Territorial male bullfrogs (*Rana catesbeiana*) do not assess fighting ability based on size-related variation in acoustic signals

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Some animals use communication signals to assess their opponent's size and fighting ability during aggressive conflicts. Male frogs assess their opponent's size based on the fundamental frequency (pitch) of advertisement calls, which is negatively correlated with body size, an important determinant of fighting ability in frogs. I conducted a field playback experiment to investigate whether territorial male bullfrogs assess the size of opponents based solely on size-related variation in fundamental frequency. I repeatedly broadcast synthetic bullfrog advertisement calls to three groups of males. Playback stimuli simulated a large male ($n = 24$), a small male ($n = 24$), or an acoustically size-matched male ($n = 34$). Neither the simulated size of the opponent, the subject's own size, nor the degree of size asymmetry between the subject and simulated intruder had significant effects on the magnitude of responses during the playback test or on the rate of habituation that occurred with repeated stimulation. Post-hoc analyses of effect sizes and statistical power indicated that the effects in this study were quite small compared to previous studies in other frogs. More important, power analyses indicated that this study had high power ($1 - \beta > 0.90$) to detect the magnitude of effect sizes observed in previous studies. Thus, territorial male bullfrogs do not appear to assess an opponent's fighting ability based solely on the fundamental frequency of acoustic signals. These results contrast starkly with theoretical predictions and previous empirical work with frogs. *Key words:* bullfrogs, communication, fighting ability, *Rana catesbeiana,* size assessment, territoriality. *[Behav Ecol 13:109–124 (2002)]*

Theoretical models of animal conflict predict that animals should attempt to assess asymmetries in fighting ability before engaging in escalated and potentially costly fights (Enquist and Leimar, 1983; Maynard Smith, 1982; Maynard Smith and Harper, 1995). Both the duration and intensity of agonistic interactions are expected to vary depending on the degree of asymmetry between opponents (Enquist and Leimar, 1983; Enquist et al., 1990). Encounters should last longer and include more dangerous behavioral elements when contestants possess similar fighting abilities and asymmetries are more difficult to detect. When the asymmetries are larger and more easily assessed, shorter, less intense encounters are expected on the grounds that weaker individuals will assess their low probability of winning and give up early in the contest. One important source of asymmetry in fighting ability is a difference in body or weapon size (reviewed in Andersson, 1994; Archer, 1988). Larger size often conveys advantages in physical contests.

Communication is common during animal conflicts, and animals often use size-dependent or condition-dependent variation in displays to assess their opponent's fighting ability during aggressive interactions (e.g., Clutton-Brock and Albon, 1979; Davies and Halliday, 1978). One property of acoustic communication signals that is constrained by body size, and is therefore expected to be a reliable size-assessment signal, is fundamental frequency, which is related to the percept of pitch (Dawkins and Krebs, 1978; Krebs and Dawkins, 1984; Morton, 1977; Wiley, 1983). Among anuran amphibians (frogs

and toads), fundamental frequency depends on the shape and mass of the laryngeal apparatus, which is related to overall body size (Martin, 1972). Fundamental frequency is negatively correlated with body size in frogs, and this call property is usually a better predictor of male body size than other call properties (Bee and Gerhardt, 2001b; Bee et al., 1999; Robertson, 1986; Wagner, 1989c). A number of studies demonstrate that large size confers advantages in physical fights between male frogs (Arak, 1983; Davies and Halliday, 1978; Given, 1988; Howard, 1978; Wagner, 1989a; Wells, 1978). Therefore, in frogs, accurate assessment of an opponent's relative size and fighting ability based on the pitch of its calls would allow contestants to estimate their likelihood of winning an escalated contest. Several studies demonstrate that male frogs base decisions about continuing or escalating vocal and physical interactions solely on size-related information conveyed by the spectral properties of an opponent's acoustic signals (Arak, 1983; Given, 1987; Ramer et al., 1983; Robertson, 1986; Wagner, 1989a,b). In these field playback experiments, male frogs were more likely to persistently attack or direct aggressive vocalizations toward the high-pitched calls of a perceived smaller frog.

Here I report results from a field playback study of acoustically mediated size assessment by territorial males of the North American bullfrog (*Rana catesbeiana,* Ranidae). During their breeding season, male bullfrogs establish territories in permanent bodies of water (Emlen, 1976; Howard, 1978). From within their territories, males emit advertisement calls, presumably to attract gravid females and to repel rival males, and these signals are by far the most common call type heard in a bullfrog breeding chorus. Territorial male bullfrogs exclude other conspecific males from calling within their territory using a combination of stereotyped aggressive movements, such as hops, jumps, and lunges toward and onto an opponent, visual displays in the form of presentations of the yellow gular sac, and vocalizations (see Emlen, 1968, 1976; Howard, 1978; Ryan, 1980; Wiewandt, 1969). Advertisement

calls, encounter calls, and a low-frequency growl (see Wells, 1978) are associated with aggressive interactions. If neither contestant withdraws during a contest, males engage in discrete wrestling bouts, in which they attempt to clasp and submerge their opponent. Such fights are not uncommon and can last more than an hour (Bee, personal observation; Howard, 1978).

Agonistic encounters between bullfrogs may be costly in terms of time, energy, and reduced vigilance for detecting predators, but the risk of sustaining serious injury during a fight is probably low. Emlen (1976) and Howard (1978) found that larger male bullfrogs win fights more frequently than smaller males. Additionally, Howard (1978) reported that the average size difference between male bullfrogs that engaged in physical fights was significantly smaller than that between males engaged in aggressive encounters that were settled by threats and displays before escalated fighting. That is, aggressive encounters were more likely to escalate to physical fighting when the size asymmetry between contestants was relatively small, as predicted by some game theory models (Enquist and Leimar, 1983). Howard's data clearly suggest that some form of size assessment occurs before escalated physical fights.

The purpose of the present study was to investigate the importance of size-related variation in the fundamental frequency of bullfrog advertisement calls as a potential size assessment cue during aggressive territorial interactions. The advertisement call is a broad-band signal that consists of a series of harmonics that are integer multiples of a fundamental frequency (range 90–135 Hz), which is usually absent from the frequency spectrum (Figure 1A). Fundamental frequency is strongly and negatively correlated with male body size (Figure 1B; see below), and fundamental frequency is the best acoustic predictor of male body size, with the exception of call properties that are highly correlated with fundamental frequency (e.g., harmonics; Bee and Gerhardt, 2001b). In previous experiments based on the habituation/discrimination paradigm, bullfrogs were shown to be capable of perceptually discriminating between two advertisement calls that differ in fundamental frequency by a magnitude typical of among-male differences in the population (e.g., 9–12 Hz; Bee and Gerhardt, 2001a,c). Hence, as in other frogs, the fundamental frequency of advertisement calls is a reliable and discriminable signal of male body size in bullfrogs.

In this study, I repeatedly broadcast synthetic advertisement calls simulating a new neighbor calling from a position adjacent to a subject's territory. Subjects heard the sounds of a simulated large male, a small male, or an acoustically size-matched male. I compared the initial and maximum aggressive responses to broadcasts of these three stimuli. I also compared the number of stimulus presentations required until males no longer responded aggressively to the playback as a measure of whether the duration of aggressive encounters depended on an assessment of an opponent's relative size and fighting ability. In previous studies (Bee, 2001; Bee and Gerhardt, 2001a), we demonstrated that the aggressive response of bullfrogs to repeated field playbacks of conspecific advertisement calls exhibits characteristics of response habituation. The primary goals of the present study were to determine whether aggressive behavior directed toward an opponent depended on the acoustically simulated size of the opponent, the size of the subject, and the degree of size asymmetry between the subject and the opponent.

