SEXUAL DIMORPHISM IN BIG BROWN BAT (*EPTESICUS FUSCUS*) ULTRASONIC VOCALIZATIONS IS CONTEXT DEPENDENT

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Although bats are well known for their use of ultrasound for echolocation, there is limited evidence for its use in a social context. We tested whether ultrasonic vocalizations in bats were contextually (roosting or flying) sexually dimorphic. During the reproductive season, we recorded ultrasonic signals of captive adult male and female big brown bats while the bats were flying on tether lines in the field, and compared these signals to ultrasonic vocalizations made while roosting in an anechoic chamber. Principal component analysis reduced 7 ultrasonic call descriptors to 2 components that related to frequency (PC1) and time or shape (PC2). While bats were roosting, ultrasonic call components related to time or shape and frequency were both sexually dimorphic, being increased in males in each instance. However, when bats were recorded while flying, these same call components were no longer sexually dimorphic. This finding suggests that bats are changing their ultrasonic calls in relation to functional context, making them monomorphic and utilitarian for activities such as foraging and navigation, but dimorphic in a situation when mating activity is likely.

Key words: bats, *Eptesicus fuscus*, mating, sexual dimorphism, ultrasonic vocalizations

Acoustic signals, ranging from infrasonic (<20 Hz) to ultrasonic (>20,000 Hz), are an important method of communication among many species including insects, fish, amphibians, birds, and mammals (review in Bradbury and Vehrencamp 1998). Additionally, it is well documented that many communication signals exhibit context-dependent variation influenced by the ecological (e.g., season, time of day, and geographic location—Baptista and King 1980; Rotella and Ratti 1988) and social (e.g., reproduction, territory defense, and predation) context of the situation (Catchpole 1979; Ehrlich et al. 1988; Ruiz-Miranda et. al. 2002). Some examples of these context-dependent changes include gray partridge (*Perdix perdix*) calling behavior and acoustic call structure exhibiting differences related to seasonal variation in call function (Rotella and Ratti 1988), calls of male yellow baboons (*Papio cynocephalus*) functioning as alarm calls or contest calls depending on the situation (i.e., aggressive encounters and male–male calling bouts—Fischer et. al. 2004), and male zebra finches (*Taeniopygia guttata*) altering song tempo in the presence of females versus when alone (Cooper and Goller 2006).

Vocalizations in the ultrasonic range have been documented to be used in intraspecific and interspecific communication for a variety of social purposes and may also be context-dependent (Sales and Pye 1974). Bats are well known for their use of ultrasound for navigation and foraging, but there is less information on the function of ultrasonic vocalizations for other purposes, especially related to mating. Although bats exhibit a wide diversity of mating systems little is known about the mechanisms involved in courtship and mating. The evidence does suggest that there are sex differences in ultrasonic vocalizations of at least some species of bats (Jones et al. 1992; Neuweiler et al. 1987; Siemers et al. 2005; Siemers and Kerth 2006; Suga et al. 1987; Thomas et al. 1979), which raises questions about the functional significance that these differences might serve.

Thomas et al. (1979) found circumstantial evidence suggesting that adult little brown bats (*Myotis lucifugus*) can determine the sex of other adult bats from the ultrasonic vocalizations they use for echolocation. More recent studies of *Eptesicus* (Kazial and Masters 2004), *Pteronotus* (Suga et al. 1987), and *Rhinolophus* (Neuweiler et al. 1987) have shown that male and female bats respond differently to ultrasonic

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Components and definitions
1. duration
2. h1start = starting frequency of fundamental (kHz)
3. h1mid = middle frequency of fundamental (kHz)
4. h1end = ending frequency of fundamental (kHz)
5. h1maxa = frequency at maximum amplitude (kHz)
6. tcmax = time to reach maximum call amplitude (ms, relative to beginning of call)
7. curvature (ranges between 0 and 1) = describes the shape of the call; a measure of the drop in the call from the starting frequency compared to a linear curve with the same starting and ending frequency

vocalizations used for echolocation. Additionally, there is recent evidence from work on *Rhinolophus* and *Myotis bechsteinii* that suggests echolocation calls may be a source of information for individual recognition (Siemers et al. 2005; Siemers and Kerth 2006).

