

## ORIGINAL ARTICLE

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**Size assessment in simulated territorial encounters between male green frogs (*Rana clamitans*)**

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**Abstract** We examined the ability of male green frogs to assess the size of an opponent based on the dominant frequency of their advertisement call, which is negatively correlated with size, using synthetic stimuli to simulate intruders of different sizes. In one field playback experiment, we broadcast a pair of stimuli representing a small and a large male; in a second experiment, we broadcast calls of a medium and a large male. In both experiments, males produced calls with significantly lower dominant frequencies in response to each stimulus. Contrast analyses revealed that males lowered the dominant frequency of their calls more in response to the large-male stimulus than in responses to the small- and medium-male stimuli. In the second experiment, males also responded to the large-male stimulus by calling at higher rates. There were no differences in mean note duration or the number of moves made toward or around the playback speaker in response to any stimulus. Thus, the frequency of an opponent's calls elicits a differential modification of calling behavior, primarily in the form of differential dominant frequency alteration, suggesting that males use dominant frequency to assess the size of opponents during aggressive encounters.

**Key words** *Rana clamitans* · Acoustic communication · Frequency alteration · Size assessment · Territoriality · Aggressive interactions

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**Introduction**

Animal communication involves an exchange of information between a sender and a receiver in the form of a signal. Communication signals often play important roles in conflict situations, such as disputes over resources, or contests over mates or territories. When these conflicts involve asymmetric contests, game theory predicts that animals can use detectable asymmetries in resource-holding potential (RHP) to settle the conflict (Parker 1974; Maynard Smith and Parker 1976; Maynard Smith 1979, 1982). The use of signals to assess the degree of asymmetry allows animals to settle conflicts through less costly displays rather than escalating each encounter to potentially injurious physical combat. In conflict situations, however, the sender and receiver may disagree about the best outcome. If assessment using communication signals plays a role in determining the outcome of asymmetric contests, selection may act on senders to inflate their apparent RHP, and signals may become deceptive. Attending to inflated signals, however, may decrease the fitness of receivers. Over time receivers should come to devalue inflated signals and attend only to unbluffable signals that honestly indicate a sender's RHP (Maynard Smith and Parker 1976; Zahavi 1977; Dawkins and Krebs 1978; Wiley 1983; Grafen 1990; Johnstone 1998), although deception may persist under some conditions, such as unavoidable receiver error or low costs of being deceived (Bond 1989; Gardner and Morris 1989; Wiley 1994).

An important source of asymmetry in RHP is the difference in body or weapon size between contestants (reviewed in Archer 1988; Andersson 1994; Reichert 1998). Contestants which are larger or possess larger weapons often win a disproportionate number of contests over smaller opponents or opponents with smaller weapons (e.g., Englund and Olsson 1990; Polak 1994; Jennions and Backwell 1996; Barki et al. 1997; Hack et al. 1997). When signals are used to settle size-asymmetric conflicts, receivers should attend only to those

unbluffable signals that are directly influenced by body size. One example of a potentially unbluffable signal that is constrained by body size, and is therefore expected to honestly indicate RHP, is the fundamental frequency of acoustic signals (Morton 1977; Wiley 1983; Krebs and Dawkins 1984; Cheney and Seyfarth 1991).

Among anuran amphibians, the frequency spectrum of acoustic signals appears to be a relatively good cue for assessing an opponent's RHP. Several studies have demonstrated that male frogs use call frequency, which is negatively correlated with body size, to assess the relative size of other males during contests over females or territories (Davies and Halliday 1978; Arak 1983; Ramer et al. 1983; Robertson 1986; Given 1987). Because large males more often win aggressive physical encounters than males of other size classes (Davies and Halliday 1978; Howard 1978; Wells 1978; Arak 1983; Given 1988; Wagner 1989), accurate assessment of an opponent's size allows contests to be settled with minimal costs to residents and intruders. Several species of frogs, however, alter the frequency of their calls during interactions with other males (e.g., white-lipped frogs, *Leptodactylus albilabris*, Lopez et al. 1988; cricket frogs, *Acris crepitans*, Wagner 1989, 1992; American toads, *Bufo americanus*, Howard and Young 1998; and wood frogs, *Rana virgatipes*, Given, in press). In an earlier study, we demonstrated that male green frogs, *R. clamitans*, decrease the dominant frequency of their calls in response to playbacks of conspecific advertisement calls in simulated territorial encounters (Bee and Perrill 1996).

