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Optimizing surveys for imperiled bat species post white-nose syndrome

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Many bat populations have plummeted in North America since the introduction of white-nose syndrome (WNS) in 2006, presenting challenges in researching population health and habitat use of affected species. Traditional survey techniques such as mist-netting and radio-telemetry have become increasingly time-consuming post-WNS due to the rarity of WNS-susceptible species in affected areas. To address the difficulty of studying imperiled bat species, we explored the use of an acoustic lure alongside mist-netting and acoustic monitoring to potentially enhance species detection in southeastern Missouri, United States. Acoustic lures elicit varying intra- and interspecific behavioral responses; thus, we explored the effects on multiple bat species' encounter rates while using tricolored bat *Perimyotis subflavus* distress vocalizations. The acoustic lure increased the number of acoustic detections for the federally endangered Indiana bat *Myotis sodalis* and non-imperiled big brown bat *Eptesicus fuscus*, and increased captures of the silver-haired bat *Lasionycteris noctivagans*, a species of conservation concern in Missouri. Additionally, only two individuals of *P. subflavus* were captured in mist-nets, and both were captured with the acoustic lure. These results suggested that acoustic lures are a valuable tool for identifying the presence of imperiled species when used in combination with mist-netting and acoustic detectors. Researchers studying bat communities that have experienced significant population declines because of WNS or other factors should incorporate multiple survey techniques, including acoustic lures, to increase capture rates and better document species distributions. Doing so should provide more accurate information on the distribution of imperiled bat species, which is relevant to conservation and management.

Key words: bioacoustics, imperiled bats, *Perimyotis subflavus*, acoustic lure, survey methods

Introduction

White-nose syndrome (WNS), wind energy, habitat loss, and human disturbance in caves during critical life events (i.e., hibernation, breeding) are among the many threats facing North American bat species (Schroder *et al.*, 2017; Smallwood *et al.*, 2020). White-nose syndrome is arguably the greatest threat facing bats, as hibernaculum surveys demonstrate this disease has dramatically reduced populations in Eastern and Midwestern North America (Cheng *et al.*, 2021). Within Missouri, United States, eight bat species are listed as state-level species of concern, and four are federally protected through inclusion in the United States Fish and Wildlife Service (USFWS) Endangered Species List (Missouri Heritage Program, 2021; USFWS, 2021). The tricolored bat *Perimyotis subflavus* has been particularly affected by WNS (Ford *et al.*, 2011; Ingersoll *et al.*, 2016; O'Keefe *et al.*, 2019; Cheng *et al.*, 2021). Since the introduction of WNS to Missouri in 2012, the *P. subflavus* population experienced a 53.2% decline by 2017, according to a statewide winter hibernacula survey (Colatskie, 2017). In response to this decline, the USFWS proposed to list *P. subflavus* as an Endangered species under the Endangered Species Act of 1973 (USFWS 2022). Population declines post-WNS make it increasingly challenging to radio-track *P. subflavus* and study this imperiled species. Consequently, limited capture success equates to less research on the roosting ecology and overall status of surviving populations.

Standard methods to assess bat populations include radio-tracking, mist-netting, and acoustic surveys. Information on roosting ecology obtained through radio-tracking individuals aids biologists in developing management practices that support the recovery of bat populations impacted by WNS. However, radio-tracking is labor intensive and requires capture methods, such as mist-netting, to tag individuals. Incorporating acoustic detectors during mist-net surveys helps detect individuals not captured in mist-nets (O'Farrell and Gannon, 1999; Flaquer *et al.*, 2007; Francl *et al.*, 2012). Furthermore, acoustic survey results can be used to assess bat activity, habitat usage, and species richness of the bat community (Hayes, 2000; Britzke *et al.*, 2013). However, acoustic monitoring presents challenges in data interpretation, such as estimating population size, since there is no method to determine if recorded bat passes represent one or multiple individuals. Because each method for studying bats has its strengths and weaknesses, combining multiple techniques within a single study may be necessary to increase effectiveness (e.g., Hill *et al.*, 2014*a*; Lintott *et al.*, 2014; Braun De Torrez *et al.*, 2017).

