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Aggressive thresholds in *Dendropsophus ebraccatus*: habituation and sensitization to different call types

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Abstract Males in many chorusing anuran species use aggressive calls during defense of calling spaces from other males. The minimal intensity of another male's vocalizations that elicits an aggressive call response has been termed the aggressive threshold. Previous studies of aggressive thresholds have shown that they are plastic: males habituated (increased their aggressive thresholds) in response to repeated presentation of stimuli above initial threshold levels. Habituation likely contributes to the stable chorus structure of these species, in which aggressive calling is rare compared to advertisement calls. I have observed high levels of aggressive calling in the treefrog *Dendropsophus ebraccatus*, suggesting that males of this species do not habituate. In this study, I investigated the plasticity of aggressive thresholds in *D. ebraccatus*. I measured the aggressive thresholds of males before and after suprathreshold stimulation by both advertisement and aggressive calls. I found that the different call types had different effects: males habituated to advertisement calls but lowered their aggressive thresholds in response to aggressive calls. I consider the latter response to be an example of sensitization, a behavior that has been documented infrequently in vocalizing anurans. Sensitization is a plausible mechanism responsible for the high levels of aggressive calling observed in this species. Given the high costs of aggressive calling, however, it is unclear why a mechanism that increases aggressive call output would be maintained.

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Introduction

Many of the social interactions of anuran amphibians are mediated by acoustic communication. Anuran acoustic communication often takes place in the context of the chorus, in which males gather, sometimes at great densities, and vocalize to attract females. Studies of calling males have revealed that many properties of their vocalizations are plastic with respect to various aspects of the social environment (Wells 1988). Males face steep competition from other males when calling to attract females and modify various aspects of their calls including the timing, complexity, rate, duration, frequency, and type of call given in response to such competition (e.g., Rand and Ryan 1981; Wells and Schwartz 1984b; Wells and Taigen 1986; Lopez et al. 1988; Bosch and Marquez 2001; Schwartz et al. 2002). These changes have consequences that affect female choice and, in some cases, may also function to repel rival males (Schwartz 1986; Wagner 1989a; Wells 1989). Thus, understanding how and why males alter their own vocalizations in response to vocal competition is a key to understanding the structure of choruses and male mating success in the complex chorus environment.

One aspect of vocal alteration that has received relatively little attention is the use of different call types by males. In particular, males in many species, in addition to giving advertisement calls that attract females and serve a role in male–male competition, also have a distinct aggressive call (=encounter call, Wells 1977). Aggressive calls are used by males at close proximity to other males and function to mediate male–male interactions. The precise message

conveyed by such signals and the mechanisms used by competitors to assess such calls are largely unknown (Wells 2007; but see Wagner 1989b, 1992; Burmeister et al. 1999).

In territorial species, aggressive calls serve to identify the territory's owner and defend territory boundaries (Wiewandt 1969). Many species of frogs, however, are non-territorial and instead defend a loosely defined calling space whose location and size can vary within and between nights depending on such factors as the density of calling males in the chorus (Telford 1985; Gerhardt et al. 1989). Aggressive calls appear to be used in the defense of calling spaces to repel intruding males. The ability of a male to maintain a calling space relatively free from interference may improve his mating success because interference from other calling males in a dense chorus can severely reduce the attractiveness and localizability of calls to females (Schwartz 1987; Grafe 1996; Wollerman 1999; Martínez-Rivera and Gerhardt 2008).Thus, the use of aggressive calls in mediating male–male interactions is an important factor that affects male mating success and that must be understood to gain a complete understanding of female mate choice in chorusing frogs.

When males defend a calling space there is presumably a boundary between tolerance and intolerance of other calling males. Males are likely to primarily assess their competitors through acoustic signals, and spacing appears to be mediated by the intensity of neighbors' calls (Brenowitz et al. 1984; Telford 1985; Wilczynski and Brenowitz 1988). Thus, the boundary can be measured in terms of the loudness of calls from neighboring males that a male is willing to tolerate. In the anuran literature, such a boundary has been termed an aggressive threshold (Lopez et al. 1988; Rose and Brenowitz 1991), which is a measure of the minimum amplitude (in decibels, sound pressure level (SPL)) of a neighboring male's call that elicits an aggressive call from the subject male.

Aggressive thresholds have been measured in several species and are well studied in some chorus frogs of the genus *Pseudacris* (Hylidae; Brzoska 1982; Brzoska et al. 1982; Robertson 1984; Telford 1985; Lopez et al. 1988; Brenowitz 1989; Rose and Brenowitz 1991, 1997; Brenowitz and Rose 1994; Marshall 2003; Marshall et al. 2003). In addition to simple measurements of thresholds in response to different call types, further observations demonstrated that the thresholds themselves are plastic in response to changes in the local chorus environment. Thresholds in *Pseudacris regilla* and *Pseudacris crucifer* were positively correlated with the density of the chorus (Rose and Brenowitz 1991; Marshall et al. 2003). In addition, these experimenters actively altered male's thresholds by broadcasting stimuli louder than their initial thresholds to them for a period of time. This resulted in a significant increase in aggressive threshold to the presented call type after a relatively brief

period of stimulation (Brenowitz and Rose 1994; Rose and Brenowitz 1997; Marshall et al. 2003). Thus, males appear to become more tolerant of neighboring males in denser choruses.

