EVALUATING THE EFFECTIVENESS OF THREE ACOUSTIC MONITORING TECHNIQUES FOR LANDSCAPE LEVEL BAT POPULATION MONITORING

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ABSTRACT

THESIS: Evaluating the Effectiveness of Three Acoustic Monitoring Techniques for Landscape Level Bat Population Monitoring

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Understanding population trends of any species is essential to its conservation and management. However, landscape level population status of many bat species is poorly understood. In an effort to resolve this issue, especially with emerging threats (e.g. White-nose Syndrome and wind energy) a national mobile acoustic monitoring protocol was developed to survey summer bat populations along roadways. However, some species are known to occur more frequently near or along river corridors, leading us to hypothesize that mobile transect conducted from boats may provide an opportunity to monitor more bat species than road based surveys. To determine the most efficient method, we compared species richness and abundance along river and road transects. We further compared species richness and sampling time of stand and landscape levels mobile methods to mist-netting and stationary acoustic detectors, respectively, to better understand the capabilities of mobile acoustic transects compared to more familiar methods.

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CHAPTER 1:

LITERATURE REVIEW

The order Chiroptera comprises approximately 20% (1116 species) of world mammalian diversity, second only to Rodentia (~42%; Wilson and Reeder 2005). While bats are not the most diverse taxa, they may be the most abundant, forming the largest aggregations of mammals in the world (Kunz 2003). Bats inhabit a diverse range of ecosystems across the planet and are only missing from a few ocean islands and polar regions (Willig et al. 2003). They show a great deal of diversity in body size, diet, and roosting habits (Patterson et al. 2003, Simmons and Conway 2003). Due to the range of habitats occupied and diverse feeding strategies, bats are a possible indicator species. Declines in bat abundance or diversity could be a signal for more widespread declines or problems in other taxa due to things such as habitat destruction and environmental containments (Jones et al. 2009).

Forty-three percent of bat species are considered threatened or near threatened by the IUCN (Hutson et al. 2001). Threats come in both focal (i.e. direct mortality) or diffuse forms. Focal threats are easily quantifiable, often one time occurrences, on an easily defined population (Weller et al. 2009). Disturbance of caves/roosts is probably the most obvious and widespread focal threat to bats (Hutson et al. 2001, Weller et al. 2009). However, most of these threats have been well mitigated through legislation, stiff civil penalties, and protective measures (e.g. cave gates/closures) enacted since the 1960's in the United States and Western Europe (Weller et al. 2009). Of more modern concern are the various diffuse threats that are difficult to quantify or observe the effects of on bat populations (Weller et al. 2009). Climate change, habitat destruction, and environmental contaminants all threaten multiple bat taxa, but are difficult to quantify and mitigate (Hutson et al. 2001, Racey and Entwistle 2003, Weller et al. 2009).

In the United States, bat populations are facing increased threats from wind energy generation, one of the fastest growing renewable energy sources in the United States (Arnett et al. 2008). In 2009, wind energy generation capabilities in the United States increased over 39 percent, while in 2008 capacity grew over 50%. Continued growth in wind energy generation is expected due to current economic and environmental concerns about fossil fuels (Arnett et al. 2008, Baerwald et al. 2009, American Wind Energy Association 2010). Estimated number of bat fatalities at wind generation facilities varies greatly, especially among geographic regions. In the Eastern United States, estimated mortality is as high as 69.6 bats killed per turbine annually, while fatalities in the Midwest varied from to 0.1-7.8 bats/turbine annually (Arnett et al. 2008). Cumulative effects on populations are poorly understood, but annual bat fatalities are conservatively estimated at 450,000 bats annually across the United States (Cryan 2011) and regionally at 33,000 – 111,000 bats annually in mid-Atlantic highlands (Kunz et al. 2007). Eighty percent of fatalities are foliage roosting bats such as the hoary bat (*Lasiurus cinereus*), Eastern red bat (*L. borealis*), silver-haired bat (*Lasionycteris noctivagans*), and Eastern pipistrelle (*Perimyotis subflavus*; Kunz et al. 2007). Most of these species are also migratory and have poorly understood population dynamics (Carter et al. 2003, Kunz et al. 2009a).

While wind turbines are killing migratory bats, White-nose Syndrome (WNS) is responsible for killing 5.7-6.7 million cave hibernating bats (USFWS 2012). Since its discovery in New York in 2006, WNS has spread to 19 states and 4 Canadian provinces, potentially killing over 90% of bats in an affected cave (USFWS 2011). To date, seven species have been infected with WNS (*Eptesicus fuscus, Myotis leibii, M. lucifugus, M. septentrionalis,* and *P. subflavus*), including the federally endangered Indiana (*M. sodalis*) and gray bats (*M. grisescens*). The associated fungus (*Geomyces destructans*) has also been detected on two additional species (*M. austroriparius, M. velifer*); however, mortality and infection characteristic of the disease has not been observed in these species (USFWS 2011). WNS is having devastating effects on hibernating bat populations in the U.S. Frick et al. (2010) modeled population of the once common little brown bat (*M. lucifugus*) and showed that the regional extirpation was possible within 20 years. This prognosis is even more troublesome for species that are less common and has led to a petition for federal listing of the northern long-eared (*M. septentrionalis*) and Eastern small-footed (*M. leibii*) bats. The outcome of the high mortality on already listed species is potentially grave.

Understanding current population trends and possible cumulative effects of threats on bat populations has long been a concern for biologists. Bats are long-lived creatures that reproduce slowly, with most species in North America producing only 1 pup a year. These factors make bats vulnerable to even small population declines (Racey and Entwistle 2000, Barclay and Harder 2003). Delayed detection of declines and resulting management actions could result in extremely long recovery times or species extinction (Crone 2001, O'Shea and Bogan 2003).

Effective population monitoring could assist managers in determining population trends, accessing species status, and setting management objectives (Thompson et al. 1998, Gibbs et al. 1999, Elzinga 2001, Joseph et al. 2006). Most population monitoring programs are intended to evaluate trends over long periods of time across large areas, most often requiring 5 or more years to produce their first results (Elzinga 2001) and best when examined over 20 years (Parr et al. 2002). Power, the ability of a monitoring program to detect a given degree of change, is affected by a variety of factors, including the length of monitoring, number of sites monitored, and variability of counts. Variation in counts is attributed to three elements: spatial (i.e., among sites), temporal (i.e., year to year population differences), and sampling variation. Sampling variation is based on the precision of a sampling method and can cause difficulties in determining trends if extremely large (Thompson et al. 1998, Elzinga 2001). One primary goal of many monitoring programs is to reduce sampling variation and thus increase power or reduce necessary sampling units or time. This can be accomplished through the standardization and selection of appropriate methodology based on the taxa or species of concern. Depending on the sampling scheme, trends can be determined through either a change in site occupancy or abundance over time (Field et al. 2005).

Occupancy methods are more easily carried out and often require less investment and skill at each site than abundance estimates (Field et al. 2005). However, to accomplish the statistical rigor necessary for population monitoring, occupancy models require a large number of monitoring sites (MacKenzie et al. 2002, Tyre et al. 2003). Additionally, this method assumes a linear relationship between occupancy and abundance, which may not be true, especially in marginal habitats (Buckland et al. 2005).

One of the most widespread monitoring programs employing occupancy modeling is the North American Amphibian Monitoring Program (NAAMP). While the organizers understand the short-comings of occupancy modeling for evaluating population trends, the effort and skill required to gather abundance measures (index or estimates) would be far to great (Weir and Mossman 2005, Weir et al. 2009). The program was not fully organized until 2001 and is just now able to analyze some of their first results. To date trends for 16 species in 10 states were analyzed, and were able to detect increasing and decreasing trends at state and regional levels. However, the magnitude of declines (or increases) required before NAAMP detects a trend is unknown (Weir et al. 2009).

Abundance measures can increase the accuracy of trend analysis in many circumstances, including occupancy analysis (Joseph et al. 2006, Dorazio 2007). In place of true abundance estimates (which are difficult to obtain), an index to abundance is often used, in which the relationship between the index measured and the actual population size may be poorly understood. In the worst case scenario, a poorly selected index may not have any relationship to abundance (Conroy 1996, Gibbs et al. 1999, Anderson 2001).

For example, many bird surveys use the number of calling birds at point counts as an index to population size. The Christmas Bird Count (CBC), the oldest and largest wildlife monitoring program in the world, uses this method (Butcher et al. 1990). Despite the data's widespread use in literature, the program was not designed to withstand statistical rigor and possess many problems for population trend analysis (Dunn et al. 2005). Even though counts of calling birds are recorded, the inconstant and unreported effort causes high sampling variability making the data mostly useful for occupancy analysis (Dunn et al. 2005). Still the CBC can be valuable for monitoring bird populations (Butcher et al. 1990).

Similarly, the Breeding Bird Survey (BBS) uses point counts as an index to population size. However, the rigorous standards and protocol of the BBS allow estimates of population change across the United States and Canada for more than 420 species (Sauer and Link 2011). The BBS uses the number of calls heard during point counts at stops along long term monitoring routes as an index to population size. Due to its consistent methodology, the BBS has been widely successful in monitoring long term national and regional trends with a variety of analysis techniques (Sauer and Link 2011).

However, the task of monitoring of many bat populations has long been difficult with the three broad survey methods most commonly implored by bat biologist: roost surveys, capture, and acoustics (O'Shea and Bogan 2003). The basic ecology of bats (e.g., nocturnal, flying, far ranging, small size, and secretive nature) interferes with much of our ability to study them and especially to estimate population sizes (Weller 2007). Consequentially, data to analyze trends and determine population status of many species is unavailable or inadequate (O'Shea and Bogan 2003).

Most inadequacies in bat population monitoring stem from the inability to use gathered information to estimate abundance of bats and high variability caused by unstandardized methods (O'Shea and Bogan 2003). The only traditional survey method capable of giving accurate insight into abundance levels is roost counts, however variability from differences in observer skill and methodology leads to uncertainty in trend analysis (Tuttle 2003, Kunz et al. 2009b, Meretsky et al. 2010). Additionally, roost surveys are only applicable to ‘conspicuous’ species which roost in easily to view locations and groups (Kunz 2003, Weller et al. 2009) and thus is only advantageous for 14 out of 45 US species (Weller et al. 2009). Furthermore, due to the inherent dangers, complications, and the expertise required in conducting these methods, existing efforts are primarily focused on species with legal protections (Ellison et al. 2003, Weller et al. 2009). Current data on inconspicuous (i.e. solitary roosting) or unprotected species are less structured and only offer themselves to highly antidotal analysis (Carter et al. 2003).