MATERIALS AND METHODS

Playback experiments

Between 10 May and 1 August 1998–2000, I conducted a field playback experiment at the Little Dixie Lake Conservation
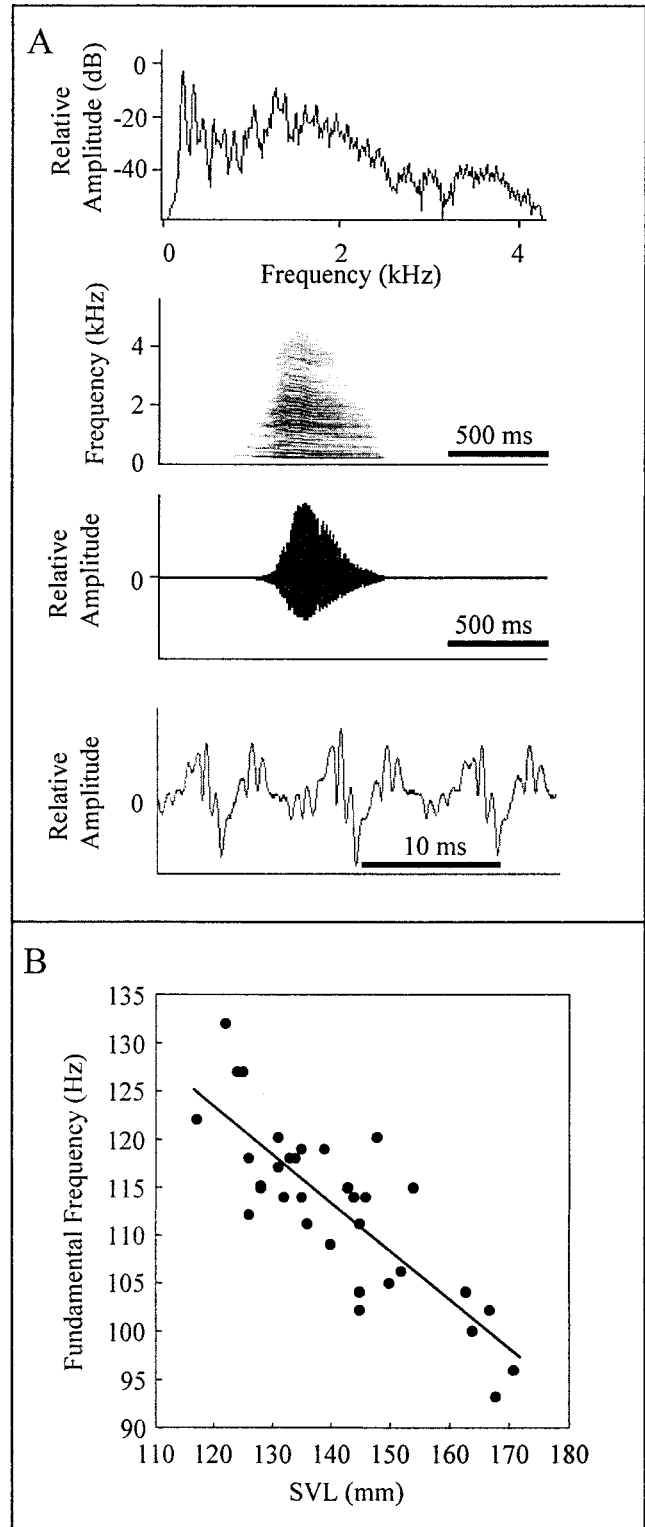
Figure 1
(A) Power spectrum, sonogram, and two oscillograms (top to bottom) of a one-note advertisement call. Horizontal bars indicate time scales. (B) Regression of the fundamental frequency of advertisement calls on male body size (snout-to-vent length, SVL). Fundamental frequency was calculated as the reciprocal of the repeated fine-temporal waveform periodicity, measured from an oscillogram of one recorded advertisement call for each of the 34 males that were tested with a size-matched stimulus in 1998 and 1999. The fundamental frequencies depicted here were also those used for stimuli in the size-matched condition.
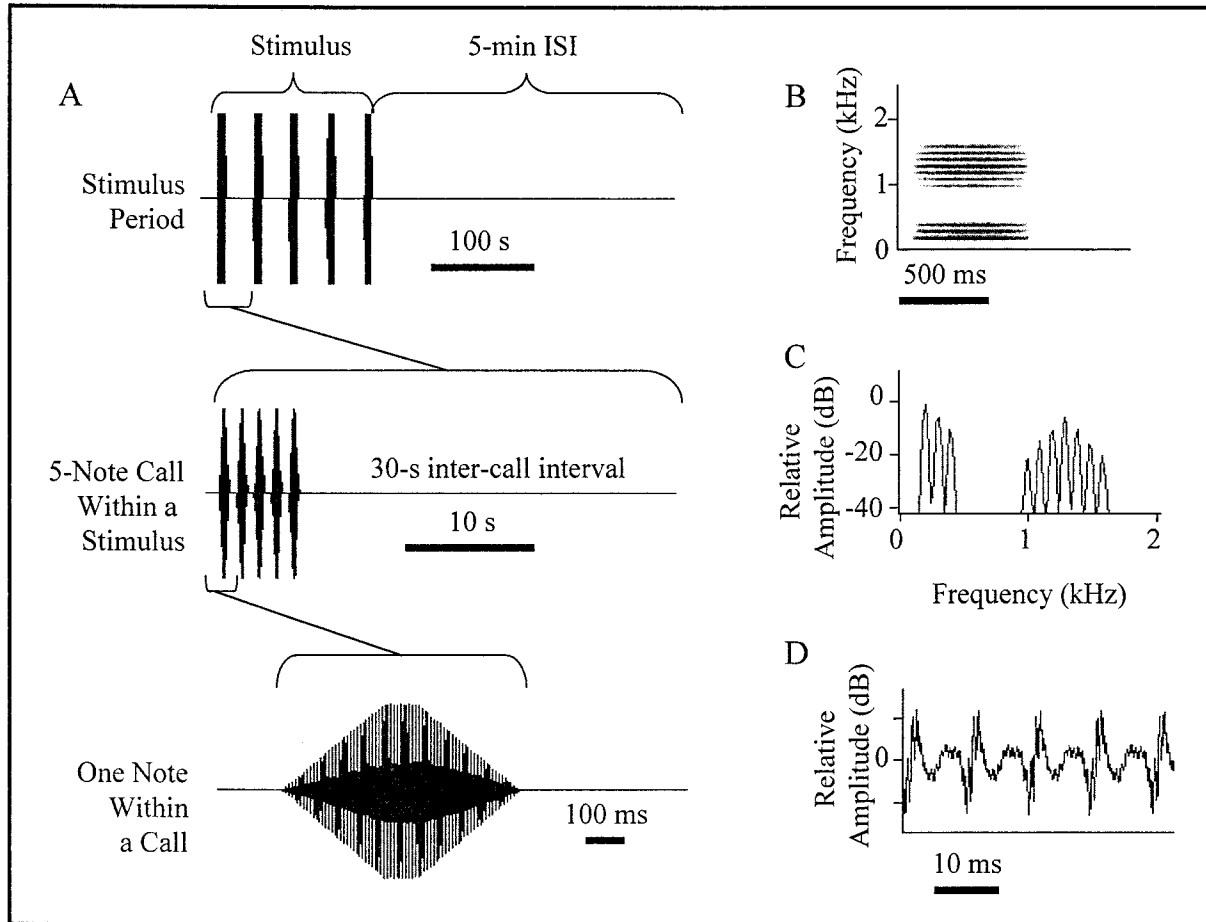
Figure 2
Synthetic bullfrog advertisement calls used as stimuli in this study. (A) Top: One stimulus period composed of one stimulus with five consecutive 5-note calls followed by a 5-min interstimulus interval (ISI). Middle: One 5-note call followed by the 30-s inter-all interval. Bottom: A single note from the stimulus. During playback tests, the stimulus period shown in the top panel was repeated as a continuous loop. (B) Sonogram of a single note from a stimulus used in playback tests ($f_1 = 99$ Hz). (C) Power spectrum of the stimulus note shown in panel B. (D) Oscillogram depicting the fine-temporal waveform of the stimulus shown in panel B. Horizontal bars indicate time scales.

Area (Callaway County, Missouri, USA) and the Thomas Baskett Wildlife Area (Boone County, Missouri; see Bee and Gerhardt, 2001a, for additional details about the study site). Playback tests were performed under ambient light between 2130 and 0930 h and usually commenced between 2200 and 0000 h each night after males began actively calling. On a night before testing, each subject was captured, and its snout-to-vent length (SVL) and mass were measured. Measurements of mass were unavailable for two individuals. Males were given individual toe clips on their hind feet, a cohort mark indicating year of testing on their forefeet, and a waistband and tag with an identifying number, which allowed visual identification of males from distances of 2–3 m.

Equipment
The digital-to-analogue output of portable notebook computers (Samsung SENS 800, Dell Inspiron 3500 or 5000) and battery-powered amplifiers (Nagra DH or Rockford Fosgate 2.6×) were used to broadcast synthetic bullfrog advertisement calls through one of four 10-inch Optimus speakers mounted in wooden boxes and floated on styrofoam platforms covered in black plastic. The frequency response of each speaker was flat (± 4 dB) over the range of frequencies in the stimuli. Playback levels were measured and calibrated in the field with

a GenRad 1982 sound-level meter or a Radio Shack sound-level meter calibrated against the GenRad meter.

Stimuli
Previous playback experiments have established that territorial males respond aggressively with encounter calls, aggressive movements, and approaches toward a speaker broadcasting both natural, prerecorded advertisement calls (Davis, 1987; Emlen, 1968; Wiewandt, 1969) and synthetic models of advertisement calls (Bee and Gerhardt, 2001a,c; Davis, 1988). I broadcast synthetic advertisement calls at a sound pressure level (SPL) of 87 dB measured at a distance of 1 m (re 20 μPa, fast RMS, C-weighted), which reflects the upper end of the range of variation in the SPL of natural calls (Bee, unpublished data; Megela-Simmons, 1984). Sound pressure levels typically varied between 86–88 dB at 1 m and between 70–72 dB at the frog's original position. Stimuli were generated at a sampling rate of 20 kHz with 16-bit resolution using custom-designed software. All values of stimulus properties fall within the range of natural variation for this species (Bee and Gerhardt, 2001b; Capranica, 1965). A stimulus consisted of five consecutive advertisement calls separated by 30-s intercall intervals (call duty cycle = 0.17; see Figure 2A). Each call within a stimulus consisted of five identical notes that were

700 ms in duration, had symmetrical linear rise and fall times of 300 ms duration, and were separated by 700-ms internote intervals (note duty cycle = 0.50). Each note within a call consisted of a series of 10 harmonics (f_2–f_4 and f_{10}–f_{16}) that were integer multiples of the fundamental frequency (f_1) and had the same starting phase relationships of $0°$ (Figure 2B–D). The dominant frequency was the second harmonic (f_2). All other harmonics were attenuated by 5–20 dB in relation to the dominant frequency. The fifth and final call of the stimulus was followed by a silent interstimulus interval (ISI) of 5 min. The combination of the stimulus and the ISI was broadcast repeatedly using the sound-editing software GoldWave 4.02. Together, a stimulus and the subsequent ISI constitute what is hereafter referred to as a ''stimulus period.'' All playback stimuli used in this study shared the properties listed above, and differed only in fundamental frequency (and correlated spectral differences).