Previous studies of the plasticity of aggressive thresholds have involved measuring threshold change in response to repeated stimulation. It is useful to examine such studies in the context of the dual-process theory of habituation (Bee 2001; Marshall et al. 2003), which proposes a general mechanism for behavioral changes in response to repeated stimulus presentation (Groves and Thompson 1970; Thompson et al. 1973). The observed behavioral response to such stimulation is hypothesized to be a summation of the independent processes of habituation and sensitization. Habituation is characterized by a decreased response to repeated stimulation, while sensitization is characterized by an initially increasing, and later decreasing, response to repeated stimulation (Thompson and Spencer 1966). Thus, previous studies demonstrating an increase in aggressive thresholds following suprathreshold stimulation are demonstrations of short-term response habituation (Brenowitz and Rose 1994; Rose and Brenowitz 1997; Marshall et al. 2003). Response sensitization has been documented infrequently in anurans (Bee 2001) and never in the context of the plasticity of aggressive thresholds.

Aggressive thresholds and their plasticity have been measured in a limited number of anuran species, and it is unclear whether habituation is a universal response to suprathreshold stimulation in chorusing anurans. Aggressive calling is infrequent in the choruses of anurans for which habituation has been demonstrated. In some anuran species, however, aggressive calls are relatively frequent, which suggests that these species do not habituate to the same degree or in the same manner. I have observed very high levels of aggressive calling during field studies of *Dendropsophus ebraccatus* (formerly *Hyla ebraccata*), a Neotropical hylid. In my primary study population in Gamboa, Panama, the density of calling males is extremely high, and males readily and frequently give aggressive calls.

The goal of this study was to examine the plasticity of aggressive thresholds in *D. ebraccatus* and to relate this to the high levels of aggressive calling observed in this species. In order to quantify the overall frequency and temporal distribution of aggressive calls, I analyzed recordings of spontaneous male calling made at different times of night. I also determined whether or not male *D. ebraccatus* habituate to conspecific calls as has been observed in other species. I used playbacks to make measurements of males' initial aggressive thresholds to both advertisement and aggressive calls. I then examined the plasticity of these thresholds by measuring them a second time following the broadcast of suprathreshold stimuli. I predicted that males

would not habituate to all stimuli. Males may not only fail to habituate but also in fact may be sensitized by supra-threshold stimulation. The dual-process theory suggests that response sensitization will occur when a stimulus is so strong in eliciting a response that the contribution of the sensitization process outweighs that of the habituation process. Thus, I predicted that if a sensitization response was observed, it would be more likely to occur in response to the presumably stronger aggressive call stimulus.

Methods

Study site and species

D. ebraccatus is a small treefrog common throughout much of Central and South America. Most calling and breeding occurs in ponds and marshes during the rainy season, which takes place from approximately late May through November at my study sites in Gamboa, Panama. I utilized two sites, a small pond and a flooded field, that were located within 1 km of each other. I did not directly measure male density, but densities can be extremely high at both locations, with males often calling within 10 cm of each other in the most concentrated areas (personal observation). Playback experiments took place nightly at the time of the most intense calling activity (2000–2400 hours) in July and August of 2007 and August of 2008.

Males have distinct advertisement and aggressive calls, which have been described in detail in several studies (Wells and Greer 1981; Schwartz and Wells 1984; Wells and Schwartz 1984a, b; Wells and Bard 1987; Wells 1989; Wollerman 1998). The advertisement call consists of a long introductory note to which shorter click notes may be appended (Fig. 1a). The introductory note of the advertisement call is pulsed, and the pulse repetition rate is static and is approximately 95 pulses/second (Wells and Schwartz 1984a; Wollerman 1998). Relatively isolated males often give calls that lack click notes; as the level of acoustic competition increases, males begin giving calls with click notes more frequently (Wells and Schwartz 1984a). The number of click notes per call is almost always between one and four. Females prefer advertisement calls with click notes to calls without clicks (Wells and Schwartz 1984a). The dominant frequency of the call is approximately 3 kHz, and there are no other major harmonics.