Capture and acoustic techniques have not allowed for abundance estimates because of their unknown detection probabilities (capture and acoustic) or inability to distinguishing individuals (acoustics; Weller 2007). Further complicating matters, individuals ability to disperse across the landscape causes a lower but unknown probability of redetection (Berry et al. 2004) and violates assumptions of traditional population estimation methodology/analysis (e.g., mark-recapture; O'Shea and Bogan 2003, O’Shea et al. 2004, Weller 2007). Therefore, these methods limit themselves to occupancy analyses. However, extreme variation in specific sampling methodologies within each technique and the expertise required to conduct these surveys even makes monitoring with occupancy analysis difficult (Weller 2007).

Mist-netting is the most widely used survey method in North America. However, it has been shown to be highly variable, bias, and labor intensive (Kunz 2003, Weller and Zielinski 2006, Kunz et al. 2009a). Additionally, some bat species, such as those that fly above the canopy or in open areas are not often caught in mist nets. Perceived community structure from mist-net surveys is greatly influenced based on subtle differences in net placement (Carroll et al. 2002, Winhold and Kurta 2008) and species morphological characteristics (Berry et al. 2004). Furthermore, height of nets, frequency of net checks, environmental conditions, and habitat use can all greatly influence mist-netting results (Carroll et al. 2002, MacCarthy et al. 2006, Robbins et al. 2008, Winhold and Kurta 2008). Inconsistent methodology (i.e., variable capture rates), unrepeated surveys, and undefined area or population of interest make it difficult to monitor trends in bat populations using capture techniques (Ellison et al. 2003, O'Shea and Bogan 2003).

The effort required to capture most species in an area with mist-netting is intensive. The only study available on detection rates of bats in mist nets showed that about 3% of bats using a corridor were captured in mist-nets (Larsen et al. 2007). This leads to the need for wide spread and consistent surveys to quantify the bat community in an area using capture techniques. Weller and Lee (2007) used a bootstrapping method on four years of extensive netting to determine the amount of mist-netting effort required to capture 8 of the 9 core species (not all species known in the area) in northwest California. They found that a mean of 26.3 surveys were required to detect this limited number of species with standard methods. If only high quality sites that were rich in diversity and abundance were selected, a mean of 11.2 survey nights were required to detect 8 species (Weller and Lee 2007). In contrast, neo-tropical studies show that 90% of species can be caught within 18 nights of surveying a variable landscape. Differences may be because Vespertillionidae, which comprise the majority of North American bats, can more easily detect nets with echolocation than Phyllostomid bats in the tropical regions which use less intense echolocation (Aldridge and Rautenbach 1987, Rautenbach et al. 1996, Moreno and Halffter 2000).

Acoustic detectors have been used to study bats since the 1970's; however, their wide spread use has only developed in last decade with increases in portability, affordability, and ability to discriminate species (Griffin 2004). Bat echolocation was not discovered until 1938, even though the mechanism ('seeing with ears') was originally hypothesized in 1790 (Griffin 1958). Over time, bat detectors have evolved from extremely large room based systems, to cars, and are now available in 3 handheld forms – heterodyne, frequency division, and full spectrum (time-expansion and direct recording); each with its advantages and disadvantages based on study questions, design, and budgets (for detailed reviews see Parsons et al. 2000, Brigham et al. 2004, Parsons and Swzeczak 2009).

In addition to changes in technology, our ability to identify the echolocation calls of bats to various taxonomic levels has evolved. The capability to identify some species by the characteristics of their echolocation calls was recognized early in bat acoustics. This ability has been refined from highly qualitative and labor intensive (e.g., audio and visual analysis; O'Farrell et al. 1999) to more refined quantitative analysis (e.g., standard filters and statistical analysis of call parameters; Britzke and Murray 2000, Murray et al. 2001, Britzke 2003, Britzke et al. 2011) and is now rapidly evolving into fully automated identification capabilities (e.g., BCID, Bat Call Identification, Inc., Kansas City, MO; Sonoat, Joe Szewczak, Arcata, CA).

Acoustic surveys, like mist-netting have inherent biases, especially when attempting to discriminate species. Some species, especially gleaning taxa, emit low intensity calls, which may result in decreased detection distance (e.g., *C. rafinesquii, M. septentrionalis*; Griffin 1958, Faure et al. 1993, Menzel 2003). Furthermore, individuals of some species can modify the structure of their calls based on habitat (Obrist 1995, Broders et al. 2004), causing great variability and overlap in species call structure. However, interspecific variation has been shown to be greater than intraspecific variation (Murray et al. 2001). Environmental factors can also have variable effects on the quality of call recordings through the reflection and attenuation of signals (Murray et al. 2001) and limit ability to identify bat calls to species level (Britzke 2003, Ford et al. 2005).

Overall, acoustic sampling detects higher species richness than mist-netting in North America (Murray et al. 1999, O'Farrell and Gannon 1999). This increased efficiency results in the ability to detect most species in less than six nights with acoustics (Hayes 1997) and sometimes in only 2-3 nights (Ahlen and Baagøe 1999). Furthermore, acoustic detection requires less time investment and equipment than capture methods (Murray et al. 1999, Ford et al. 2005, Weller and Zielinski 2006). However, results from other regions of the world are mixed with both capture and acoustic methods favored in different conditions and with different taxa causing most biologist to recommend a combination of sampling methods for a full understanding of bat communities (Duffy et al. 2000, Flaquer et al. 2007, MacSwiney et al. 2008).

Acoustic methodologies involve either passive or active sampling. Passive (stationary) sampling, the more commonly used method, allows simultaneous sampling of many points for long periods of time (sometimes up to months) with relatively little researcher effort. However it also requires many sets of expensive equipment (Hayes 2000, Britzke 2004). Active sampling can allow the collection of higher quality calls leading to a greater percentage of calls being able to be identified (Britzke 2004, Milne et al. 2004). Most often this method is deployed along transects where a researcher walks a predetermined route and orients the detector toward bats either while walking or at stopped locations (e.g., Allyson and Harris 1996, Ellison 2005, Georgiakakis 2010, Berthinussen and Altringham 2011, Boughey et al. 2011). If recorded bats are seen, flight characteristics, color, and body size can aid in identification (Limpens 2004). This method can also allow a large variety of habitats to be sampled in one area, actually allowing a greater variety of habitats to be sampled than passive methods, especially when equipment is limited (Ford et al. 2005). However, the method requires the observer to choose which bats to follow, introducing bias not present in passive recording (Murray et al. 1999). Study question, sample size needed, and available resources determine which method is best on an individual basis (Britzke 2004).

In 2003, Bat Conservation Ireland developed a car-based acoustic sampling method to monitor bat populations. The concept is to mount a bat detector to a car and record bat activity along a predetermined route. The speed of the car allows each bat echolocation sequence to represent one individual bat, providing and index to abundance (Roche et al. 2005). The method allows for efficient large scale sampling across landscapes by volunteers (Jones et al. in press). The program has been widely successful and preliminary results indicated that this method could be used to successfully monitor population trends of three species across Ireland (Roche et al. 2011). It was quickly adopted by the European community and expanded worldwide to include over 19 countries and 733 routes by 2011 (Jones et al. in press). In 2009, the program was adopted with a national protocol in the United States and, if repeated over a large area for many years, could allow for region wide monitoring of many species – especially inconspicuous migratory ones that are often most lacking in population data.

The program has been analyzed for its ability to detect 25% and 50% population declines over 25 years (amber and red alerts, respectively) at an 80% power level and alpha (probability of false detection) of 0.05. In Ireland, with 25 routes conducted twice a year, anywhere from 14-25 years (red alert) or 8-12 years (amber alert) are required for sufficient power in each of the three species (Roche et al. 2011). In the UK, amber alerts in the common pipistrelle (*Pipistrellus pipistrellus*) can be detected in as little as 7 years with 20 routes conducted twice annually. However, less encountered species and less dramatic trends (i.e., red alerts) took as long as 20 or more years even with 100 routes (Jones et al. in press).

The primary reason for the long time periods required for this monitoring program is the great variation of bat counts. However, this is a common difficulty encountered with bat studies (Gibbs et al. 1998). Bats have high temporal variation in their foraging areas (Kunz 1973), possibly do to availability of insects (Hayes 1997). However, the use of transects (walking and car based) have some ability to limit this variation by sampling large areas in one night (Britzke 2004, Ellison 2005); still the variation causes some problems for population monitoring as the trend must be greater than the 'noise' (i.e., sampling variation) to be detected (Elzinga 2001).

Use of roads for sampling may also bias the species observed. The noise from passing cars is a barrier to movement (Kerth and Melber 2009) and reduces foraging efficiency of gleaning bats (Siemers and Schaub 2011). However, effects on species with different morphology or aerial foraging strategies appear to be less (Kerth and Melber

2009). Noise may not be the only import factor. Berthinussen and Altrnigham (2011) found that noise effects did not extend beyond 25 m yet overall bat activity is progressively reduced within 1 km of a major roadway. However, these studies examined the effects of multiple lane highways with high traffic volume. The effects of smaller roads or less frequent traffic noise (where US surveys are conducted) was not considered.

Some species may be attracted to forested roadways (Zimmerman and Glanz 2000), which create edge habitats and open areas preferred by many foraging bats (Verboom and Spoelstra 1999, Menzel et al. 2002, Boughey et al. 2011). However, bats may perceive cars as a threat and avoid them when encountered (Zurcher et al. 2010). Roads can also provide locally abundant insect densities under street-lights which attract some species (Rydell 1992, Blake et al. 1994), yet others may avoid the increased light intensity along roadways (Rydell 1992, Stone et al. 2009).