I examined differences in aggressive responsiveness to simulated intruders of different sizes by broadcasting one of three types of acoustic stimuli to territorial males. For playback tests conducted in 1998 and 1999, subjects ($n = 34$) were presented with an acoustically size-matched opponent, for which the fundamental frequency of the stimulus was the same (± 1 Hz) as that of the subject's own advertisement calls. On the night each subject in this group was tested, prior to the beginning of the playback test, I recorded one or more of its advertisement calls with an HHB PDR 1000 DAT recorder (sampling rate = 32 kHz) or one of the notebook computers (sampling rate = 22.05 kHz), using Sennheiser MKH 70 or ME 66 directional microphones mounted on a tripod placed 1–2 m from the subject. Calls were digitized and stored on the notebook computer as 8-bit sound files at a sampling rate of 22.05 kHz. I used GoldWave 4.02 software to determine the fundamental frequency of one randomly chosen advertisement call from each subject by taking the reciprocal of the average period of 10 repetitions of the fine-temporal waveform from an oscillogram [fundamental frequency (Hz) = 1/waveform periodicity (s)]. I selected an appropriate stimulus from a series of synthetic stimuli with fundamental frequencies spanning the range of natural variation (90–135 Hz) that had been previously generated offline and stored on disk. The fundamental frequencies of the subjects' advertisement calls were strongly negatively related to male body size (Figure 1B). The average fundamental frequency of the size-matched stimuli was 113 Hz (SD = 9 Hz; range = 93–132 Hz).

In 2000, I broadcast synthetic advertisement calls that simulated either a large intruder with a low fundamental frequency ($f_1 = 95$ Hz; $n = 24$ males) or a small intruder with a high fundamental frequency ($f_1 = 125$ Hz; $n = 24$ males). The large and small stimuli were presented in random order during the 2000 breeding season. Previous work has established that territorial males can perceive a frequency difference of this magnitude (Bee and Gerhardt, 2001a). Based on the regression of fundamental frequency on SVL calculated for the 34 males that heard a size-matched stimulus (Figure 1B), the large stimulus acoustically represented a male with an SVL of 177 mm, and the small stimulus represented a male with an SVL of 117 mm. Subjects in this study had SVLs ranging from 115 to 171 mm (mean $\pm$ SD = 141 $\pm$ 13.5 mm; population range = 109–181 mm; Bee, unpublished data).

Protocol

To begin a playback test, I positioned the playback speaker at a distance of 6 m along the pond bank from an actively calling target male, in the direction of the male's most distant neighbor. If there were no nearby neighbors (e.g., < 15–20 m away), I determined speaker position (left or right relative to the frog) by flipping a coin. Typical distances between adja-

cent territorial male bullfrogs range between 3 and 16 m (Bee, personal observations; Emlen, 1968, 1976). I preferentially tested males that did not have a nearest neighbor within 10 m in at least one direction along the pond bank to avoid interference from other territorial males. I started playbacks 15–30 min after subjects resumed normal calling behavior after speaker placement.

During each stimulus period of a playback test, I counted the number of advertisement calls, encounter calls, and aggressive movements toward, around, and away from the playback speaker; measured the maximum distance advanced toward the speaker; and determined the latency to the first encounter call with a stop watch. If no encounter call was produced during a stimulus period, I assigned a latency value of 452 s, which was equivalent to the duration of the stimulus period. For a male to be included in the data set, it had to give at least one encounter call during the first stimulus period of the test. Twelve males did not respond during the first stimulus period with an encounter call in response to the size-matched ($n = 3$), small ($n = 1$), large ($n = 8$) stimuli. Ten of these males were successfully retested at a later date, and eight males were retested with the same stimulus to which they did not respond on the first test (size-matched = 2, small = 1, and large = 5). Although there was a trend for males to be nonresponsive when they heard the large stimulus ($\chi^2_2 = 17.1$, $p < .05$), most of these males (five of eight) were successfully retested with the same stimulus at a later date (one male was retested with the small stimulus, and two males were not retested). Some factor other than stimulus size probably influenced the behavior of these nonresponsive males.

I examined the magnitude of the aggressive response during a habituation phase that consisted of repeating the stimulus period as a continuous loop. In 1998 and 1999, the stimulus period was repeated until subjects met an arbitrary criterion of asymptotic response decrement, defined as no movement and no production of encounter calls during three consecutive stimulus periods (hereafter ''response decrement criterion''). In 2000, I used a slightly different protocol that consisted of repeatedly broadcasting the small or large stimulus for a total of 30 stimulus periods (= 3.75 h).

Data analysis

I made between-group comparisons of three primary measures of response strength: initial responses during the first stimulus period, maximum responses during any stimulus period, and the rate of aggressive response decrements. Initial responses were determined as the numbers of advertisement calls, encounter calls, and movements, the distance advanced toward the speaker, and the latency to the first encounter call during the first stimulus period. Maximum aggressive responses were determined as the maximum numbers of advertisement calls, encounter calls, and movements, the maximum distance advanced toward the speaker and the minimum latency to an encounter call during any stimulus period. The rate of habituation of the aggressive response was measured as the number of stimulus periods required to reach the response decrement criterion, including the three consecutive stimulus periods without an encounter call or movement (Bee, 2001). In response to the size-matched stimulus, 30 of 34 males (88.2%) reached the response decrement criterion after experiencing 30 or fewer stimulus periods. In response to both the large stimulus and the small stimulus in 2000, 20 of 24 males (83.3%) reached the response decrement criterion in 30 or fewer stimulus periods. To make meaningful between-group comparisons, I did not consider responses occurring after the 30th stimulus period for the 12 of 82 males (14.6%) that had not yet met the response decrement crite-

rion. Eight of these 12 males were not tested after the 30th stimulus period; the remaining 4 males tested with the size-matched stimulus met the criterion after hearing 31, 36, 41, and 81 stimulus periods. This procedure was necessary to make comparisons between groups that experienced slightly different habituation training phases. For testing differences in the rates of habituation, a maximum value of 30 stimulus periods to criterion was assigned to the 12 males that had not yet met the criterion in 30 periods.

Following log-transformations $[Y = \log_{10}(Y + k)$, where k = constant], most response variables met the requisite assumptions of parametric statistical analyses. However, the variance in the maximum number of advertisement calls was not homogenous between groups, and the maximum approach distance departed from normality. Because parametric tests are generally regarded as robust to moderate violations of normality and homogeneity of variance at large sample sizes (Rosenthal and Rosnow, 1991), I chose to use parametric statistical methods. Statistical significance was set at the $\alpha = 0.05$ level.

To examine the effects of a resident's size and the size of the simulated opponent, I divided subjects into three size classes: small (SVL $\leq$ 134 mm, $n = 29$), medium (135 mm $\leq$ SVL $\leq$ 146 mm, $n = 26$), and large (SVL $\geq$ 147, $n = 27$). I performed 3 (stimulus) $\times$ 3 (size class) multivariate analyses of variance (MANOVA) to examine between-group differences in the magnitude of the initial response and the maximum response. Subsequent 3 $\times$ 3 ANOVAs were used to separately analyze between-group differences in the five response variables for initial and maximum aggressive responses. I performed a 3 $\times$ 3 ANOVA to compare the number of stimulus periods required to reach the response decrement criterion. These two-way ANOVAs were designed to answer three questions (Bee et al., 2000; Wagner, 1989b): First, do males respond differently to different-sized opponents (stimulus effect)? Second, do males of different sizes respond differently to opponents (size-class effect)? Third, does a male's aggressive response depend on his own size and the size of his opponent (stimulus $\times$ size class interaction)?

To directly examine the importance of relative size differences in determining male responses, I computed an index of size asymmetry (AI; Enquist and Leimar, 1983; Enquist et al., 1990):

$$AI = \ln[(\text{subject's SVL, mm})/(\text{simulated opponents's SVL, mm})].$$

The opponent's SVL was based on the regression of fundamental frequency on SVL for males in the size-matched group (see above; Figure 1B). For subjects in the size-matched group, the AI was zero [= ln(1)]. A negative AI indicates that the subject was smaller than the simulated opponent, and a positive AI indicates that the subject was larger than the simulated opponent. One-way MANOVAs and ANOVAs were used to compare responses of three groups of males having AIs that were negative ($n = 30$), positive ($n = 18$), or equal to zero ($n = 34$). I also calculated the Pearson product-moment correlation between response variables and the AI.

Because males tested with the large and small stimuli in 2000 experienced the same number of stimulus periods (30), I was able to directly compare changes in the responses of small, medium, and large males (between subjects) that occurred with repeated presentations (within subjects) of the large and small stimuli (between subjects). I performed 2 (stimulus) $\times$ 3 (size class) $\times$ 30 (stimulus period) repeated-measures ANOVAs on each of the five response variables. I used the Greenhouse and Geiser (1959) method to adjust the degrees of freedom for omnibus repeated-measures effects.

Two important caveats regarding the interpretation of the data presented here deserve consideration. First, although no males were tested multiple times in the same year, there is some small, but unknown, probability that males tested in 1998 or 1999 were also tested in a subsequent year. Individual toes clipped from the hind feet regenerated between breeding seasons and were indistinguishable from toes that had not been clipped. Therefore, identifying individuals between years was not possible. Toes clipped from the forefeet as cohort marks, however, did not regenerate between years. Hence, I could identify individuals that were marked in previous years, but I could not determine whether those individuals had also been tested in that year because only a subset of frogs marked each year were also tested that year. However, the number of males unknowingly tested twice could not exceed the total number of individuals that were tested in one year and also marked in a previous year, which was small (6 of 82 males; 7.3%). Therefore, the maximum potential for pseudoreplication in this study was rather small. For purposes of statistical analyses, I assume that any response to a stimulus in one year is independent of whether the male was also tested in a previous year.