Acoustic lures represent an underutilized tool that can increase captures of imperiled bat species (e.g., Hill and Greenaway, 2005; Goiti *et al.*, 2007; Hill *et al.*, 2014*a*; Braun De Torrez *et al.*, 2017; Preble *et al*., 2021). Acoustic lures for bats function similarly to those used to attract birds (Conway and Gibbs, 2005), except that bat lure speakers broadcast at ultrasonic frequencies. However, given their relative novelty, many unknowns remain concerning the use of acoustic lures in studying imperiled bats. For instance, much of the research to date has focused on whether acoustic lures increase mist-netting capture success (e.g., Quackenbush *et al.*, 2016; Braun De Torrez *et al.*, 2017), while fewer studies have examined the impact of lures on acoustic detections (e.g., Russ *et al.*, 2004; Hill and Greenaway, 2005; Loeb and Britzke, 2010). Additionally, some research suggests that certain broadcasted vocalizations could alter bat behavior (Russ *et al.*, 2004; 2005), thus careful consideration is required when selecting ultrasonic lure calls (i.e., species-specific broadcasts, social foraging calls or distress calls). For example, Rafinesque's big-eared bat *Corynorhinus rafinesquii* was not attracted to broadcasted intraspecific social calls (Loeb and Britzke, 2010), while Bechstein's bat *Myotis bechsteinii* and Natterer's bat *M. nattereri* were (Schöner *et al.*, 2010). The literature describing the effects of an acoustic lure is dominated by studies that utilized a non-distress social call (e.g., Hill and Greenaway, 2005; Braun de Torrez *et al.*, 2017). Even so, we hypothesized that distress calls may also increase the capture and detection of bats. Similar to birds, distress calls may be an adaptive alarm signal to provoke a mobbing or inspection behavior near the broadcasted call (Bradbury and Vehrencamp, 1998; Russ *et al.*, 2005; Carter *et al.*, 2015), increasing a target species' potential capture and detection rates. Lastly, more information is needed to determine how certain species, especially those of conservation concern such as *P. subflavus*, respond to acoustic lures. As such, more research is needed to address knowledge gaps related to the influence of acoustic lures on bat behavior, detections, and captures in a post-WNS world.

We evaluated whether an acoustic lure influenced mist-netting captures and acoustic detection of imperiled bat species. Specifically, we focused on populations of WNS-impacted species, particularly *P. subflavus*, in the Ozarks region of southeastern Missouri, United States. At the time of our surveys, *P. subflavus* had experienced slightly lower levels of decline compared to other WNS-impacted species (e.g., northern long-eared bat *M. septentrionalis* and little brown bat *M. lucifugus*; Colatskie, 2017). Therefore, we reasoned that *P. subflavus* would serve as an ideal imperiled study species since it was not yet too rare on the landscape to effectively study. Secondly, our study assessed the lure's effect on the capture success and acoustic activity of other bat species occurring within the study area. We predicted that the lure would increase both mist-net captures and acoustic detections of *P. subflavus*, and potentially other imperiled bat species.

Materials and Methods

Our study was conducted in the state of Missouri, United States, and encompassed nine Missouri Department of Conservation (MDC) conservation areas in the southeastern counties of Shannon, Carter, and Reynolds (Fig. 1). The landscape was characterized by dense mixed oak (*Quercus* spp.), hickory (*Carya* spp.), and pine (*Pinus* spp.) forests, extensive river systems, and hilly terrain (Steyermark and Yatskievych, 2006). Study sites included forested streams and ponds within MDC conservation areas. We aimed to select a minimum of two study sites for mist-net and acoustic surveys at each conservation area; however, only one site was used for either mist-netting or acoustic surveys at three of our smaller conservation areas which had limited waterbodies for sampling. Study sites were at least one mile (1.6 km) apart.

We collected data during the bat maternity season of May–August across three study years (2018–2020), although we did not employ the acoustic lure during 2018. Surveys involving the capture of bats with mist-nets were suspended in 2020 due to the SARS-CoV-2 (COVID-19) pandemic to avoid the risk of infecting bats (MDC, 2020), thus during the final study year we only surveyed bats with acoustic detectors. Mist-netting and acoustic detector surveys were conducted on different nights, with or without the acoustic lure (2019 for both surveys, 2020 for acoustic surveys only), separated by at least one week. Capture