Roads may also not provide the appropriate habitat for many specialist species that require specific foraging habitats. In the UK, Daubenton's bat (*M. daubentonii*) is almost exclusively found along water ways and is monitored with a separate European monitoring program from the car-based program (Walsh et al. 2003). In the United States, gray bats (*M. grisescens*) are also known to forage almost exclusively along waterways (Tuttle 1976, Tuttle and Stevenson 1977, Tuttle 1979). Furthermore, roadways are often centered around increased urbanization, which has been shown to have a negative effect on activity of some species (Duchamp and Swihart 2008). However urban areas may provide the only forested habitat in agricultural landscapes and result in higher bat activity (Gehrt and Chelsvig 2004). Variability in responses by different species to road factors causes some species to be over-sampled while others are under-sampled (Linton 2009).

In general, overall bat activity and diversity is considered greater over both lentic and lotic waterways. While higher activity levels over water are well established, diversity of species over water has not been examined thoroughly. The increased activity is often attributed to higher insect densities and availability of water (Grindal 1999, Owen et al. 2004, Fukui et al. 2006); however, additional factors such as habitat structure and proximity to roost sites are likely to play significant roles. Bats forage in 'uncluttered' environments that limit interference with echolocation calls. Gaps created by watercourses or ponds often provide the types of areas favored by bats (Owen et al. 2004, Ford et al. 2005, Ober and Hayes 2008) and the presence of a smooth water surface can further simplify the acoustic environment. Rivers and streams also often provide a forested buffer, which may prove the only habitat available in some agricultural and urban settings (Medley et al. 1995). Flooding along these areas provides suitable snags with solar radiation for cavity roosting bats (Kalcounis-Rüppell et al. 2005). Additionally, rivers may be used as landmarks for long distance migrations (Furmankiewicz and Kucharska 2009). Increased use of riparian habitat suggests that conducting acoustic monitoring on a river instead of the roadway may increase the number of recorded calls and species detected, allowing managers to monitor more species with greater power.

GOALS AND OBJECTIVES

Due to the known and theorized higher levels of bat activity and diversity above or near water sources, could conducting mobile acoustic transects by boat provide increased opportunity for land managers to efficiently monitor multiple bat species over the traditional mobile car acoustic transects?

Specifically to answer this question we will:

1. Determine which acoustic sampling method (car, boat, or stationary) detects the greatest species richness.

    Hypothesis- Stationary detectors will detect more species than both mobile methods, but boat mobile acoustic transects will detect higher species richness than car transects.

2. Determine which acoustic sampling method (car or boat) indicates the highest diversity.

    Hypothesis- Boat acoustic transects will yield the highest Simpson's and Shannon-Weiner diversity indexes.

3. Determine which mobile acoustic sampling method (car or boat) collects the highest bat activity.

    Hypothesis- Boat acoustic transects will collect the highest number of calls.

4. Compare the bat community as determined by road and river transects to mist-netting data.

Hypothesis- Boat mobile acoustic transects will yield the highest richness and diversity.

5. Compare the overall time investment for stationary detectors, and mobile transects (car and boat).

Hypothesis- Car acoustic transects cars will take the least amount of time.

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CHAPTER 2:

EVALUATING THE EFFECTIVENESS OF THREE ACOUSTIC MONITORING TECHNIQUES FOR LANDSCAPE LEVEL BAT POPULATION MONITORING

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ABSTRACT

Understanding population trends of any species is essential for conservation and management. However, population status of many bat species at a landscape level is poorly understood due to the difficulty sampling these species. In an effort to resolve this issue, especially with emerging threats (e.g. White-nose Syndrome and wind energy) a national mobile acoustic monitoring protocol was developed to survey summer bat populations. Since bats vary their habitat use, we compared species richness and abundance along car and boat transects to identify the most efficient mobile method. We further compared species richness to mist-netting and stationary acoustic detectors at the stand and landscape levels, respectively, to better understand the capabilities of mobile acoustic transects compared to traditional survey methods. Using sample-based rarefaction, there was no difference at the 95% confidence level in species richness (species/individual), density (species/sample), or diversity (Shannon-Weaver and Simpson's indices) between transect methods. However, car transects tended to show slightly higher measures. While over 1.5 as many calls were recorded and identified along boat transects, there were no clear advantages to boat transects (except for *Myotis grisescens*). Additionally, car transects were least variable and time consuming, leading us to conclude that car transects are the most efficient mobile acoustic method to monitor species. However, only two species (*Perimyotis subflavus* and *Lasiurus borealis*) were likely in sufficiently high abundance using either method to allow detection of small trends. Nonetheless, mobile acoustic transects offer the only measure of summer abundance and car transects likely provide opportunity to monitor 2-4 species in the eastern United States.

INTRODUCTION

Population monitoring is an essential part of wildlife management. Understanding the population status, trend, and distribution of a species allows managers and policy makers to evaluate management actions and provide appropriate legal protections (Gibbs et al. 1998, Elzinga 2001). The power of a monitoring program to detect trends is influenced by initial abundance and sampling variation, which can mask trends in populations by introducing 'noise' that is greater than the trend to be detected (Gibbs et al. 1998, Elzinga 2001, Meyer et al. 2010). A well-developed sampling methodology that is consistently repeated is an efficient method to reduce sampling variability and increase the statistical power of trend analysis (Thompson et al. 1998, Elzinga 2001). However, designing and implementing programs with high power to detect trends are often resource intensive and beyond the capacity of many budgets (Field et al. 2005).

The recent emergence of two large scale threats to bat populations in the mid-2000's has emphasized the need for large scale bat population monitoring in the United States. White-nose Syndrome (WNS) was discovered in 2006 and this fungal infection has killed 5.7-6.7 million hibernating bats across 19 states (USFWS 2012), threatening once abundant species with extinction (Frick et al. 2010). Additionally, bats are increasingly threatened by collisions and possible barotrauma at wind energy facilities (Cryan and Barclay 2009). Mortality at sites varies greatly across the United States, anywhere from 0.1-69.6 bats per turbine per year, primarily migratory foliage-roosting bats (Arnett et al. 2008). Despite the ability to document mortality at some sites, the cumulative effects across populations are difficult to quantify since population estimates exist for very few species (primarily listed species). Additionally, poorly understood diffuse threats such as habitat destruction and environmental contamination could further affect populations on a large scale (Weller et al. 2009).

Despite a recognized need, the monitoring of North American bat populations has been a difficult task with few solutions. A 1999 workshop on bat monitoring determined that current methodologies are inconsistent and biased, offering little opportunity for true bat population monitoring (O'Shea and Bogan 2003). One reason for these difficulties is that bat populations are notorious for extremely high temporal variation in activity levels (Hayes 1997, Gibbs et al. 1998), adjusting their foraging area across the landscape in response to unknown or unpredictable variables (e.g., insect abundances; Fukui et al. 2006) and environmental factors (Duchamp et al. 2007, Lacki et al. 2007, Weller 2007). The workshop participants concluded that new methodologies needed to be developed in order to achieve monitoring goals (O'Shea et al. 2003).

In response to the urgent need to collect baseline data and monitor the cumulative impact of threats, a national mobile acoustic monitoring program was established in 2009. The protocol is a modified version of a monitoring program originally designed by Bat Conservation Ireland in 2003 that rapidly spread across Europe under the indicator bats (iBats) program (Jones et al., in press). The U.S. protocol calls for driving a vehicle ~48km (30 mile) transect at 32 kph (20 mph) with an ultrasonic bat detector mounted on the roof (Britzke and Herzog 2009). Because echolocation calls are recorded while the vehicle is moving faster than most bats flight speed, each call is assumed to represent a single bat, producing an index to species abundance (Roche et al. 2011). The program has spread across the United States (especially the eastern US), and is currently implemented in at least five statewide programs, three National Parks, and 20 National Forests.

While the established mobile method along roads has been effective for some species, only 3 out of 9 species are monitored with the program in Ireland (Roche et al. 2011, Jones et al., in press). The ability to detect trends along roadways of less abundant species (encountered at <0.1 sequences per min) is masked by variability in detections (Roche et al. 2011). Furthermore, certain bat species may avoid roadways due to anything from the perceived threat of traffic to increased lighting (Linton 2009, Berthinussen and Altringham 2011, Stone et al. 2009, Zurcher et al. 2010). Therefore, placement of transects on or within proximity of certain roadways may not allow the monitoring of all species occupying an area (Roche et al. 2011, Jones et al., in press).

Monitoring from rivers, may provide opportunity to monitor more bat species. Bat activity is higher above water than land, possibly due to increased opportunities to drink and feed on emerging aquatic insects (Grindal 1999, Holloway and Barclay 2000, Fukui et al. 2006, Hagen and Sabo 2011). Additionally, riparian habitats may create the habitat structure favored by bats (i.e., forested corridors and gaps; Ford et al. 2006, Loeb and O'Keefe 2006). Furthermore, some species only occur within close proximity to waterways (e.g., *Myotis grisescens*; Tuttle 1976, LaVal et al. 1977) while others likely prefer these habitats for foraging (e.g., *M. lucifugus, Perimyotis subflavus*; Ford et al. 2005) and possibly use them for migratory routes (Furmankiewicz and Kucharska 2009). These factors have caused some states and agencies to employ a boat-based mobile acoustic sampling methodology along rivers and lakes (e.g., Wisconsin DNR). However, the assumed advantages of this sampling technique are not confirmed.

We decided to compare the results of car and boat based monitoring efforts to test if one method provides the opportunity to monitor more species. We evaluated differences at two scales: the stand level by comparing a portion of river and road directly adjacent to one another, and the landscape level by comparing transects designed by the national mobile-acoustic monitoring protocol to nearby river transects. We hypothesized that boat-based mobile acoustic monitoring along rivers will provide the opportunity to monitor more species than the traditional car-based mobile acoustic sampling.

STUDY AREA

Our study was in the southern 11 counties of Illinois, south of IL Rt 13 within or around the Shawnee National Forest (SNF). The SNF is a patchwork of privately and federal owned property, with a few core areas of expansive forest (Figure 1). This part of southern Illinois consists of the Ohio and Mississippi floodplains with oak-hickory forests in the Shawnee-hills and Illinois Ozarks.