Second, because size-matched stimuli were presented in 1998 and 1999 and the large and small stimuli were presented in 2000, there is some potential for differences in behavior to result from differences in any number of factors that varied between years and produced differences between the males tested with the size-matched or large and small stimuli. I believe the impact of such factors was minimal in the present study for the following five reasons. First, there were no between-group differences in SVL ($F_{2,79} = 0.14$, $p = .8685$), mass ($F_{2,77} = 0.60$, $p = .5496$), or an index of physical condition ($F_{2,77} = 1.78$, $p = .1748$; after Baker, 1992). Second, with the single exception of minimum response latency ($F_{2,79} = 7.64$, $p = .0009$), there were no significant differences in response variables based on year as a main effect ($0.10 < F_{2,79} < 2.45$, $.09 < p < .91$). Response latencies were significantly lower in 1999 compared to both 1998 and 2000 (Scheffé's multiple comparisons test: $p < .02$), and latencies in 1998 and 2000 were not different ($p = .2814$). Third, playback tests were conducted over the duration of the breeding season each year, which lasted between mid-May to late July in all 3 years, and tests were started at similar times of night in all 3 years. Fourth, based on maps of territory positions made nightly in all 3 years, bullfrogs established territories in similar locations in the same ponds across years. Fifth, based on these territory maps, there were no obvious differences in male density and intermale spacing between years, although precise measurements are unavailable. Hence, there is little evidence to suggest that physical, temporal, spatial, and social variables covaried with experimental treatments, and I assume that their impact on the data presented here was negligible.

Power analysis and meta-analysis
For between-subjects comparisons based on ANOVA, I computed effect sizes and the statistical power of the test following Cohen (1988) and Rosenthal and Rosnow (1991). The effect size for the F statistic from ANOVA is η^2, which is the proportion of variance explained by membership in two or more experimental groups. The variable η^2 is a generalization of the more familiar coefficient of determination (r^2) associated with tests of differences between two groups (see Cohen, 1988, for an extensive discussion of this topic). Following Rosenthal and Rosnow (1991), I calculated η^2 as:

$$\eta^2 = [(F)(\text{df effect})]/[(F)(\text{df effect}) + (\text{df error})].$$

I also calculated the effect sizes from five other studies of acoustically mediated size assessment in frogs to determine how the effect sizes in the present study compared to previous

Table 1

Results for main effects of stimulus in playback studies of size assessment by male frogs based on size-related variation in advertisement call properties

| Study	Manipulation of opponent size	Analysis (ANOVAs)	Response variable	df	F	p	η^2	Power
Davies and Halliday (1978)	small vs. large	2 (stimulus, within) × 2 (opponent size class, between)	no. of attacks	1,22	11.8	< .01	.35	0.93
			% time attacking	1,22	20.7	< .01	.48	> 0.97
Robertson (1986)	small vs. medium vs. large	one-way (between-subjects)	intensities evoking encounter calls	2,32	21.52	< .01	.57	> 0.99
			intensities evoking fight or flight	2,32	7.8	< .01	.33	> 0.99
Given (1987)	small vs. large (622 v 524 Hz)	one-way (within subjects)	single-note aggressive calls/min	1,12	9.99	< .01	.45	> 0.98
			total notes/min	1,12	7.08	.02	.37	0.96
Wagner (1989b)	small vs. large (3700 vs. 3000 Hz)	2 (stimulus, between) × 2 (subject size class, between)	call rate	1,33	4.64	.04	.12	0.59
			call group rate	1,33	8.63	< .01	.21	0.84
			call rate within a call group	1,33	5.74	.02	.15	0.70
			calls per call group	1,33	5.64	.02	.15	0.68
			call group duration	1,33	8.06	< .01	.20	0.84
Bee et al. (2000)	small vs. large (450 vs. 350 Hz)	2 (stimulus, within) × 2 (subject size class, between)	frequency alteration	1,28	7.34	.01	.21	0.76
	medium vs. large (400 vs. 350 Hz)	2 (stimulus, within) × 2 (subject size class, between)	frequency alteration	1,28	7.01	.01	.20	0.76
this study	small vs. large (125 vs. 95 Hz)	one-way (between-subjects)	initial advertisement calls	1,46	1.33	.25	.03	
			initial encounter calls	1,46	0.07	.79	.00	
			initial movements	1,46	0.51	.48	.01	
			initial distance	1,46	0.25	.62	.01	
			initial latency	1,46	0.48	.49	.01	
			maximum advertisement calls	1,46	2.47	.12	.05	
			maximum encounter calls	1,46	0.00	.96	.00	
			maximum movements	1,46	0.87	.36	.02	
			maximum distance	1,46	0.38	.54	.01	
			minumum latency	1,46	0.07	.79	.00	
			habituation rate	1,46	0.33	.57	.01	

studies that have addressed similar questions (Bee et al., 2000; Davies and Halliday, 1978; Given, 1987; Robertson, 1986; Wagner, 1989b). These studies, which included five species from four families, were chosen because they met the following criteria: they reported results from acoustic playback experiments; they explicitly tested hypotheses about the function of spectral call properties as assessment signals; and they either used ANOVA to make statistical comparisons and reported F ratios and degrees of freedom, or they provided raw data (Given, 1987), from which I generated F ratios using ANOVA. In Table 1, I report effect sizes for the variables in these studies that exhibited significant differences in responses to playback stimuli differing in size-related spectral properties (e.g., fundamental frequency or dominant frequency). The average effect size for the significant effects from these studies was η^2 = .29 (range: η^2 = .12–.57). For comparison, I include results from one-way ANOVAs and effect sizes for a between-subjects comparison of bullfrog responses to the large and small stimuli presented in this study.

Statistical power ($1 - \beta$, where β = probability of a type II statistical error) for a given effect size, sample size, and α level (α = 0.05) was calculated using the power tables in Cohen (1988). For most of the statistical tests in the present study that yielded nonsignificant results, I report the statistical power of the test to detect both the observed effect size ($1 -$ β_{actual}) and an expected effect size ($1 - \beta_{expected}$), determined as the average effect size from the five previous studies listed in Table 1. Reports of the power to detect the expected effect size are limited to univariate analyses of variance.

RESULTS

In response to broadcasts of the stimuli, males oriented toward the speaker, produced advertisement calls and encounter calls, and approached the speaker using stereotyped aggressive movements. Males often repeatedly charged toward the speaker during the broadcast of the stimulus and returned to their original calling site during a subsequent interstimulus interval. Similar aggressive behavior was originally described by Emlen (1968; see also Wiewandt, 1969). The proportions of males that "attacked" the speaker by approaching the entire 6 m distance were similar in responses to the small stimulus (14/24, 58.3%), size-matched stimuli (20/34, 58.8%), and the large stimulus (14/24, 58.3%; χ^2_2 < .01, p > .99). As habituation training proceeded, aggressive responses exhibited marked decrements, and the majority of males eventually returned to within 0.5 m of their original calling position and resumed producing exclusively advertisement calls. Previous work has ruled out sensory adaptation and effector fatigue as explanations for aggressive response decrements (Bee and