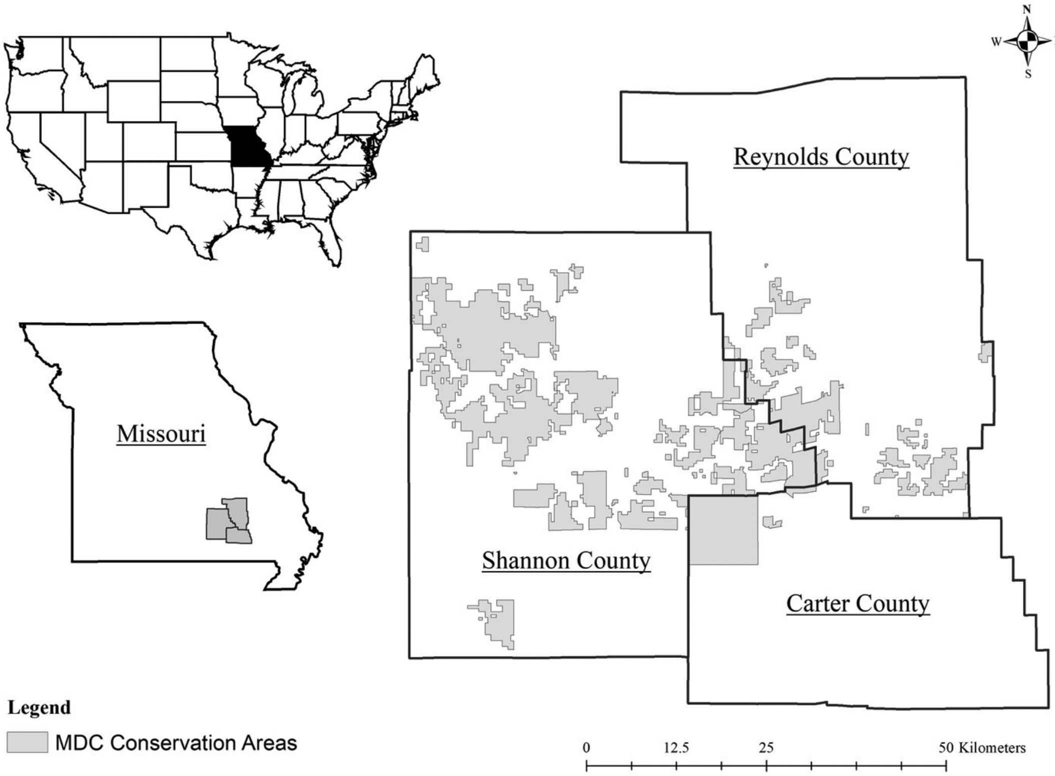

FIG. 1. Map of Missouri Department of Conservation (MDC) Conservation Areas included in the study

and acoustic data collected in 2018 served as a preliminary field season to document the limited detections of imperiled species and justify the use of the acoustic lure.

Mist-nets were deployed over waterbodies (i.e., ponds, ephemeral and permanent streams) across our study area. In our preliminary mist-net survey in 2018, we surveyed 13 unique locations, visiting each site between two to three times. To test the effects of the acoustic lure on mist-net captures in 2019, we visited 12 mist-net locations two times, with one visit utilizing the lure and one visit without the lure. The survey effort across 2018 and 2019 included 47 mist-net nights (23 in 2018 and 24 in 2019). Mist-net surveys were standardized to last for five hours, beginning at sunset (USFWS, 2019). We positioned mist-nets (38 mm polyester mesh nets ranging in size from 4, 6, 9, 12, to 18 m in length by 2.6 m to 7.3 m in height; Avinet Research Supplies, Maine, United States) across and adjacent to waterbodies, resulting in the optimal mist-net coverage for each site. We attempted to capture as many imperiled species as possible; thus, we utilized multiple mist nets at a site. To encompass the majority of the waterbody at which we were sampling, we used at least one triple high net (7.3 m tall net) and a combination of two to three other smaller nets. The same number and size of mist nets were used in each site's subsequent visit to standardize mist-netting effort at each site. We followed methods described by Kunz and Parsons (2009), such as separating mist-net attempts at a study site by at least a week to minimize the effect of net avoidance. We followed procedures and recommendations for wildlife handling under the most current version of the 'USFWS Range-wide Indiana Bat Survey Protocol' (USFWS, 2019) and through the MDC's Institutional Animal Care and Use Committee. Additionally, we adhered to the most recent WNS decontamination protocol procedures to prevent the spread of WNS (WNS Decontamination Team, 2018). Finally, we only utilized mist-nets for capturing bats as our survey was limited to locations with water, making the use of harp traps unsuitable for sampling. Harp traps are only used at sites where we expect a large amount of bat activity, such as cave entrances. In the United States, summer surveys are completed with mist-nets unless there is special permission for harp trapping at locations, such as cave entrances, narrow corridors, and near known maternity trees. Use of harp traps must be approved as a separate activity on state and federal permits, which we did not obtain due to the scope of our project and our survey locations.

