect ( $P \leq 0.05$ ).



**Figure 16.6.** The effects of burning and insect herbivory on the growth of white oak seedlings at Mammoth Cave National Park. Different letters within a data series depict a significant means separation for the effect of burning ( $P \leq 0.05$ ).

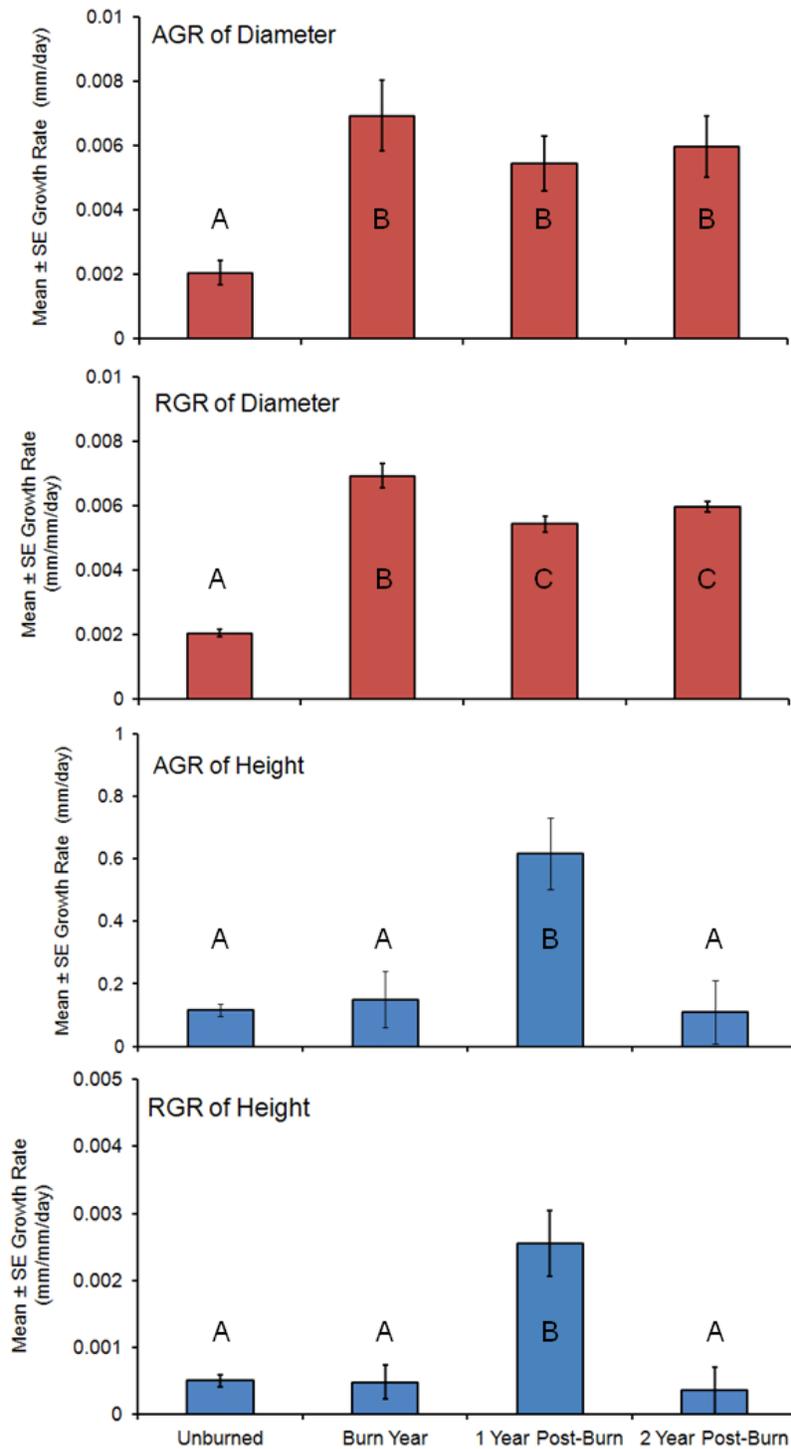


Figure 16.7. Effects of burning on growth of white oak seedlings over multiple years at Mammoth Cave National Park. Different letters within a data series depict a significant means separation for the effect of year since burn ( $P \leq 0.05$ )