Original Article



Acoustic Lure Allows for Capture of a High-Flying, Endangered Bat

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ABSTRACT Mist nets are commonly used to capture free-flying bats; however, some bat species are very difficult to capture because of their flight behavior, habitat preferences, and ability to avoid nets. High-flying, open-space foragers are especially underrepresented by mist-net surveys. Few studies have investigated the effectiveness of using acoustic lures (playbacks of conspecific vocalizations) to increase capture success of bats in mist nets. We tested the efficacy of an acoustic lure to capture a high-flying rare molossid, the endangered Florida bonneted bat (Eumops floridanus), which had been captured only once away from a known roost prior to our research. We used a crossover experimental design with 2 lure treatments (nets with lures playing social call recordings from 2 different roosts) and 2 control nets (no lures) in 6 sites for 2 nights each. We captured 15 Florida bonneted bats in our treatment nets and 0 in our control nets. One lure had greater capture success (n=13) than the other (n=2), with a trend for greater captures of males (n=11) than females (n=4). We suggest that these differences were due to the social context in which the calls used in the lures were recorded. Our study demonstrated the utility of acoustic lures to capture Florida bonneted bats and expands research opportunities critical to the species' conservation, such as the ability to use radio telemetry to track captured bats to unknown roosting and foraging areas. Our study also lays the foundation for future research into social call playbacks as a technique to lure other high-flying and elusive bat species into mist nets. © 2017 The Wildlife Society.

KEY WORDS acoustic lure, conservation, conspecific playbacks, endangered species, *Eumops floridanus*, Florida bonneted bats, mist net, social behavior.

Bats are an ecologically diverse and beneficial order of mammals found worldwide (Kunz et al. 2011), yet are challenging to study because they are small, nocturnal, and can fly (Kunz and Parsons 2009). Free-flying bats are commonly captured using mist nets to investigate ecological patterns, such as community composition, population dynamics, and habitat associations, and collect biological samples (e.g., tissue for DNA, hair for isotopes, guano for diet; Hayes et al. 2009). However, surveying with mist nets tends to be biased against certain species, especially highflying, open-space, and edge foragers that fly above nets (Francis 1989, Kalko et al. 2007). Further, some species

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exhibit net shyness whereby individuals detect and learn to avoid mist nets (Kunz and Kurta 1988, Waldien and Hayes 1999, Hill and Greenaway 2005, MacCarthy et al. 2006, Robbins et al. 2008). Acoustic surveys using ultrasonic detectors can be used as an alternative to detect species that are difficult to capture and quantify the relative activity of particular species at different locations. However, limitations of acoustic surveys include the inability to differentiate certain species with similar echolocation call characteristics, estimate abundance, evaluate relative differences in activity among species, and infer details on individuals such as sex, reproductive status, morphological measurements, or health (Hayes et al. 2009, Parsons and Szewczak 2009). Additionally, techniques to locate new roosts typically require capturing an individual, attaching a radio transmitter, and tracking it back to a roost (Amelon et al. 2009). To address pressing research questions and conservation concerns for species rarely captured in mist nets, it is necessary to develop methods to increase capture success.

The use of acoustic lures, or broadcasting playbacks of conspecific vocalizations, is a common approach to attract and increase captures of birds (Podolsky and Kress 1992, Sydeman et al. 1998, Schaub et al. 1999). There is also evidence that bats respond to acoustic lures due to their tendency to eavesdrop on other bats for ecological and social information (Barclay 1982, Fenton 2003, Hill and Greenaway 2005, Gillam 2007). Acoustic lures for bats typically consist of natural or synthetic auditory stimuli (often in the ultrasonic range) that are broadcast to attract conspecifics and heterospecifics. Using acoustic surveys, researchers have documented increased activity levels of several species of bats in response to playbacks of echolocation calls, social calls, feeding buzzes, and distress calls (Barclay 1982, Gillam 2007, Arnold and Wilkinson 2011, Carter et al. 2015). However, only a few published studies have investigated acoustic lures as a means to increase bat captures in mist nets and the response has varied by species both in direction (attraction or avoidance) and magnitude (Hill and Greenaway 2005, Loeb and Britzke 2010, Lintott et al. 2013). Furthermore, to our knowledge, the effect of lures on capture success has never been tested on high-flying, aerial hawking species, often the most challenging bats to capture with mist nets.