During the fall and winter, big brown bats (*Eptesicus fuscus*, Vespertilionidae) congregate at the hibernacula and engage in mating activity. In these conditions of little to no light, it has been suggested that vocalizations would be important for sex recognition (Kazial and Masters 2004). One way to assess the functional significance of sexually dimorphic vocalizations is to measure vocalizations used in different situations. We hypothesize that the ultrasonic call components of big brown bats are sexually dimorphic in the mating season when they are being used in a social context. To test our hypothesis, we investigated if sexual dimorphism in the ultrasonic call characters of big brown bats was context dependent. We recorded the ultrasonic vocalizations of adult male and female big brown bats to test whether components of the signal would differ when bats were flying in the field (i.e., echolocating) versus when bats were roosting in a laboratory colony setting (i.e., social context).

MATERIALS AND METHODS

Study species.—Big brown bats were selected as the model species because they are locally abundant, thrive in captivity, and can be observed year-round. Relatively little is known about the mating system of big brown bats, but they, as well as other species of vespertilionids, have been suggested to be promiscuous and random maters, with males and females having multiple partners (Fenton 1984; Thomas et al. 1979; Wai-Ping and Fenton 1988). Big brown bats exhibit a dissociated pattern of reproduction, mating in the fall and winter months while the gonads are regressed (males) or in stasis (females—Oxberry 1979).

All bats used in this study were adult wild-caught males and females from Alabama and Georgia that had been acclimated to captivity for months previous to the experiment (see description below). All bats were individually tagged with wing bands, and implanted with passive integrated transponder tags for identi-

fication. Bats were maintained on a diet of mealworms and water ad libitum, supplemented with vitamins and minerals. Methods used in this study met guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). All protocols were approved under Auburn University Institutional Animal Care and Use Committee #2005-0827.

Bats were collectively housed in environmentally controlled chambers (2.1 × 4.0 × 2.1 m; EJS Limited, Inc., Stafford, Texas), and allowed to interact at will during the mating season. The environmental chambers allowed researchers to alter temperature and photoperiod in order to induce desired behaviors or conditions. During spring and summer months, chambers were kept at 23°C with natural photoperiod. In November, bats were exposed to periods (e.g., 4 days) of low temperature (5°C) to induce hibernation, followed by 4- to 7-day periods of 23°C. The alternating temperature pattern ends in early spring (March–April). This temperature regime has been documented to stimulate a high degree of mating behavior and mimics the natural seasonal variation during these months in Alabama (Mendonça et al. 1996; Mendonça and Hopkins 1997).

Recordings.—Each bat was recorded individually in an experimental anechoic chamber and while flying outside on a tether line. Ultrasonic calls were detected with a U30 bat detector (sensitivity typically 10 dB sound presseure level [SPL] at 50 kHz; >20 dB SPL at 20–120 kHz; Ultra Sound Advice, Inc., London, United Kingdom). A custom-built 6× amplifier (designed by W. M. Masters, The Ohio State University, Columbus, Ohio) was used to amplify output of the microphone. We used CBDisk (Engineering Design, Belmont, Massachusetts) to acquire high-frequency signals direct-to-disk at sample rates up to 330 kHz. We used a Toshiba laptop with a Pentium III processor, 128 MB of RAM, and a 6.4 GB hard disk (Toshiba Corporation, Tokyo, Japan). The recordings consisted of a single file that contained many different calls. The calls were extracted from the large files so they could be analyzed individually (Burnett and Masters 2001). The individually extracted calls were analyzed with a custom program written in Matlab (version 6.5; Mathworks, Inc., Natick, Massachusetts) that automatically measures the value of different variables that are used to describe the calls (e.g., duration, starting frequency, etc.; a complete description is provided by Burnett and Masters [2001]). After individual calls were extracted and analyzed by the Matlab program, we picked 7 variables (Table 1; Fig. 1) that are direct descriptors of the call and have been used to discriminate individual bats (Burnett et al. 2001). The requirements for choosing calls were as follows: signals must be loud enough to start computer acquisition, signals must be of sufficient length (>0.2 ms) to be useful, and signals must contain enough information to determine the value for the 7 descriptors (a complete description is provided by Burnett et al. [2001]).

