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An Acoustic Survey of Bats at Radford Army Ammunition Plant's New River Unit, Virginia

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ABSTRACT

White-nose Syndrome (WNS) has caused significant declines in cave bat populations in western Virginia. At the Radford Army Ammunition Plant, New River Unit (Pulaski County, Virginia) pre-WNS bat surveys were minimal and preliminary in nature. Therefore, we completed a large-scale acoustic survey to understand what species of bats occur at the site, and their relative activity. We deployed 12 acoustic detectors at 14 sites for up to 88 nights, May-August 2016. Two automated identification programs recognized 119,600 and 150,391 valid echolocation call files (Kaleidoscope v. 4.0 and EchoClass v. 3.1, respectively). Kaleidoscope identified 60% of bat calls as belonging to the Big Brown Bat (*Eptesicus fuscus*)/Silver-haired Bat (*Lasionycteris noctivagans*) group, 28% as Eastern Red Bat (*Lasiurus borealis*), 5% as *Myotis* species, 3% as Tricolored Bat (*Perimyotis subflavus*), 2% as Hoary Bat (*Lasiurus cinereus*), and 2% as unidentified. EchoClass identified Eastern Red Bat (23%), Big Brown Bat/Silver-haired Bat (21%), Hoary Bat (2%), *Myotis* species (0.2%), and Tricolored Bat (0.3%). Unidentified bat calls represented 53% of call files. We also investigated false-positive identifications of rare species (*Myotis* spp. and Tricolored Bats) in these automated identification programs using manual verification. Calls auto-identified as *Myotis* spp. more often keyed out to Eastern Red Bat, but Tricolored Bat calls appeared to be accurately identified. The apparent misidentification by both programs emphasizes the continued need for visual (manual) confirmation of any *Myotis* spp. calls, coupled with netting efforts at suspect *Myotis* spp. sites. We find sparse evidence of *Myotis* spp. and convincing evidence of Tricolored Bats as a continued presence but in low numbers at the ammunition plant.

Key words: automated identification, EchoClass, false positive, Kaleidoscope, *Myotis*, *Perimyotis*.

INTRODUCTION

White-nose Syndrome (WNS) is a fungal disease that has caused extreme declines in cave-hibernating bat populations in the eastern United States since its discovery in New York in 2006. The current estimate of bat deaths due to the fungus, *Pseudogymnoascus destructans,* exceeds 6 million and it has been confirmed in 31 states (USFWS, 2017). In Virginia, precipitous declines in cave bat populations have been documented since WNS was first detected in the state in 2009 (Powers et al., 2015). For example, Reynolds et al. (2016) reported that summer captures of Northern Long-eared Bats (*Myotis septentrionalis*) in western Virginia declined by 95.1% in 2013 as compared to pre-WNS capture rates. Furthermore, the juvenile capture rate decreased from 40% pre-WNS to <10% in 2013 (Reynolds et al., 2016). Counts from hibernacula conducted in western Virginia in 2013 showed declines of 99.0% for Little Brown Bats (*Myotis lucifugus*), 89.5% for Tricolored Bats (*Perimyotis subflavus*), and 33.5% for Indiana Bats (*Myotis sodalis*) compared to pre-WNS counts (Powers et al., 2015). The persistence of *P. destructans* in Virginia hibernacula suggests

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surveys of summer bat activity will be needed to monitor changes in bat assemblages and improve future management of rare species.

The Radford Army Ammunition Plant, New River Unit (Pulaski County, Virginia) is a military installation in southwestern Virginia that lacked data describing the bat community before WNS. Two nights of preliminary mist net surveys in summer 2012 at the ammunition plant confirmed the presence of Big Brown Bats (*Eptesicus fuscus*), Eastern Red Bats (*Lasiurus borealis*), and Tricolored Bats (Appendix 1). Concurrent acoustic surveys across eight sites additionally identified calls as Hoary Bats (*Lasiurus cinereus*), Silver-haired Bats (*Lasionycteris noctivagans*), and Northern Long-eared Bats (Appendix 1). Following these limited survey efforts, we launched a large-scale acoustic survey of the property in summer 2016. This included the deployment of acoustic detection units across a mosaic of habitat types.

