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## Context-dependent plasticity of aggressive signalling in a dynamic social environment

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Frog choruses are dynamic social environments in which males must balance the conflicting demands of attracting a female and defending their calling site. The optimal trade-off is probably one that adaptively tracks temporal and spatial changes in the local social environment. Previous studies have indicated that a male frog's propensity to interact aggressively with its nearby neighbours reflects patterns of plasticity that could allow for such tracking. We presently do not know the mechanisms underlying plasticity in aggressive signalling in frogs, nor do we understand how plasticity depends on variation in social context. In this field playback experiment with spring peepers, *Pseudacris crucifer*, we investigated how differences in the type of signalling interactions (a form of social context) experienced by calling males influence the plasticity of aggression directed towards nearby neighbours in the chorus. We used the habituation–discrimination experimental paradigm to present males with calls simulating a new neighbour that was initially either aggressive (producing a mixture of advertisement and aggressive vocalizations) or nonaggressive (producing only advertisement calls). We found that interacting with an initially aggressive versus nonaggressive neighbour had negligible effects on the plasticity of a male's aggressive response, but that subsequent changes in social context (e.g. a switch to aggressive calling by a previously nonaggressive neighbour) greatly influenced a male's aggressive responsiveness. We discuss our results in terms of short-term habituation as a mechanism for the plasticity and specificity of aggressive signalling behaviour in frog choruses.

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Many animal behaviours occur in dynamic social environments that show high degrees of spatial and temporal variability in both density and composition (Parrish & Hamner 1997). Among the most impressive examples are the often large and dense breeding choruses of many insects and anurans (Gerhardt & Huber 2002; Greenfield 2005; Wells 2007). In frogs, choruses typically form when males aggregate in suitable breeding habitats and produce acoustic signals that function to attract females and repel rival males (Wells 1977; Gerhardt & Huber 2002; Wells & Schwartz 2007). Frog choruses are dynamic in the sense that male calling activity shows both diel and seasonal patterns of variation (Runkle et al. 1994; Murphy 1999; Bridges & Dorcas 2000; Oseen & Wassersug 2002). Moreover, the attendance and calling activity of particular individuals in the chorus can be highly variable within and between nights (Murphy 1994; Runkle et al. 1994; Friedl &

Klump 2005). Variability in the local social environments encountered by signalling male frogs has potentially important implications for the evolution of frog communication systems.

Sexual selection in the form of female choice and male–male competition has played a fundamental role in the evolution of anuran communication (Ryan 1991; Ryan & Rand 1993; Gerhardt 1994; Gerhardt & Huber 2002). The dynamic nature of choruses poses trade-offs between attracting mates (i.e. female choice) and defending calling sites and territories against rivals (i.e. male–male competition). Within a chorus, calling male frogs typically switch from producing exclusively advertisement calls to producing a combination of advertisement calls and aggressive calls in response to males that intrude into their calling site or territory (Fellers 1979; Wells & Schwartz 1984; Schwartz 1989; Bee et al. 1999), or when the perceived amplitude of another nearby male's signals exceeds some threshold level (Brenowitz & Rose 1994; Stewart & Bishop 1994; Rose & Brenowitz 1997; Marshall et al. 2003). Aggressive signalling has important benefits and costs for calling male frogs. On one hand, the production of advertisement and aggressive calls allows males to maintain some minimum distance between themselves and their nearby neighbours in the chorus (Whitney & Krebs 1975; Robertson 1984; Telford 1985;

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Wilczynski & Brenowitz 1988; Brenowitz 1989; Murphy & Floyd 2005). The maintenance of intermale spacing, in turn, can benefit signalling males by improving the ability of females to recognize and locate potential mates and to exercise preferences for particular call properties (Telford 1985; Dyson & Passmore 1992; Schwartz & Gerhardt 1995). On the other hand, however, males may risk reduced mate attraction during episodes of aggressive signalling because females typically prefer advertisement calls over aggressive calls (Oldham & Gerhardt 1975; Schwartz 1986; Wells & Bard 1987; Brenowitz & Rose 1999; Marshall et al. 2003).

In light of the trade-off between attracting mates and repelling rivals in the dynamic environment of a frog chorus, we might expect natural selection to favour the evolution of flexible signalling behaviours that optimize the ratio of benefits (intermale separation) and costs (reduced mate attraction) of aggressive signalling within any particular chorus. This hypothesis is supported by the discovery that male frogs use aggressive calls in ways that depend on recent experience and that dynamically track changes in the local social environment (Brenowitz 1989; Rose & Brenowitz 1991; Burmeister et al. 1999a; Bee & Gerhardt 2002; Marshall et al. 2003). For example, in several species of treefrogs (Hylidae), the thresholds at which males switch from advertisement calling to aggressive signalling can be modified by recent experience with a nearby calling neighbour and can depend on factors related to social context, such as acoustic correlates of local density in the form of the perceived amplitude of their nearest neighbours' calls (Brenowitz 1989; Rose & Brenowitz 1991, 1997; Brenowitz & Rose 1994; Marshall et al. 2003). At present, we do not know the neurophysiological mechanisms that generate plasticity in aggressive behaviour, nor do we understand well how various factors related to social context might influence such plasticity in variable social environments (but see Bee 2001).