We used SM2BAT+ bat detectors (Wildlife Acoustics, Massachusetts, United States) to monitor bat activity at each site (n = 40). We deployed acoustic detectors for up to three nights in 2018 and for two nights per site in 2019–2020. We initially used SMX-US microphones (Wildlife Acoustics) in 2018, and then upgraded to the SMM-U1 microphones (Wildlife Acoustics) for 2019 and 2020. We verified the microphone's sensitivity and functionality with the manufacture's ultrasonic calibrator before deploying detectors. We deployed detectors ≥ 3 m from the water's edge and vegetation to minimize acoustic interference from vegetative clutter (Reichert *et al.*, 2018). We standardized our acoustic detection surveys to record bat activity for five hours, starting at sunset. Recorded calls were identified to species using an automatic classifier in Kaleidoscope Pro v.5.1.9 (Wildlife Acoustics, Massachusetts, United States; Szewczak *et al.*, 2017). We manually reviewed calls to verify the automatic species identifications. We used reference libraries and a key, Sonobat Echolocation Call Characteristics of Eastern U.S. Bats v.4x, during the manual vetting process. We defined a 'bat pass' as three or more echolocation pulses within the search phase that originated from a single species passing by the detector (Britzke *et al.*, 2013; Loeb *et al.*, 2015; Reichert *et al.*, 2018). Ambiguous bat passes that could not be identified to species were grouped with similar species (e.g., 40–45 kHz *Myotis*, 20–30 kHz bats — Reichert *et al.*, 2018).

We used a digital acoustic bat lure (Apodemus BatLure, Apodemus Field Equipment, Mheer, Netherlands) alongside our mist-net (2019) and acoustic detector surveys (2019 and 2020) for one night per survey and study site. We used a coin toss to determine which survey night at a site received the lure treatment. During mist-net surveys, the lure was angled upward 120 degrees towards the center of a triple high mist-net with < 30.5 cm of space between the lure and mist-net (Loeb and Britzke, 2010; Braun De Torrez *et al.*, 2017). Since we mist-netted over water, we either positioned the lure on the surface of a pond with a modified foam boogie board (Supplementary Data) or we used a fence t-post to elevate the lure over shallow streams. The lure was placed at a height of 127 cm. During acoustic surveys, we positioned the lure on a t-post at least three meters away from the detector. Similar to our mist-netting methods, we used a coin toss to determine which survey night a site received the lure treatment. To differentiate lure call recordings from wild bat passes, we recorded the lure during the day and aurally verified suspicious recordings by alternating between the 'real-time' and 'compressed' processing modes in Kaleidoscope Pro (Reichert *et al.*, 2018). Any lure calls were removed from the analysis.

We used distress calls from captured *P. subflavus* (n = 11♂♂ and 5♀♀) as the lure call, following methods of Quackenbush *et al*. (2016) as their bat community was the most similar to the one within our study location. We recorded the distress vocalizations with a SM4BAT detector and SMM-U2 microphone (Wildlife Acoustics, Massachusetts, United States) during fall swarm and spring emergence surveys at hibernacula during 2018 and 2019 in Oklahoma, Missouri, and Kansas. We created a ten-minute recording of five unique tracks made from 44 *P. subflavus* distress calls that were saved to an SD card. We randomized the order of the distress calls to maintain relative novelty of the lure throughout the deployment period. We used 'Audacity' (v. 2.3.2, 2018) to create this lure file. The manufacture's (Apodemus Field Equipment, Mheer, Netherlands) accessory timer kit controlled the lure's start and end times and programmed the lure to run ten minutes on and ten minutes off (Eckenweber and Knörnschild, 2016; Quackenbush *et al.*, 2016; Braun De Torrez *et al.*, 2017). The lure's volume and other audio settings were set according to the manufacturer's recommendations to avoid clipping, which causes disruptions in the sound produced by the lure.

