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Effect of passive acoustic sampling methodology on detecting bats after declines from white nose syndrome

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Concomitant with the emergence and spread of white-nose syndrome (WNS) and precipitous decline of many bat species in North America, natural resource managers need modified and/or new techniques for bat inventory and monitoring that provide robust occupancy estimates. We used Anabat acoustic detectors to determine the most efficient passive acoustic sampling design for optimizing detection probabilities of multiple bat species in a WNS-impacted environment in New York, USA. Our sampling protocol included: six acoustic stations deployed for the entire duration of monitoring as well as a 4 x 4 grid and five transects of 5-10 acoustic units that were deployed for 6-8 night sample durations surveyed during the summers of 2011-2012. We used Program PRESENCE to determine detection probability and site occupancy estimates. Overall, the grid produced the highest detection probabilities for most species because it contained the most detectors and intercepted the greatest spatial area. However, big brown bats (*Eptesicus fuscus*) and species not impacted by WNS were detected easily regardless of sampling array. Endangered Indiana (*Myotis sodalis*) and little brown (*Myotis lucifugus*) and tri-colored bats (*Perimyotis subflavus*) showed declines in detection probabilities over our study, potentially indicative of continued WNS-associated declines. Identification of species presence through efficient methodologies is vital for future conservation efforts as bat populations decline further due to WNS and other factors.

Key words: White-nose syndrome, detection probability, Indiana bat, little brown bat.

INTRODUCTION

White-nose syndrome (WNS) is a disease of cave hibernating bats caused by the fungal agent, *Pseudogymnoascus destructans,* first documented in North America in 2006 (Blehert et al., 2009). Since its onset, WNS has caused the deaths of >6 million bats (USFWS, 2013) and has rapidly spread from central New York to at least 22 states and 5 Canadian provinces. *P. destructans* has been documented from four additional states, but infection in bats with discernible lesions remains uncertain, that is, presence of *P. destructans* of DNA has been discovered without concomitant disease. In the context of severe bat population declines, sampling methodologies must be modified to account for these lower chances of detection. As WNS continues to spread and bat populations decline further, biologists invariably will need to rely more on acoustic detection, as a matter of necessity, as the primary

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method of monitoring bats on the landscape.

Acoustic monitoring is a non-invasive sampling technique that has become commonplace over the last decade for investigating ecology, species assemblages and relative abundance of bats within landscapes and/or relative to land management therein (Johnson et al., 2002; Milne et al., 2004; Ford et al., 2011; Johnson et al., 2011b). Use of acoustics has allowed the detection of greater overall species richness more quickly and over greater spatial extent than traditional capture methodologies such as mist-netting (Murray et al., 1999; O'Farrell and Gannon, 1999). Although most research suggests that use of both capture and acoustic techniques provides the more complete assessment of a bat community, the additive benefit of netting to increase detection probability is lessened in a post-WNS landscape because of low capture rates. Furthermore, in these severely impacted areas, the need for very high levels of sampling effort to detect declining species simply will be logistically prohibitive in that the efforts required may approach the entire duration of when temperate forest bats are present on the landscape (Coleman, 2013). As managers begin to incorporate acoustical methods, questions remain on the most effective deployment strategies in terms of duration and configuration of multiple detectors.

The Anabat acoustic detector (Titley Electronics Ballina, New South Wales, and Australia) is a type of frequency-divi-sion detector that has been used widely to evaluate species-specific habitat use and foraging activity (Betts, 1998; O'Farrell and Gannon, 1999; Britzke et al., 2002; Britzke, 2003; Ford et al., 2006). Although Anabats have been field evaluated (Johnson et al., 2002; Britzke, 2003; Milne et al., 2004; Brooks and Ford, 2005; Britzke et al. 2011), no research has assessed multiple passive sampling arrays for detecting numerous species in the context of WNS-impacted landscapes. Accordingly, the objective of our study was to assess detection probabilities and occupancy estimates of bats among differing spatial and temporal arrangements of Anabat detectors in a post-WNS landscape.

MATERIALS AND METHODS

This study was conducted at the Fort Drum Military Installation (Fort Drum) in Jefferson and Lewis counties in northern New York (44°00'N, 75°49'W). Fort Drum is a U.S. Army installation of approximately 43,000 ha that lies at the intersection of the St. Lawrence-Great Lakes Lowlands, the foothills of the Adirondack Mountains, and the Tug Hill Plateau ecoregions within the Black River and Indian River drainages. The nearby Niagara Escarpment (10 -15 km west) contains karst formations with caves that support hibernating bats (Fenton, 1966). Largely undeveloped except in the Cantonment area, much of the Fort Drum landscape is a forested habitat dominated by northern hardwood types of varying successional stages. Approximately 20% of the installation area is comprised of wet meadows and beaver (*Castor canadensis*) impacted streams and ponds.