Because body size and call frequency are strongly negatively correlated in male green frogs (Bee and Perrill 1996), and larger green frogs win fights more often than smaller males (Wells 1978), the ability to produce calls with lower frequencies during aggressive encounters provides a potential means of bluffing actual size and RHP during territorial contests (Wagner 1992). If frequency alteration represents a dishonest signal, we would expect receivers to devalue frequency as containing any useful information about body size. Therefore, we asked whether male green frogs use call frequency to assess the size of an opponent during aggressive interactions. In this study, we report results from two field playback experiments in which we presented males with two synthetic green frog advertisement calls that differed only in fundamental frequency, simulating two males of different sizes and, presumably, RHP. We recorded males' response calls and determined the mean dominant frequency, mean note duration, and the calling rate for responses to both stimuli. In addition, we counted the number of moves a male made toward or around the playback speaker as an indicator of his propensity to fight. We tested the hypothesis that some or all of these call properties and a male's fighting propensity would differ between responses to each conspecific stimulus against the null hypothesis that responses to the two stimuli would not differ. Hence, we predicted that if males rely on an opponent's call frequency to estimate his size or

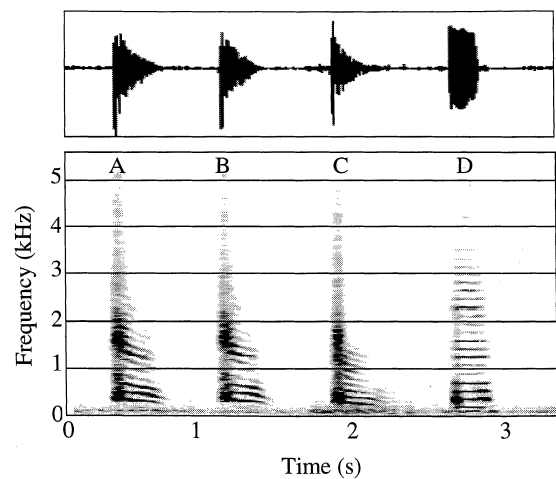
RHP, we should observe differences in behavior in responses to the two stimuli in each experiment.

## Methods

### Study site and organism

We studied the vocal behavior of male green frogs during June and July of 1995 and 1996 in four ponds in Indianapolis, Indiana, where they breed from mid-May through late August. Males typically call from aquatic territories in shallow water around the perimeter of the pond. Females have been shown to choose males with territories having the highest-quality oviposition sites (Wells 1977). Male green frogs have a complex vocal repertoire with five call types (Wells 1978; Bee and Perrill 1996). The advertisement call (type I) is best described as a short-duration signal (120–240 ms) that is typically produced once or twice per minute as a single-note call or a multiple-note call with up to six consecutive notes. Both the rate of calling and the number of notes comprising each call tend to increase with general chorus activity during the night, and calling rates of three to four calls per minute are not uncommon during periods of active chorusing (personal observation). The type I advertisement call is a broad-band signal with a variable number of harmonics (usually 10–20). Sound energy is concentrated into two distinct bands centered around 400 Hz and 1600 Hz (Fig. 1, calls A and B). The dominant frequency, defined here as the single frequency of greatest relative amplitude, is in the lower band and corresponds to the second harmonic of the fundamental frequency. Mean values of dominant frequency and note duration of type I advertisement calls in our study population are approximately 400 Hz and 160 ms, respectively (Bee and Perrill 1996).

Males use encounter calls (type III) in close-range territory defense against intruding males (Wells 1978; Bee and Perrill 1996), and aggressive interactions can escalate to intense wrestling bouts (Wells 1978; personal observation). Type III encounter calls are spectrally and temporally very similar to type I advertisement calls (cf. Fig. 1, calls B and C), but are marked by lower frequency and longer durations, and are produced at lower sound pressure levels (SPLs) and increased calling rates (Bee and Perrill 1996). Males



**Fig. 1** Oscillograms (*top*) displaying the amplitude-time envelope of the calls and sonograms (*bottom*) displaying the relative amplitudes of the harmonics in the calls (*darker shading* corresponds to higher intensity). *A* and *B*: two representative type I advertisement calls from a male green frog; *C*: a type III encounter call from the same male given in response to the 350-Hz stimulus; *D*: the 350-Hz stimulus used in playback tests. The additional harmonics above the first nine are a result of harmonic distortion