The spectral characteristics of aggressive and advertisement calls are similar, but aggressive calls have a higher and more variable pulse rate, ranging between 200 and 500 pulses/second (Fig. 1b). There is gradation in many characters of the aggressive call that seems to be associated with the level of escalation (increase in intensity) of the conflict, but this gradation does not encompass the range of
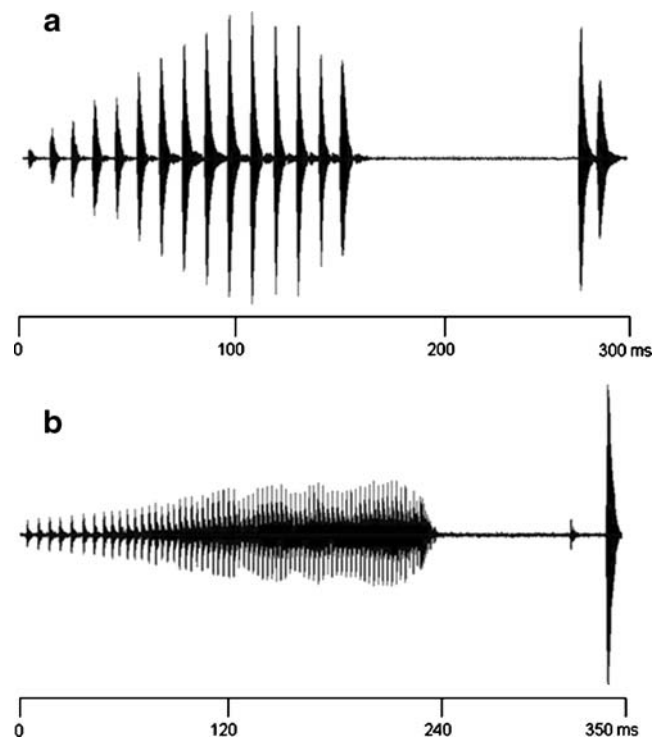
Fig. 1 Waveform displays showing change in amplitude over time (in ms) of typical *D. ebraccatus* advertisement and aggressive calls. **a** Advertisement call with long introductory note and a single click note. **b** Aggressive call with single click note typical of a highly escalated interaction. As level of aggression escalates, aggressive calls increase in duration, while decreasing in pulse rate and number of click notes (Wells and Schwartz 1984b)

the advertisement call. In playback tests, when males are challenged with a louder simulated competitor, they respond by increasing the duration and decreasing the pulse rate and number of click notes of the aggressive call (Wells and Schwartz 1984b; Wells 1989). Although aggressive calls are common, physical fights are rare and appear fairly benign (personal observation). Aggressive calls have been shown to be less attractive to females than advertisement calls in this species (Wells and Bard 1987).

Frequency of aggressive calling

Observations of the *D. ebraccatus* chorus suggested that males give high levels of aggressive calls throughout the nightly calling period. In order to quantify this, I analyzed recordings of spontaneous male calling I had made prior to this experiment. Recordings were made in the field during June and July of 2006 and July of 2007, from the same population described in this study. I used a Marantz PMD-660 digital audio recorder and a Sennheiser SE-67 directional microphone to record approximately ten calls from each male (*n*=104). Density of the chorus was not

noted, but the recordings encompass the variation in chorus densities observed nightly and over the breeding season. I analyzed recordings using a computer program (Audacity 1.2.4) and counted the number of advertisement and aggressive calls in order to calculate the proportion of each male's calls that were aggressive calls. I also noted the time of night at which the recording was made so that I could examine how the level of aggressive calling changes within the nightly calling period.

Playback stimuli

I broadcast synthetic calls representative of different *D. ebraccatus* call types to males in order to measure their aggressive thresholds. Single calls (16-bit digital files with a sampling rate of 20 kHz) were synthesized using a program provided by J. Schwartz. Pulses of both call types were shaped with linear rise and fall times constituting 45% each of pulse duration, and pulse duty cycle (ratio of pulse duration to pulse period) was 50%. Two basic call types were used: a typical advertisement call and an aggressive call with properties typical of a high level of aggression (see above). Parameters of the synthetic stimuli were based on computer analysis of calls from my previous recordings (Audacity 1.2.4) and published work describing the call characters of males from this population (Wells and Schwartz 1984a, b). Temporal and spectral parameters of the synthetic stimuli, along with average values of these properties from recordings made at the study site, are given in Table 1. There is little variation in nightly temperature at the study sites; thus, it was not necessary to correct any of the features of the stimulus calls to account for changes in temperature.

The playback stimuli were created by repeatedly copying and pasting the calls in an audio-editing computer program (Cool Edit Pro 2.0, Syntrillium 2002) to create the appropriate spacing between calls. Both advertisement and aggressive calls were repeated with a call period of 6 s, which approximates the typical *D. ebraccatus* rate of calling. I used the program's amplification function to decrease the SPL of the stimuli in steps of 2 dB. I then arranged these stimuli on a single track so that there was a 2-dB increase in amplitude every 30 s. The tracks used for the playbacks ultimately increased in amplitude by 40 dB. By gradually increasing the intensity over time, I was able to measure the male's aggressive threshold as the intensity at which he first gave an aggressive call. Stimuli were recorded onto a compact disk for playback in the field. I used a portable sound-level meter (Radio Shack 33-2055) to confirm that the stimuli broadcast from the playback system reproduced the range of SPLs and increase in SPL over time generated by the computer program.

Playback procedure

I performed playbacks on calling males in the field. Because male *D. ebraccatus* repeatedly enter into aggressive calling interactions with neighbors, I only performed playbacks on males that were either naturally calling in relative isolation or on males that I transported from denser areas of the chorus to isolated areas. Any male that was moved from a denser chorus was allowed to call for at least 15 min in isolation prior to testing. As males generally enter into aggressive calling interactions much more often than every 15 min (personal observation), I considered this time interval sufficient to allow males' calling behavior to recover from the effects of being in a dense chorus. Performing playbacks with isolated males was necessary to ensure that any aggressive response was to the playback stimulus and not to a neighboring male, but precluded comparisons of aggressive thresholds and chorus density. Although aggressive interactions tend to be less common in low-density areas than in very dense choruses, they are still frequent. In addition, aggressive calling interactions escalate to high levels even in interactions between otherwise isolated males (personal observation). Thus, I consider my choice of an aggressive call stimulus typical of a highly aggressive male to be reasonable.