In an environment where cave bat populations have dramatically declined, acoustic techniques are most often used as a "first pass" to detect potential netting sites to confirm (or fail to confirm) the presence of *Myotis* spp. (hereafter, "myotids"; USFWS, 2016a). Although netting is the ideal choice for positively confirming species' presence, short-term netting efforts may not capture all species and therefore falsely suggest absence for some (O'Farrell & Gannon, 1999). Alternately, passive acoustic detectors placed at multiple sites for longer periods can provide increased detection probabilities in areas where WNS has caused declines (Coleman et al., 2014). Ultimately, we chose to first employ acoustic monitoring because our primary goal was to survey for all species of bats, especially rare species, at this military installation.

Following federal surveying guidelines for Indiana Bats (USFWS, 2016a), we utilized two acoustic auto-identification programs, Kaleidoscope v. 4.0 (Wildlife Acoustics, Inc., Concord, MA) and EchoClass v. 3.1 (ERDC, Vicksburg, MS), to assess presence of rare species. *A priori*, we understood that EchoClass is more conservative in its identifications than Kaleidoscope, with the former typically determining more bats calls as unidentifiable. This is likely a combination of pre-set choices made for each program, and acknowledged, inherent variation in call identification between programs (USFWS, 2016b).

As a secondary goal, we compared each acoustic auto-identification program's rate of detection for regionally rare species: all members of the genus *Myotis* and Tricolored Bats. We manually verified all *Myotis* spp. and Tricolored Bat identifications and compared the number of suspected false positives of each program for these calls. There is a long-standing argument about the merits of manual verification versus automated identification software (e.g., varying opinions about call parameters of each species, intraspecific geographic differences in call parameters, observer bias [Betts, 1998]). Multiple researchers suggest that automated programs have particularly limited reliability for identifying rare species and species that have similar calls (Russo & Vaught, 2016; USFWS, 2016b). Therefore, we chose not to rely entirely on automated programs for identification and relative activity calculations of rare species.

MATERIALS AND METHODS

Site Description

The Radford Army Ammunition Plant's New River Unit encompasses 1,142 ha in Pulaski County, Virginia (37°6′6′′N, 80°39′11′′W; datum NAD83). The primary function of this government-owned facility is the storage of military-grade ammunition. A mosaic of habitat types is represented at this site, including grasslands (some fire-maintained, others mowed or established as wildlife food plots), mature forest stands (deciduous, coniferous, mixed), waterbodies (man-made ponds, vernal pools, first-order streams), and buildings (mainly ammunition storage magazines that are partially earth-covered). We selected sites that could attract foraging bats and therefore focused efforts on all bodies of water (permanent or vernal, lentic or lotic; e.g., Grindal et al., 1999; Zimmerman & Glanz, 2000; Francl, 2008), which comprised five sites. We then added a sampling of closed-canopied/late successional (five sites) and open-canopied/early successional habitats (four sites; Table 1).

Field Methods

From 9 May to 5 August 2016 (88 survey nights), we surveyed 14 sites using SMZC and SM4BAT ZC Song Meters (Wildlife Acoustics, Maynard, MA). We spaced sites at least 250 m apart to ensure that individual bats were not sampled on two detectors simultaneously (Ford et al., 2005). Acoustic devices recorded continuously from 1800 h to 0600 h with SMZC units using internal, omnidirectional microphones and SM4BAT ZC units requiring external, directional microphones. We secured detectors mainly on trees or stand-alone PVC poles ca. 2-3 m high to prevent attraction by other wildlife that could disturb equipment. We monitored Song Meter recorders for proper functionality approximately every 2.5 weeks when we replaced batteries. To maximize habitats surveyed and detection of species across the survey, we

Table 1. Fourteen sites acoustically surveyed for bats at the Radford Army Ammunition Plant, New River Unit, Pulaski County, Virginia in summer 2016. Presented are habitat description, grouped habitat types (ES = early successional/open canopied grassland, LS = late successional/closed canopied forest; W = water) and the number of detector nights each site was acoustically surveyed (1 detector for 1 night = 1 detector night). GPS coordinates are available upon request.