Bat species of the family Molossidae are particularly difficult to capture (Best et al. 1996, Emmons and Feer 1997, Sampaio et al. 2008) likely because of their flight at high altitudes (Norberg and Rayner 1987, McCracken et al. 2008) and in open areas that provide little vegetative cover typically used to funnel bats into mist nets (Kunz and Parsons 2009). Worldwide, molossids constitute a very small fraction (often <1%) of total mist-net captures (Ammerman et al. 2012), despite documentation of colonies consisting of millions of individuals (e.g., Brazilian free-tailed bats [Tadarida brasiliensis]; Betke et al. 2008). Thus, many studies of molossids rely instead upon counts and captures at roost sites (Hristov et al. 2010, Noer et al. 2012) or acoustic surveys (Davidai et al. 2015, Deshpande and Kelkar 2015).

The endangered Florida bonneted bat (Eumops floridanus) is a molossid that is extremely difficult to capture away from roosts, and very few known roosts exist at which to focus capture efforts (Florida Fish and Wildlife Conservation Commission 2013, U.S. Fish and Wildlife Service [USFWS] 2013, Braun de Torrez et al. 2016, Ober et al. 2017). Because of its recent designation as federally endangered in the United States (USFWS 2013) and our limited understanding of its basic biology (Ober et al. 2017), empirical data to inform recovery plans and conservation actions for the species is needed. Prior to this study, only one free-flying Florida bonneted bat (a juvenile) had ever been captured away from a known roost despite documentation of the species flying above mist nets (USFWS 2013). Because of the distinct challenges and conservation and research needs, we selected Florida bonneted bats as a model to test the effectiveness of an acoustic lure at increasing capture success of high-flying bat species. We took an experimental approach to test if playbacks of conspecific social calls

recorded around known roosts could lure Florida bonneted bats into mist nets. Based on frequent recordings of social calls around roost sites (Braun de Torrez et al. 2016) and observations of social chatter between individuals in flight, we hypothesized that Florida bonneted bats would be attracted to playbacks of conspecific social calls and captures at mist nets with playback calls (treatments) would be greater than at mist nets without playback calls (controls).

STUDY AREA

We conducted this study in 3 conservation areas within the core range of Florida bonneted bats in south Florida, USA (USFWS 2013): 1) Fred C. Babcock-Cecil M. Webb Wildlife Management Area (BWWMA) in Charlotte County, managed by Florida Fish and Wildlife Conservation Commission; 2) Florida Panther National Wildlife Refuge (FPNWR) in Collier County, managed by the USFWS; and 3) Fakahatchee Strand Preserve State Park (FSPSP) in Collier County managed by Florida Department of Environmental Protection (Fig. 1). All sites contained a mix of mesic and hydric slash pine (*Pinus elliottii*) flatwoods, cypress (*Taxodium ascendens* and *T. distichum*) communities, freshwater prairies, ponds, and hardwood hammocks. Prior to this study, we detected high Florida bonneted bat activity in all 3 conservation areas using acoustic surveys.

METHODS

Acoustic Lures

Using SM3BAT ultrasonic detectors (Wildlife Acoustics, Maynard, MA, USA), we recorded full-spectrum ultrasonic recordings of Florida bonneted bat calls from 2 roosts (Fig. 1). Because there is evidence of colony-specific and individual-specific vocalizations in bats (Fenton 2003), we used recordings from roosts in 2 distinct locations to control for any local effect in which individuals may respond to the lure only because they recognize the callers. The first roost was located within BWWMA in a bat house containing at least 15 bats the date we made recordings. The second roost was located within FPNWR in a slash pine snag containing at least 12 bats the date we made recordings. We mounted detectors on 3-m poles located 10 m away from each roost. We visually examined spectrograms of recordings using Sonobat Software for Bat Call Analysis (Sonobat, Arcata, CA, USA) and identified social calls by their low frequency, U- or W-shaped pulses in the call sequence (Pfalzer and Kusch 2003; Fig. 2). Social calls sharing similar characteristics to those we documented have been described for many other species (Fenton 2003). Although we do not know the significance of these calls to Florida bonneted bats, we previously recorded many similar calls around these roosts (Braun de Torrez et al. 2016), suggesting they have a social function. From each roost, we selected 5 15-s files that contained clear, high-quality social calls (call features were sharply defined and showed little attenuation) within a sequence of echolocation calls.