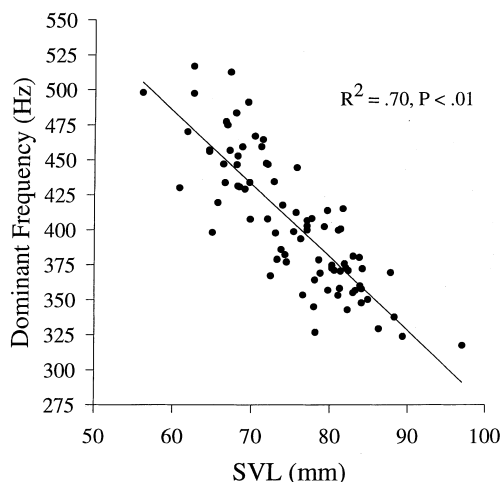
also produce a "high intensity advertisement call" (type II), which is very distinct from type I advertisement calls and type III encounter calls. Wells (1978) reported that this call was most commonly produced during aggressive encounters across territorial borders between neighbors, but in a previous study (Bee and Perrill 1996), we suggested that the function of this call is not clear.

### Experimental design

We performed two experiments in a repeated-measures design with three treatment levels (Table 1). In both experiments, the first treatment was always a 4-min no-stimulus period during which we recorded unsolicited vocalizations to serve as controls. We then broadcast a different synthetic type I advertisement call in each of two consecutive 4-min stimulus periods following the 4-min no-stimulus period. In experiment 1 ( $n = 30$ ), the stimuli had dominant frequencies of 450 Hz and 350 Hz. The dominant frequencies for the stimuli used in experiment 2 ( $n = 30$ ) were 400 Hz and 350 Hz. In both experiments, the sequence of stimulus presentation was counterbalanced between subjects to reduce the potential effects of presentation order on male responses (Table 1). Because snout-vent length (SVL) is negatively correlated with dominant frequency, the 450-Hz, 400-Hz, and 350-Hz stimuli correspond to the natural calls of males with approximate SVLs of 66.6 (small), 76.2 (medium), and 85.7 mm (large), respectively, based on the regression of dominant frequency on SVL for all males recorded in the population between 1994 and 1996 (Fig. 2). The mean ( $\pm$  SD) SVLs of males tested in experiments 1 and 2 were  $74.0 \pm 8.0$  mm and  $77.9 \pm 8.0$  mm, respectively, and were not significantly different

**Table 1** Design of stimulus presentation order for experiments 1 and 2. Each stimulus period was 4 min

Treatment order	Sequence 1 ( $n = 15$ )	Sequence 2 ( $n = 15$ )
Experiment 1: 450 vs 350 Hz		
1	No stimulus	No stimulus
2	450 Hz	350 Hz
3	350 Hz	450 Hz
Experiment 2: 400 vs 350 Hz		
1	No stimulus	No stimulus
2	400 Hz	350 Hz
3	350 Hz	400 Hz



**Fig. 2** Regression of the mean dominant frequency of two to ten calls on snout-vent length (SVL) for 83 males recorded in the Indianapolis, Indiana population between 1994 and 1996  $Y = -5.24X + 799.11$

( $t_{57} = 1.87$ ,  $P = 0.07$ ). No male was tested in the same experiment twice or in more than one experiment on the same night. Only two males were tested in both experiments.

### Synthetic stimuli

The synthetic stimuli consisted of a series of sine waves, which we generated from digital files with 8-bit resolution at a sampling rate of 20 kHz using a custom-designed sound synthesis program (available from J.J. Schwartz). The 450-Hz, 400-Hz, and 350-Hz stimuli consisted of the first nine harmonics, including the first harmonic (fundamental), which were integer multiples of fundamental frequencies of 225 Hz, 200 Hz, and 175 Hz, respectively. The relative amplitudes of each of the nine harmonics in the synthetic stimuli were based on our analyses of natural type I advertisement calls and were the same for each stimulus (Fig. 1, call D). Each stimulus had a note duration of 160 ms. Thus, the frequency spectrum was the only call property that varied between the pair of synthetic stimuli used in an experiment. We recorded the synthetic stimuli onto separate cassette tapes from an IBM-compatible computer equipped with a SoundMan 16 sound board.

### Playback and recording procedures

A Marantz PMD430 recorder was used to broadcast stimuli through a Sony APM-090 Amplifier Powered Monitor speaker mounted on a styrofoam platform floated at 1 m from the test subject. Each stimulus call was on a separate continuous tape and was repeated at 15-s intervals. We broadcast stimuli at a SPL of 84 dB (re 20  $\mu$ Pa, 'fast' RMS) at 1 m, which corresponds to the SPL of natural calls at that distance (Bee and Perrill 1996). SPLs were calibrated with a Brüel and Kjær Precision Integrating Sound Level Meter Type 2230. We recorded male vocalizations during all three test periods with a second Marantz PMD430 cassette recorder and an Audio-technica AT815 condenser microphone.