We evaluated the influence of the acoustic lure on mist-netting captures (2019 season) and acoustic detections (2019 and 2020 seasons) using statistical analyses performed in R Studio version 1.1.463 (R Core Team, 2020). Herein, we report the 2018 season data to provide additional information as to the status of these species' populations. We used generalized linear models (GLM; 'glm' function from R base package — R Core Team, 2020) with a Poisson distribution and log link, where acoustic lure (yes or no) was the predictor variable, and number of mist net captures or acoustic bat passes were the response variables. Model significance (α = 0.05) was assessed using Wald χ^2 tests ('Anova' function from the 'car' package — Dunn and Smyth 2018; Fox *et al*. 2020). If the models demonstrated overdispersion (i.e., ratio of residual degrees of freedom to residual deviance > 1.5), we used the negative binomial distribution ('glm.nb' function from the 'MASS' package — Dunn and Smyth, 2018; Ripley *et al.*, 2020). The 'ggplot2' package used to visualize results with bar charts with error bars (Wickham *et al.*, 2020). Finally, because we had low detection success for all imperiled bat species individually, we aggregated species into four species groupings to better assess lure effectiveness on capture rates and acoustic bat passes with a larger number of detections (Table 1). Group 1 included all bats except for the frequently observed eastern red bat *Lasiurus borealis* and evening bat *Nycticeius humeralis*. These two common species were excluded from Group 1 analyses because they distracted from our main objective of evaluating lure effectiveness in increasing detection success of rare species. Group 2 included Missouri's bat species of conservation concern, Group 3 included all individuals captured or recorded, and Group 4 included all species sampled individually as long as there were ≥ 10 captured individuals per species and ≥ 10 documented sites

TABLE 1. The acoustic lure's effect on all four species groups' captures and bat passes. Missouri species of concern (Group 2) include the silver-haired bat (*L. noctivagans*), hoary bat (*A. cinereus*), Seminole bat (*L. seminolus*), Indiana bat (*M. sodalis*), tricolored bat (*P. subflavus*), gray bat (*M. grisescens*), northern long-eared bat (*M. septentrionalis*), and the little brown bat (*M. lucifugus*). Only species with sufficient detections (≥ 10 captured individuals; ≥ 10 unique capture sites for acoustic detections) are listed for Group 4. * — Indicates statistical significance (P < 0.05 — marked in bold) for non-imperiled species, and ** — indicates statistical significance of a federally- or state-protected species. Blank values represented an insufficient number of captures for statistical analysis

Group or species	Capture (P)	Acoustic (P)
Group 1: All bats except *L. borealis* and *N. humeralis*	0.13	0.46
Group 2: Missouri species of conservation concern	0.12	0.54
Group 3: All individuals	0.16	0.29
Group 4: Species with sufficient detections:		
Lasiurus borealis	0.30	0.38
Nycticeius humeralis	0.53	0.53
*Lasionycteris noctivagans***	**0.02**	0.80
Myotis grisescens	–	0.46
Aeorestes cinereus	–	0.42
*Eptesicus fuscus**	–	**0.02**
*Myotis sodalis***	–	**0.04**
40 kHz *Myotis*	–	0.69

with acoustic species detections; fewer detections do not provide valid statistical inference. We combined capture results for juveniles and adults.

Results

We captured 354 individuals of nine bat species during our mist-netting efforts in 2018 and 2019 (Table 2). The most commonly captured species was *L. borealis*, accounting for 74% of the total captures across both years (n = 262 of 354 total captures — Table 2). In 2019 (n = 263 captures), the lure did not increase the total captures of all species excluding *L. borealis* and *N. humeralis* (Group 1; $\chi^2 = 2.31$, $d.f. = 1$, $P = 0.13$), Missouri species of conservation concern (Group 2; $\chi^2 = 2.44$, $d.f. = 1$, $P = 0.12$), or all species combined (Group 3; $\chi^2 = 1.98$, $d.f. = 1$, $P = 0.16$ — Table 1). Of the species analyzed for Group 4, *L. noctivagans* was the only species that exhibited a significant lure effect ($\chi^2 = 5.27$, $d.f. = 1$, $P = 0.02$ — Fig. 2), as more individuals were captured when using the lure than without it (Table 2). Finally, while the number of *P. subflavus* captures was too small to analyze statistically, the only two *P. subflavus* individuals captured were caught using the lure (Table 2).