In the summers of 2011 and 2012, we deployed acoustic bat detectors across Fort Drum in various sampling arrays. We used a mix of Anabat II detectors connected to compact flash-storage Zero-Crossings Analysis Interface Modules and wholly-contained SD1 and SD2 units (Titley Electronics, Ballina, New South Wales, Australia)[1]. We calibrated all units using an ultrasonic insect deterring device prior to use in the field (Larson and Hayes, 2000). We placed Anabat units in weatherproof boxes with polyvinyl chloride (PVC) tubes attached that contained a small hole in the bottom for water drainage (O'Farrell, 1998). Boxes were placed on 1.5 m tripods aligned in a manner that allowed sound to enter the PVC tubes at a 45° reflective angle to be received by Anabat transducers perpendicularly (Britzke et al., 2010).

Randomly selected acoustic monitoring stations may not successfully detect endangered Indiana bats (*Myotis sodalis*) or little brown bats (*M. lucifugus*) due to recent WNS declines (Ford et al., 2011). Therefore, we focused monitoring efforts on these species by placing arrays of detectors near a little brown bat maternity colony in an artificial bat house (Dobony et al., 2011) and known historic Indiana bat maternity areas at Fort Drum (Johnson et al., 2011a). We deployed detectors to record passively at permanent stations that remained in the field for the entire summer season, linear stream transects of 5-10 detectors deployed for 6-8 nights at a time, and a 4 x 4 "grid" of detectors that also were deployed for 6-8 days per sampling session (Figure 1).

To ensure that more than one Anabat did not collect data on the same bat simultaneously, we separated individual detector sites by 200-250 m. The exception to this was a double transect of five sites where two detectors were pointed in opposite directions at each site and data from both units were combined. We chose deployment locations and the azimuth of microphone direction at each site to maximize call quality. For example, we specifically targeted sites with abundant open space such as forest canopy gaps, forested trails with open corridors, or over open water where many bat species would be expected to forage (Ford et al., 2005). We set Anabats to record data continuously from approximately 1900 to 0700 h over our sampling sites, once in 2011 for each sampling array and 4 times each for the arrays in 2012 for a total of 5 survey events. We changed batteries and memory cards as needed and downloaded data to a laptop computer using the CFCread program (Titley Scientific, Ballina, Australia).

We analyzed call files using EchoClass (v1.1, U.S. Army Engineer Research and Development Center, Vicksburg, MS, USA). Although the ability to identify bat calls to the species level has been criticized (Barclay, 1999), research has suggested that good quality calls of eastern North American bats can be identified both qualitatively (O'Farrell et al., 1999) and quantitatively (Britzke et al., 2002; Britzke et al., 2011). To minimize the impact of species identification when accuracy is less than 100%, we considered species of bats to be present at a site (detector station) if the maximum likelihood *P*-value estimate for an individual species' identified call was ≥ 90%.

We created nightly presence-absence detection histories from the acoustic data for the 9 possible species at Fort Drum (Gorresen et al., 2008). We considered each nightly survey independent due to the separation of sites and break in sampling during daylight hours. Because double transect detectors may have recorded the same bats simultaneously, both Anabats at each site were considered a single unit for computing detection histories, that is, if a species was detected by either Anabat at a site on a given night it was considered to be present at that site. For each bat species, we attempted to fit a candidate set of 15 models to determine whether sampling habitat ("wet" = riparian zone or lake, wet meadow, or beaver swamp or "dry" = upland forest or field), year, or time of season (Table 1) impacted estimates of overall occupancy or detection (MacKenzie et al., 2002) using program PRESENCE (version 2.4, Hines and Mackenzie, 2008). We ranked models using Akaike's

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[1] Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