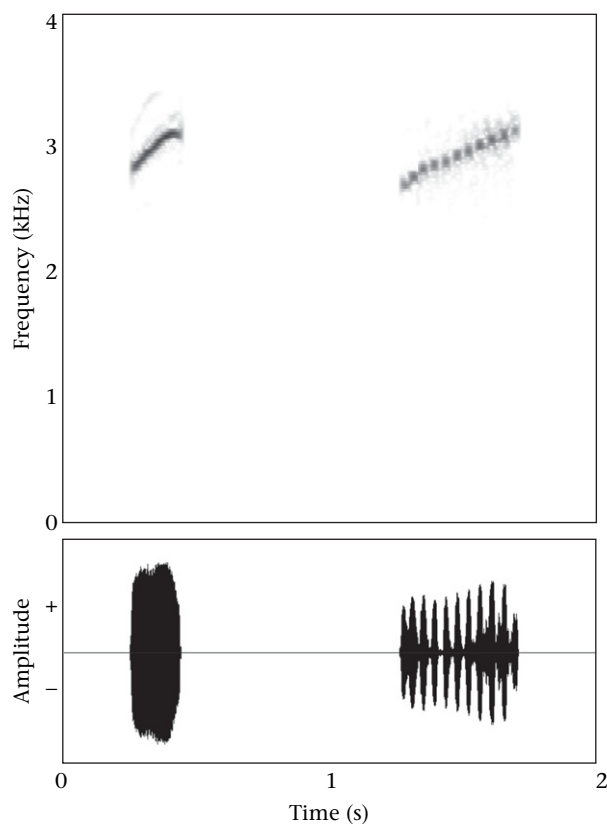
Here, we report results from a study of spring peepers, *Pseudacris crucifer* (Hylidae), in which we tested the general hypothesis that plasticity in aggressive signalling by calling males depends on factors related to the social context of signalling in a chorus. In an earlier study of this species, we showed that patterns of plasticity in aggressive signalling are related to acoustic correlates of the local density of other nearby signalling males, which is one important aspect of social context (Marshall et al. 2003). In the present study, we tested the hypotheses that plasticity in aggressive signalling depends on two additional factors related to social context: (1) the type of signals produced by new nearby neighbours and (2) the persistence with which new neighbours produced those signal types. Because habituation appears to be an important learning mechanism involved in mediating aggressive interactions among neighbouring male frogs (Owen & Perrill 1998; Bee 2001, 2003; Bee & Gerhardt 2001a, b; Marshall et al. 2003), we tested our hypotheses using field playback experiments based on the habituation–discrimination paradigm. During the habituation phase, we compared the responses of males interacting with a neighbour that persistently produced either only advertisement calls (simulating an initially nonaggressive neighbour) or a mixture of advertisement calls and aggressive calls (simulating an initially aggressive neighbour). Our prediction was that the magnitude of the subjects' responses would depend on the initial signalling behaviour of the simulated new neighbour (nonaggressive versus aggressive), but that all subjects would eventually habituate with repeated exposures to the new neighbour's signals. In contrast to these expectations, differences in a neighbour's initial behaviour did not influence the responsiveness of focal males during the habituation phase. During the transition from the habituation phase to the subsequent discrimination phase, our playbacks simulated conditions under which the neighbour either maintained its initial calling behaviour or changed its behaviour by switching from

nonaggressive to aggressive signalling. Our prediction was that a change in the neighbour's signalling behaviour from nonaggressive to aggressive (i.e. an escalation) would elicit recovery of the habituated response. This prediction was confirmed, indicating that plasticity of aggressive signalling depended on features of social context related to changes in signalling behaviours shown by nearby neighbours, such as an aggressive escalation. We discuss our findings in relation to possible mechanisms that would allow male frogs to track changes in their local social environment in a chorus.