Experiment 1: ultrasonic signals used when roosting in an anechoic chamber.—We recorded individual adult male and female bats in the 2003 ($n = 8, 8$, respectively) and 2004 ($n = 12, 14$, respectively) reproductive seasons (January–March). There were no significant differences in calls between
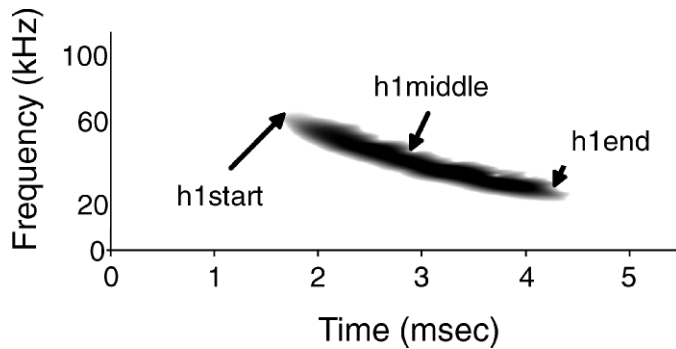
Fɪɢ. 1.—Sonogram of a typical ultrasonic call of a big brown bat (*Eptesicus fuscus*) roosting in an anechoic chamber. Three of the 7 call descriptors are labeled for descriptive purposes (see Table 1 for description of h1maxa, tcmax, and curvature).

Fɪɢ. 2.—Tether line system for recording bats flying in the field.

years (see below); therefore, data were combined for analysis. To obtain accurate ultrasonic signals under controlled conditions, bats were recorded while in an experimental anechoic room (2.1 × 2.1 × 2.1 m). The experimental room was lined on the walls and ceiling with 12.7-cm-thick mattress foam, and covered with 5.1-cm-thick corrugated mattress padding. The floor was covered with 5.1-cm-thick corrugated mattress padding. Bats were placed individually in the experimental room, and we recorded the ultrasonic vocalizations that each individual made during the experimental period. Bats were recorded before peak feeding times because prior feeding tends to decrease activity level and calls (Boughman and Wilkinson 1998). Bats were introduced to the experimental room and given a brief period of time (~10 min) to acclimate. When a bat began emitting ultrasonic vocalizations from a stationary position, we oriented the microphone to face the bat. We recorded at least 5 files, 8 s in duration, from each bat (therefore, each bat was recorded for at least 40 s) to attain at least 150 calls for each bat. Data from these calls were averaged, and mean values were used in the statistical analyses.

Experiment 2: ultrasonic signals used when flying.—In the 2004 reproductive season (January–March), we recorded a different set of big brown bats (16 adult males and 16 adult females) while flying singly on a tether line in the field (Szewczak 2000). The field was located on the Auburn University campus next to the bat-housing facility. The bat-housing facility is located in an open field (100 × 200 m) with a woodline running along the southern and western borders. The tether line system provided the opportunity to acquire seminatural field recordings of the bats' ultrasonic vocalizations while flying. Recordings took place just after sunset when bats would typically be leaving the roost to forage. The tether line consisted of a 25-m run line with a 10-m zip line (Fig. 2). The 25-m run line was placed running north to south (30 m from the bat-housing facility and 45 m from the southern woodline) because preliminary trials showed that bats consistently flew in this direction. Therefore, we placed the microphone and recording apparatus at the southern end of the 25-m run line to record the echolocation signals emitted by the subject. A collar was placed around the bat's neck and then
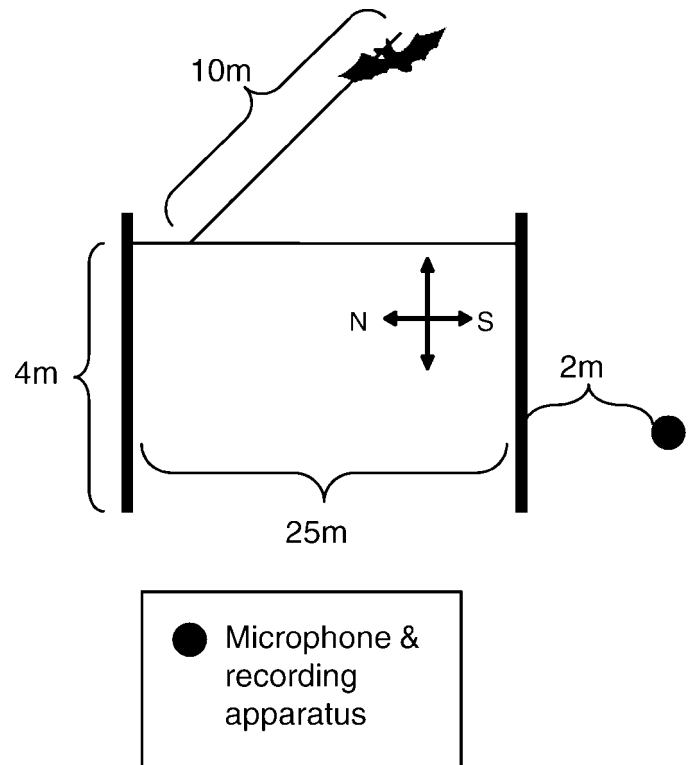
attached to the 10-m zip line. All bats were released from the same point and facing the direction of the recording microphone. Bats were hand released and recorded while flying alone. We recorded at least 5 files, 15 s in duration, from each bat (therefore, each bat was recorded for at least 75 s) to attain at least 150 calls for each bat. Data from these calls were averaged, and mean values were used in the statistical analyses.