Playback stimuli were broadcast from a portable compact disk player (Panasonic SLSW940S) through a battery-powered amplified speaker (Saul Mineroff Electronics, SME-AFS) mounted on a tripod. The speaker was adjusted such that the amplitude of the loudest call broadcast from the speaker would have an SPL of 95 dB at 1 m, measured by the portable sound-level meter. I was unable to consistently position the speaker 1 m from the male due to variation in elevation of males at their calling sites and water depth, so that the actual SPLs of the loudest calls that could have been presented to each male varied from 89 to 100 dB SPL at the focal male's position. The initial SPL of playback presentation was 40 dB less than the loudest call that could have been presented. This is presumably near the threshold of hearing in *D. ebraccatus* (Wilczynski et al. 1993), and I considered it unlikely that males' aggressive thresholds would be at a lower SPL than that of the initial playback presentation in most cases.

A series of playbacks was used to measure each male's aggressive threshold and response to suprathreshold stimulation to the advertisement and aggressive call stimuli. All males in the dataset presented here received both the aggressive call and advertisement call playbacks, presented in random order. It could be argued that males' responses to the playback of a second call type could be altered by their having already experienced a playback with a different call type. I thus allowed a 15-min timeout period between playbacks of the two different call types. I used statistical tests to ensure that any effects of order on the magnitude

Table 1 Properties of synthetic stimuli used in playback experiments

	Synthetic stimuli		Natural calls		
	Advertisement	Aggressive	Advertisement average	Aggressive average	Aggressive range
Total call duration (ms)	301	427	245.2 (50.0)	350.7 (70.6)	138–587
Introductory note duration (ms)	175	300	169.8 (18.4)	143.7 (32.8)	79–399
Pulse number	17	72	16.4 (1.6)	49.5 (17.4)	19–122
Pulse rate (pulse/second)	97.1	240	96.9 (3.3)	340.0 (112.6)	202–565
Dominant frequency (KHz)	3.1	3.1	3.05 (0.13)	3.04 (0.22)	2.54–3.51
Number of click notes	1	1	0.60 (0.35)	1.63 (0.54)	0–4

For comparison, the average values of these call parameters from recordings made in 2007 of naturally calling males are also shown (advertisement call, $n=67$; aggressive call, $n=38$; columns show mean (standard deviation)). Values chosen for the call properties were based on previous recordings and were designed to represent a typical advertisement call and an aggressive call characteristic of a highly escalated interaction. Many of the characters of aggressive calls are graded (see text for details) and mean values do not correspond to those of a highly aggressive call. Thus, I also show the range of values for these call parameters in recordings of natural aggressive calls

of aggressive thresholds or their direction of change following suprathreshold stimulation were accounted for (see below).

The playback methodology followed that of Brenowitz and Rose (1994). Three playback sessions per stimulus were performed with each male. The first measured his initial aggressive threshold to either the advertisement or aggressive call. I broadcast the appropriate call type, which increased in SPL in steps of 2 dB every 30 s. I stopped the playback at the point at which he first gave an aggressive call. The SPL at which this occurred was his "initial aggressive threshold." Immediately following this playback, I presented the male with a second playback consisting of a suprathreshold stimulus. This allowed me to determine how males' aggressive thresholds change in response to calls broadcast above their initial threshold. This playback consisted of the same call type being broadcast at an SPL of 4 dB SPL greater than the initial threshold for 5 min, followed immediately by one minute of the same call at 8 dB SPL above the initial threshold. The final playback was a remeasurement of the aggressive threshold using the method described above. I again noted the point at which the male first gave an aggressive call and the SPL at which this occurred was his "final aggressive threshold." Following a timeout period of 15 min, I repeated these three steps with the other call type. Following playbacks, I measured the SPL of the playback at the position of the frog. I collected males for weighing and measuring and gave each male a unique toeclip to ensure individual identification.

In order to be included in the dataset, males were required to give an aggressive call in response to both attempts to measure his aggressive threshold for at least one call type. Some males ($n=6$) responded to one call type but ceased calling or moved away during the playback of the second call type. These data are included for statistical comparisons within a given call type but are not included for comparisons between the two call types. Occasionally ($n=3$) males instantly gave an aggressive call at the lowest playback level presented. I did not consider this to be his aggressive threshold but instead immediately ceased playback for 5 min and resumed playback at a lower amplitude.

Statistical analysis

Descriptive statistics of sound pressure levels were calculated from the absolute sound pressures in µPa (0 dB SPL= 2×10^{-5} Pa) rather than relative sound pressures in dB SPL because the dB scale is logarithmic. Means and standard errors of aggressive threshold values were calculated from these converted absolute pressure measurements. These values were then reconverted to the dB scale, resulting in standard errors asymmetrical about the mean. Nonparametric inferential statistics, however, were calculated from the unconverted relative dB SPL values.