Site Name	Habitat Description	Habitat	Detector Nights
Bagging Plant	Concrete building, overlooking open grassland	ES	66
Coyote Creek	First-order stream, open canopy	W	46
Dirt Road	Primitive gravel road through closed-canopied mature pines	LS	46
EPFU Alley	Paved road lined with mature mixed forest	LS	88
Fishing Pond	0.2-ha pond	W	66
Food Plot	VA. Dept. of Game & Inland Fisheries food plot	ES	23
Ginseng Hollow	Mowed grassy plot surrounded by mature forest	LS	88
Hibernaculum	Grass-covered storage building	ES	66
Ignitor	Concrete wall structures. scattered mature pines	LS	35
Pond 2	0.2-ha pond	W	88
Pwamp	0.8-ha pond/swamp	W	88
Road S of Hazel Hollow	Open, shrubby grassland	ES	66
Vernal Pool	Vernal pool/grassland surrounded by mature deciduous forest	W	88
White Pines	Primitive gravel road through mature pines	LS	18

moved individual detectors that had recorded negligible bat activity in the previous 2.5-week monitoring period; for this reason, we sampled sites for 18-88 detector nights (1 detector set for 1 night = 1 detector night) per site.

Acoustic Analysis

We initially employed the automated programs Kaleidoscope and EchoClass to identify bat call files. For Kaleidoscope, we set parameters to identify calls from 16-120 kHz, with a minimum number of three call pulses, and selected nine known species in the region from Kaleidoscope's classifier version 3.1.0 (Big Brown Bat, Silver-haired Bat, Eastern Red Bat, Hoary Bat, Indiana Bat, Northern Long-eared Bat, Little Brown Bat, Eastern Small-footed Bat [*Myotis leibii*], and Tricolored Bat). Sensitivity was set at a more liberal level (-1), following a similar regional study by Austin (2017). In EchoClass, we selected species Set 2, which included the same species selected in Kaleidoscope. Sensitivity levels are predetermined in this program. In AnalookW software version 4.1t (Corben, 2015), using a standard noise filter (Clement et al., 2014), H. Custer manually vetted all myotid and Tricolored Bat calls detected by both programs, searching for false positives. Further analysis of all calls recorded from two randomly-selected sites

(Fishing Pond and Hibernaculum [Table 1]) were manually vetted by K. Powers to seek out additional myotids and Tricolored Bat species (false negatives). We used dichotomous keys (M. A. Menzel, S. Owen, and J. B. Johnson, West Virginia University, unpubl. data) and classification trees (A. Silvis, Virginia Tech, unpubl. data) to detect calls that were, with author certainty, false positives or false negatives. Furthermore, due to a recognized overlap in call signatures, we grouped Big Brown Bat and Silver-haired Bat identifications into one species group in our results (Betts, 1998; Austin, 2017). After re-assigning false positives and false negatives to what we considered to be the correct species identification, we calculated each program's identification accuracy for myotids and Tricolored Bats.

RESULTS

Both automated identification programs categorized at least some calls as belonging to each of the nine bat species we had selected as *a priori* possibilities. The 872 detector nights included 376 at sites with water, 275 at late-successional sites, and 221 at early-successional sites. Across all detector-nights, Kaleidoscope recognized 119,600 bat call files whereas EchoClass recognized 150,391 (Table 2). Both programs determined that Big Brown Bat/Silver-haired Bat and Eastern Red Bat were most commonly detected (Table 2), together comprising 44% (EchoClass) to 88% (Kaleidoscope) of all calls. If unidentifiable bat calls were removed, the combined percentages of these three species (90.0% for Kaleidoscope, 94.4% for EchoClass) were comparable.