Data analysis.—We measured 7 call descriptors (Table 1) that have been used to discriminate individual bats (Burnett et al. 2001). Then, we performed a principal component analysis on the mean call values (i.e., calls from an individual bat were averaged) from all the bats used in the study (i.e., anechoic chamber and flying recordings: $n = 36$ from males, $n = 38$ from females) because it was likely that certain call characteristics were correlated, and because this analysis allowed us to reduce the number of call variables into a more manageable number. We used the 75% variance rule as our extraction criteria for the number of components that would be retained in the principal component analysis. Bat calls recorded in the anechoic chamber were collected in 2 years; therefore, we tested if call components obtained in 2003 and 2004 differed. We used separate analyses of variance (ANOVAs) on the components of the principal component analysis to test for variation in the ultrasonic vocalizations associated with sex and situation. Separate ANOVAs were justified because principal component analysis axes are independent (Johnson and Wichern 1988). We conducted statistical analyses using StatView for Windows (version 5.0.1; SAS Institute Inc., Cary, North Carolina).
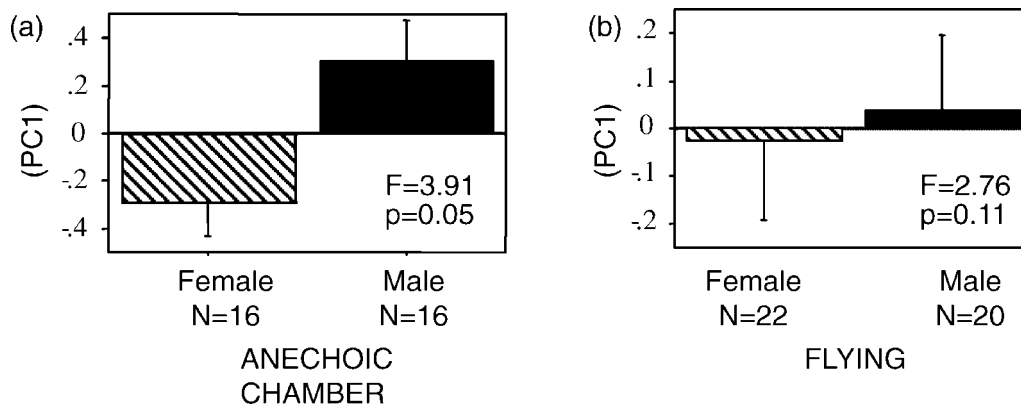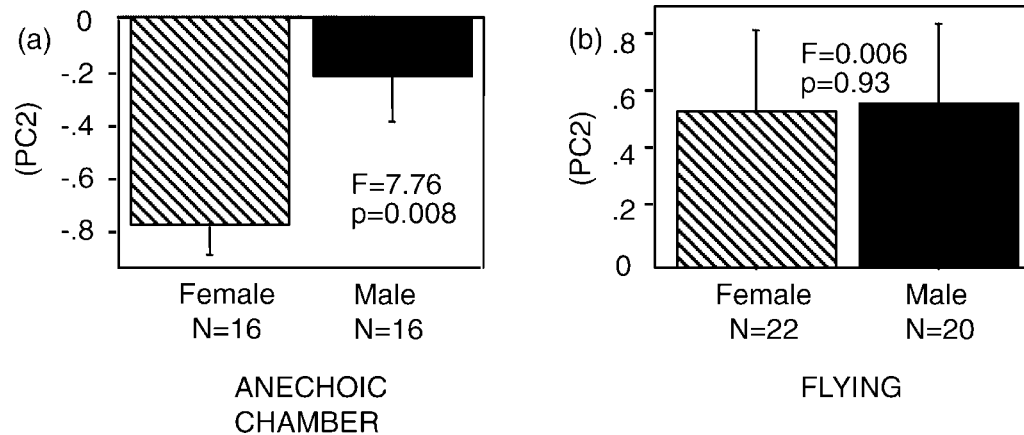