Wells (1978) observed movements and splash displays associated with aggressive interactions that commonly preceded physical encounters. During playbacks, males often jumped toward and around the speaker. As a behavioral indicator of a male's propensity to fight, we counted the number of times it moved during the playback of each stimulus. After each playback test, the male was captured, toe-clipped, weighed (to the nearest 1 g), and SVL was measured (to the nearest 1 mm). All playback trials were conducted under ambient light levels between 2000 and 0330 hours EST.

### Analysis of recorded vocalizations

From our recordings, we determined the dominant frequency (i.e., the second harmonic) and the note duration of all type I advertisement calls and type III encounter calls. We limited our analyses to the first note if a call was a multiple-note call. Because type II high-intensity advertisement calls rarely occurred during playback trials, and their significance is ambiguous (Bee and Perrill 1996), they were excluded from all analyses. The calling rate was determined as the number of type I and type III calls produced during each 4-min period. The spectral and temporal features of recorded vocalizations were analyzed using SoundEdit 2.0 (for calls recorded in 1995;  $\pm 10$  Hz) or a Kay DSP Sonograph Model 5500 (for calls recorded in 1996;  $\pm 5$  Hz). A subset of the calls recorded in 1995 and originally analyzed using SoundEdit were reanalyzed with the Kay sonograph to confirm that the two analysis methods yielded similar results. We determined the dominant frequency from a spectrogram, generated by averaging the spectrum over a thin time section (approximately 50 ms) taken from the beginning to middle of the call. Note duration was determined to the nearest millisecond from an oscillogram.

### Statistical analysis

Mean dominant frequency, mean note duration, and calling rate (calls/4-min period) for calls produced by each male in each

treatment period served as dependent response variables in a repeated-measures multivariate analysis of covariance (MANCOVA) in which SVL was the single covariate. A preliminary analysis indicated that SVL was significantly correlated with dominant frequency in all three treatment periods in both experiments, with note duration in experiment 1, and with the number of moves in experiment 2 (Table 2). The effects of relative and absolute body size on the responses of males to different sized intruders are examined elsewhere (M.A. Bee, S.A. Perrill, P.C. Owen, unpublished data). Because we were primarily interested in the size-independent differences in male responses to two different-sized intruders, we used SVL as a covariate to examine differences in responses to the two stimuli. We tested the assumption of normality for each response variable in both experiments using one-sample Kolmogorov-Smirnov tests (all  $P$ -values  $> 0.05$ ). Separate univariate repeated-measures analyses of covariance (ANCOVA) were computed for each response variable, using SVL as a covariate. Because repeated-measures analyses often violate the assumption of sphericity, thereby increasing the risk of type I errors (Keselman and Rogan 1980), we used the Greenhouse and Geisser approach to adjust the degrees of freedom for the  $F$ -tests in each omnibus ANCOVA (Greenhouse and Geisser 1959). We used planned contrasts (Rosenthal and Rosnow 1985) to compare mean dominant frequency, mean note duration, and calling rate in the responses to the two stimuli in each experiment to test the hypothesis that males differentially modify properties of their calls based on differences in the frequency of the stimulus. We did not compute the contrasts of each stimulus period versus the no-stimulus period for two reasons: (1) we have already demonstrated that each call property is altered in responses to natural conspecific advertisement calls compared to no-stimulus and heterospecific controls (Bee and Perrill 1996), and (2) we were primarily interested in differences in the responses to the two different stimuli representing males of different sizes. Significance was set at  $\alpha = 0.05$  for all analyses and the results from each experiment were analyzed separately. For each statistical test, we also calculate the effect size, which measures the degree of departure from the null hypothesis (Cohen 1988). The effect size index for the analysis of variance ( $f$ ) is defined as the standard deviation of standardized means (Cohen 1988). As such,  $f$  measures the departure of mean differences from the hypothesized expectation of no difference. We provide estimates of the Greenhouse and Geisser correction for violations of sphericity ( $\epsilon$ ), post hoc measures of effect size ( $f$ ), and the statistical power ( $1-\beta$ ) of treatments yielding non-significant results (after Cohen 1988).