We recorded bat passes from seven species in 2018 and 10 species each in 2019 and 2020. The most frequently recorded species across the three years was *L. borealis* (31.4% of the total recordings across three seasons; n = 11,891 total recordings). For the 2019 and 2020 data collection seasons, the lure increased the recorded number of bat passes for *Eptesicus fuscus* ($\chi^2 = 5.14$, $d.f. = 1$, $P = 0.02$) and *Myotis sodalis* ($\chi^2 = 4.19$, $d.f. = 1$, $P = 0.04$ — Fig. 3). However, total bat activity remained unchanged between treatments for the community of bats without *L. borealis* and *N. humeralis* (Group 1; $\chi^2 = 0.53$, $d.f. = 1$, $P = 0.46$), Missouri's species of conservation concern (Group 2; $\chi^2 = 0.36$, $d.f. = 1$, $P = 0.54$), all individuals (Group 3; $\chi^2 = 1.13$, $d.f. = 1$, $P = 0.29$), and the 40 kHz *Myotis* ($\chi^2 = 0.16$, $d.f. = 1$, $P = 0.69$) (Table 1).

Discussion

We found that the use of an acoustic lure attracted three bat species, increasing either their

Table 2. Total captures and bat passes observed between 2018 and 2020, sorted by species most to least commonly detected. We sampled bats via mist-nets and acoustic detections in 2018 prior to incorporating the lure for the 2019 season. We were unable to mist-net in 2020 due to the COVID-19 sampling restrictions. Sampling effort is indicated by trap nights for mist-netting and nights when we deployed acoustic detectors, with and without the lure. While mist-net area differed across sites, the number and size of mist nets used at each location were identical with and without the lure to standardize effort. All sites were sampled with mist-nets and acoustic monitors for the same duration (i.e., five hours per night). †Data for the 2018 season were not incorporated into the analysis of lure effectiveness, as this season provided preliminary data to highlight the limited sample sizes of the target species. * — Indicates statistical significance for non-imperiled species, and ** — indicates statistical significance of a federally- or state-protected species. Statistically different cells ($P < 0.05$) are marked in bold

Species	Captures			Bat passes		
	2018† No Lure	2019 No Lure	2019 Lure	2018† No Lure	2019 & 2020 No Lure	2019 & 2020 Lure
Lasiurus borealis	85	74	103	372	1465	1895
Nycticeius humeralis	16	10	13	3	228	304
*Lasionycteris noctivagans***	0	**1**	**11**	8	110	123
Myotis grisescens	9	3	4	13	558	767
Lasiurus seminolus	1	0	1	0	N/A	N/A
Aeorestes cinereus	7	2	4	73	51	84
*Eptesicus fuscus**	1	2	3	35	**37**	**105**
Perimyotis subflavus	0	0	2	44	19	2
*Myotis sodalis***	1	1	0	0	**6**	**16**
Myotis septentrionalis	0	0	0	0	9	14
Myotis lucifugus	0	0	0	0	16	2
Species Group: 40 kHz Bat	N/A	N/A	N/A	1187	319	313
Species Group: 40 kHz *Myotis*	N/A	N/A	N/A	78	627	733
Species Group: *N. humeralis* / *L. borealis*	N/A	N/A	N/A	0	443	652
Species Group: *E. fuscus* / *L. noctivagans*	N/A	N/A	N/A	274	230	541
Species Group: Low-frequency bat	N/A	N/A	N/A	43	32	29
Species Group: *M. sodalis* / *M. lucifugus*	N/A	N/A	N/A	0	14	17
Totals	120	93	141	2130	4164	5597
Trap nights	23	12	12	53	40	40

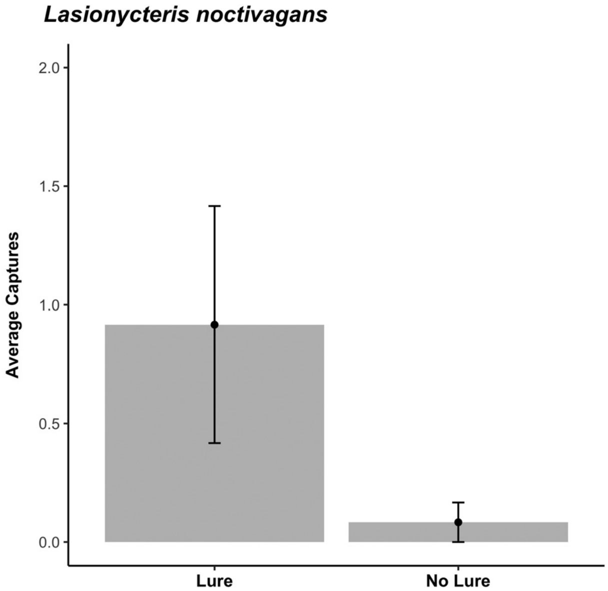

FIG. 2. *Lasionycteris noctivagans* capture results with and without the presence of an acoustic lure in 2019. Captures were averaged by sampling location, with error bars representing the standard error of the mean