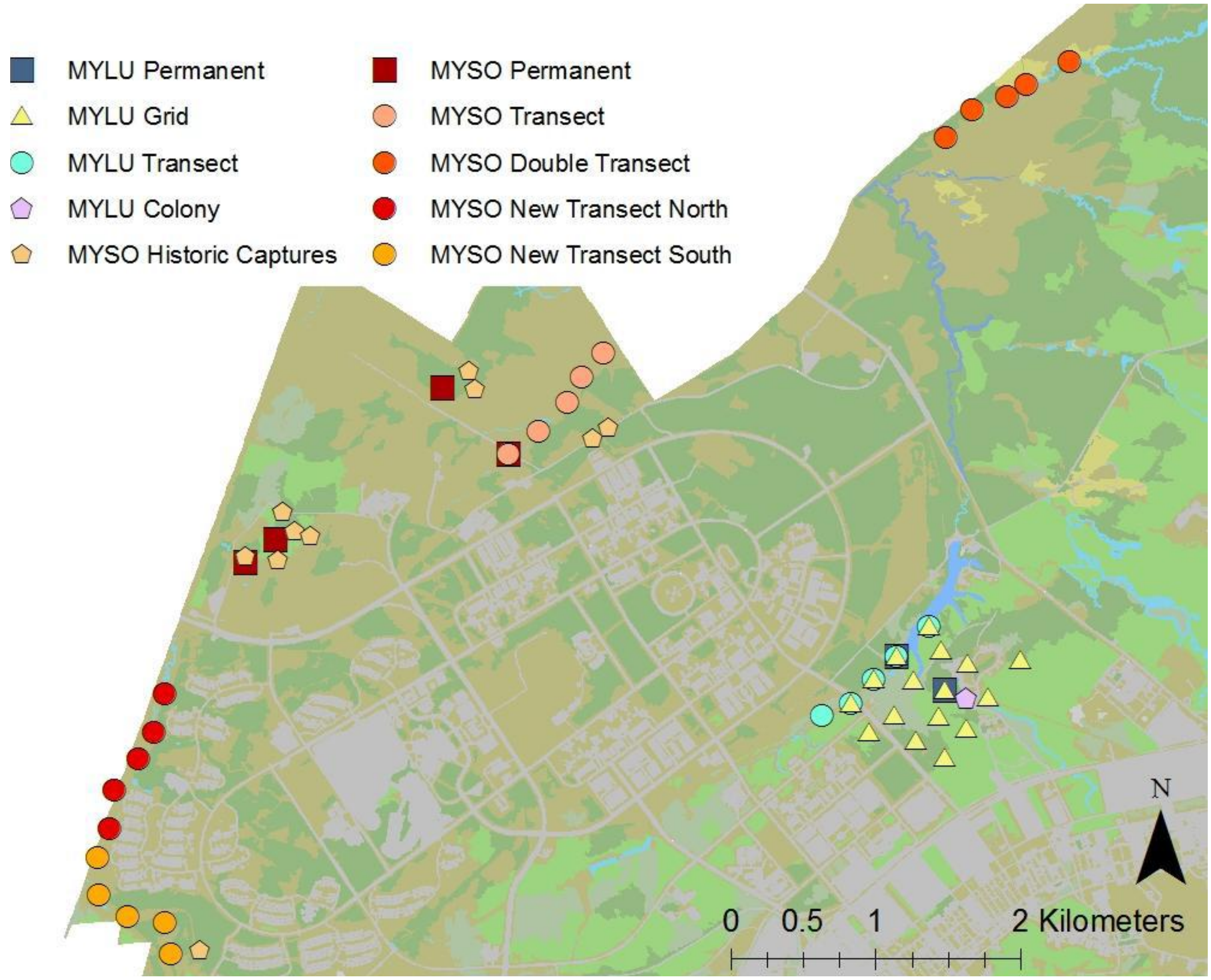

Figure 1. Passive acoustic sampling arrays in known maternity use areas of little brown bats (*Myotis lucifugus;* MYLU) and Indiana bats (*M. sodalis;* MYSO) at Fort Drum Military Installation Cantonment Area. Each point represents the site of a one passively deployed Anabat, except MYSO Double Transect which represents 2 Anabats per site. MYSO New Transect North and MYSO New Transect South deployed twice for 6-8 day sampling periods each in summer 2012. All other arrays sampled 4-5 times for 6-8 sampling periods in summers 2011 and 2012.

Information Criterion (AIC) corrected for small sample size and compared the weight of evidence among candidate models using Akaike weights (Burnham and Anderson, 2002). Following those analyses and retaining the significant covariates for the top approximating models, we collapsed detection histories so that each sampling array in its entirety was considered a single "site" for a given night. For example, if a species was detected at any of the 16 grid sites in a given night, it was considered present at that grid on that night. Because all sites in a particular sampling array were collapsed into a single representative site for the second set of models, we adjusted the covariate for habitat to a continuous variable representing the percent of wet sites in an array. We applied these covariates as a starting point for a single, additional model for each species to determine detection probability estimates among the varying sampling arrays. Included in the covariate set for sampling arrays were permanent, grid, transect and double transect sites. If the anticipated model failed to converge, we attempted to remove covariates from the previous candidate set in a step-wise fashion until the best possible model was reached to describe detection probabilities based on sampling array. Finally, for WNS-impacted species, we calculated the effort required (in sampling nights) to determine true absence based on detection probabilities derived from occupancy models following Wintle et al. (2012).