Fourteen species of bats occur within this portion of Illinois. Eight are common in the study area: *Eptesicus fuscus, Lasiurus borealis, L. cinereus, M. grisescens, M. lucifugus, M. septentrionalis, M. sodalis, Nycticeius humeralis,* and *P. subflavus.* Additionally, *Corynorhinus rafinesquii*, *M. austroriparius,* and *M. leibii* occur in isolated areas in lower abundances. *Lasionycteris noctivagans* and *Tadarida brasiliensis* have been observed infrequently in the study area only during migration period (T. Carter, unpublished data).

Stand level comparison.– A stand level comparison was conducted near the Oakwood Bottoms area of SNF, in Jackson and Union Counties, IL. We sampled approximately 12.5 km transects by boat and car along the Big Muddy River and an associated levee (Figure 2). The distance from the river to the levee is approximately 200 m (max ~500m) and within view of each other, except for a few portions. The surrounding landscape is a mix of bottomland hardwood forest, floodplain, and agriculture. The close proximity of these stand level transects eliminated difference in bat occupancy that may result from habitat variation at larger scales. The majority of the levee used for the car route is a portion of a landscape level national acoustic monitoring route.

Landscape level comparison.– A landscape level analysis was designed around the three car-based mobile acoustic transects established by SNF in 2009 under the nationwide monitoring program. Each car transect was paired with the nearest navigable river (Figure 1). The Mississippi Bluffs study area was located in the western portion of SNF (Jackson and Union counties). The car transect was 65 kilometers from Murphysboro to Reynoldsville, IL. The boat transect was the lower 57 km of the Big Muddy River (Figure 1). The Garden of the Gods study area was located in northeast SNF (Harding and Gallatin counties). The car transect consisted of 50 km from Equality, Illinois to Eichorn, Illinois (Figure 1). The boat transect was conducted along the lower 31 km of the Saline River and 20 km along the Ohio River to Cave in Rock, IL (Figure 1). The Southern Pope County study area (southeast SNF) was located entirely in Pope County, IL. The road transect ran approximately 45 km from north of Brookport, IL to Hamletsburg, IL (Figure 1). The closest suitable navigable river was Lusk Creek, 9 km north. This river transect began at the Rocky branch of Lusk creek and continued 9 km to the Ohio River, the transect continued on the Ohio River for 15 km ending at Barren Creek (Figure 1).

METHODS

Study design

Stand level comparison.– In May-July 2010 and 2011we sampled the river and road of the stand level comparison simultaneously. We coordinated the start and speed throughout each sample using two-way radios and visual signals to maintain the same approximate location along the car and boat transect, reducing potential temporal variation in sampling. We analyzed 89 nights of mist netting capture data from *M. sodalis* monitoring efforts conducted between 1999 and 2011 (excluding 2002, 2004, 2005, and 2007) at nearby Oakwood Bottoms Greentree Reservoir, SNF, Jackson County, IL (Figure 2; Carter 2003, Carter and Feldhamer 2005, Feldhamer et al. 2006, Carter et al. 2008, Carter et al. 2009, Carter et al. 2010, Whitby et al. 2011), to allow comparison to a standard sampling protocol.

Landscape level comparison.– On the landscape level, car and boat transects were conducted on the same night, starting at the same time in May-July 2010 (n=2) and 2011 (n=4). We used standard stationary bat detectors, mounted on a tripod within a weatherproof container and PVC microphone opening (Britzke et al. 2010) for comparison to a conventional sampling approach. We randomly selected, without replacement, four locations (two river and two road) from ten *a piori* selected locations along the transects (five river and five road; Figure 1). We deployed detectors with an unobstructed field of detection perpendicular to the selected travel corridor. For each stationary sample period, we sampled for four consecutive nights, including the night that sites transects were conducted. We recorded the travel time, equipment establishment and removal time, and person-hours for each method in the landscape level comparison to create an index of financial cost.

Acoustic sampling

Data collection.– We sampled transects following the national mobile acoustic sampling guidelines (Britzke and Herzog 2009) using Anabat SD2 detectors (Titley Electronics, Ballina, NSW, Australia). Detectors were calibrated throughout the season to assure similar sensitivities (Larson and Hayes 2000). The detector was mounted vertically on the car roof and was placed on a tripod at a 30 degree angle on the front of the boat. Docking lights were used on the boat to simulate the car headlights and to avoid hitting things and dying. We also reduced the boat transect speed from the recommended 32 kph to ~20 kph. For the stand level comparison, the car speed was also reduced, as described above; however, for the landscape level comparison, the car speed was maintained at the recommended ~32kph. For both comparisons, we sampled both the river and road simultaneously, beginning 30 min after sunset on nights with low wind.

Call identification.– Calls were downloaded and analyzed using ANALOOK (version 4.7j, Titley Electronics). We used a screening filter to eliminate noise and another filter to identify sequences with one or more high-quality calls (Britzke and Murray 2000). Parameters from sequences with three or more calls were then exported and identified using a mixture discriminate function analysis and a 12 species call library collected across the eastern United States (Britzke et al. 2011). Because both *C. rafinesquii* and *T. brasiliensis* are infrequently encountered in Illinois and the call library lacked reference calls for these species, they were excluded from analysis. Species counts with <2 sequence identified in a night were excluded due to the possibility of identification error (Britzke et al. 2002, Shirmacher et al. 2007). With mobile acoustic transects, we assume that each sequence represents an individual bat and therefore the number of sequences recorded provides an index of abundance. For landscape level activity analysis, the number of sequences was divided by the duration of recording in order to account for differences in sampling speed, time, and distance (Roche et. al 2011). For stationary bat detectors, we converted the sequence counts to presence-absence data.

Species Richness and Diversity

We used the program EstimatesS (version 8.2 ; R. K. Colwell, University of Connecticut) to compare species accumulation curves (richness and density), shared species, and diversity (Shannon-Weaver and Simpson's index) of sampling methods. We used sample-based rarefaction (Colwell et al. 2004) to interpolate expected species richness at sample and individual levels (Gotelli and Colwell 2001, Colwell et al. 2004). Sample-based rarefaction uses presence-absence data to account for non-random association of species occurrence (Gotelli and Colwell 2001). We chose to use the computational method instead of the classic resampling method to calculate more rigorous confidence intervals (Colwell et al. 2004). We scaled each rarefaction curve to individuals (except for stationary data, where abundance levels do not reflect individuals) to estimate true species richness (species in relation to number of observations) and samples to estimate species density (number of species in the sample area). Estimates of species density does not account for differences in sample effort or area between methods

(Gotelli and Colwell 2001). However for our study it is a relevant estimate of the differences between the standard application of methodologies.

We examined the similarity of samples using the quantitative Morisita-Horn similarity index for transects (and mist-netting data at the stand level) and classic Sorensen incidence-based index for comparing similarity of landscape level transects and stationary acoustic data (Magurran 2004). Each shared species index express similarity between samples with a value between 0 (no shared species) and 1 (all species present in both samples, and at equal abundance with Morista-Horn). We further compared communities with abundance data using rarefied diversity indexes (exponential Shannon-Weaver, inverse Simpson's) over 100 repetitions with replacement. The exponential Shannon-Weaver and inverse Simpson's expressions of the diversity indices represent the number of species required at even abundances to reach the observed index value and can be interpreted as the number of abundant and very abundant species in a sample, respectively (Ludwig and Reynolds 1988). Rare species are given less weight with species richness (rarefaction), Shannon-Weaver, and Simpson's index and combined are the useful indicators of community diversity (Hill 1973, Jost 2006).

Activity Rates

To test if there was an overall difference in the transect sampling methodology we used a permutation-based nonparametric multivariate analysis of variance (perMANOVA; Anderson 2001) in PC-ORD ver. 5 using Sorensen distances (McCune and Mefford 1999). For the stand level comparison we used a complete randomized block design (blocked by method [n=2] and grouped by sample date [n=22]). In the landscape level analysis, we used a two-level nested perMANOVA to test overall differences in species abundance between site and method. Replicates (n=6) were nested within method (n=2) nested within site (n=3). Following a significant perMANOVA, we compared the pairwise abundance using PAST ver. 2.14 (Hammer et al. 2001). To compare species abundance, we used paired t-tests for species with normally distributed differences and the Wilcoxon-Sign Rank test for nonparametric distributions. Variation of each species was compared with the coefficient of variation.

To control for false discovery rates, we converted all p-values to Q-values using QVALUE version 1.0 (Storey et al. 2004) in the program R version 14.2 (R Development Core Team 2012). All tests were two-tailed and were considered significant if Q-values ≤ 0.1.

RESULTS

Stand level Comparison

The stand level comparison was replicated 4 times in 2010 and 18 times in 2011. We sampled 246.9 km and 287.6 km from the car and boat, respectively. A total of 737 and 1170 call sequences were recorded from the car and boat, respectively. Of these, 549 (74%; car) and 913 (78%; boat) consisted of three or more high-quality identifiable calls. Mist-netting was conducted for a total of 89 nights from 1999-2011 with a total of 715 captures. Of these, 228 captures and 17 nights were from 2010 and 2011.

Species Richness and Diversity.– Eight species were identified along the boat transect, nine species on the car transect, and nine species netting (7 species in 2010 and 2011; Table 1). Despite differences in observed species richness, rarefaction indicated that there was no difference between species richness and density of the three sampling methods at the 95% confidence level. However, car transects tended to have slightly higher species richness and density (Figure 3; Table 2).

The Sorensen and Morisita-horn shared-species indices, useful measures for comparing the similarity between methods, indicated that the car and boat transects were most similar and transects and netting were less similar (Table 3). The Shannon-Weaver and Simpson's diversity index values the boat and car transects did not result in significant differences in diversity (Table 2, Figure 4). However, mist-netting produced higher indices than both transect methods, indicating more abundant and very abundant species (Table 2, Figure 4).

Activity.– Based on the perMANOVA we found that sample method accounted for over 79% of variation. There were significant differences between sampling from the car and boat (F_1 4.91, P=0.007) and across sampling time (F_{21} 2.03, P=0.010) on species abundance levels. The boat recorded 19.6(±13) more total sequences per transect than the car (Q=0.009). This was likely primarily due to 12.3(±8.5) more *Perimyotis subflavus* sequences along each sample of the boat-based transect (Q=0.014). *Lasiurus borealis* (Q=0.009) and *M. grisescens* (Q=0.014) also were detected at higher abundances along the boat transect (Table 4). *Eptesicus fuscus, L. cinereus, L. noctivagans, and N. humeralis* did not differ in abundance between transect types (Table 4). *Myotis* species besides *M. grisescens* were excluded from pairwise analysis because no sequences were identified (*M. austroriparius, M. leibii, and M. sodalis)* or they were only detected with a single method (*M. septentrionalis*; Table 1). Coefficient of variation was greater along the boat transects for five species and average abundance was greater along the car for two species (Table 4).