## Results

### Experiment 1: 450 Hz versus 350 Hz

MANCOVA revealed significant differences between calls produced during the three treatment periods in this

**Table 2** Correlations ( $r$ ) between snout-vent length and the dependent variables in experiments 1 and 2

	Dominant frequency	Note duration	Calling rate	Moves
Experiment 1				
No stimulus	-0.88**	0.15	-0.15	
450 Hz	-0.88**	0.51*	0.08	0.24
350 Hz	-0.90**	0.61**	-0.08	0.25
Experiment 2				
No stimulus	-0.72**	0.22	0.08	
400 Hz	-0.66**	0.05	0.08	0.72*
350 Hz	-0.62**	0.02	0.11	0.64*

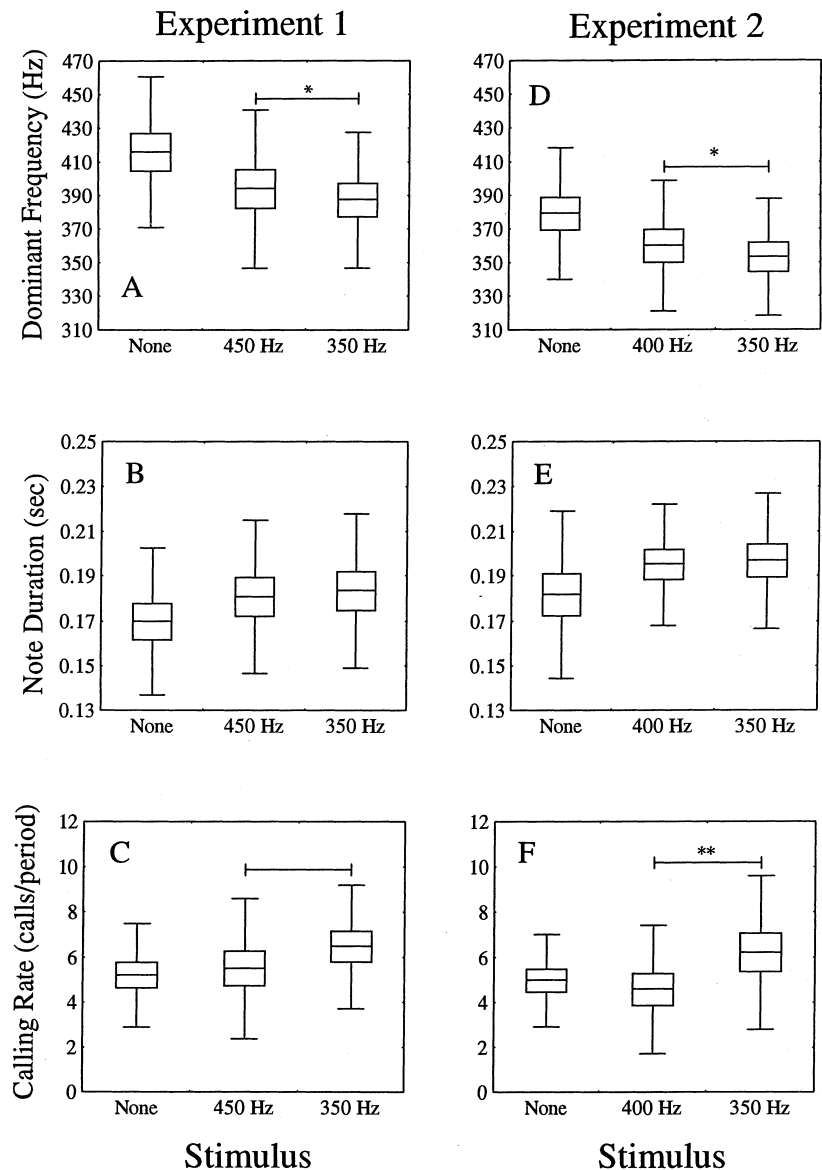
\* $P < 0.01$ ; \*\* $P < 0.001$

experiment (Wilk's  $\lambda = 0.32$ ,  $P < 0.01$ ; Fig. 3). Subsequent univariate ANCOVAs revealed a significant difference in dominant frequency ( $F_{2,58} = 35.32$ ,  $P < 0.01$ ,  $\epsilon = 0.82$ ,  $f = 1.09$ ; Fig. 3A). A contrast analysis revealed that the mean ( $\pm$  SD) dominant frequency of responses to the 350-Hz stimulus ( $387.2 \pm 40.3$  Hz) was significantly lower than the mean dominant frequency of responses to the 450-Hz stimulus ( $393.8 \pm 47.0$  Hz) ( $F_{1,29} = 6.36$ ,  $P = 0.02$ ,  $f = 0.47$ ; Fig. 3A). The mean changes in dominant frequency in response to the 450-Hz and 350-Hz stimuli were  $-22.0 \pm 22.3$  Hz and  $-28.6 \pm 20.9$  Hz, respectively. Nineteen of 30 males (63.3%) lowered the dominant frequency of their responses more to broadcasts of the 350-Hz stimulus than to the 450-Hz stimulus. There were no significant differences in note duration ( $F_{2,58} = 3.15$ ,  $P = 0.07$ ,  $\epsilon = 0.68$ ,  $f = 0.33$ ,  $1-\beta = 0.61$ ; Fig. 3B) and calling rate ( $F_{2,58} = 2.66$ ,  $P = 0.08$ ,  $\epsilon = 0.97$ ,  $f = 0.30$ ,  $1-\beta = 0.52$ ; Fig. 3C). The mean note durations of responses to the 450-Hz and 350-Hz stimuli were  $180.6 \pm 34.2$  ms and  $183.3 \pm 34.4$  ms, respectively, and were not significantly different ( $F_{1,29} = 0.67$ ,  $P = 0.42$ ,  $f = 0.15$ ,  $1-\beta = 0.12$ ; Fig. 3B). Males did not call at significantly different rates in response to the 450-Hz stimulus ( $1.4 \pm 0.8$  calls/min) and the 350-Hz stimulus ( $1.6 \pm 0.7$  calls/min) ( $F_{1,29} = 3.09$ ,  $P = 0.09$ ,  $f = 0.33$ ,  $1-\beta = 0.45$ ; Fig. 3C). The mean number of moves that were made during responses to the 450-Hz ( $2.0 \pm 3.7$ ) and 350-Hz ( $2.3 \pm 3.4$ ) stimuli were not significantly different ( $F_{1,29} = 0.50$ ,  $P = 0.48$ ,  $f = 0.13$ ,  $1-\beta = 0.10$ ).