RESULTS

We detected 9 species that occur at Fort Drum at least once during the 2011 and 2012 monitoring seasons. Two transects, "MYSO New Transect North" and "MYSO New Transect South" were only deployed once each in 2012. We removed one permanent site placed near the installation's artificial bat house from detection histories for each species because of high sound distortion caused by multiple bats being detected simultaneously. We were able to fit candidate models for all species to determine impacts of habitat, year and time of season on occupancy

Table 1. Candidate set of models for assessing impacts of habitat, year, or time of season on bat occupancy (*Ψ*) and detection probability (*p*) estimates at Fort Drum Military Installation using passive acoustic sampling; "." = constant, "habitat" = wet vs. dry sites, "year" = 2011 vs. 2012, "season" = 15 May-15 July/pre-volancy of young vs. 15 July-15 Aug/post-volancy of young.

Model	*Ψ*	*p*
1	.	.
2	.	Habitat
3	.	Season
4	.	Year
5	.	Habitat + Season
6	.	Habitat + Year
7	.	Year + Season
8	.	Habitat + Season + Year
9	Habitat	Habitat
10	Season	Season
11	Year	Year
12	Habitat + Season	Habitat + Season
13	Habitat + Year	Habitat + Year
14	Season + Year	Season + Year
15	Habitat + Season + Year	Habitat + Season + Year

and detection probability estimates (Table 2).

Placement of detectors at wet or dry habitats had an effect on occupancy estimates for silver-haired (*Lasionycteris noctivagans*), big brown (*Eptesicus fuscus*), and hoary bats (*Lasiurus cinereus*), with higher occupancy rates at wet versus dry sites (Table 2). Habitat also had an effect on eastern eastern small-footed bat (*Myotis leibii*) occupancy, but the opposite trend was observed. The detection probabilities of all 9 species were greater at wet sites versus dry sites. Year had an effect on eastern red (*Lasiurus borealis*), hoary, little brown, Indiana, eastern small-footed and tri-colored bats (*Perimyotis subflavus*). Hoary and eastern small-footed bats showed slight increases in detection probabilities from 2011 to 2012 whereas the other species showed moderate to large declines from 2011 to 2012. Finally, time of season affected detection probabilities of eastern red, northern (*Myotis septentrionalis*), and eastern small-footed bats such that values increased in the late, post-volancy season when "new" bats are added to the landscape.

For array-specific detection histories, we created additional models to determine changes in detection probability based on a sampling array for 7 species (Table 3). We were unable to successfully model northern and eastern small-footed bats due to very low actual detections for these species. The percent-wet habitat covariate for the collapsed representative sites of permanent locations and the grid were 60 and 40%, respectively. Transects with single units and the double transect were all located at wet sites (100%).

Silver-haired bats were detected widely regardless of sampling array, year, or season. However, the detector grids produced the highest detection probability estimates (Figure 2). Eastern red bats were also detected widely regardless of sampling array. The highest detection probability estimates were recorded at the grid, and an increase in detection probability was observed from early to late season. Hoary bats detection probability estimates were highest at the grid relative to other sampling arrays, though overall species' detection probabilities were high regardless of array, year, or season. Big brown bats were detected at all sampling arrays, but showed the highest detection probability at the double transect regardless of year and season (Figure 2). Both little brown bats and Indiana bats were detected at higher levels at the grid relative to other array types. Finally, the tri-colored bat was detected at transects, the grid, and permanent stations in 2011 and 2012, however the species was not detected at the double transect in either year.

DISCUSSION

Even prior to the advent of WNS, occupancy modeling and detection probability estimates to assess bat species assemblages, relative activity, and habitat use with Anabat acoustic detectors had been gaining wider attention and use. Yates and Muzika (2006) reported an effect of year on Indiana bat detectability in the Ozark Mountain region of Missouri, pre-WNS. Similar to our within-year observations for eastern red bats, Hein et al. (2009) reported an increase in activity of eastern red bat over the sampling season, but also with little brown, and tri-colored bats in the South Carolina Coastal Plain, an area not been impacted by WNS at that time. Weller (2008) used

Table 2. Top (within 2.00 ∆AICc) models for assessing impacts of habitat, year, or time of season on bat occupancy (*Ψ*) and detection probability (*p*) estimates at Fort Drum Military Installation using passive acoustic sampling at 40 sites; "." = constant, "habitat" = wet vs. dry sites, "year" = 2011 vs. 2012, "season" = 15 May-15 July/pre-volancy of young vs. 15 July-15 Aug/post-volancy of young.