Landscape level comparison

Each landscape level comparison was conducted two times in 2010 and four times in 2011 at each study site (total 18 paired car and boat transects). We sampled 650 km of river (37 h 6 min of recording) and 933 km of roadways (34 h 43 min of recording). Across the boat transects, 4,233 and 3,528 (83%) sequences were recorded and identified, respectively. On the car transects, 2,490 and 1,779 (71%) sequences were recorded and identified, respectively. Stationary detectors recorded and identified 89,303 and 66,485(74%) sequences, respectively, over 180 detector nights at 50 detector sites.

Species Richness and Diversity.– All 12 species were identified along the car transects and at stationary locations while boat transects detected 9 species (Table 1). Rarefied species richness of transects was not different along car transects, however, car transects tended to show a higher species richness than boats (Figure 5). When transects were compared to the stationary acoustic data using sample-based rarefaction species density was higher at stationary acoustic locations compared to both transect types (Figure 5). While car transects generally documented a higher species density than boat transects, these two methods almost completely overlapped at the 95% confidence level (Figure 5). By 11 sampling events 95% confidence levels for all three methods overlapped indicating they could document all species (Figure 5). However, at all study sites stationary locations detected all species with a high confidence (i.e., smaller confidence intervals) at a low number (<4) of sampling events, while both transects methods did not (Figure 5; Table 1).

Similar to the stand level comparison, we used the Sorensen and Morisita-horn shared species indices to compare communities along transects and stationary locations. Based on incidence (Sorensen index) the car was identical to stationary sites while species composition along the boat transects was only marginally different from both stationary sites and car transects (Table 5). Boat and car transects had similar species and abundances (Morisita-Horn; Table 5). Based on the Shannon-Weaver (SW) and Simpson's index (SI), abundant and very abundant species occurred more frequently on car transects than boat transects at the 95% confidence interval for two study sites (Figure 6). Across the landscape, 1.63 and 1.04 more abundant (SW) and very abundant (SI) species were detected along car transects than boat transects, respectively (Table 2; Figure 6).

Activity.– Based on the perMANOVA, there was a significant difference in bat communities between sampling methods (P=0.04) but not between sites (P=0.61). Because there was no difference in sites, we pooled data across sites by method to test mean and variance of species abundance. We did not compare transect abundances to stationary data, as there is no way to estimate abundances from the later. *Lasionycteris noctivagans, L. cinereus, M. austroriparius, M. leibii, M. septentrionalis,* and *M. sodalis* were excluded from pairwise comparisons because all occurred along less than half the transects (<9) within each method (Table 6). Only *L. borealis* and *P. subflavus* were encountered every time car and boat transects were sampled (Table 6). Total activity was 0.6(±0.45) sequences per min greater along the boat transect (Q <0.001). Two species were more abundant on the boat transect, while 3 were more abundant on the car transect (Table 6). Coefficient of variation was greater for two species on the boat (Table 6). Only *M. grisescens* was encountered more frequently and showed a lower coefficient of variation along the boat transect (Table 6).

Time Investment.–For the landscape level comparison the car transects required the least total time (travel, preparation, transect sampling, clean-up) to survey (Table 7). Car transects also required less than half the time per km sampled compared to boat transects (Table 7). Four stationary detectors required 1.77 and 1.42 times as long to establish and remove per session than car and boat transects respectively (Table 7).

DISCUSSION

A great deal of grey literature is available from Europe on the development and preliminary results of car-based mobile acoustic transects (see Jones et al., in press). However, only one peer-reviewed paper is available examining the method's data (Roche et al. 2011). To our knowledge this is the first study of mobile based acoustic data in the United Sates and the first comparison of mobile acoustic methods and more traditional methods for studying bats.

Stationary acoustic detectors quickly accumulate and detect bat species richness (Ford et al. 2005, Murray et al. 1999). Our results were similar, with all 12 species identified after sampling four stationary locations for four nights each across the Southern Illinois landscape (Figure 5). However, both mobile acoustic transect methods did not have similar ability to detect species. Neither car nor boat transects detected all 12 species within a study area. Species richness was similar between mist-netting and stand level car-transects; however, mist-netting required over three times the effort (65 nights) to achieve the same number of individual observations as 21 stand level car transects (Figure 3A), illustrated by the lower species density per sampling event (Figure 3B). Overall, observed species density (number of species per sample) decreased from stationary locations to car-based transects to boat-based transects and to mist-netting.

Differences between methods are further demonstrated by the steepness of the rarefaction curve, which can be used as an indicator of the evenness of species presence across samples (Gotelli and Colwell 2001). The steeper initial rarefaction curve (and higher diversity indices for mist-netting) suggests that stationary detectors and mist-netting more consistently and evenly detect species than both mobile acoustic transect methods (Figures 3-5). Furthermore, the wide confidence intervals along both transect types, at the stand (Figure 3) and landscape levels (Figure 5) demonstrate the infrequent detection of many species, especially from the genus *Myotis*, with these methods.

As expected bat activity was greater along boat transects than car transects; however, we found that species richness and density does not follow the same trend. While not significant at the 95% confidence level, car transects detected a greater species richness and density at both the stand and landscape levels. Additionally, the higher diversity indices along car transects indicates that car transects more evenly detect species than boat transects, and can likely monitor ~1 more species. Bell (1980) similarly observed decreasing richness and diversity over water, while other studies indicate that these measures are greater at water sites compared to land sites (Ellison 2005, Winhold and Kurta 2008). However, none of these studies account for differences in sample size (i.e., rarefaction). We did see differences in within-species abundances between car and boat transects (Table 4 & 6); however, they were not nearly as universal as expected.

Car and boat transects at the landscape level (Morisita Horn 0.898) were not as similar to parallel boat and car transects (i.e., stand level; Morisita-Horn 0.991). Therefore, it is likely that car transects designed to closely parallel water bodies will be able to detect a similar bat community to a boat transect without the added effort required to conduct boat transects. How close a car-based transect has to be to a river is likely based on site specific factors, but within 1km is likely beneficial and within 0.65 km ideal, as aquatic insects disperse 650-1845 m from water (Kovats et al. 1996).

The average and range of variability observed in bat populations is possibly the highest of vertebrates (Gibbs et al. 1998). This variability has made bat monitoring efforts difficult since the ability to successfully monitor species is driven by higher initial abundance and lower sampling variation (Gibbs et al. 1998; Meyer et al. 2010). The overall variation in bat activity that we observed for both the boat and car (coefficient of variation [CV] 54% & 55%, respectively) at the landscape scale was below the average variation for bat studies (CV 95%) and similar to other small mammal studies (60%; Gibbs et al. 1998). This seems to indicate that mobile acoustic transects may be able to lower the high variation that typifies historical bat monitoring efforts and provide increased opportunity for population monitoring compared to more traditional methods.

Agency reports from Europe (especially Ireland) have shown that mobile-acoustic transects can provide useful information on trends and distribution of bats. However, in Ireland where the program has been implemented since 2004, only 3 of 9 species were encountered frequently enough (>0.1 sequences per min) for statistical analysis of population trends (Roche et al. 2009, Roche et al. 2011). Assuming similar requirements in the United States, *L. borealis*, *P. subflavus*, and *M. grisescens* were the only species to be encountered frequently enough for trend analysis. Although *Perimyotis subflavus* was over twice as abundant on the landscape scale boat transects than car transects, the lower variance along the car and added effort required to conduct boat transects likely means that car-based monitoring is the most efficient way to monitor both *L. borealis* and *P. subflavus,* especially considering they are still more abundant along roads then the most common Irish species (Roche et al. 2011). *Eptesicus fuscus* and *N. humeralis* may be able to be monitored from cars better than boats, but would likely require a longer time period to confidently detect trends since we observed mean encounter rates less than 0.1 sequences per min. *M. grisescens* had both higher abundance and lower variation on the boat and would likely be able to be monitored via boat transects but not car transects.

Habitat and time partitioning between bat species may account for low encounter rates of some species (Kunz 1973, LaVal et al. 1977, Swift and Racey 1983, Aldridge and Rautenbach 1987, Arlettaz 1999, Adams and Thibault 2006, Nicholls and Racey 2006). Abundance of bat species changes throughout the night (Kunz 1973, Winhold and Kurta 2008). Extending transects or including replicates that start later in the evening could increase encounters of some species such as *L. cinereus* and *L. noctivagans* which may not reach peak foraging activity until 4-8 hours after sunset (Kunz 1973). Additionally, assuring transects are designed to stratify available habitat and therefore target certain species may add to monitoring ability (Buckland et al. 2005) However, if transects are not randomly selected from the landscape, observed changes along these routes may not be indicative of overall population trends (Buckland et al. 2005, Roche et al. 2011).

Mobile acoustic sampling from the car required the least time-investment. Preparing for and conducting these routes was also the simplest of all methods. Besides requiring added preparation time, boat transects introduced sampling variation that could cause problems for the long term analysis. Log-jams caused two transects to have to be altered throughout the 2011 season and access to rivers via boat ramps was inconsistent. Furthermore, simply operating a boat at night is inherently dangerous, and required that two people participate in sampling. In addition, a third person was required to drive the vehicle and trailer to pick-up the boat at the end of the transect (or a doubling of the sampling time in order to return the boat to the original location). So while the boat transect only appears to add about one hour of time to car transects it can easily be over three times the time investment in total person hours. Conducting boat transects along lake edges with improved access may reduce variation from changing sampling methodology and reduce man-hours, but does not account for other possible sampling variation with insect abundances. However, these factors were not tested and should be explored.