We used 2 BatLuresTM (Apodemus Field Equipment, Mheer, Netherlands) to broadcast calls, which include an

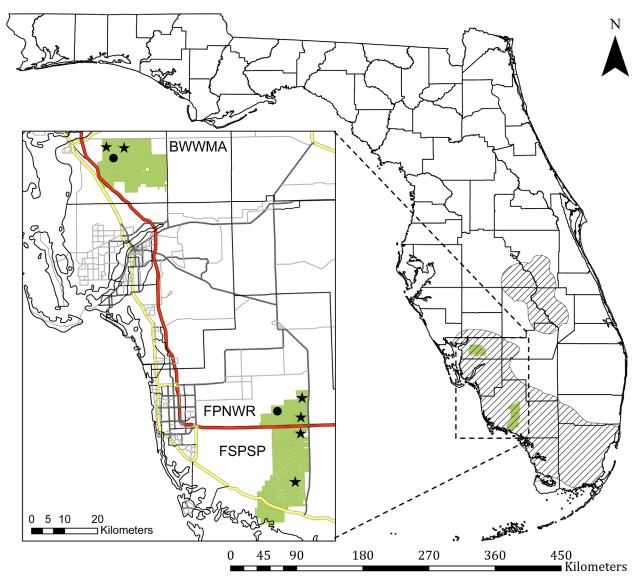


Figure 1. Three conservation areas (green shading) within the range of the Florida bonneted bat, *Eumops floridanus*, (diagonal hashed lines) where we conducted an experiment testing the effect of acoustic lures on capture success from April–May 2016 in Florida, USA: 1) Fred C. Babcock-Cecil M. Webb Wildlife Management Area (BWWMA); 2) Florida Panther National Wildlife Refuge (FPNWR); and 3) Fakahatchee Strand Preserve State Park (FSPSP). Stars = capture sites. Dots = locations of social call recordings for lure 1 (BWWMA) and lure 2 (FPNWR).

ultrasonic speaker, a programmable timer, and a secure digital card that can be loaded with user-selected .wav files. One BatLure TM, hereafter lure 1, contained 5 social call files from the BWWMA roost. The second BatLure TM, hereafter lure 2 contained 5 social call files from the FPNWR roost. We programmed each file to repeat 3 times with a 1-s break between recordings for a total playback time of 4 min. Following manufacturer recommendations, we set the volume 1 increment below the level at which clipping of the signal occurred, indicated by a red light on the BatLure TM. Based on anecdotal evidence of habituation to the lure in other species (S. T. Samoray, Copperhead Environmental Consulting, personal observation), we programmed a period of silence for 3 min and 48 s (i.e., the amount of silence allowed by the software) between each 4-min playback session.

Experimental Design

To test the effect of acoustic lures on capture success of Florida bonneted bats, we conducted a crossover study in April and May 2016. We selected 2 sites within each of the 3 conservation areas based on consistent acoustic detections of Florida bonneted bats, for a total of 6 sites (Fig. 1). At each site, we set up 4 pairs of 7.3-m triple-high mist-net systems (Bat Conservation and Management, Carlisle, PA, USA), hereafter net pairs (Fig. 2). To improve capture success, we deployed each net pair in a V-configuration (Kunz et al. 2009): one set of 3 12-m mist nets and one set of 3 9-m mist nets (38-mm mesh, Avinet, Freeville, NY, USA) placed at 90° angles to each other. Each net pair was between 200 m and 600 m from all other net pairs. We located all net pairs in open areas (pine flatwoods, fresh water prairies, large quarry ponds), which is in contrast to locations where mist nets are

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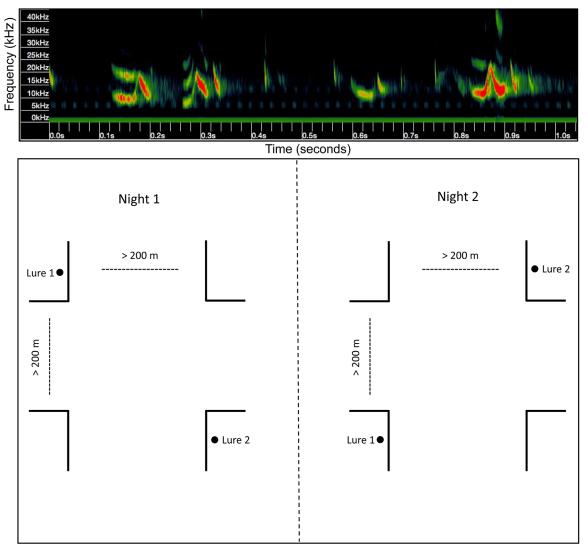


Figure 2. Example of Florida bonneted bat (*Eumops floridanus*) social calls broadcast with an acoustic lure (BatLureTM, Apodemus Field Equipment, Mheer, Netherlands) in Florida, USA, during April–May 2016 (top panel). Spectrogram is visualized with Kaleidoscope Viewer (Wildlife Acoustics, Maynard, MA, USA). Diagram depicting experimental design of mist-net configurations at each of 6 sites (bottom panel). We placed lures at 2 net pairs (1 12-m and 1 9-m triple high net systems) on the first night and switched lures to the remaining 2 net pairs on the second night.