Models	***K***	***AICc***	***∆AICc***	***ωi***
Silver-haired				
Ψ (habitat), p (habitat)	4	1798.21	0.00	0.6480
Eastern red				
Ψ (.), p (habitat + year + season)	5	2342.49	0.00	0.4967
Ψ (.), p (habitat + season)	4	2343.13	0.64	0.3607
Hoary				
Ψ (habitat), p (habitat)	4	1990.24	0.00	0.3756
Ψ (habitat + year), p (habitat + year)	6	1990.48	0.24	0.3332
Big brown				
Ψ (habitat), p (habitat)	4	1961.30	0.00	0.7888
Little brown				
Ψ (.), p (habitat + year)	4	1028.32	0.00	0.6225
Ψ (.), p (habitat + year + season)	5	1029.83	1.51	0.2926
Indiana bat				
Ψ (.), p (habitat + year)	4	998.16	0.00	0.6853
Northern				
Ψ (.), p (season)	3	176.63	0.00	0.2318
Ψ (.), p (.)	2	177.26	0.63	0.1692
Ψ (.), p (habitat + season)	4	178.00	1.37	0.1169
Ψ (.), p (habitat)	3	178.57	1.94	0.0879
Eastern small-footed				
Ψ (.), p (year + season)	4	84.92	0.00	0.2717
Ψ (.), p (habitat + year + season)	5	86.03	1.11	0.1560
Ψ (habitat), p (habitat)	4	86.60	1.68	0.1173
Tri-colored				
Ψ (habitat + year), p (habitat + year)	6	191.16	0.00	0.3879
Ψ (.), p (habitat + year)	4	191.97	0.81	0.2587

occupancy modeling as a monitoring tool for assessing the effectiveness of a multiple-species conservation plan for bats in the Pacific Northwest. He found occupancy modeling that related species presence to habitat factors was most effective for common species but equivocal for rare species unless sampling effort is high. Those findings were consistent with ours for eastern small-footed and northern bats in that we were unable to model these species due to very low detection data.

Although previous research has focused on occupancy modeling for multiple bat species conservation and management, no prior studies have assessed differences in multiple species detectability at various passive acoustic sampling arrays in the context of WNS-associated declines. Overall, detection probabilities were the highest for most species at the grid of detectors, regardless of year or time of the season. Each array type was represented by a unique value for percent-wet.

Therefore, our simple characterization of habitat did not have an impact on the probability of detecting a species at a particular array type, although it may have influenced the probability of detecting particular species at one array

Table 3. Occupancy (*Ψ*) models for determining impacts of passive acoustic sampling arrays on the detection probability (*p*) estimates of bat species at 40 sites at Fort Drum Military Installation, summers 2011 and 2012; “.” = constant, “array” = permanent, grid, transect, double transect, “habitat” = wet vs. dry sites, “year” = 2011 vs. 2012, “season” = 15 May-15 July/pre-volancy of young vs. 15 July-15 Aug/post-volancy of young.

Bat species	**Model**
Silver-haired	*Ψ* (habitat), *p* (habitat + array)
Eastern red	*Ψ* (.), *p* (season + array)
Hoary	*Ψ* (.), *p* (array)
Big brown	*Ψ* (.), *p* (array)
Little brown	*Ψ* (.), *p* (habitat + year + array)
Indiana	*Ψ* (.), *p* (habitat + year + array)
Tri-colored	*Ψ* (habitat + year), *p* (year + array)