MANAGEMENT IMPLICATIONS

Decisions about bat monitoring will have to be made based on project goals and resource availability. If simply establishing species presence/absence at sites is the goal and many bat detector units are available, then traditional stationary acoustic detectors may be the best approach. However, if one needs to monitor abundance levels, then car based mobile acoustic transects provide an index to abundance for the most species across a landscape with the least amount of effort, and can be implemented by volunteers with little training (Jones et al., in press). However, if goals include monitoring species that are associated with water (e.g., *M. grisescens*) then boat based transects may be necessary. Nonetheless, boat-based surveys did not offer the clear advantages to monitoring that we hypothesized. Variability of activity within sampling areas and low encounter rates for some important species with mobile acoustic transects make it clear that this is not a universal approach to bat monitoring and that mobile acoustic transects likely can only monitor 2-4 species, including species with no applicable traditional monitoring method. The great diversity in bats, even in temperate climates, makes a single universal monitoring protocol unlikely (O'Farrell and Gannon 1999, Duffy et al. 2000, Flaquer et al. 2007).

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FIGURES

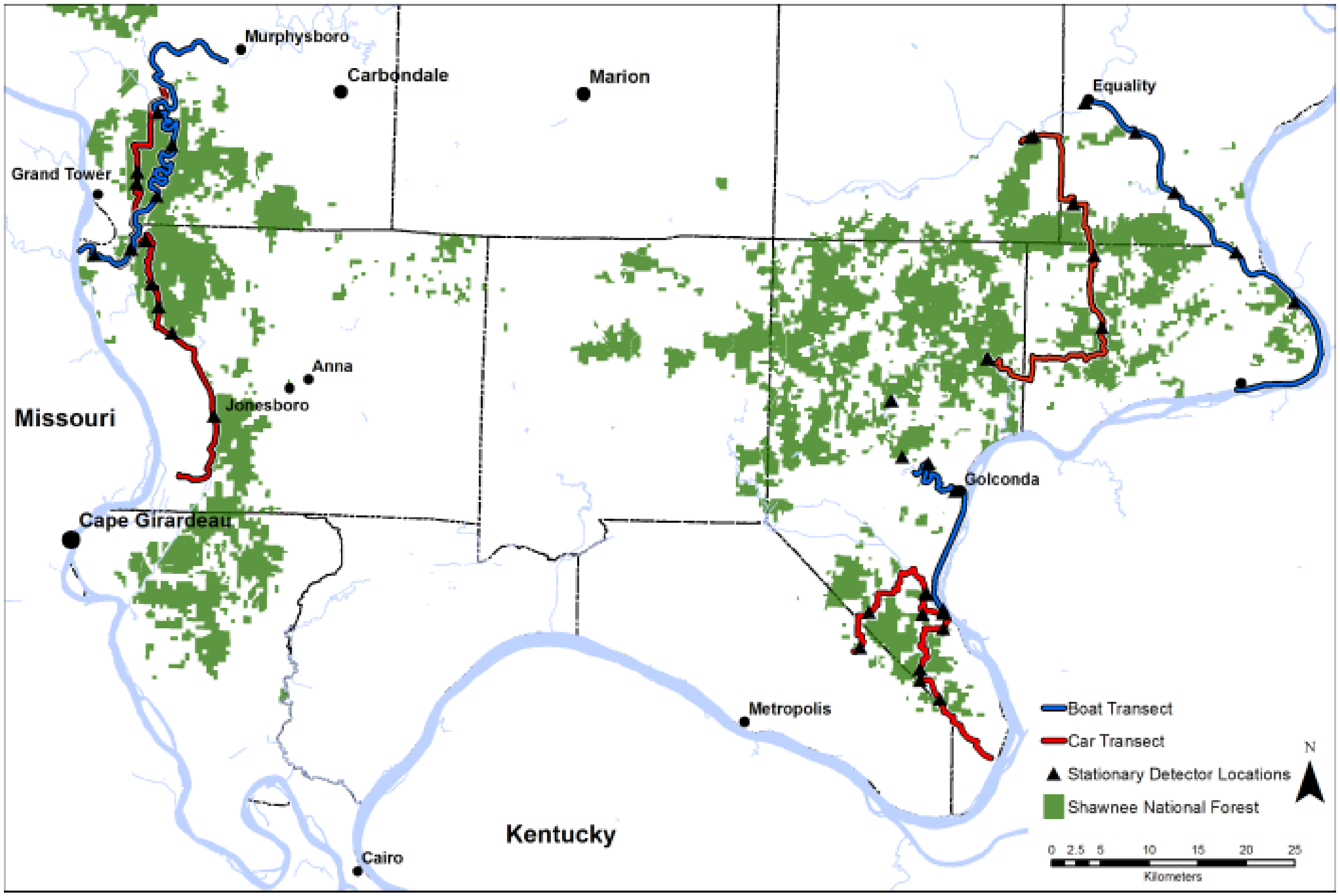

Figure 1. Landscape level mobile acoustic transects (Red=Car, Blue = Boat) and stationary detector locations (triangles) sampled May-July 2010 and 2011 across Shawnee National Forest, Illinois, USA.

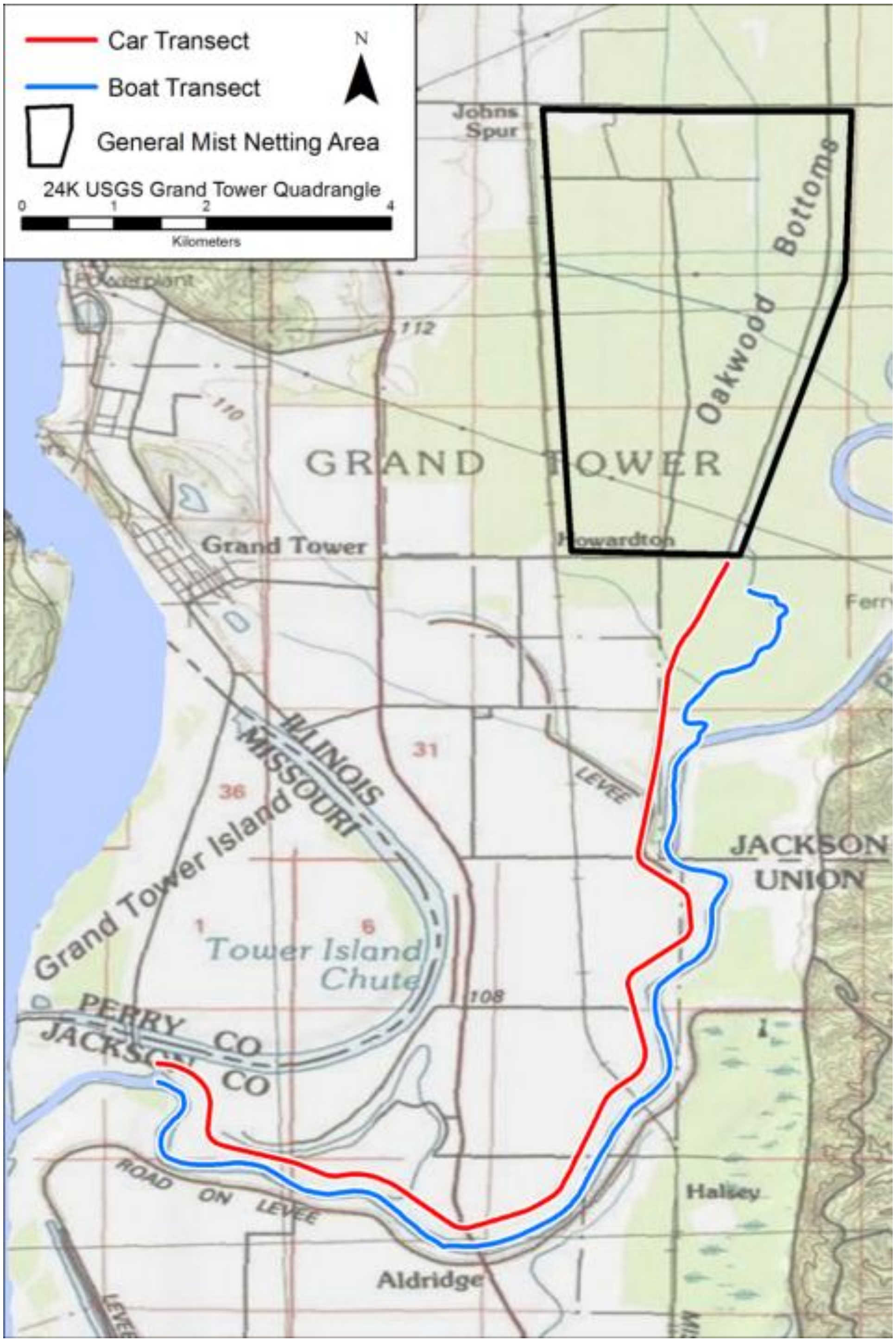

Figure 2. Stand level boat and car based acoustic transects and mist-netting area (Oakwood bottoms) in Jackson and Union counties, Illinois, USA sampled May-July 2010 and 2011.