Initial Responses
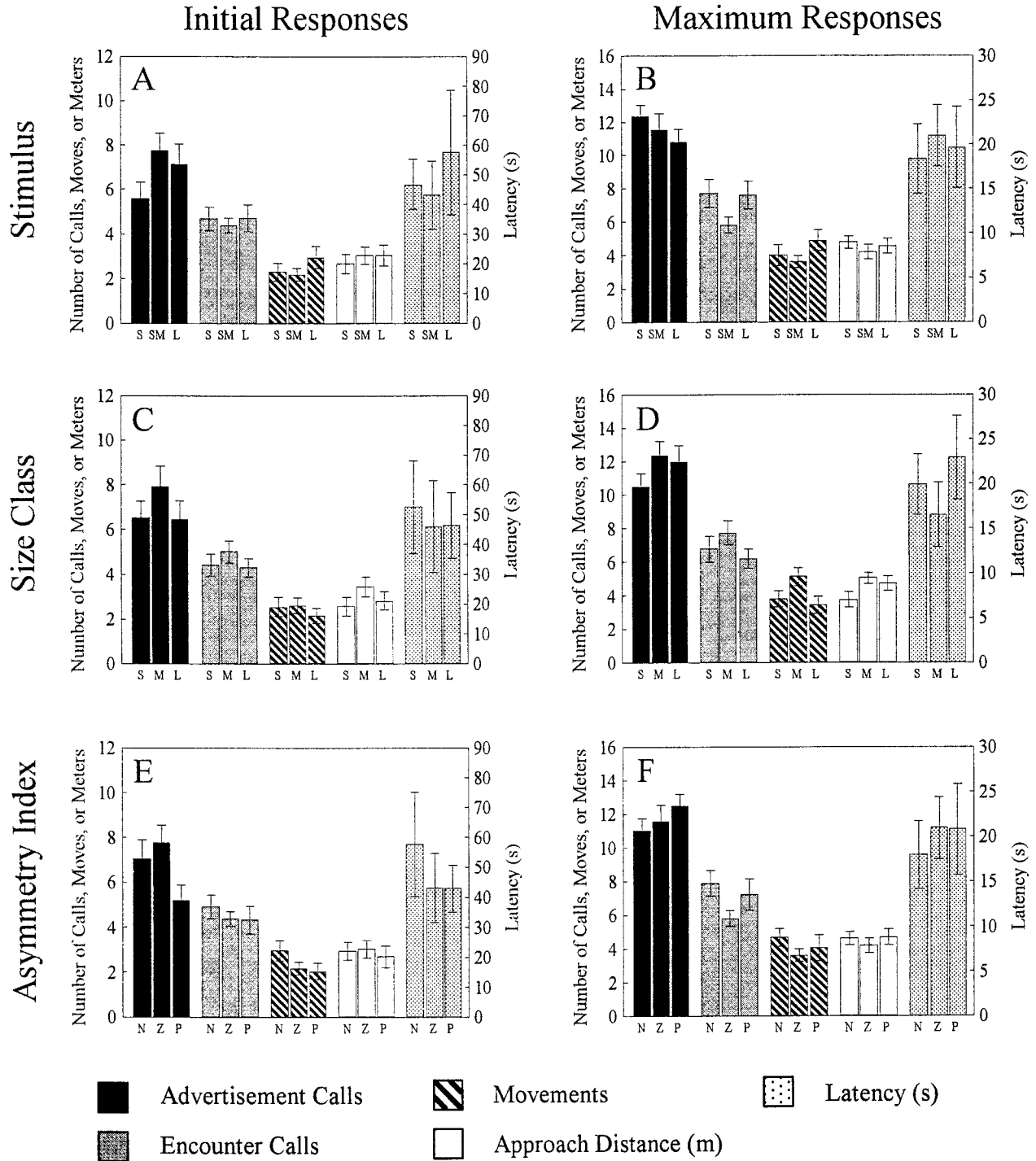
Maximum Responses

Figure 3

Mean (± SE) numbers of advertisement calls, encounter calls, movements, and meters approached toward the speaker, and the mean latency to the first encounter call. (A) Initial and (B) maximum responses for the effect of stimulus (S = small male, SM = size-matched male, L = large male). (C) Initial and (D) maximum responses for the effect of size class (S = small males, M = medium-sized males, L = large males). (E) Initial and (F) maximum responses for the effect of asymmetry index [N = negative (subject smaller), Z = zero (same size), P = positive (subject larger)].

Gerhardt, 2001a,c), which result from stimulus-specific habituation.

Initial and maximum responses

MANOVA did not reveal any significant differences in the magnitude of initial responses that were due to the main ef-

fect of stimulus (Wilks's λ = 0.94, $R_{10,138}$ = .40, p = .9443), the main effect of size class (Wilks's λ = 0.89, $R_{10,138}$ = .86, p = .5690), or the stimulus × size class interaction (Wilks's λ = 0.72, $R_{20,229}$ = 1.22, p = 0.2402). Subsequent univariate ANOVAs also failed to reveal any significant differences for these effects (Figure 3A,C; Table 2). Similar results were obtained for the maximum aggressive response, for which there

Table 2
Results of 3 (stimulus) × 3 (size class) ANOVAs on the magnitude of initial responses

Source	df	MS	F	p	η^2	Power for observed effect size	Power for expected effect size[a]
Advertisement calls							
Stimulus	2	0.169	2.29	.1090	.06	0.48	> 0.995
Size class	2	0.067	0.91	.4078	.02	0.18	> 0.995
Stimulus × size class	4	0.141	1.90	.1203	.09	0.57	> 0.97
Error	73	0.074					
Encounter calls							
Stimulus	2	0.002	0.04	.9610	.00	0.00	> 0.995
Size class	2	0.022	0.53	.5924	.01	0.10	> 0.995
Stimulus × size class	4	0.082	1.97	.1088	.10	0.63	> 0.97
Error	73	0.042					
Movements							
Stimulus	2	0.014	0.21	.8143	.01	0.10	> 0.995
Size class	2	0.020	0.31	.7362	.01	0.10	> 0.995
Stimulus × size class	4	0.091	1.39	.2443	.07	0.45	> 0.97
Error	73	0.066					
Approach distance							
Stimulus	2	0.009	0.33	.7227	.01	0.10	> 0.995
Size class	2	0.012	0.41	.6667	.01	0.10	> 0.995
Stimulus × size class	4	0.066	2.26	.0706	.11	0.68	> 0.97
Error	73	0.029					
Response latency							
Stimulus	2	0.069	0.45	.6377	.01	0.10	> 0.995
Size class	2	0.056	0.37	.6950	.01	0.10	> 0.995
Stimulus × size class	4	0.008	0.05	.9953	.00	0.00	> 0.97
Error	73	0.153					

MS, mean square; η^2, effect size; power = $1 - \beta$.

[a] $\eta^2 = .29$

was no multivariate effect of stimulus (Wilks's $\lambda = 0.79$, $R_{10,138} = 1.72$, $p = .0813$), size class (Wilks's $\lambda = 0.81$, $R_{10,138} = 1.58$, $p = .1187$), or a stimulus × size class interaction (Wilks's $\lambda = 0.81$, $R_{20,229} = .74$, $p = .7830$). There were also no significant differences in univariate comparisons of maximum responses (Figure 3B,D; Table 3).

MANOVA also did not reveal any significant differences, nor were there univariate differences (Table 4), depending on whether the AI was positive, negative, or equal to zero for initial responses (Wilks's $\lambda = 0.92$, $R_{10,150} = .62$, $p = .7914$; Figure 3E) and maximum responses (Wilks's $\lambda = 0.86$, $R_{10,150} = 1.22$, $p = .2831$; Figure 3F). In general, there were no linear or nonlinear trends relating the degree of size asymmetry to the initial and maximum values of the response variables (Figure 4). However, with a large sample size of 82 individuals, the initial number of movements was significantly correlated with the size asymmetry index ($r = -.23$, $p = .036$), but the degree of asymmetry explained less than 6% of the variation in the initial number of movements. This correlation was not significant following a Bonferroni correction procedure to account for multiple comparisons of the AI. All other correlations had absolute values less than $|r| = .19$ ($p > .10$; $1 - \beta_{actual} < 0.43$).

Rates of habituation

ANOVA did not detect any significant differences in the rate of habituation related to the main effects of stimulus ($F_{2,73} = 1.12$, $p = .3315$, $\eta^2 = .03$, $1 - \beta_{actual} = .26$, $1 - \beta_{expected} > .98$; Figure 5A) or size class ($F_{2,73} = 0.47$, $p = .6255$, $\eta^2 = .01$, 1

$- \beta_{actual} = .10$, $1 - \beta_{expected} > .98$; Figure 5B), and there was no significant interaction ($F_{4,73} = 1.54$, $p = .1982$, $\eta^2 = .08$, $1 - \beta_{actual} = .53$, $1 - \beta_{expected} > .95$). The number of stimulus periods to reach criterion also did not depend on whether the AI was positive, negative, or equal to zero ($F_{2,79} = 1.09$, $p = .3412$, $\eta^2 = .03$, $1 - \beta_{actual} = .27$, $1 - \beta_{expected} > .98$; Figure 5C), and there was no correlation between the AI and the number of periods to criterion ($r = -.13$, $p = .23$, $1 - \beta_{actual} = .23$; Figure 4F).

The results from the 2 (stimulus) × 3 (size class) × 30 (stimulus periods) ANOVA, which compared responses to the large and small stimuli broadcast to males in 2000, are presented in Table 5. There were no significant effects of stimulus, size class, or stimulus × size class interactions for any response variables. The repeated measure of stimulus period was highly significant for all five response variables. For advertisement calls, there was also a significant stimulus period × stimulus interaction. As Figure 6A illustrates, the number of advertisement calls started moderately high, then exhibited a sharp decrease over the first four periods, suggesting the rapid habituation of evoked advertisement calling during initial presentations of the stimulus. For males that heard the large stimulus, advertisement calling remained at this reduced level, while the rate of advertisement calling by males that heard the small stimulus exhibited a general increase over periods 5–24, and then a final decrease during the last few periods (Figure 6A). The trend for males presented with the small stimulus is explained by an increase in advertisement calling concomitant with an overall increase in nightly chorus activity, which begins between 2100 and 2300 h, peaks around

Table 3
Results of 3 (stimulus) × 3 (size class) ANOVAs on the magnitude of maximum responses

Source	df	MS	F	p	η^2	Power for observed effect size	Power for expected effect size[a]
Advertisement calls							
Stimulus	2	0.012	0.62	.5394	.02	0.18	> 0.995
Size class	2	0.014	0.75	.4777	.02	0.18	> 0.995
Stimulus × size class	4	0.022	1.14	.3429	.06	0.39	> 0.97
Error	73	0.019					
Encounter calls							
Stimulus	2	0.085	2.26	.1111	.06	0.48	> 0.995
Size class	2	0.047	1.25	.2937	.03	0.26	> 0.995
Stimulus × size class	4	0.052	1.39	.2460	.07	0.46	> 0.97
Error	73	0.038					
Movements							
Stimulus	2	0.102	1.40	.2533	.04	0.32	> 0.995
Size class	2	0.213	2.91	.0608	.07	0.55	> 0.995
Stimulus × size class	4	0.012	0.16	.9590	.01	0.09	> 0.97
Error	73	0.073					
Approach distance							
Stimulus	2	0.065	0.85	.4298	.02	0.18	> 0.995
Size class	2	0.116	1.52	.2255	.04	0.32	> 0.995
Stimulus × size class	4	0.069	0.91	.4654	.05	0.33	> 0.97
Error	73	0.076					
Response latency							
Stimulus	2	0.003	0.02	.9770	.00	0.00	> 0.995
Size class	2	0.095	0.65	.5262	.02	0.18	> 0.995
Stimulus × size class	4	0.073	0.50	.7388	.03	0.20	> 0.97
Error	73	0.147					

MS, mean square; η^2, effect size; power = $1 - \beta$.

[a] $\eta^2 = .29$

0000–0200 h, and then declines steadily until dawn (Bee and Gerhardt, 2001a). Why males presented with the large stimulus did not also exhibit this nightly trend in calling activity is unclear.