The primary focus of this study was to determine whether there was a difference in aggressive thresholds measured prior to and following suprathreshold stimulation for the two call types. I used Wilcoxon matched-pairs signed ranks (WSR) tests to test the null hypothesis that there is no difference in the magnitude of aggressive threshold levels before or after suprathreshold stimulation. Sign tests were used to test the null hypothesis that there is no difference in the number of positive and negative deviations when the difference between the final and initial thresholds was calculated. A consistent trend for final aggressive thresholds to be larger than initial aggressive thresholds would constitute evidence for habituation.

I was also interested in testing whether initial aggressive thresholds differed depending on whether the playback

stimulus was an advertisement or aggressive call. I used a Wilcoxon matched-pairs signed ranks test to test the null hypothesis that there is no difference in the magnitude of aggressive threshold levels in response to the aggressive and advertisement call stimulus. I only compared initial aggressive thresholds for this test. Finally, I examined the effect of playback order and tested whether males responded differently depending on whether they were first exposed to the aggressive call or the advertisement call playback. I used Wilcoxon–Mann–Whitney (WMW) tests to compare the initial aggressive thresholds and any change in aggressive thresholds to the two different call types between the different playback orders. Statistical tests were performed with SPSS 16.0.1 (SPSS Inc., 2007) software on a PC computer. All statistical tests were carried out at $\alpha=0.05$.

Results

Frequency of aggressive calling

Aggressive calls were given throughout the night. Although the modal proportion of aggressive calls in a recording was zero, the majority of recordings contained at least one aggressive call and the median proportion of aggressive calls was 12.1% (Fig. 2). In 17.3% of recordings, over 50% of males' calls were aggressive. A linear least-squares regression analysis showed that the proportion of calls which were aggressive declined significantly with time of night ($n=104$, $R^2=0.038$, $P=0.049$, $y = -1.527 \times 10^{-5}x + 1.407$, where x is the time in seconds from 0000 hours), although time of night only explained 3.8% of the variation in levels of aggressive calling (Fig. 3). The proportion of calls which were aggressive declined from 20.6% for recordings made
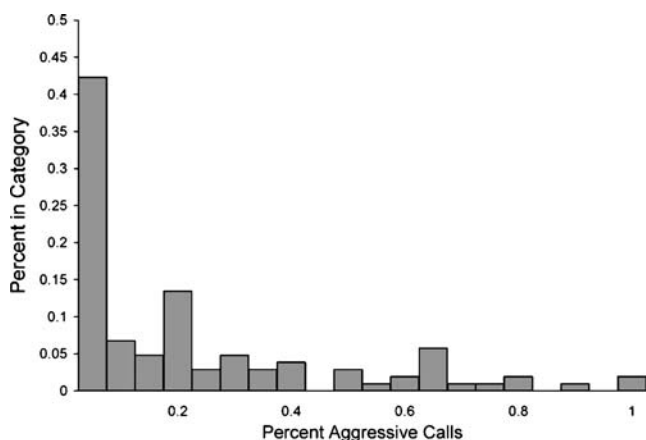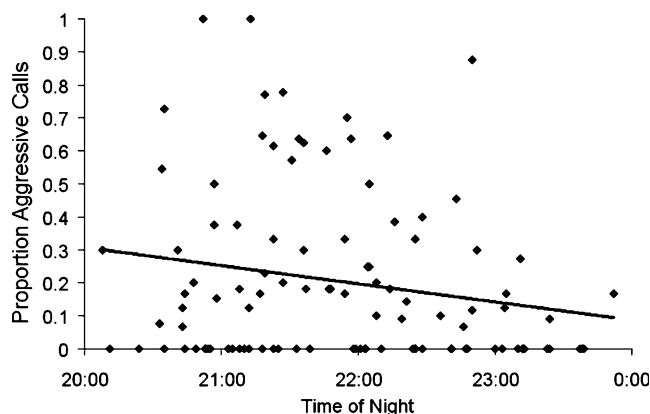
Fig. 3 Scatterplot showing the proportion of calls during baseline recordings that were aggressive calls versus time of night in which the recording was made. Trend line was generated by a linear least-squares regression
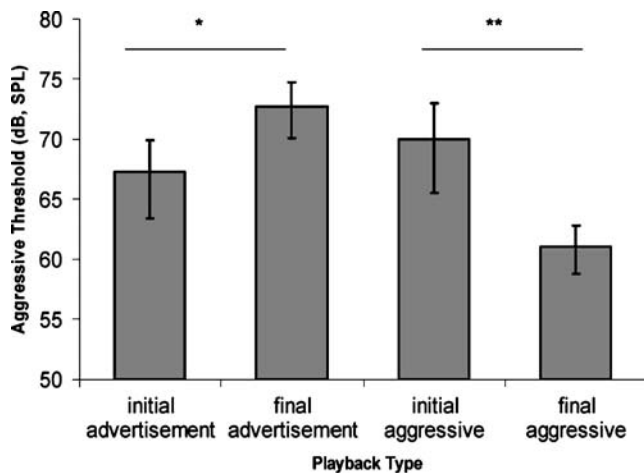
between 2000 and 2100 hours to 5.1% for recordings made between 2300 and 2400 hours.