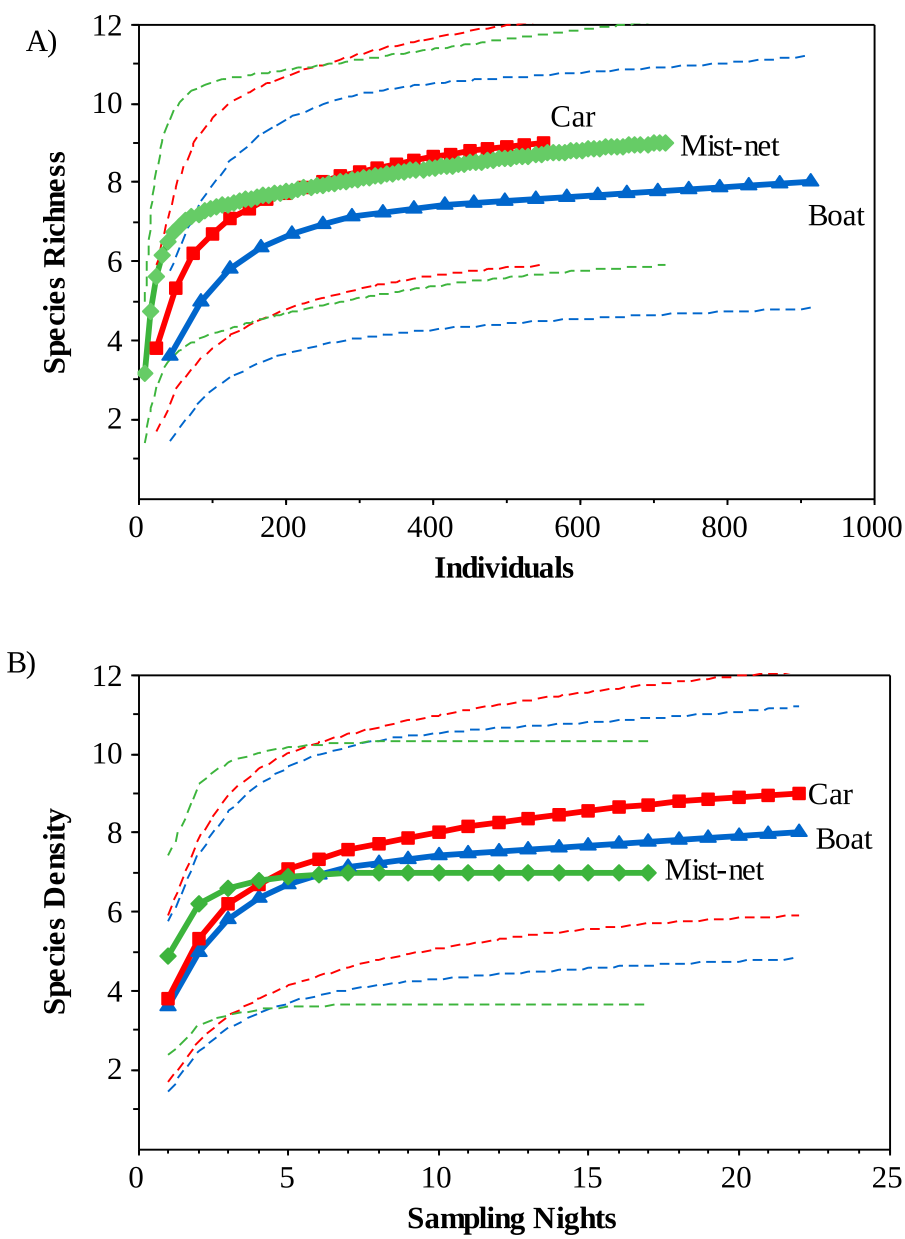

Figure 3. Stand level sample-based rarefaction curves and 95% confidence intervals (dotted lines) for three sample methods (car [red squares], boat [blue triangles], and mist-netting [green diamonds]) in Shawnee National Forest, Jackson and Union counties, Illinois, USA. Acoustic sampling and mist-net samples from May-July 2010-2011; mist-netting individuals from Oakwood Bottoms 1999-2011.

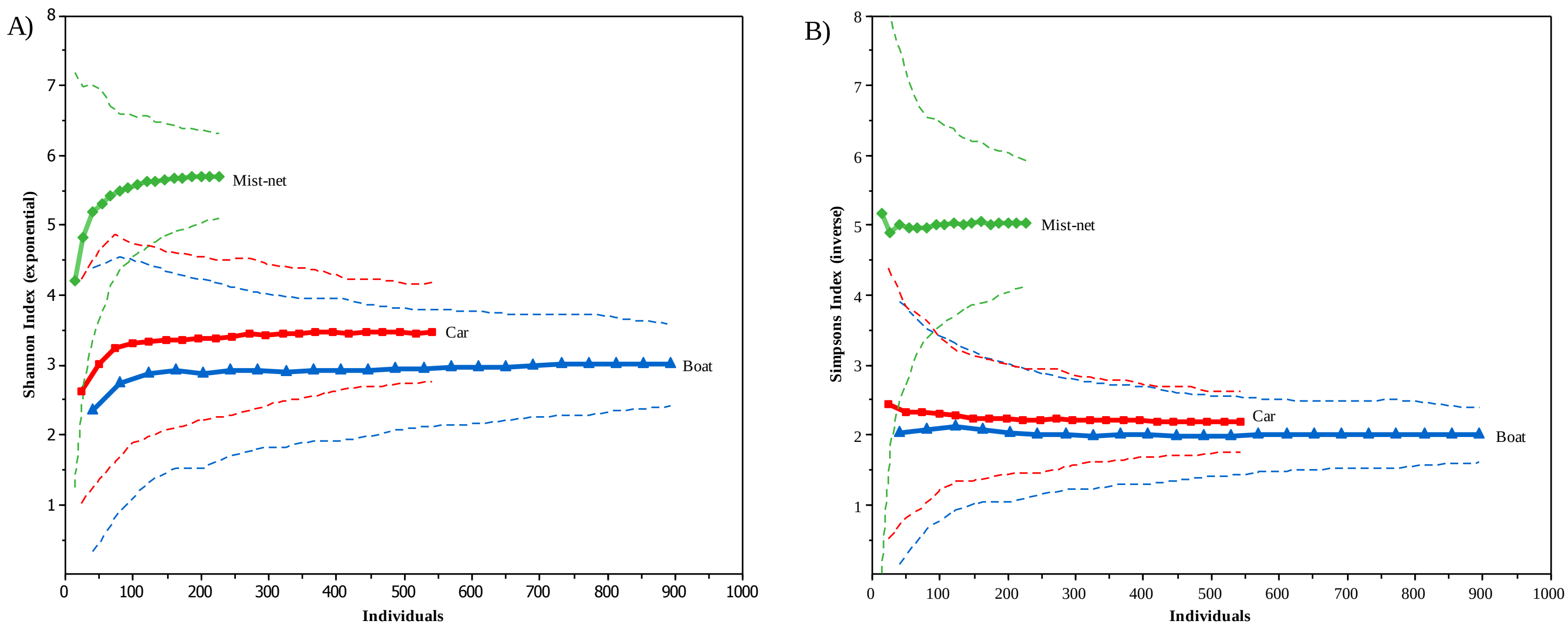

Figure 4. Stand level Shannon-Weaver index (exponential) and Simpsons index (inverse) for three sample methods (mist-netting, car, and boat transects) across individuals (symbols represent samples: nights mist-netting or transects run [car, boat]) in Shawnee National Forest, Jackson and Union counties, Illinois, USA May-July 2010 & 2011.

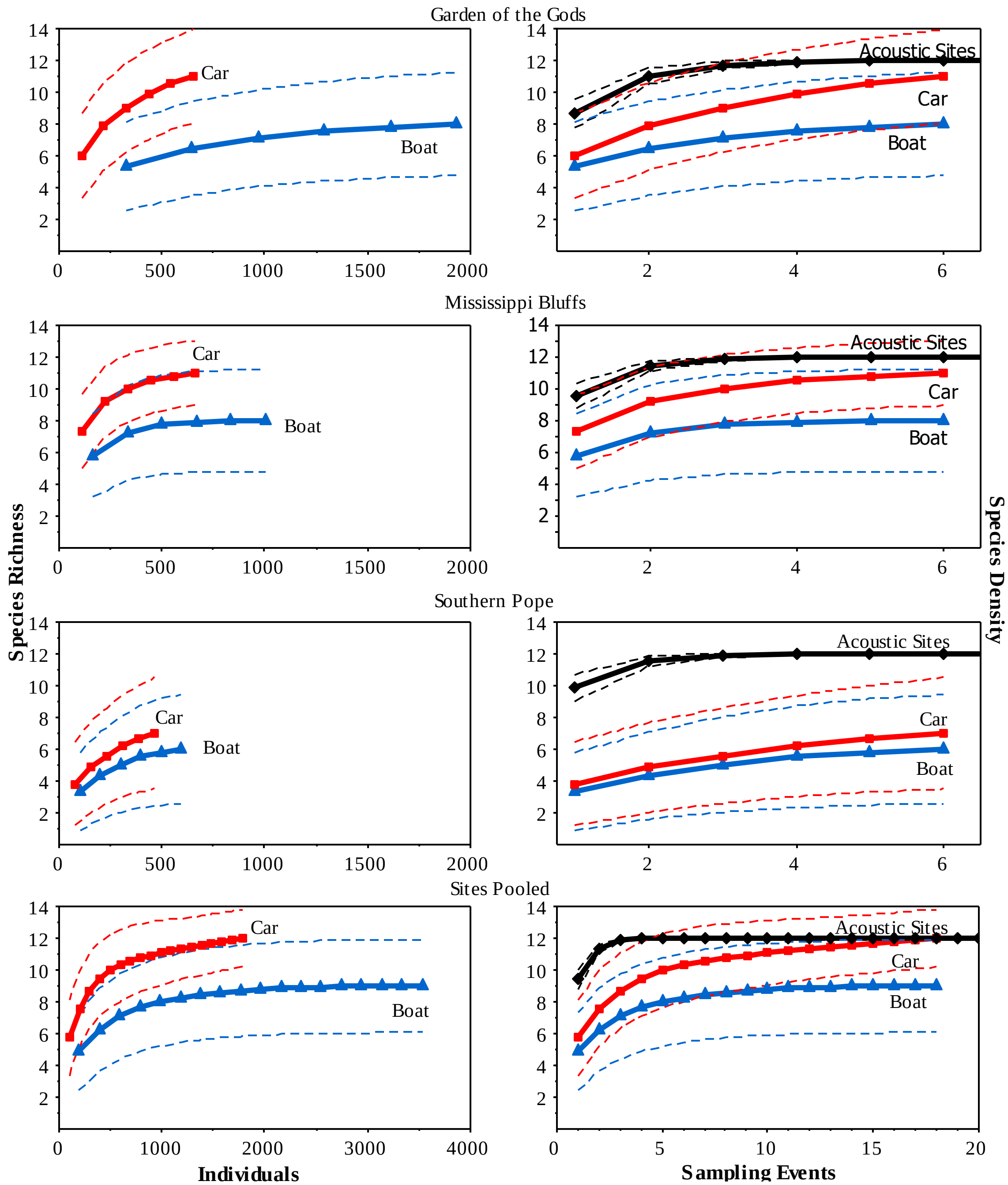

Figure 5. Landscape level sample-based rarefaction curves (density and richness) and 95% confidence intervals (dotted lines) for car (red squares) and boat (blue triangles) mobile acoustic transects and stationary detector sites (black circle) in Shawnee National Forest, Jackson and Union Counties, Illinois May-July 2010-2011