### Experiment 2: 400 Hz versus 350 Hz

One male was excluded from the following statistical analyses because of our failure to measure SVL. The MANCOVA revealed significant differences between calls produced during the three treatment periods (Wilk's  $\lambda = 0.23$ ,  $P < 0.01$ ; Fig. 3). The subsequent univariate ANCOVAs revealed significant differences in dominant frequency ( $F_{2,56} = 32.13$ ,  $P < 0.01$ ,  $\epsilon = 0.86$ ,  $f = 1.06$ ; Fig. 3D) and calling rate ( $F_{2,56} = 3.55$ ,  $P = 0.05$ ,  $\epsilon = 0.82$ ,  $f = 0.35$ ; Fig. 3F), but no significant differences in note duration were detected ( $F_{2,56} = 3.23$ ,  $P = 0.06$ ,  $\epsilon = 0.70$ ,  $f = 0.34$ ,  $1-\beta = 0.64$ ; Fig. 3E). The analysis of planned contrasts revealed that the mean dominant frequency of responses to the 350-Hz stimulus ( $353.0 \pm 34.8$  Hz) was significantly lower than that of responses to the 400-Hz stimulus ( $359.7 \pm 38.8$ ) ( $F_{1,28} = 5.30$ ,  $P = 0.03$ ,  $f = 0.44$ ; Fig. 3D). The mean changes in dominant frequency in response to the 400-Hz and 350-Hz stimuli were  $-19.2 \pm 20.9$  Hz and  $-25.9 \pm 18.2$  Hz, respectively. Twenty of 30 males (66.7%) lowered the dominant frequency of their responses more to the 350-Hz stimulus. Males did not produce calls of significantly different durations in their responses to the 400-Hz stimulus ( $195.0 \pm 27.0$  ms) and the 350-Hz stimulus ( $196.7 \pm 30.1$  ms) ( $F_{1,28} = 0.16$ ,

**Fig. 3** Box plots showing the means (line),  $\pm 2$  SE (box),  $\pm 1$  SD (whiskers) of dominant frequency (A), note duration (B), and calling rate (C) during the no-stimulus period and the 450-Hz and 350-Hz stimulus periods of experiment 1, and of dominant frequency (D), note duration (E), and calling rate (F) during the no-stimulus period and the 400-Hz and 350-Hz stimulus periods of experiment 2 (\* $P < 0.05$ , \*\*  $< 0.01$ ;  $P$ -values are from planned contrasts)



## Discussion

In a previous study, we reported that male green frogs responded to broadcasts of natural conspecific advertisement calls almost exclusively with type III encounter calls (Bee and Perrill 1996). Males also direct type III encounter calls toward opponents in close-range interactions during aggressive contests over territories (Wells 1978; personal observation). Although type I advertisement calls and type III encounter calls are spectrally and temporally very similar (Fig. 1), type III encounter calls have lower dominant frequencies and longer note durations, and are produced at higher rates and lower SPLs (Bee and Perrill 1996). We observed similar responses to the synthetic stimuli used in this study (Fig. 3). Thus, the important temporal and spectral changes in vocalizations that occur during simulated

territorial contests, which include a decrease in dominant frequency and an increase in note duration and calling rate, appear to be consistent within and between years and can be elicited with natural and synthetic stimuli. Notably, however, change in dominant frequency is the only behavioral modification consistent between the two experiments in this study and between this and a previous study (Bee and Perrill 1996).