In contrast to advertisement calling, repeated stimulus broadcasts resulted in decreases in encounter calling, move-ments, and approach toward the speaker and an increase in response latency, reflecting the overall trend for aggressive responses to habituate with repeated playbacks (Figure 6). The changes in these four variables that occurred with re-peated stimulation did not depend on the size of the simu-lated opponent (nonsignificant stimulus period × stimulus

Table 4
Results of one-way ANOVAs on the effects of asymmetry index on the magnitude of initial and maximum responses and the number of stimulus periods to criterion

Response variable	F	p	η^2	Power for observed effect size	Power for expected effect size[a]
Initial response					
Advertisement calls	1.57	.2146	.04	0.33	> 0.995
Encounter calls	0.11	.8994	.00	0.00	> 0.995
Movements	0.65	.5231	.02	0.19	> 0.995
Approach distance	0.08	.9196	.00	0.00	> 0.995
Response latency	0.15	.8570	.00	0.00	> 0.995
Maximum response					
Advertisement calls	1.03	.3621	.03	0.27	> 0.995
Encounter calls	2.47	.0910	.06	0.47	> 0.995
Movements	0.80	.4525	.02	0.19	> 0.995
Approach distance	0.83	.4390	.02	0.19	> 0.995
Response latency	0.11	.8999	.00	0.00	> 0.995
Periods to criterion	1.09	.3412	.03	0.27	> 0.995

[a] $\eta^2 = .29$.
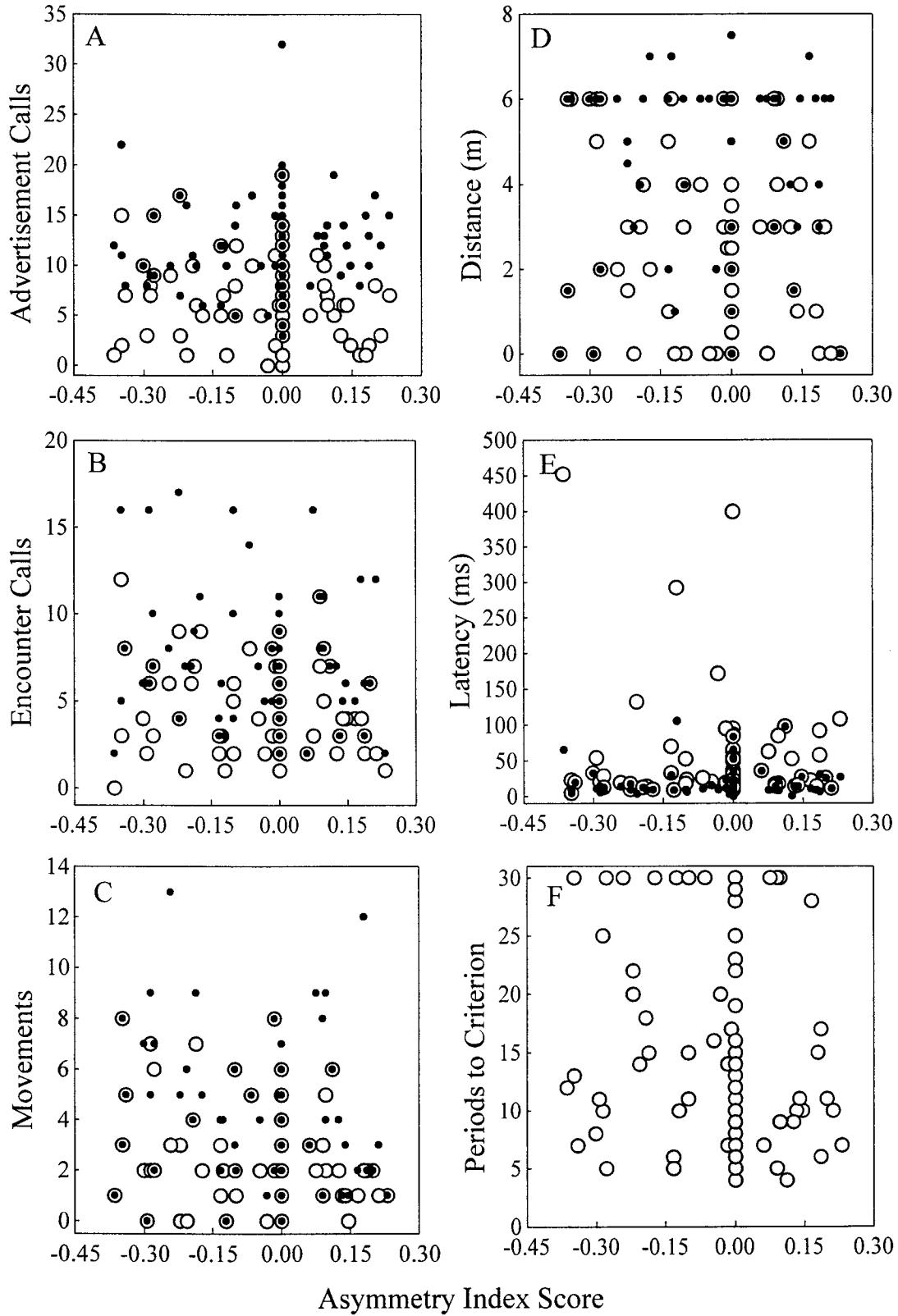
Figure 4
Scatterplots depicting the relationship between asymmetry index scores and the initial values (open circles) and maximum values (filled circles) of (A) advertisement calls, (B) encounter calls, (C) aggressive movements, (D) approach distance, (E) latency, and (F) the number of stimulus periods required to reach the response decrement criterion.
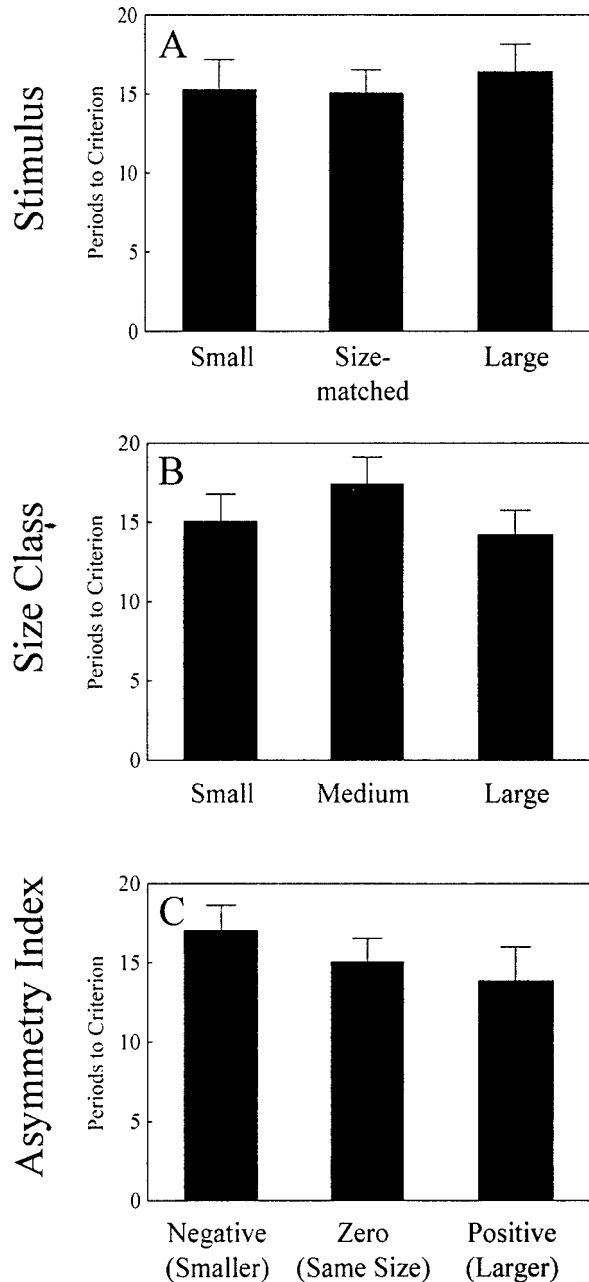
Figure 5
Mean (+ SE) number of stimulus presentations required by males
to reach the response decrement criterion for the effect of (A)
stimulus, (B) size class, and (C) asymmetry index.

interactions) or the size of the subject (nonsignificant stimu-
lus period × size class interactions), and there were no sig-
nificant three-way interactions between stimulus, size class,
and the repeated measure (Table 5).

DISCUSSION

The results of the present study strongly suggest that the ag-
gressive response of territorial male bullfrogs does not de-
pend solely on the opponent's acoustically simulated size, the
size of the resident male, or the degree of size asymmetry.
Because previous work has shown that male bullfrogs can per-
ceive differences in fundamental frequency as small as 9–12
Hz (Bee and Gerhardt, 2001a,c), we can be confident that the

fundamental frequencies used to simulate a large male and a
small male in the present study were perceived as different.
Hence, the male bullfrogs in this study appeared to ignore
size-related variation in fundamental frequency. Based on re-
sults from this field study, I conclude that, unlike many other
frogs, male bullfrogs do not assess their opponent's size based
solely on information conveyed by the fundamental frequency
of advertisement calls.

A comment on statistical power

The conclusion that male bullfrogs do not use fundamental
frequency as an assessment signal is, of course, equivalent to
accepting the null hypothesis of no difference between treat-
ment groups. Therefore, a comment on the power of the sta-
tistical tests reported above is in order. Statistical power refers
to the probability that a statistical test will yield a significant
result (Cohen, 1988). Obtaining statistical significance de-
pends on the specified type I error rate (α), the size of the
study (i.e., the sample size), and the size of the effect, which
is a measure of the degree to which the null hypothesis is
false (Cohen, 1988; Rosenthal and Rosnow, 1991). Based on
an analysis of effect sizes from this study, the different treat-
ments in the playback tests had small or negligible effects on
the territorial aggressive response of male bullfrogs compared
to the effects demonstrated in other frogs ($.00 \leq \eta^2 \leq .11$;
see Table 1). The effects of the subject's body size and the
degree of size asymmetry had similar small effects.