Advertisement call playback

Males showed a habituation response following presentation of suprathreshold advertisement calls (Fig. 4). The mean initial aggressive threshold of males to the advertisement calls was 67.2 dB (+2.7, −3.9 dB). Following playback of suprathreshold advertisement calls, the mean final aggressive threshold of males to the advertisement call was 72.7 dB (+2.0, −2.6 dB). This increase in aggressive threshold following suprathreshold stimulation was significant (WSR, $n=24$, $z=-2.53$, $P=0.012$). Sixteen of 24 individuals increased aggressive thresholds in response to suprathreshold stimulation, and five individuals showed no change in threshold. The proportion of individuals habituating to suprathreshold advertisement calls was significant (Sign test, $n=24$, $P=0.027$).

Aggressive call playback

Males did not habituate to suprathreshold stimulation in the form of a highly aggressive call. In fact, males appear to be sensitized by aggressive calls: males significantly decreased their aggressive thresholds following suprathreshold stimulation by this call type (Fig. 4). The mean initial aggressive threshold of males to the aggressive call was 70.0 dB (+3.0, −4.6 dB). Following playback of suprathreshold aggressive calls, the mean final aggressive threshold of males to the aggressive call was 61.0 dB (+1.8, −2.2 dB). This decrease in aggressive threshold following suprathreshold stimulation was significant (WSR, $n=18$, $z=-2.63$, $P<0.01$). Fourteen of 18 individuals decreased aggressive thresholds in response to suprathreshold stimulation, and three individuals showed no

Fig. 2 Percentage histogram showing the proportion of calls given by males during baseline recordings that were aggressive calls. Bin size= 0.05, N=104

Fig. 4 Mean aggressive thresholds for each playback type (advertisement, $n=24$; aggressive, $n=18$). Means and standard errors were converted into dB, SPL after being calculated from absolute pressures in μPa (see text); therefore, standard errors are asymmetric about the mean. *Horizontal bars* show significant differences between initial and final aggressive thresholds for each call type. Wilcoxon signed ranks test, *$P<0.05$, **$P<0.01$. There was no significant difference between the initial aggressive thresholds to the advertisement and aggressive call (WSR, $P>0.5$)

change in threshold. The proportion of individuals sensitized by suprathreshold aggressive calls was significant (Sign test, $n=18$, $P<0.001$).

Initial thresholds to different call types

There was no evidence that the initial aggressive thresholds to advertisement and aggressive calls differed (WSR, $n=18$, $z=-0.524$, $P>0.5$; Fig. 4). Although males responded in quite different ways to suprathreshold stimulation by the two call types, their initial responses were similar.

Effects of playback order

Although direct experimentation is required to rule out effects of playback order (e.g., Brenowitz and Rose 1994), I found no evidence that a male's aggressive threshold to one call type was influenced by previous exposure to the other call type. There was no statistically significant difference in initial aggressive thresholds to either the advertisement (WMW, $n=16$, $n'=8$, $U=51$, $P=0.425$) or aggressive call (WMW, $n=12$, $n'=7$, $U=33.5$, $P=.471$) between the two playback orders. Furthermore, there was no statistically significant difference in the change in aggressive threshold to either the advertisement (WMW, $n=16$, $n'=8$, $U=54$, $P=0.539$) or aggressive call (WMW, $n=12$, $n'=7$, $U=35$, $P=0.924$) between the two playback orders. Thus, the responses of males to the two different playback types appear to be independent of playback order.

Discussion

High levels of aggressive calling were observed in *D. ebraccatus*. Repeated bouts of aggressive calling took place throughout the nightly calling period. With the exception of Stewart and Rand (1992), most descriptions of anuran calling in other species imply much lower levels of aggressive calling than that seen in *D. ebraccatus* (Wells 1988). Studies of gross temporal patterns of calling within the chorus suggest that for many species, aggressive calling primarily takes place in the initial stages of nightly chorus formation and calling stabilizes to nearly pure advertisement calling thereafter (Wells 1988). The gross temporal distribution of aggressive calling I observed in *D. ebraccatus* does not match this pattern (but see Wells and Bard 1987). Although levels of aggressive calling did decline as the night progressed, a relatively large proportion of male calls were aggressive throughout the night, even near the end of the nightly calling period.

Behavioral habituation is a decrement in response to repeated stimulation (Thompson and Spencer 1966). Habituation has been invoked as an explanation of the relative lack of aggressive calling following initial chorus formation that has been observed in many species (Marshall et al. 2003). In these species, males give aggressive calls in the early stages of chorus formation because neighbors are calling louder than their initial aggressive thresholds. Later in the evening, however, males habituate to the repeated calls of neighboring individuals, resulting in increased aggressive thresholds and little aggressive calling. This has been demonstrated convincingly in *P. regilla*: males show habituation responses to all call types (Rose and Brenowitz 1997), and choruses are stable, with only rare bouts of aggressive calling beyond the initial stages of chorus formation (Allan 1973). Because male *D. ebraccatus* continually give aggressive calls throughout the night, my primary prediction in this study was that males would not habituate to suprathreshold stimulation.