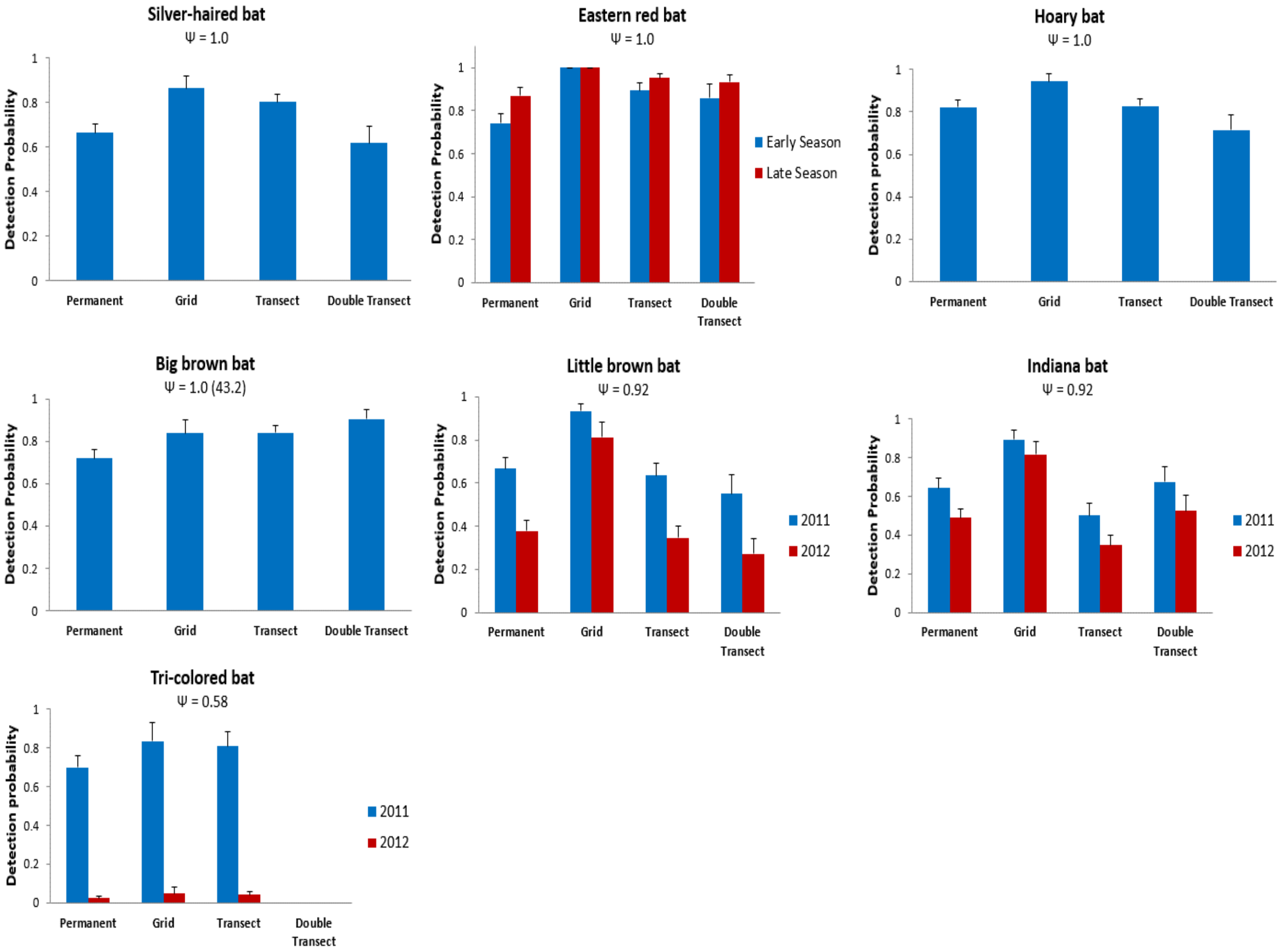

Figure 2. Detection probabilities of bats at various passive acoustic sampling arrays at Fort Drum Military Installation, summers 2011 and 2012. Time of season (early/late) and sampling year (2011/2012) included for important covariates affecting detection probability estimates from single species occupancy models; Ψ = proportion of sites occupied (naive estimate of occupancy).

type over another when included as a covariate. Indiana, little brown, and tri-colored bats were always detected at the highest probabilities at the grid of detectors despite overall declines from 2011 to 2012. However, species not impacted by WNS and the big brown bat were detected with high probabilities regardless of the sampling array, year, or time of season and should presently be expected to be detected under most sampling circumstances at Fort Drum.

Although the grid generally produced the highest detection probabilities, it is important to consider the varying levels of effort that were required for each array. Our grid placement required the highest number of available Anabat units relative to other arrays at 15 units, followed by the double transect requiring 10 units, and the permanent and transect sites that each required five units at a time. For many managers, limited fiscal resources may prohibit the use of large numbers of acoustical detectors simultaneously. In such cases, permanent stations or transects probably are the most feasible alternatives. However, it is important to note that MacKenzie and Royle (2005) suggested that the optimal strategy for detecting rare species is to focus effort at more sites at the expense of longer sampling duration at fewer sites. Relative to the endangered Hawaiian hoary bat, Gorresen et al. (2008) observed that reasonable precision of most parameter estimates of detection and occupancy were not achieved until 15 individual sites were sampled with acoustical detectors.