In Table 1, I compare results from previous studies to an
analysis of the effect of stimulus size in the present study. For
the present study, I computed a one-way (between-subjects)
ANOVA on each response variable for males that heard the
large and small stimuli in 2000. This analysis provides for the
most direct comparison of my results with those of earlier
studies. All of these studies share the fact that stimuli simu-
lated large and small males from near the ends of the range
of natural variation in body size. Notice in Table 1 that the
effect sizes associated with a difference in opponent size in
the present study are smaller than those found in other stud-
ies, while the sample size of the present study (indicated by
the degrees of freedom) is larger than previous studies. Clear-
ly, manipulating a simulated opponent's size by varying fun-
damental frequency had smaller effects on behavior in bull-
frogs than it did in other frogs.

The nonsignificant results reported here are unlikely to
represent type II statistical errors, in which I failed to reject a
false null hypothesis. Although the present study lacked suf-
ficient statistical power to detect the small effects reported
above ($.00 \leq 1 - \beta \leq .68$), this study had extremely high
power ($1 - \beta \geq .97$; Tables 2–5) to detect the magnitude of
effects reported in previous studies that have demonstrated
size assessment in frogs ($\eta^2 = .29$; see above). This point is
best illustrated by comparing the power of the test from the
present study reported in Table 1, in which I compared re-
sponses to the large and small stimuli in 2000. Given a sample
size of $n = 24$ males per treatment (total $n = 48$), the power
of the statistical test in this study (at $\alpha = 0.05$) ranged from
$1 - \beta = .67$ for the smallest effect from other studies reported
in Table 1 ($\eta^2 = .12$; Wagner, 1989b), to $1 - \beta > .99$ for the
largest effect ($\eta^2 = .57$; Robertson, 1986). The power to de-
tect the average effect from the previous studies in Table 1
($\eta^2 = .29$) was greater than $1 - \beta = .98$. Perhaps a more
accurate summary of the evidence from this study is that the
effects of size-related variation in acoustic signals are quite
small in bullfrogs relative to the effects found in other frogs.

Table 5
Results from a 2 (stimulus, between) × 3 (size class, between) × 30 (stimulus periods, within) ANOVA of responses to the large and small stimuli

Source	df	MS	F	p	η^2	Power for observed effect size	Power for expected effect size[a]
Advertisement calls							
Stimulus	1	4.559	2.89	.0967	.06	0.38	> 0.97
Size class	2	0.184	0.12	.8903	.01	0.08	> 0.95
Stimulus × size class	2	0.810	0.51	.6024	.02	0.12	> 0.95
Error	42	1.580					
Stimulus period	29	0.277	4.57	.0000	.10		
Stimulus period × stimulus	29	0.140	2.31	.0254	.05		
Stimulus period × size class	58	0.053	0.88	.5864	.04		
Stimulus period × stimulus × size class	58	0.052	0.85	.6111	.04		
Error	1218	0.061					
Encounter calls							
Stimulus	1	0.440	0.26	.6100	.01	0.09	> 0.97
Size class	2	2.276	1.37	.2660	.06	0.29	> 0.95
Stimulus × size class	2	0.799	0.48	.6222	.02	0.12	> 0.95
Error	42	1.665					
Stimulus period	29	0.966	15.96	.0000	.28		
Stimulus period × stimulus	29	0.060	0.99	.4650	.02		
Stimulus period × size class	58	0.073	1.21	.2114	.05		
Stimulus period × stimulus × size class	58	0.084	1.38	.0908	.06		
Error	1218	0.060					
Movement							
Stimulus	1	0.801	0.85	.3629	.02	0.15	> 0.97
Size class	2	1.430	1.51	.2326	.07	0.34	> 0.95
Stimulus × size class	2	0.171	0.18	.8352	.01	0.08	> 0.95
Error	42	0.947					
Stimulus period	29	0.442	12.29	.0000	.23		
Stimulus period × stimulus	29	0.043	1.19	.2864	.03		
Stimulus period × size class	58	0.030	0.83	.7123	.04		
Stimulus period × stimulus × size class	58	0.040	1.13	.3072	.05		
Error	1218	0.036					
Distance							
Stimulus	1	0.012	0.05	.8170	.00	0.00	> 0.97
Size class	2	0.204	0.91	.4123	.04	0.20	> 0.95
Stimulus × size class	2	0.139	0.62	.5431	.03	0.17	> 0.95
Error	42	0.225					
Stimulus period	29	0.059	7.65	.0000	.15		
Stimulus period × stimulus	29	0.010	1.31	.2492	.03		
Stimulus period × size class	58	0.008	1.07	.3863	.05		
Stimulus period × stimulus × size class	58	0.009	1.10	.3565	.05		
Error	1218	0.008					
Latency							
Stimulus	1	1.112	0.36	.5528	.01	0.09	> 0.97
Size class	2	2.422	0.78	.4651	.04	0.20	> 0.95
Stimulus × size class	2	2.300	0.74	.4831	.03	0.17	> 0.95
Error	42	3.106					
Stimulus period	29	2.616	17.23	.0000	.29		
Stimulus period × stimulus	29	0.155	1.02	.4315	.02		
Stimulus period × size class	58	0.154	1.02	.4429	.05		
Stimulus period × stimulus × size class	58	0.207	1.37	.0999	.06		
Error	1218	0.152					

MS, mean square; η^2, effect size; power = $1 - \beta$.

[a] $\eta^2 = .29$
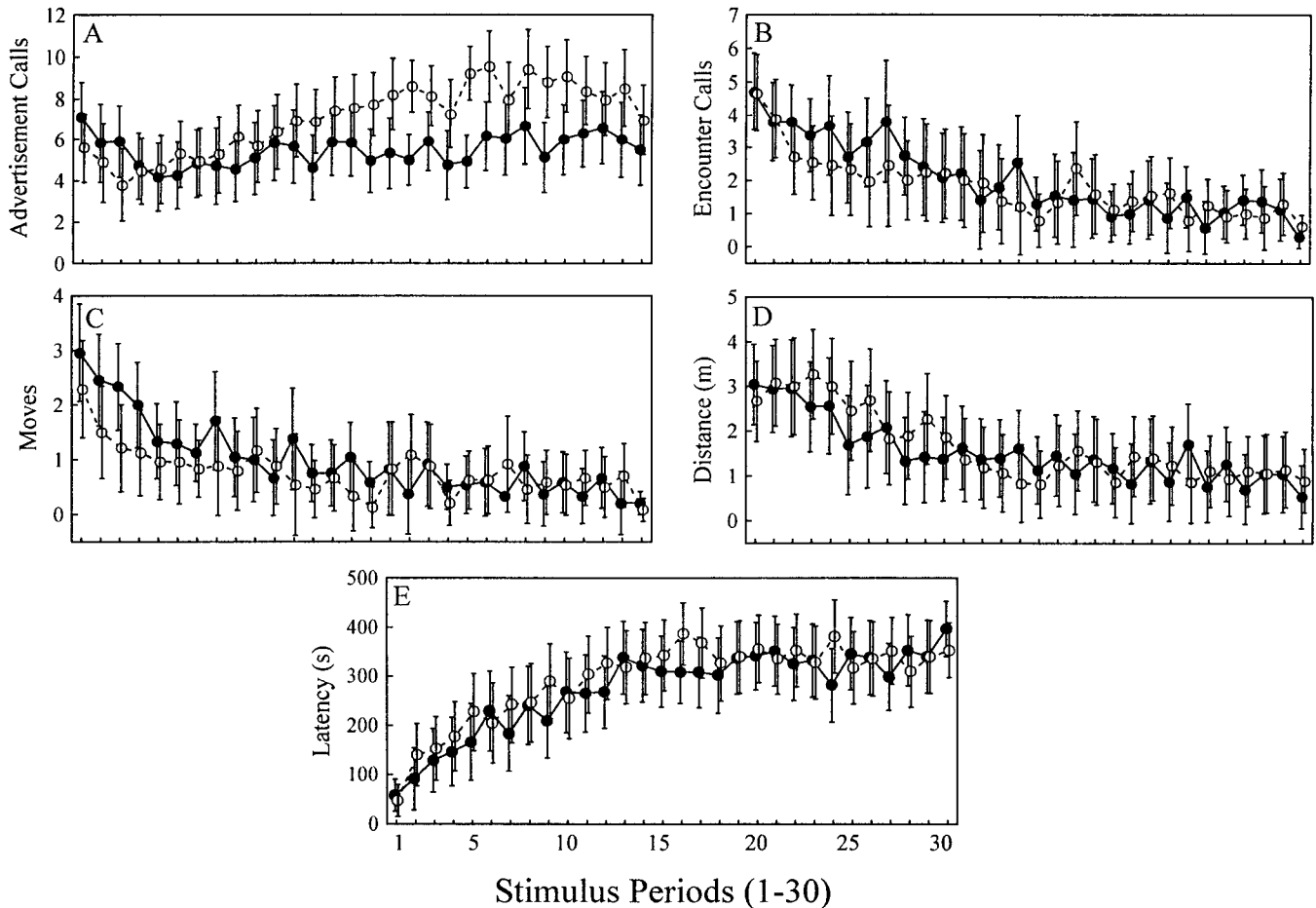
Figure 6
Mean (± 95% CI) responses of males that heard the large stimulus (filled circles, solid lines) and the small stimulus (open circles, dashed lines) during 30 stimulus periods of habituation training in 2000. Responses include the number of (A) advertisement calls, (B) encounter calls, and (C) movements, (D) the maximum approach distance, and (E) the latency to the first encounter call.