We discovered a stark contrast in *Myotis* spp. calls identified by the two programs. Kaleidoscope identified 5,975 call files as *Myotis* (5% of all identified bat files), but our manual vetting suggested that >99% of these were Eastern Red Bat calls. EchoClass was much more conservative in its *Myotis* identifications, with only 367 call files, but 89.1% of these were likely Eastern Red Bat calls (Table 2). After manual verification, *Myotis* spp. calls comprised <0.1% of all calls for both programs, and calls were not concentrated in any particular habitat type on the property. Although not presented in Table 2, more than one-third of identified

Table 2. Number of call files per species, as identified from acoustic surveys of bats at Radford Army Ammunition Plant, New River Unit, Pulaski County, Virginia in summer 2016. Presented are species detected by the automated identification programs Kaleidoscope and EchoClass ("Original"), and results after manual vetting of *Perimyotis subflavus* (Tricolored Bat) and *Myotis* species ("Modified") with relative proportion of calls by species (percentage, in parentheses). Calls listed as "No ID" were recognized as bat calls but were further unidentifiable by the automated programs or manual vetting. Reductions in the number of Total, Modified calls compared to Total, Original calls are due to additional "noise" files being erroneously identified as bat calls.

	Kaleidoscope		EchoClass	
Species	Original	Modified	Original	Modified
E. fuscus/L. noctivagans	72,072 (60%)	72,097 (61%)	31,718 (21%)	28,845 (20%)
Lasiurus borealis	33,892 (28%)	40,510 (34%)	35,086 (23%)	35,419 (24%)
Lasiurus cinereus	2,546 (2%)	2,547 (2%)	3,083 (2%)	3,085 (2%)
Myotis spp.	5,975 (5%)	45 (<0.1%)	367 (0.2%)	40 (<0.1%)
Perimyotis subflavus	3,282 (3%)	1,992 (2%)	487 (0.3%)	443 (0.3%)
"No ID"	1,833 (2%)	1,961 (2%)	79,650 (53%)	79,681 (54%)
Total	119,600	119,152	150,391	147,513

Myotis calls keyed out to that of *M. leibii*. After manual vetting, we recognized myotids at eight of the 14 sites, and the total number of calls was <0.05 call sequences per detector night for any *Myotis* species across the entire survey period.

We classified call sequences as Tricolored Bats at 13 of the 14 survey sites, with generally higher call volume at permanent open-water sites (primarily at Fishing Pond, Table 1). After manual vetting, total number of Tricolored Bat calls were 0.5 and 3.7 call sequences per detector night for EchoClass and Kaleidoscope, respectively. Kaleidoscope appeared to correctly categorize 60% of Tricolored Bat calls, and EchoClass correctly classified 91% of these calls. Calls misidentified as Tricolored Bat better fit the parameters of Eastern Red Bat.

Our searches for false negatives did not reveal extreme cases of misidentification. For example, manual vetting of >70,000 calls from one open water site (Fishing Pond) detected 15 additional calls of *M. leibii*. However, because these 15 calls had been correctly identified to the genus *Myotis* by both programs, they did not alter our grouped myotid trends (Table 2). No additional Tricolored Bat calls were recognized in an examination of these calls.

DISCUSSION

The bat species that had been previously confirmed via mist-netting and detected through acoustic techniques in 2012 were all identified in our 2016 acoustic surveys (Appendix 1). Not surprisingly, Big Brown Bat/Silver-haired Bat and Eastern Red Bat were the most commonly-detected species. Although both automated identification programs recognized unusually high detections of Silver-haired Bat calls (which we grouped with Big Brown Bat), this species typically is caught only in May when it migrates through Virginia (Cryan, 2003; Powers, pers. obs.). It is likely that nearly all autoclassified Silver-haired Bat calls are actually those of Big Brown Bats (Betts, 1998).

The presumed near-absence of Little Brown Bats, Northern Long-eared Bats, and Indiana Bats was expected, given their federal or state listings and documented rarity on the landscape in western Virginia (Powers et al., 2015; Reynolds et al., 2016; Austin, 2017). The presumed presence of Eastern Small-footed Bats is not entirely surprising; we suspect that they use cliff faces and other vertical exposures along the New River (ca. 4.5 km E of the ammunition plant) as day roosts, and may forage at the study site. Netting efforts would be required to confirm their presence.