FIG. 4.—Comparison of mean ultrasonic time components (PC2) for male versus female big brown bats (*Eptesicus fuscus*) a) when signals were used in a social context compared to b) when signals were used for navigation. Error bars represent the standard error.

than females (Grilliot 2007). The appearance of the dimorphism only within the mating season suggests it has a reproductive role. Seasonal variation in vocal behavior is known to play a role in mating behaviors in a variety of taxa (Bosch et al. 2002; Catchpole 1982). In the non-mating season, female big brown bats form maternity colonies, whereas males form separate colonies, so interaction between males and females is limited. In the mating season, male and female big brown bats congregate at the hibernacula to engage in reproductive behaviors and to hibernate. If ultrasonic vocalizations are useful in a social context, then sexual dimorphism in vocalizations should be important at this time of year.

Additionally, in the roosting situation, the fact that calls of males showed significantly greater values than calls of females for PC2, the component associated with time or shape, suggests that the signals are not being used for echolocation. Bat species that process echoes in the time domain (i.e., frequency-modulated, low-duty-cycle species) must limit the duration of their echolocation signal to prevent overlap of the emitted pulse and returning echo. For these bats (big brown bats included), overlap of the emitted pulse with the returning echo must be avoided because of neural mechanisms used for interpretation of the echoes (Jones 1999). We suggest that the signal used in the roosting situation is being used for social purposes, and this would eliminate the need to interpret the returning echo. Thus, when bats are flying, and males and females presumably are using their signal for similar purposes (i.e., navigation), there is no apparent difference in ultrasonic components related to time or shape (PC2). However, when bats are roosting, we found that ultrasonic components related to time or shape differed significantly between sexes, and that females decreased time or shape components more than males. Although the components were shorter for both sexes when the bats were roosting than when the bats were flying, the signal produced by males was still longer and possibly more expensive than that of females. Therefore, these differences in call components might not only identify the sender as male, but have the potential to provide information on the condition or reproductive status of the male.

Because males do not decrease variables associated with time or shape and frequency to the same extent as females

when roosting, males may be incurring an energetic cost. For example, cost of producing echolocation calls can be 9.5 times the resting metabolic rate (Speakman et al. 1989). In addition, for some taxa (particularly birds and amphibians), increasing duration, frequency, call rate, or a combination of these proportionally increases energy expenditure (Andersson 1989; Eberhardt 1994; Ryan 1988; Taigen and Wells 1985). During winter hibernation (i.e., mating season), one would expect bats to conserve energy as much as possible. Therefore, when ultrasonic signals are being used for navigation and foraging, the signals are nondimorphic and utilitarian. When ultrasonic vocalization is being used in a social context, it may be that producing more energetically expensive signals suggests to a potential female that a male is of high quality and may be a suitable mate.

Our study provides support for the possibility that ultrasonic vocalizations commonly regarded to function in navigation and foraging also may play a role in social communication. There is considerable variation in the sonic and ultrasonic vocalizations both within and between species, and signals that are acoustically variable have the potential to communicate a great deal of information concerning the sender and the situation (Fenton 1985). Furthermore, the noticeable differences in call structure between species suggests that bats might recognize when conspecifics are present (Fenton 1985) and studies indicate that the ultrasonic vocalizations emitted by one bat could be heard by other individuals at moderate distances (5–15 m) as in a roost situation (Barclay 1982; Obrist 1995). Additionally, Kazial and Masters (2004) found that female big brown bats could differentiate between the echolocation signals of male and female bats, although they did not observe a sexual dimorphism in call characteristics (in that study, animals were not housed communally nor under reproductively stimulatory conditions). If a variety of information is carried in the ultrasonic vocalizations of bats, then the signals could function in a social context.

Our results indicate that there is contextual variation in the ultrasonic vocalizations of male and female big brown bats in a manner that suggests that this variation is used to signal the sex of the bat and, potentially, some aspect of male condition or quality. Indeed, further experiments using playbacks confirm

differential response between sexes to specific components of the ultrasonic vocalization (Grilliot 2007). The evidence from our study suggests that there are acoustic differences between the vocalizations of male and female big brown bats, but much work remains to be done in regards to the function of high-frequency vocalizations in situations other than for navigation and foraging.

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