Comparison with other frogs

Behavioral discriminations between two conspecific signals based solely on differences in size-related spectral call properties has been demonstrated in field playback experiments in five species of frogs from four families. Male natterjack toads (*Bufo calamita,* Bufonidae) and male cricket frogs (*Acris crepitans,* Hylidae) were more likely to abandon calling or retreat in response to broadcasts of low-pitched calls, while a higher proportion of males attacked a speaker broadcasting high-pitched calls (Arak, 1983; Wagner, 1989a,b). Robertson (1986) demonstrated differences in the threshold playback amplitude required to evoke encounter calling and fighting or retreat that depended on the size of the simulated opponent in *Uperoleia rugosa* (Myobatrachidae). In the present study, the same proportion of bullfrogs "attacked" the playback speaker in response to all three stimuli.

Previous field playback studies conducted with territorial male carpenter frogs (*Rana virgatipes,* Ranidae) and green frogs (*Rana clamitans,* Ranidae) have also demonstrated that males respond differently to calls simulating males of different size. Like bulllfrogs, both carpenter frogs and green frogs have several distinct call types in their repertoire (Bee and Perrill, 1996; Given, 1987; Wells, 1978). Given (1987) found that male carpenter frogs produced more single-note aggressive calls and more total call notes in responses to the calls of a small male (dominant frequency = 622 Hz) compared to the call of a large male (dominant frequency = 524 Hz). In green frogs, the type II high-intensity advertisement call is considered an agonistic signal (Ramer et al., 1983; Wells, 1978). In response to the calls of a small male (dominant frequency = 505 Hz), other small males produced more type II calls, but large males did not. Large males produced more type II calls in response to the calls of another large male (dominant frequency = 288 Hz), whereas small males failed to increase their rate of type II calling in response to the large opponent (Ramer et al., 1983). Bee and Perrill (1996) demonstrated that male green frogs lower the dominant frequency of their calls during simulated territorial intrusions. In follow-up studies, Bee et al. (1999, 2000) found that the magnitude of frequency alteration depended on both the size of the simulated intruder and on the size of the subject. Males lowered their frequency more in response to simulated large intruders (dominant frequency = 350 Hz versus 400 Hz or 450 Hz), and this trend was most pronounced for smaller males. Wagner (1989a,b) also found that the properties of a male cricket frog's calls in response to playbacks depended on the simulated size of the opponent.

Both carpenter frogs and green frogs are closely related to bullfrogs (Hillis and Davis, 1986), and the vocal repertoire of all three species includes distinct aggressive vocalizations. However, I failed to demonstrate that bullfrogs respond with encounter calls differently depending on either their own

size, their opponent's size, or the degree of size asymmetry. Thus, it is interesting that both of these close relatives exhibit behavioral differences in elicited vocalizations depending on the size of a simulated opponent, while the bullfrogs in this study did not.

Comparison with game theory predictions

The results of this study are particularly interesting in light of expectations based on theoretical models of animal communication that predict the evolution of assessment signaling to settle asymmetric conflicts (e.g., Maynard Smith 1982). In bullfrogs, large body size confers an advantage in physical fights (Howard, 1978), and the fundamental frequency of advertisement calls is the best acoustic predictor of male body size (Bee and Gerhardt, 2001a). At a general level, game theory would predict that the size-related information conveyed in bullfrog advertisement calls should function as an assessment signal during aggressive encounters, as it does in other frogs. However, there was no evidence that the fundamental frequency of advertisement calls functioned as an assessment signal in bullfrogs.

Enquist's sequential assessment game (Enquist and Leimar, 1983; Enquist et al., 1990) makes predictions about the influence of asymmetries in fighting ability on the duration and intensity of aggressive encounters. For example, in conflict situations where the asymmetry in relative fighting ability is large and, presumably, clear to both contestants, the weaker individual is expected to quickly realize its low likelihood of winning the contest and decide to give up early in the interaction. The relatively stronger opponent should be more likely to persist and escalate to more aggressive behaviors. In contrast, when the asymmetry in relative fighting ability is small and more difficult to discern, interactions are expected to last longer and include a higher number of repetitions of aggressive behaviors.

Based on these model predictions, two predictions could be made for the aggressive response of male bullfrogs to repeated playbacks simulating intruders of different sizes. First, in cases where the asymmetry index is negative (e.g., when small males responded to the large stimulus), we might expect the duration of interactions to be relatively shorter because smaller males would give up early in the conflict. Second, in cases where the asymmetry in fighting ability was small (e.g., when males responded to an acoustically size-matched stimulus), we might expect interactions to be relatively more intense and to last relatively longer. However, there were no indications that the magnitude of the aggressive response and the number of stimulus periods until males stopped responding aggressively to the stimulus were related to the degree of size asymmetry.

Possible explanations for the apparent lack of size assessment in bullfrogs

Although there are no a priori reasons that bullfrogs should differ from other frogs in terms of acoustically mediated size assessment, there are at least three possible explanations for why this study failed to demonstrate acoustically mediated size assessment. First, this study can be criticized for using synthetic stimuli that may not have included acoustic elements necessary for size assessment and, therefore, did not elicit aggressive responses equivalent to those evoked by vocalizations of real intruders. Although this valid criticism is difficult to dismiss, I point out that bullfrogs respond aggressively to these stimuli, and some males continue to respond for several consecutive nights and for several consecutive hours each night (Bee, 2001; Bee and Gerhardt, 2001a). Differences in the harmonic structure of natural calls and the synthetic stimulus (cf.

Figure 1A and Figure 2) probably had negligible effects on size assessment. The bullfrog auditory periphery does not encode individual spectral components using a rate-place code, but instead appears to extract spectral information using a temporal code of phase-locked responses to the fundamental frequency and spectral components primarily below the fourth harmonic (Schwartz and Simmons, 1990; Simmons et al., 1992, 1993).

Second, the stimuli in this study were presented from speakers positioned 6 m from the subject, whereas other studies have presented stimuli from much smaller distances (e.g., 25 cm to 3 m; Ramer et al., 1983; Robertson, 1986). Aggressive responses in frogs are known to vary depending on the perceived proximity of another calling male (Given, 1987; Robertson, 1986; Schwartz, 1989; Wagner, 1989b), and thus it is conceivable that stimuli presented at a distance of 6 m were perceived as relatively nonthreatening. However, individual male bullfrogs exhibit repeated, aggressive approaches toward playback speakers positioned 6 m away, both within a night and across multiple nights of testing (Bee and Gerhardt, 2001a). Moreover, males discriminate between familiar territorial neighbors and strangers over similar distances (Davis, 1987), and interactions between adjacent territorial males sometimes occur over these distances (Bee, personal observation). These observations suggest that male bullfrogs perceive a new male calling from a distance of 6 m to be a threat to territory ownership and that males are willing to engage an opponent over this distance. Whether size assessment in bullfrogs and other species varies as a function of perceived proximity is a question open to further experimental study (see Robertson, 1986).

Another explanation stems from the recent finding that male bullfrogs actively lower the fundamental frequency of their advertisement calls in the context of male–male aggression (Bee and Bowling, in press). In sequences of consecutively recorded calls, males produced advertisement calls with significantly lower fundamental frequencies following the production of an encounter call, a signal associated with territory defense. A number of other frog studies have also demonstrated socially mediated reductions in spectral properties of advertisement calls during male–male vocal interactions (Bee and Perrill, 1996; Bee et al., 1999, 2000; Given, 1999; Howard and Young, 1998; Wagner, 1989a, 1992). Because spectral properties are negatively related to body size, which often determines fighting ability, male frogs might lower the frequency of spectral properties of their calls during aggressive encounters as an attempt to acoustically inflate their apparent size (Wagner, 1989a, 1992; Bee et al., 2000).

If frequency alteration represents a dishonest signal of size in frogs, then natural selection would favor receivers that devalue fundamental frequency as reliable size assessment cues, especially when other forms of assessment are not too costly. It is interesting to speculate that in bullfrogs, the costs of probing an opponent in a close-range interaction to more directly assess its fighting ability using visual or tactile cues may be sufficiently low to have permitted some devaluation of fundamental frequency as a reliable size assessment signal. This is not to say, of course, that size-related variation in advertisement calls does not play some role in size assessment. But there is little evidence to suggest that males use this information before more escalated encounters in which assessment could also be based on additional visual or tactile information. It is interesting that Davies and Halliday (1978) found that male common toads (*Bufo bufo*, Bufonidae) were more likely to persistently attack a small male in amplexus when that male was paired with broadcasts of a small male's high-pitched calls than when paired with the low-pitched calls of a large male. However, no such discrimination occurred when these play-

back stimuli were paired with a large opponent. Presumably, visual and tactile information also contributed to the assessment of an opponent's size and fighting ability.

The pitch of frog calls is often cited as a classic example of an unbluffable assessment signal in animal communication (e.g., Alcock, 1998; Bradbury and Vehrencamp, 1998; Krebs and Dawkins, 1984; Wiley, 1983). However, the results from recent studies demonstrating socially mediated plasticity in fundamental frequency during agonistic encounters call into question the reliability of call pitch as an assessment signal during aggressive interactions between male frogs. The importance of investigating whether call pitch functions as a size assessment cue is highlighted by the present study, which demonstrates the absence of size assessment based solely on size-related variation in acoustic signals. Clearly, frequency alteration and the use of fundamental frequency as an assessment signal by male frogs deserve additional experimental and theoretical consideration.

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