Indeed, male *D. ebraccatus* did not always habituate to suprathreshold stimulation. As with previous studies of aggressive thresholds, males did habituate to the advertisement call by increasing their aggressive thresholds following suprathreshold stimulation. In response to aggressive calls, however, males actually decreased their aggressive thresholds. I consider this an example of sensitization, an effect that has been documented infrequently in anuran communication (Bee 2001). I had predicted that response sensitization was more likely to be observed in response to the aggressive call stimulus. The aggressive call is a stronger stimulus in terms of eliciting an aggressive calling response: males are more likely to give aggressive calls in response to playbacks of aggressive calls compared to playbacks of advertisement calls (Wells and Greer 1981).

Thus, if response to repeated stimulation in this species follows the dual-process theory, the aggressive call, but not the advertisement call, presumably has a stronger effect on the sensitization process than on the habituation process, resulting in the sensitization response of a decreased aggressive threshold. This pattern of response may explain the extreme level of aggressive calling often heard in *D. ebraccatus* choruses, just as the habituation response has been suggested to be responsible for the lack of sustained aggressive calling in choruses of other species (Marshall et al. 2003). Clearly, studies on other species are needed to firmly establish this pattern, but I suggest that for species in which aggressive calling takes place frequently throughout the chorusing period, males are likely to be sensitized by aggressive calls and possibly to advertisement calls as well. However, as discussed below, it is unclear why males would have a mechanism that does not allow them to habituate to all call types.

Habituation in this scenario implies an increased tolerance to the stimulus that initially elicited an aggressive response after repeated, suprathreshold presentation of that stimulus. Although few studies directly measure the thresholds that evoke an aggressive response in frogs, several studies involving repeated stimulus presentation have found that males habituate over time and their aggressive response decreases in response to repeated presentation of a stimulus (Megela and Capranica 1983; Brenowitz and Rose 1994; Rose and Brenowitz 1997; Owen and Perrill 1998; Bee 2001; Marshall et al. 2003). In the most extensively studied species, *P. regilla*, males habituated to both advertisement and aggressive calls, although habituation was slow in response to aggressive calls presented at a very high magnitude (Brenowitz and Rose 1994; Rose and Brenowitz 1997).

Plasticity of aggressive thresholds in the form of habituation has been interpreted as an adaptive response to adjust levels of aggression to varying chorus densities. In particular, if thresholds were fixed, at high densities, males would repeatedly engage in aggressive interactions with nearby neighbors. This in turn could severely reduce a male's ability to attract a mate because females are less attracted or repelled by aggressive calls (Oldham and Gerhardt 1975; Schwartz and Wells 1985; Wells and Bard 1987; Backwell 1988; Grafe 1995; Brenowitz and Rose 1999). Thus, in situations where reductions in spacing are unavoidable, the ability to raise thresholds to neighboring males' calls allows males to balance the ability to defend their calling space while maximizing their time spent giving attractive signals to females.

A similar argument could be made for the response to advertisement calling I observed in male *D. ebraccatus*. In this species, aggressive calls are less effective in attracting females than advertisement calls (Wells and Bard 1987). In two-choice phonotaxis tests, females preferred advertise-ment calls to aggressive calls and although females showed phonotaxis towards aggressive calls in two-choice tests in which both alternatives were aggressive calls, they responded at a much lower rate to these tests than to tests involving advertisement calls (Wells and Bard 1987). The latter result indicates a lower general motivation to respond to aggressive calls. Therefore, habituation in response to advertisement calls in *D. ebraccatus* would seem a reasonable response to avoid the heavy costs in terms of reduced attractiveness to females of unnecessary extended aggressive calling interactions.

This explanation of habituation as an adaptive response to changes in chorus density does not hold for *D. ebraccatus* once other aspects of its call repertoire are examined. In response to the aggressive call, I observed a decrease in the aggressive threshold following suprathreshold stimulation relative to the initial threshold. Thus, suprathreshold aggressive calls have a sensitizing effect on males: they became more willing to engage in aggressive calling following exposure to loud aggressive calls. Sensitization has not been documented previously in studies of aggressive thresholds in anurans but has been described in the response of *Lithobates catesbianus* to repeated presentations of conspecific advertisement calls at constant intensity (Bee 2001). Similar sensitization responses have been observed in species in other taxa including three-spined sticklebacks, *Gasterosteus aculeatus* (Peeke 1982) and white-crowned sparrows, *Zonotrichia leucophrys* (Petrinovich and Patterson 1981).

Not only does the direction but also the absolute magnitude of the response to suprathreshold stimulation in *D. ebraccatus* differs from that seen in other species. When aggressive thresholds have been measured in other species, aggressive thresholds to aggressive calls were lower than those to advertisement calls (Robertson 1984; Lopez et al. 1988; Rose and Brenowitz 1991). In this study, by contrast, the initial aggressive thresholds to advertisement and aggressive calls in *D. ebraccatus* did not differ. Thus, male *D. ebraccatus* appear to have equal initial tolerances for advertisement and aggressive calls. Repeated suprathreshold stimulation may be necessary for differences in the aggressive response to aggressive calls relative to advertisement calls to emerge, particularly at the somewhat low sound pressure levels used in this study.