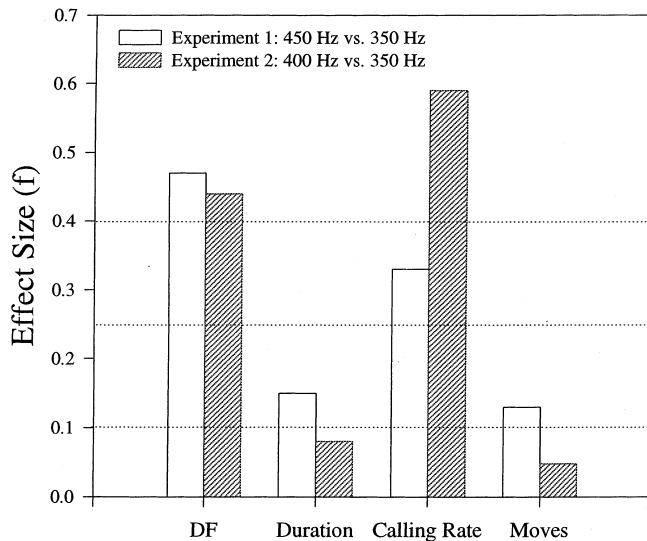
Wells (1978) demonstrated that SVL is a good predictor of fighting ability in male green frogs. In our playback experiments, the only difference between test stimuli was the fundamental frequency of the call, which is negatively correlated with male SVL in green frogs (Fig. 2). Therefore, our stimuli acoustically simulated males with different RHPs. Because male green frogs have previously been shown to alter dominant frequency, note duration, and calling rate during simulated territorial contests (Bee and Perrill 1996), we asked whether males altered these properties differentially to stimuli representing males of different sizes and RHP. Males lowered the dominant frequency of their response calls significantly more to the large-male stimulus compared to the small-male and medium-male stimuli. Similarly, males increased their rate of calling significantly more in response to the large-male stimulus in experiment 2. These results indicate that males modify their vocal behavior in different ways or to different degrees depending on the frequency of an opponent's call (see also Ramer et al. 1983). This observation held when the difference in dominant frequency between the two stimuli was as small as 50 Hz, corresponding to a SVL difference of about 1 cm (Fig. 2). Surprisingly, we failed to find any differences in the number of moves made toward or around the speaker during the 4-min broadcasts of each stimulus. Thus, the differences in behavior elicited by broadcasts of stimuli representing males of different sizes are manifest only in the aggressive vocalizations males produce in response to a perceived intruder, particularly in the magnitude of frequency alteration.

An important distinction in animal communication is the difference between broadcast and transmitted information (Wiley 1983; Krebs and Dawkins 1984). Broadcast information is measured as an increase in the predictability of a sender's identity, condition, or behavior after it produces a signal, and refers to the information encoded in the signal by the sender. Transmitted information, on the other hand, is measured as an increase in the predictability of a receiver's behavior after it receives a signal and refers to the information actually decoded from the signal by the receiver. Differences between broadcast and transmitted information can result from propagation distortion and receiver error (Wiley 1994). The strong inverse relationship between body size and fundamental frequency in the calls of green frogs (Fig. 2) indicates that unsolicited type I advertisement calls contain broadcast information about the size of the caller. This information is encoded by many acoustically signaling anurans as a

result of constraints imposed by the shape and mass of the laryngeal apparatus, which determine the fundamental frequency of acoustic signals (Martin 1972; Duellman and Trueb 1986). Our observation that male green frogs responded differently to synthetic stimuli differing only in fundamental frequency suggests that some information about an opponent's body size is transmitted to receivers during aggressive interactions. The data presented here thus support the hypothesis that male green frogs use the frequency of advertisement calls to assess the size of competitive rivals during agonistic encounters. Because green frogs attend to the information about body size conveyed in the fundamental frequency, the production of lower-frequency calls during aggressive interactions represents a potential means of producing deceitful signals of size and the expectation that fundamental frequency is an unbluffable signal may be compromised.