acoustic activity (*M. sodalis* and *E. fuscus*) or capture rates (*L. noctivagans*). Two of these species are imperiled (i.e., *M. sodalis* and *L. noctivagans*); thus, our results highlight the utility of acoustic lures in enhancing encounter success of rare bat species post-WNS, augmenting knowledge gain for subsequent conservation actions. Similar to our findings, Quackenbush *et al.* (2016) found that acoustic lures increased mist-net captures of a group of *Myotis* species (i.e., *M. lucifugus*, *M. septentrionalis*, and *M. sodalis*), although Samoray *et al*. (2019) did not find a lure effect on *M. sodalis*. Quackenbush *et al.* (2016) and Samoray *et al*. (2019) did, however, come to similar conclusions of acoustic lure effectiveness on *E. fuscus* captures, as neither found an effect of the lure on the capture rate of this species. The effect of an acoustic lure consequently seems to vary by species and the behaviors it elicits, either attracting bats to a sampling location or increasing echolocation pulses. Thus, results between our study and those of Quackenbush *et al*. (2016) and Samoray *et al*. (2019) concerning *M. sodalis* and *E. fuscus* highlight the need for further research on the impacts of acoustic luring on these two species specifically, and for other imperiled bat species more generally.

Lasionycteris noctivagans was more likely to be captured using the acoustic lure. We are unaware of any other acoustic lure study that resulted in increased captures of *L. noctivagans*, but other studies have demonstrated increased capture rates of other *Lasiurus* species (e.g., *Lasiurus borealis* — Samoray *et al.*, 2019). These findings suggest that bats in the genus *Lasiurus* are attracted to at least some heterospecific vocalizations; therefore, it may be useful to incorporate lure calls from different species for their capture. Acoustic lures may be increasingly useful in the study of *L. noctivagans* due to their population declines resulting from wind energy development (Arnett *et al.*, 2008, 2016).

While we found that the acoustic lure increased detections or captures of three bat species, our

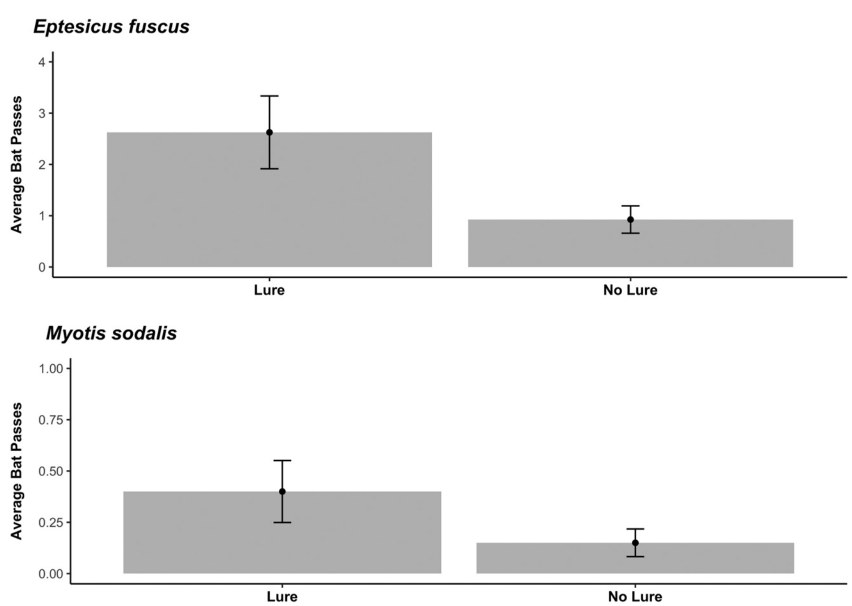

FIG. 3. *Eptesicus fuscus* and *M. sodalis* acoustic activity with and without the presence of an acoustic lure across the 2019 and 2020 data collection seasons. Bat passes were averaged by sampling location, with error bars representing the standard error of the mean