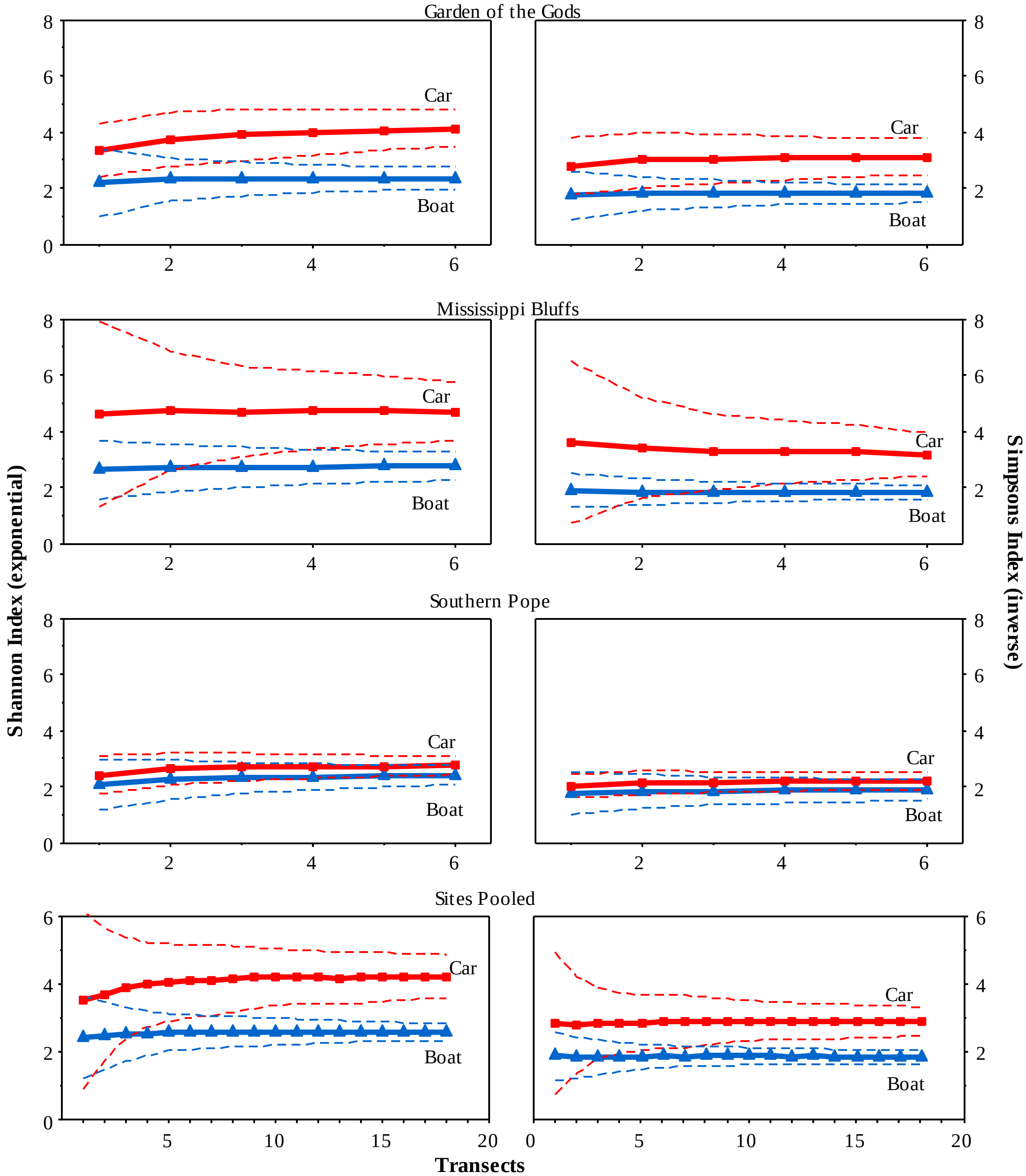

Figure 6. Landscape level Shannon-Weaver (exponential form) and Simpson's (inverse) for car (red squares) and boat (blue triangle) mobile acoustic transects at three study sites and pooled across sites in Shawnee National Forest, Jackson and Union counties, Illinois, USA May-July 2010 & 201

TABLES

Table 1. Species observations for car and boat transects and mist-netting conducted in Jackson and Union Counties, IL and landscape level car and boat mobile acoustic transects across Shawnee National Forest, May-July 2010-2011. Mist-netting from 1999-2011 in parentheses.

	Stand-Level			**Landscape-Level**		
	Boat Transect	**Car Transect**	**Mist-Netting**	**Boat Transect**	**Car Transect**	**Stationary Detectors**[1]
Eptesicus fuscus	20	32	68 (114)	30	112	3079
Lasiurus borealis	112	46	54 (157)	260	432	8493
Lasiurus cinereus	16	20	0 (2)	10	68	947
Lasionycteris noctivagans	36	37	0 (1)	26	30	1715
Myotis austroriparius	0	0	0 (0)	0	2	519
Myotis grisescens	50	7	0 (0)	604	53	7224
Myotis leibii	0	0	0 (0)	0	15	962
Myotis lucifugus	2	2	23 (53)	22	28	2004
Myotis septentrionalis	0	11	21 (186)	0	2	424
Myotis sodalis	0	0	24 (109)	4	15	2026
Nycticeius humeralis	40	27	8 (28)	64	94	2516
Perimyotis subflavus	637	367	30 (65)	2508	928	36576
TOTAL[2]	1170	737	-	4233	2490	89303

[1]Number of sequences for stationary detectors can only be used as an index to activity and does not represent number of individuals

[2]Total represents all sequences of one or more pulses that were recorded, but not necessarily identified

Table 2. Richness and diversity indices (Shannon-Weaver and Simpson's) for car and boat transects and mist-netting conducted in Jackson and Union Counties and landscape level car and boat mobile acoustic transects across Shawnee National Forest, Illinois, USA May-July 2010-2011.

		Richness	Shannon-Weaver Index (abundant species)	Simpson's Index (very abundant species)
Stand	Car Transect	9±3.1	3.8±1.18	2.20±0.74
	Boat Transect	8±3.2	2.91±0.90	2.01±0.88
	Mist-netting	7±3.4	5.71±0.61	5.03±0.90
Land-scape	Car Transect	12±1.84	4.22±0.65	2.90±0.43
	Boat Transect	9±2.94	2.59±0.27	1.86±0.22
	Stationary Detectors	12±0	-	-

Table 3. Shared-species indices based on abundance (Morisia-Horn) and species presence/absence (Sorensen's) for stand level car and boat transects and mist-netting conducted in Jackson and Union Counties, Illinois, USA May-July 2010-2011

	Morisita-Horn	**Sorensen's**
Car & Boat	0.99	0.95
Mist-Netting & Car	0.41	0.78
Mist-Netting & Boat	0.38	0.71

Table 4. Average abundance of 7 bat species (W= Paired Wilcoxon Signed Rank Test; T= paired T-test) and coefficient of variation along simultaneous 12.5 km stand level mobile acoustic transects conducted by boat and car in Jackson and Union Counties, IL May-July 2010-2011. * denotes a significant Q-value (*<0.1, **<0.01)

	Transects Present		**Average Abundance / Transect**				**Coefficient of Variation**	
	Boat	Car	Boat		Car	(Test)	Boat	Car
Eptesicus fuscus	5	9	0.91		1.45	(W)	201%	165%
Lasiurus borealis	17	14	5.09	**	2.09	(T)	84%	93%
Lasiurus cinereus	6	9	0.73		0.91	(W)	171%	127%
Lasionycteris noctivagans	8	12	1.64		1.68	(W)	158%	101%
Myotis grisescens	9	3	2.27	*	0.32	(W)	206%	264%
Nycticeius humeralis	11	11	1.82		1.23	(T)	112%	109%
Perimyotis subflavus	22	22	28.96	*	16.68	(W)	74%	71%
Total Sequences	-	-	53.18	**	33.5	(T)	62%	49%

Table 5. Shared-species indices based on abundance (Morisia-Horn) and species presence/absence (Sorensen's) for landscape level car and boat transects and stationary acoustics conducted across Shawnee National Forest, IL May-July 2010-2011

	Morisita-Horn	Sorensen's
Car & Boat	0.898	0.857
Stationary & Car	-	1.000
Stationary & Boat	-	0.857

Table 6. Presence, average abundance per minute, Q-values for comparisons of mean (W= Paired Wilcoxon Signed Rank Test; T= paired T-test), and coefficient of variation of bat species along 3 landscape level mobile acoustic transects conducted by boat and car in Shawnee National Forest, Illinois, USA May-July 2010-2011. Gray rows indicate species that were detected <50% of sampled transects (18 per method). * denotes a significant Q-value (*<0.1, **<0.01, ***<0.001)

	Transects Present		**Avg sequences/minute**			**Coefficient of Variation**	
	Boat	Car	Boat	Car	(Test)	Boat	Car
Eptesicus fuscus	6	11	0.012	0.051 **	(W)	180%	144%
Lasionycteris noctivagans	5	6	0.012	0.013			
Lasiurus borealis	18	18	0.119	0.213 **	(T)	64%	60%
Lasiurus cinereus	4	5	0.005	0.036			
Myotis austroriparius	0	1	0.000	0.001			
Myotis grisescens	14	8	0.263 **	0.026	(W)	124%	130%
Myotis leibii	0	4	0.000	0.007			
Myotis lucifugus	8	9	0.010	0.014	(T)	148%	116%
Myotis septentrionalis	0	1	0.000	0.001			
Myotis sodalis	2	6	0.002	0.007			
Nycticeius humeralis	12	16	0.029	0.043 *	(T)	93%	59%
Perimyotis subflavus	18	18	1.068 ***	0.457	(T)	56%	50%
Total Abundance	-	-	1.825 ***	1.224	(T)	54%	55%

Table 7. Time-investment for landscape level car and boat transects and stationary acoustics conducted across Shawnee National Forest, Illinois, USA May-July 2010-2011

	Total Time(h)	**Range**	**Sampling effort**
Car Transect	4.8	3.5-5.5	5.6 min/km
Boat Transect	6.0	3.4-7.7	13.0 min/km
Stationary Acoustics	8.5	5.4-11.7	31.3 min/detector night