Male *D. ebraccatus* clearly are sensitized by exposure to conspecific aggressive calls, and this mechanism may partially explain why they are observed to engage in such high levels of aggressive calling. It is important to keep in mind that the responses described here as habituation and sensitization are short-term measures of the responsiveness to a single presentation of repeated suprathreshold stimulation. The strengths of the habituation and sensitization processes are known to change over time; in particular, the

sensitization process generally shows an initial increase but ultimately a decrease in response. The goal of this study was to measure short-term aggressive threshold plasticity, but in order to fully explain gross temporal patterns of the levels of aggressive calling, longer-term stimulus presentations will be necessary.

Nonetheless, my data suggest that complete response habituation does not take place and that sensitization is important in determining the level of aggressive calling in *D. ebraccatus*. This raises some difficult questions. First, if males are sensitized by aggressive calls, it is unclear how advertisement calling resumes once males begin giving aggressive calls. The cyclical nature of *D. ebraccatus* choruses may be a clue. The pattern of chorusing in this species can be roughly described as unison-bout chorusing, in which bouts of calling are separated by bouts of silence (Rosen and Lemon 1974; Whitney and Krebs 1975; Schwartz and Wells 1983; Schwartz 1991). Unison-bout chorusing has been well described in the closely related *Dendropsophus microcephalus*, although the use of different call types within call bouts was not described for this species (Schwartz and Wells 1983; Schwartz 1991). Within bouts of calling in *D. ebraccatus*, callers and the chorus as a whole typically progress from initially giving advertisement calls to almost entirely giving aggressive calls to silence (personal observation). The strength of sensitization is known to decay over time with the lack of stimulation (Thompson and Spencer 1966; Thompson et al. 1973). Thus, although it is not clear what cues these transitions, the period of silence should result in reducing the effect of sensitization, allowing males to resume advertisement calling when the chorus restarts.

The more difficult question is why males are sensitized by aggressive calls. Aggressive calling is unquestionably costly. Females are far less responsive to aggressive calls than to advertisement calls, so males would be expected to reduce their levels of unnecessary aggressive calling (Oldham and Gerhardt 1975; Schwartz and Wells 1985; Wells and Bard 1987; Backwell 1988; Grafe 1995; Brenowitz and Rose 1999). Aggressive calling may have additional energetic costs. Energetic costs of calling have been implicated as limiting factors in the ability of males to attract females in several species (Ryan 1988). Although the mechanism of call production and the energetic costs of calling are not known for *D. ebraccatus*, their aggressive calls have a similar structure and rate of production but are generally of longer duration than advertisement calls. Thus, it is possible to speculate that producing a typical aggressive call involves a greater calling effort than an advertisement call, although this will depend on whether the mechanism of amplitude modulation is shared by both call types. Absolute energy expenditure increases with call effort in a variety of frog species (e.g., Bucher et al. 1982;

Taigen and Wells 1985; Wells and Taigen 1986; Wells and Taigen 1989; Grafe 1996); therefore, the aggressive calling of *D. ebraccatus* could conceivably be more energetically expensive than advertisement calling. All speculation aside, even if aggressive calls are less costly than advertisement calls, they undoubtedly have nontrivial energetic costs, and I suggest that such calling is energetically costly because it reduces the energy budget available for advertisement calling that is far more effective at attracting females.

In addition to these costs, it is not clear that there are any strong benefits to aggressive calling in this species. In extensive field observations, I have observed very few adjustments in male spacing following an aggressive calling interaction. Both males generally resume normal advertisement calling after a short amount of time. Thus, aggressive calls appear ineffective in their presumed function of repelling rival males, although more subtle changes in calling behavior following an aggressive interaction have not been examined. Additionally, physical fights are extremely rare, of brief duration, and do not appear to entail a significant risk of injury to either combatant.

We do not understand the communicative significance of the aggressive call or its potential use in the resolution of disputes in this species, but based on the available evidence, it does not seem that males benefit from high levels of aggressive calling. Thus, it is difficult to explain why a sensitizing mechanism, which seems to increase the likelihood of aggressive calling, would be present. It is possible that the sensitization response is simply a consequence of the general stimulus–response system with the aggressive call being particularly effective at producing a strong change in state, as predicted by the dual-process theory (Thompson et al. 1973) and was not selected for its role in adjusting the aggressive thresholds of males to changing social conditions. A sensitization response to aggressive calls combined with high-chorus densities could conceivably result in the gross temporal patterns of aggressive call use observed in *D. ebraccatus*. The cyclical nature of chorusing may in part be an adaptation to allow sensitization to decay following the inevitable bouts of aggressive calling. Conceivably, this pattern could maximize, to the extent that is possible, the time spent advertisement calling. These conclusions would be improved by a better general understanding of the function of aggressive calling in this, and other, anuran species.

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