In our study, permanent sites did not detect little brown bats any better and only detected Indiana bats slightly better than did our transect sites. Permanent sites were never moved to different locations, whereas transect sites were placed repeatedly along four different streams at different time intervals. Of course, transect deployment and removal required much more effort than permanent stations, but batteries and storage cards did not need to be exchanged during recording cycles as occurred at permanent sites. More importantly, transects sampled a much greater area of the landscape. The flexibility in sampling a greater number of sites, with greater variation in habitat over a larger area in less time using transects probably makes this design the preferable method for reasonably high detectability for multiple species on most landscapes as compared to fixed units.

For focal monitoring of Indiana bats, benefits of long sampling durations and coverage over a wide area may both be necessary for optimally determining presence. As such, deploying detectors for greater than 6-8 days in transects along streams where the expectation of foraging use would be high probably is required. For example, based on the detection probabilities derived from our study, true absence of Indiana bats can be determined at the grid in 5-6 sampling nights or in 8-10 sampling nights using transects (Wintle et al., 2012). Similar patterns may be observed for determining probable absence of little brown bats. For tri-colored bats, the grid and the transect could both determine probable absence in 4-12 sampling nights relative to detectability of this species in 2011 and in 2012 using the grid, but a much longer sampling duration would be required based on detection estimates from transects in 2012 (Figure 3).

Although longer-duration transects may be necessary for detecting Indiana bats when equipment is limited, the double transect detected Indiana bats mostly as well as our grid design. Duchamp et al. (2006) suggested that having two detectors at a site increased the probability of detecting bat species in Indiana and Missouri, consistent with our findings for Indiana bats relative to other array types. However, for little brown bats, the double transect was no better than permanent stations or regular transects. This may indicate that although little brown bats are declining at Fort Drum (Ford et al., 2011), the spatial extent of their foraging may not be as limited or altered as other WNS-impacted species. For example, tri-colored bats were never detected at the double transect, despite easy detectability of this species at other arrays in 2011. However, even pre-WNS, tri-colored bats were scarcely observed in comparison to the once common little brown bat at Fort Drum, and it is uncertain whether they continue to occupy the landscape in stable numbers post-WNS.

As a species that was once found in colonies of hundreds to thousands of individuals in the summer across their range (Davis and Hitchcock, 1965) and at Fort Drum (Dobony et al, 2011), little brown bats are now infrequently observed in the Northeast, as many documented colonies have collapsed (Frick et al., 2010; Dzal et al., 2011; Turner et al., 2011). Indiana bats have also exhibited population declines regionally and locally as a result of WNS mortality (Ford et al., 2011; Ingersoll et al., 2013; USFWS, 2013) and were known to have a distribution restricted largely to the Cantonment area on the Fort Drum landscape even pre-WNS (Johnson et al., 2011a). Therefore, randomly selected locations are unlikely to be suitable for detecting these species at Fort Drum- and potentially elsewhere- when severe declines have been observed. Use of completely random sites may markedly increase survey effort required even over those shown here. Indiana bats and little brown bats were detected with high probabilities at all sampling arrays and at much higher detection rates than tri-colored, northern, and eastern small-footed bats, probably because efforts were focused on known historic maternity areas for these species in this study. On landscapes where historic capture information is not available, efforts can be focused at these species by focusing on areas with suitable habitat for these species or other target species.

Although the Indiana bat is the only species at Fort Drum that is presently subject to regulatory mandates of the Endangered Species Act (ESA 1973, as amended), northern bats have been proposed for endangered status by the USFWS (2013) and little brown bats are under review to assess their candidacy for federal listing as a result of WNS-associated declines (Kunz and Reichard, 2010). Furthermore, the tri-colored bat is believed to be one of the most severely impact species by WNS (Turner et al., 2011) and may also be subject to status reviews or listing proposals in the near future. With potential legal status changes probable, the ability to detect these bat