Although male green frogs alter dominant frequency and calling rate by significantly different magnitudes in response to simulated intruders with different RHPs, it is unclear whether the magnitude of the differences between responses to the different stimuli is biologically relevant. It was beyond the scope of this study to determine whether the seemingly small differences between the magnitudes of frequency and calling rate alterations to each stimulus documented here have any effect on the subsequent behavior of the receiver of the altered signal. That is, whether the difference between the magnitudes of frequency and calling rate alteration to large and small males, for example, functions in communication is unknown. We do not claim that these differences are behaviorally significant. The results of these two experiments, however, clearly demonstrate that males alter certain call properties to significantly different degrees based on the perceived size of the intruder.

Based on his survey of effect sizes in the behavioral sciences, Cohen (1988) suggested three conventional levels of  $f$  for small ( $f = 0.10$ ), medium ( $f = 0.25$ ), and large ( $f = 0.40$ ) effect sizes. The statistically significant and consistently directional differences in dominant frequency and calling rate alteration to stimuli representing males of different sizes, coupled with an examination of the effect sizes ( $f$ ) associated with the differences in the perceived size of the broadcast stimuli, suggests that the effects of an opponent's body size on a resident's vocal responses are medium to large for changes in dominant frequency and calling rate (Fig. 4). Because statistical power increases as a function of effect size and sample size at a specified  $\alpha$ -level (Cohen 1988), our inability to detect a statistically significant difference in calling rate during responses to the 450-Hz and the 350-Hz stimuli in experiment 1 results from our low power ( $1-\beta = 0.45$ ) to detect medium effect sizes with our sample size and not from the lack of an effect. We believe that accepting the null hypothesis that calling rate does not differ in responses to the 450-Hz and 350-Hz stimuli would represent a type II error resulting from low power associated with an insufficient sample



**Fig. 4** Cohen's (1988) effect size index ( $f$ ) for the differences in dominant frequency ( $DF$ ), note duration, calling rate, and the number of moves made in responses to the 450-Hz and 350-Hz stimuli of experiment 1 and the 400-Hz and 350-Hz stimuli of experiment 2. Cohen's (1988) conventional levels of small ( $f = 0.10$ ), medium ( $f = 0.25$ ), and large ( $f = 0.40$ ) effect sizes are indicated by dotted lines

size (Rosenthal and Rosnow 1991). A slightly larger sample size would have sufficiently increased our ability to detect these differences at the conventional  $\alpha = 0.05$  level (Cohen 1988). It is not clear why the effect size associated with the difference in calling rate in response to the larger absolute difference between stimuli in experiment 1 is smaller than that in experiment 2. Nonetheless, these effect sizes suggest that the perceived size of an intruder, based on dominant frequency, has a strong effect on the degree to which males modify their vocalizations during agonistic encounters. Figure 4 also illustrates that differences in the dominant frequency of the stimuli had small effects on differences in note duration and the number of moves made toward or around the speaker, suggesting that differential responses to intruders of different sizes are manifest in changes in dominant frequency and calling rate.

Other studies examining the ability of anurans to assess an opponent's size during contests have examined the probabilities of attack or retreat associated with encountering a large or small opponent (e.g., Davies and Halliday 1978; Arak 1983; Wagner 1989). The implications of these studies are clear: males attack smaller opponents and retreat from larger opponents. Thus, male anurans make decisions about whether or not to escalate conflicts after assessing the size of their opponents based on their call frequency. The results reported here are less straightforward. We have not demonstrated that male green frogs base decisions about escalating contests on their perception of the size of their opponent. In fact, the differences in the fundamental frequencies of the stimuli had negligible effects on determining the number of moves a male made

toward or around a speaker (Fig. 4). This difference between green frogs and other anurans may reflect some level of devaluation of the size information conveyed in the fundamental frequency of the signal. We have, however, demonstrated that perceived differences in opponent size elicit differences in calling behavior during contests, primarily in the form of differential reduction of dominant frequency. An interesting question is why male green frogs produce advertisement calls with dominant frequencies higher than the lowest frequency they can produce. One possibility is that the decrease in SPL that is concurrent with the decrease in dominant frequency (Bee and Perrill 1996) represents a cost to producing lower-frequency calls that is paid in the form of a reduction in the effective distance of signal propagation. The role of frequency alteration in male-male communication and how it relates to the perceived relative and absolute RHP of an opponent are two additional questions that are open to further investigation. Male green frogs do not appear to use frequency alteration to signal more accurate information about their body size or their size-independent fighting ability during aggressive interactions (Bee and Perrill 1996; M.A. Bee, S.A. Perrill, P.C. Owen, unpublished data), and the hypothesis that frequency alteration represents a means of intraspecific deception remains an interesting possibility.

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