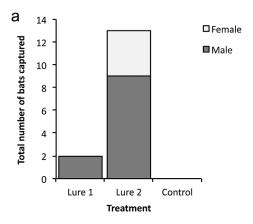
traditionally set to increase capture success (Kunz et al. 2009). We sampled each site for 2 nights for a total of 12 nights (48 net nights). On the first night, we randomly selected 2 net pairs as treatments (1 lure each) and 2 as controls (no lure). On the second night, we switched treatments and controls. At each treatment net pair, we placed 1 lure on a 1-m-high tripod approximately 1 m from the center of the 12-m net. We opened nets for 4 hr starting 30 min after sunset and checked nets every 15 min for the presence of bats.

We placed each captured bat in a numbered cotton bag and examined the bat to determine age (adult or sub-adult), reproductive status, and sex. We released each bat near the site of capture. We used chi-square goodness-of-fit tests to test for differences in captures between treatments and controls, the 2 lures, and sex ($\alpha = 0.05$), and adjusted P values with a Bonferronni's correction for multiple comparisons. All capture and handling procedures followed American Society

of Mammalogists guidelines for research on live animals (Sikes et al. 2016) and were in accordance with the following approved permits: University of Florida Institutional Animal Care and Use Committee (no. 201308070), USFWS (no. TE 23583B-1), Florida Panther National Wildlife Refuge (no. 41545-2016-IR), Florida Department of Environmental Protection (no. 12161424), and Florida Fish and Wildlife Conservation Commission (no. SUO-49616).

RESULTS

Over 12 mist-net nights of our experiment, we captured 15 adult Florida bonneted bats and found an effect of treatment on the number of captures ($\chi^2_1 = 15$, P < 0.001; Fig. 3a). Florida bonneted bats were never captured in our control net pairs. We captured Florida bonneted bats at all 6 sites and on 9 of the 12 nights of the study ($\bar{x} = 1.25 \pm 0.32$ [SE] bats per night). We captured more Florida bonneted bats in net pairs using lure 2 (n = 13) than with lure 1 (n = 2; $\chi^2_1 = 8.07$,



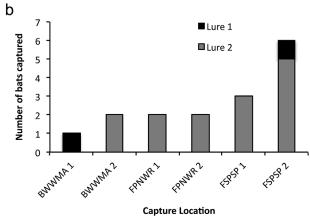


Figure 3. Total number of Florida bonneted bats (*Eumops floridanus*) captured in: a) each treatment (lure 1 and lure 2) and control mist-net pairs across 6 sites and 12 nights (48 total net nights); and b) number of captured Florida bonneted bats broken down by capture site in Florida, USA, during April–May 2016. Lure 1 = recordings from BWWMA, Lure 2 = recordings from FPNWR.

P=0.014; Fig. 3). Although qualitative, we did not find evidence that the response by bats to lures was location-dependent (nets with lure 1 captured bats in BWWMA and FSPSP, nets with lure 2 captured bats in all 3 sites; Fig. 3b). Bats did not respond more to calls recorded from bats in their respective conservation areas; rather, captures in all 3 conservation areas were greater with the set of lure calls recorded at the roost in FPNWR (lure 2) than with the set recorded at the roost in BWWMA (lure 1). More males were captured (n=11) than females (n=4, all with lure 2), but this was not statistically significant ($\chi^2_1=3.27$, P=0.21; Fig. 3a).

DISCUSSION

Our results demonstrate the efficacy of an acoustic lure to capture a high-flying, endangered bat species that had been extremely difficult to capture using standard mist-netting techniques. Previously, 465 mist-net nights (using triple-high mist-net sets) yielded only one Florida bonneted bat capture, despite nets being set in areas where the species had been documented to occur (K. N. Smith, Florida Fish and Wildlife Conservation Commission, unpublished report; E. C. Braun de Torrez, University of Florida, unpublished data). Many molossids forage in open areas, which are very

challenging to mist net because of a lack of flight corridors and overhanging vegetation typically used to channel bats into nets (Norberg and Rayner 1987). Additionally, nets in open areas are easily detected and avoided by bats, particularly on moonlit or windy nights (Kunz et al. 2009). Despite our sites having little to no canopy cover, we captured Florida bonneted bats on nearly every night of the study using the acoustic lure. The ability to capture free-flying Florida bonneted bats expands many research opportunities including investigations into roost ecology, foraging behavior, and population genetics that can be used to direct conservation actions across the species' range.