Despite state-listed Tricolored Bats being nearly ubiquitous across our 14 sites, the number of call sequences recorded at any given site was relatively low. Based on 2012 captures at Fishing Pond (Appendix 1), coupled with manual vetting of several hundred Tricolored Bat calls at this site in 2016, we confirm their continued presence with some confidence. The Radford Army Ammunition Plant has several characteristics that may explain why Tricolored Bats may be present and more abundant than myotids on the property. Forest successional stages vary widely across the property, and all stages are within 1 km of at least one of the open water sites on the property, providing the bats' preferred foraging habitat (Center for Biological Diversity and Defenders of Wildlife, 2016). Given that Tricolored Bat roost selection varies by female reproductive stage (Veilleux et al., 2004), the variety of open- and closed-canopied habitats may be an attractive feature of the property. We suggest further netting efforts to confirm the continued presence of Tricolored Bats and identify habitat features at the ammunition site that are relatively important for this species.

Our acoustic survey results mirror those of other regional acoustic surveys conducted after the onset of WNS. Austin (2017) also surveyed bats in the central Appalachian highlands in summer 2015 and 2016, and found a similar species composition, and proportionally similar dominant species. Small-bodied species like Tricolored Bats and myotids also were poorly represented on the landscape. Although published acoustic surveys in Virginia prior to the onset of WNS are scarce, other regional surveys around the advent of WNS noted declines in particular species of *Myotis* (Dzal et al., 2011; Reynolds et al., 2016) and in Tricolored Bats (Dzal et al., 2011) during summer months.

Although our acoustic survey was intended as a first step in assessing the structure of this WNS-affected environment, the frequent misidentifications made by automated programs could have negatively affected management of bat populations (Russo & Vaught, 2016). In this case, the over-identification of *Myotis* spp. at the ammunition plant might have led to resource allocations that could or should have been used for other rare animal or plant species with a confirmed presence (Ford, 2014). Identification of acoustic calls will always have some degree of subjectivity. The overlap in call characteristics among bat species and the influence of habitat clutter on calls during foraging activities has been well documented (e.g., Wund, 2006). Therefore, we support continued manual vetting of all *Myotis* calls (USFWS, 2016b).

A management goal of the Radford Army Ammunition Plant is to monitor rare species; our acoustic surveys provide a springboard for future combined acoustics and netting projects. Surveys should focus on netting locations with the highest detection rates for Tricolored Bat (particularly Fishing Pond) and *Myotis* spp. to confirm their (continued) presence on the military installation. If netting and subsequent radiotelemetry efforts successfully document Tricolored Bat or *Myotis* spp. maternity roosts or bachelor colonies on the property, these data would contribute to the Best Management Practices already in place on the property.

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Appendix 1. Number of call files per species, as identified from acoustic surveys of bats at Radford Army Ammunition Plant, New River Unit, Pulaski County, Virginia in summer 2012. Call results assessed by EchoClass v. 3.1, and calls identified as *Myotis* spp. or *Perimyotis subflavus* were manually vetted by K. Powers. After vetting all *Myotis* spp. calls, only *M. septentrionalis* remained. Calls listed as "No ID" were recognized as bat calls but were further unidentifiable by the automated programs or manual vetting. Four sites with (*) were included in 2016 project; remaining four sites were open-canopied sites not selected for continued surveys. GPS coordinates are available upon request. Superscript ([1]) indicates individuals of this species were captured in concurrent mistnetting efforts.

Species	Bagging Plant*	EPFU Alley*	Fishing Pond*	Pwamp*	Maga-zine 1521	Maga-zine 1523	Maga-zine 1618	Pole Barn	TOTAL
E. fuscus[1]/*L. noctivagans*	2	23	146	49		1	5		226
Lasiurus borealis[1]	2	18	58	31		1	7		117
Lasiurus cinereus		1		11		2		1	15
Myotis septentrionalis							1		1
Perimyotis subflavus[1]			41	1					42
"No ID"	4	40	108	139	1	8	22	1	323