results were inconclusive for our primary target species, *P. subflavus*. Our small sample size and the cancellation of our mist-netting efforts in 2020 limited our evaluation of the lure's efficacy on this species' capture and acoustic detection. Even so, we captured two *P. subflavus* while a distress call lure was broadcasted, which was particularly important for the study of this declining species and identification of its roosting habitat (Hammesfahr *et al*., 2022). This species roosts in small numbers (Fujita and Kunz, 1984; Perry and Thill, 2007) and switches roosts frequently (Veilleux and Veilleux, 2004), which along with the range-wide population declines resulting from WNS, made finding and recording free-flying *P. subflavus* challenging. More research should be done on this species regarding its response to acoustic luring, especially in regions where this species is still abundant.

Our acoustic lure results highlight several important considerations, including the types of calls used for the lure, how various species respond to these lures, and the environments in which lures may be used successfully. We tested the effects of an acoustic lure that broadcasted *P. subflavus* distress calls rather than non-distress social calls. While we recognize that the bulk of the literature describes increased bat detections or captures utilizing non-distress social calls (e.g., Hill and Greenaway, 2005; Braun de Torrez *et al.*, 2017), we acknowledge the importance of testing different types of acoustic lures. Distress calls have been used successfully to lure bats in some studies (e.g., Russ *et al*., 2004; 2005), while in others results were equivocal or species-specific (Quackenbush *et al.*, 2016), thus necessitating further study into the comparative effects of non-distress versus distress social calls in attracting bats.

The *P. subflavus* lure was effective at increasing captures or acoustic activity for three non-target species, which may indicate that some bats still investigate calls even if they originate from other bat species (Schöner *et al*., 2010). Acoustic lures may also be more effective for bat species that roost in larger social groups. This may explain why we detected a greater acoustic response to our lure from the large colony roosting species *M. sodalis* (Silvis *et al.*, 2014) and *E. fuscus* (Kurta and Baker, 1990), compared to *P. subflavus* that roost in smaller groups (Fujita and Kunz, 1984; Perry and Thill, 2007). We only tested the effects of the lure during the breeding and maternity seasons, which may have also resulted in a seasonal impact with how individuals respond to social calls.

Additionally, more information is needed regarding how species respond to acoustic luring across different habitats, as it unknown how far a bat can detect noise (Schnitzler and Kalko, 2001) and how the density of forested habitats may impact the lure's effective range for different species (Law *et al.*, 2018; Hill *et al.*, 2014*b*, Freeze *et al.*, 2021). Thus, experiments evaluating a combination of different types of *P. subflavus* calls and calls originating from heterospecifics may be necessary to understand how *P. subflavus* responds to acoustic luring, especially in differing seasons and habitats across this species' range.

Capturing imperiled species is often challenging in areas affected by WNS, due to low capture success rates despite high levels of time and effort. The reduced capture success of imperiled bat species hinders the development of evidence-based management decisions that could help protect impacted populations. We discovered that capturing *P. subflavus* in Missouri post-WNS via mist-nets is a slow and unreliable method for their detection. While a variety of survey methods are still necessary to monitor individual's reproductive status and find their roosts, the selection of this species to test the impacts of an acoustic lure was problematic due to their small population size. Our research underscores the need to test the efficacy of acoustic lures on rare species in areas where they are still abundant. In particular, surveying for *P. subflavus* in regions where they are more abundant, especially across habitat types (e.g., cave maternity roosts, forested waterbodies) using multiple survey methods (e.g., harp traps at cave entrances, mist-nets, acoustic detectors) with and without an acoustic lure may be necessary to yield sample sizes large enough to analyze.

The outcomes from our analysis contribute to a growing number of acoustic lure studies that seek to understand acoustic lures' function and their incorporation into mist-net surveys for various species. Our findings suggest that bat surveys should utilize multiple tools and methods, such as acoustic lures, to increase the odds of capturing and acoustically detecting rare bat species. Even so, information is generally lacking for the vast majority of bat species regarding the efficacy of acoustic lures. Further research should explore how a variety of bat species respond to different types of lure calls (i.e., social calls at the roost vs. distress calls; intra- vs. interspecific calls) across diverse habitat types and seasons. Additional research contributions can extend the understanding of how acoustic lures can be easily incorporated into North American bat studies, to prevent failures in the detection of imperiled species when they are still present.

Supplementary Information

Contents: Supplementary Data: Acoustic lure methods. Supplementary Information is available exclusively on BioOne.

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