Previous work has shown that Brazilian free-tailed bats and velvety free-tailed bats (Molossus molossus), high-altitude foraging molossids, were attracted to playbacks of feeding buzzes (Barclay 1982, Gillam 2007) and distress calls (Carter et al. 2015). Nonetheless, we are unaware of any studies demonstrating that acoustic lures can effectively lure these species, or any other high-flying bat species, into a mist net. Experiments using acoustic lures to increase capture success of forest dependent bats have resulted in mixed responses. Similar to our findings, greater capture rates were documented in the United Kingdom for Bechstein's bat (Myotis bechsteinii), a woodland species rarely captured in mist nets, by using playbacks of conspecific social calls (Hill and Greenaway 2005, Goiti et al. 2007). Acoustic lures using a variety of synthesized bat calls increased capture rate of 4 bat species in the United Kingdom by 2- to 12-fold (Lintott et al. 2013). In contrast, capture rate of Rafinesque's bigeared bat (Corynorhinus rafinesquii) in the United States decreased in response to social call playbacks, suggesting that these calls repel conspecifics and may reduce intraspecific competition during foraging (Loeb and Britzke 2010).

In our study, it is not clear why one set of lure calls was more effective at attracting Florida bonneted bats into mist nets than the other. If there was a local effect in which individuals were attracted to calls from a colony they recognized, we would have expected more captures with lure 2 in FPNWR, more captures with lure 1 in BWWMA, and no difference between lures in FSPSP. Instead, we captured more bats with lure 2 in all 3 conservation areas, with only 1 individual being captured with lure 1 in BWWMA and FSPSP. We suggest that this difference is due to the unknown social context in which the calls used in our playbacks were recorded. We did not know the sex, reproductive status, or social status of the bats that produced the calls in our recordings. These demographic attributes may influence the response of bats, which are capable of recognizing individual-specific information from echolocation calls (Fenton 2003, Bohn 2015). For example, big brown bats (Eptesicus fuscus) are able to differentiate sex, age, and family affiliation of conspecifics based on echolocation (Masters et al. 1995). Additionally, we do not know if bats responded to certain calls more than others within the sets of lure calls. Differences in the time of night, seasonality, and significance of social calls used in the playbacks may influence the strength of response from conspecifics. Many bird species have songs that both attract and repel other individuals depending on the social context and receiver (Catchpole and Slater 1995). Similarly, the cause of a trend toward male-biased captures is not clear. This could be a result of mate attraction to female callers or territoriality in response to male callers (Wilkinson 1995). In spotted antbirds (Hylophylax naevioides), for example, males responded more strongly to playbacks of male songs than did females, suggesting different roles in territorial defense (Bard et al. 2002). In 2 species of European pipistrelle bats (Pipistrellus spp.), vocalizations by males attract females to roosts but repel other males during the mating season (Lundberg and Gerell 1986, Sachteleben and von Helversen 2006, Russ and Racey 2007), and structurally similar vocalizations made by both sexes repel conspecifics from foraging areas (Barlow and Jones 1997). Future research should explore the importance of social context in which calls destined for use in the lures are recorded and should determine which calls are most effective at attracting freeflying bats.

We suggest that acoustic lures can effectively attract highflying bats to an altitude low enough to be captured in mist nets in areas where Florida bonneted bats are already known to occur. On several occasions, we observed a Florida bonneted bat fly over the nets, then make a 180° turn in response to the lure playbacks before being captured. Similarly, we observed Florida bonneted bats flying around mist nets and emitting social calls in response to the lure playbacks. Not all bats that investigated lures were captured in our mist nets, suggesting that individuals responded differently to lures or were able to avoid nets. Our study lays the foundation for investigating social communication in Florida bonneted bats and provides a basis to further explore individual responses to lures. We recommend research into ways to further increase capture rate by optimizing variables such as types of social calls, spacing of playbacks, amplitude, and net configuration. Using an acoustic lure, Florida bonneted bats can now be captured in areas where no known roost sites exist and tracked back to new roost locations using radiotelemetry; identification and characterization of new roost structures has been identified as a key research priority by the USFWS and FWC (FWC 2013, USFWS 2013). However, until we understand the possible biases associated with individual responses to the lures in a particular context (e.g., social, ecological, reproductive), we caution against using lures to draw conclusions that require an unbiased sample of the population, such as habitat associations and population dynamics. Finally, we suggest that social call playbacks be investigated as a technique to increase captures of other high-flying and elusive molossids that have been noted to be very difficult to capture (e.g., Guianan bonneted bat [Eumops maurus], Sampaio et al. 2008; Underwood's bonneted bat [E. underwoodi], Emmons and Feer 1997; and Western mastiff bat [Eumops perotis], Best et al. 1996).

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