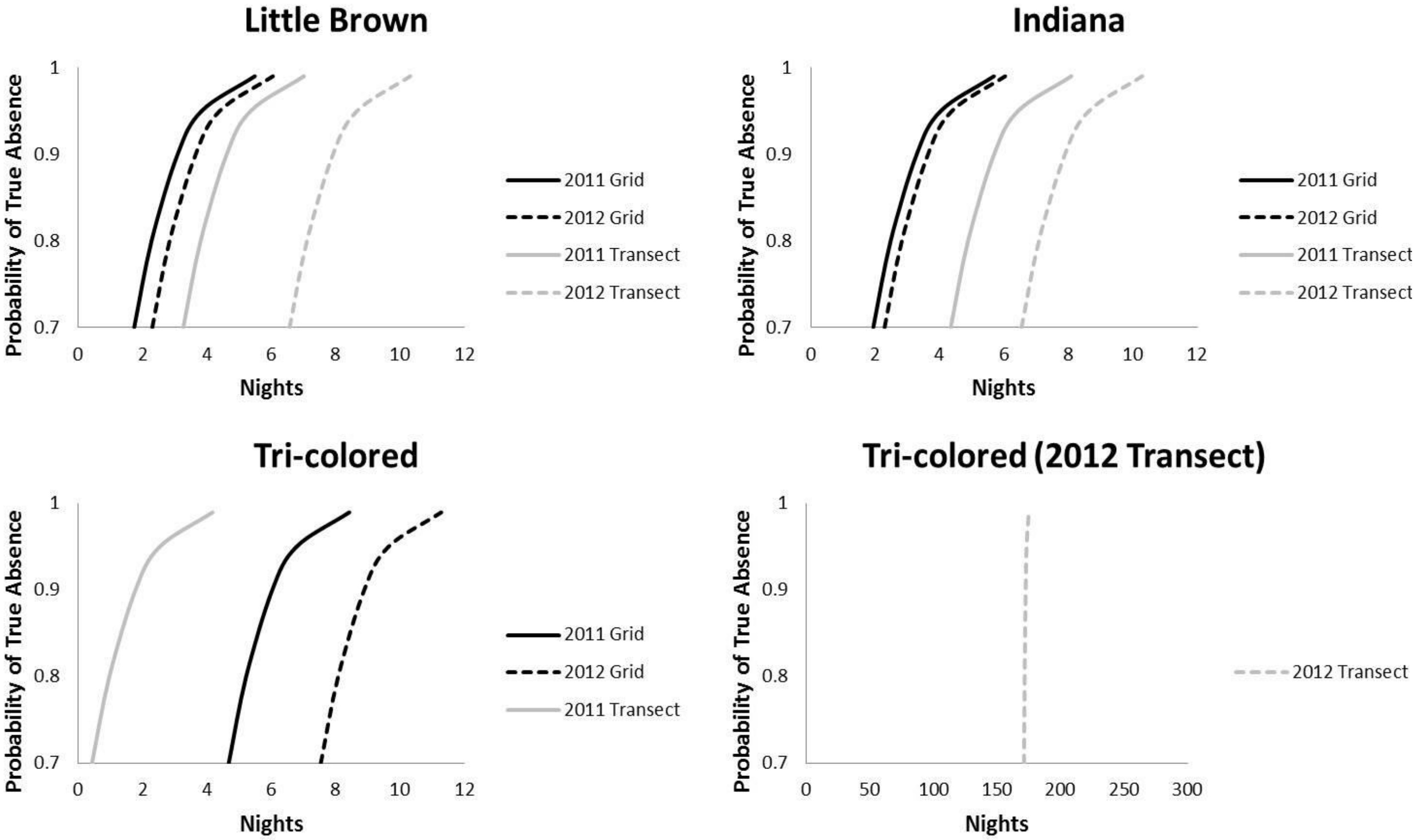

Figure 3. Suggested effort of passive acoustic sampling for determining true absence of Indiana (*M. sodalis*), little brown (*M. lucifugus*), and tri-colored (*Perimyotis subflavus*) bats using statistical probability according to the methods of Wintle et al. (2012). Detection probabilities derived from single species occupancy models at a grid of 15 Anabat detectors and four transects each of five Anabat detectors at Fort Drum Military Installation, New York, summers 2011 and 2012.

species when present will be critical from a regulatory perspective whereby managers can employ monitoring programs and mitigation activities to avoid or minimize potential take (ESA 1973, as amended). Following the suggestions of Weller (2008), future efforts to assess optimal sampling conditions for northern bats, eastern small-footed bats, and tri-colored bats are needed and perhaps should be focused on known historic areas of occupancy to determine if these species still occupy the Fort Drum landscape following WNS declines. For managers seeking an optimal strategy for determining presence or probable absence of Indiana bats or little brown bats, our study suggests that a grid of detectors in an expected area of use is most effective, possibly due to its inherent ability to survey a wider spatial area than the other methods presented here. However, in situations where high numbers of Anabats or other detectors are not available, the other sampling arrays presented here may be viable options for detecting the focal species in our study, that is, the Indiana and little brown bats, when placed in areas of anticipated use. However, deploying detectors across the widest area possible in areas of know previous use, expected use, or suitable habitat is likely more effective than deploying detectors for very long periods at permanent stations or in random locations. Nonetheless, determining the best sampling design for additional WNS-impacted species will warrant further investigation, particularly for those species that may obtain federal listing in the near future.

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