## METHODS

### Study System

Spring peepers are a small hylid treefrog common throughout eastern North America. Males commonly form dense aggregations in and around ponds and marshes during their spring breeding season. The advertisement call of this species consists of a single short note that sweeps upward in frequency from about 2800 to 3200 Hz (Fig. 1). The aggressive call spans a similar frequency range but is longer and amplitude modulated and thus has a different temporal structure compared to the advertisement call (Fig. 1). Previous studies have shown that calling males respond to playbacks simulating other nearby calling males with mixtures of advertisement calls and aggressive calls (Schwartz 1989; Marshall et al. 2003). In a previous study, we showed that a male's threshold for producing aggressive calls in response to playbacks of advertisement calls is directly related to the amplitude of his nearest



**Figure 1.** The advertisement call and aggressive call of the spring peeper. The top (spectrograms) and bottom (oscillograms) panels depict one advertisement call (left) and one aggressive call (right) from one exemplar used in playback experiments. Note the similarity in spectral content and differences in temporal structure between the two calls.

neighbour's calls and can be modified by experience (Marshall et al. 2003). Neighbouring males that do not direct aggressive calls towards each other may still engage in acoustic interactions in which one male's calls are entrained to those of a neighbour (Rosen & Lemon 1974; Gerhardt et al. 1989).

Our field playback tests were conducted during the breeding seasons (March and April) of 2000, 2004 and 2006–2008. We performed tests under ambient light conditions between 2100 and 0200 hours in shallow ponds located in the Thomas Baskett Wildlife Area and the Three Creeks Conservation Area in Boone County, Missouri, U.S.A. Our experiments were approved by the Institutional Animal Care and Use Committees of the University of Missouri (no. 1910) and the University of Minnesota (no. 0602A81890).

#### *Playback Protocol*

Our acoustic stimuli consisted of the natural advertisement calls and aggressive calls of five males recorded from our study population during the 1999 breeding season at substrate temperatures near 15 °C ( $\pm 1$  °C). These recordings of 'stimulus donors' were made on cassette tape using a Sennheiser ME66 shotgun microphone (Sennheiser Electronic Corporation, Old Lyme, CT, U.S.A.) and portable Sony TC-D5M recorder (Sony Electronics Inc., San Diego, CA, U.S.A.). We used Macromedia SoundEdit 16 (Adobe Systems Inc., San Jose, CA, U.S.A.) to digitize (44.1 kHz, 16-bit resolution) and bandpass-filter (2400–3400 Hz) one advertisement call and one aggressive call per stimulus donor to serve as the calls for generating five exemplars of each of the various stimuli used in the treatment conditions (see *Experimental Design*) (Fig. 1). We chose calls that were recorded with relatively low background noise and no overlap by the calls of nearby conspecific males. Using the copy/paste functions of sound-editing software, we created stimulus exemplars in which calls were broadcast at a constant rate of 1 call/s. Although there is a positive relationship between call rate and temperature in spring peepers (Wells et al. 1996), the rate of approximately 1 call/s can be observed across a wide range of temperatures at which these frogs breed.

We broadcast the stimuli through a tripod-mounted Pyle Driver H-3910 horn speaker (Harman International Industries, Inc., Woodbury, NY, U.S.A.) using a Dell Inspiron 3500 notebook computer (Dell Inc., Austin, TX, U.S.A.) and a Rockford Fosgate 2.6 $\times$  amplifier (Rockford Fosgate, Tempe, AZ, U.S.A.). Stimulus amplitudes could be adjusted in 2 dB steps using the playback software (Cool Edit 2000, Syntrillium Software Corporation, Phoenix, AZ, U.S.A.) or a custom-built attenuator. Our field playback system was flat ( $\pm 1$  dB) over the frequency range of our stimuli. On each night of the study, prior to beginning playback tests, we calibrated the sound pressure levels of the advertisement calls in our stimuli to be approximately 95 dB SPL (C-weighted, fast RMS, re. 20  $\mu$ Pa) at a distance of 50 cm by broadcasting the stimuli in habitat similar to that from which frogs were normally observed calling. This calibrated value falls within the range of SPLs of natural advertisement calls recorded at a distance of 50 cm (Gerhardt 1975). The amplitude of any aggressive calls in a stimulus exemplar preserved the natural relative amplitude between the advertisement call and aggressive call in our original recordings of the stimulus donor male (see Fig. 1). Following each playback test, we measured the actual sound level of the stimulus at the position from which the subject had been calling. These values typically ranged between 94 and 99 dB SPL (C-weighted, fast RMS, re. 20  $\mu$ Pa). All sound pressure level measurements were made with a General Radio 1982 (IET Labs Inc., Westbury, NY, U.S.A.) or a CEL-383 (CEL Instruments Ltd., Hitchin, U.K.) sound level meter.

Prior to commencing a playback test, we placed the speaker and a Sennheiser ME66 microphone approximately 50 cm from an actively calling male. Although adjacent males in the chorus are often seen calling with intermale spacing of 50 cm, we attempted to minimize the influence of neighbouring males on a subject's responses by selectively testing males with calling neighbours located more than 1 m away, but usually less than 2–3 m away. We aborted tests when a nonfocal frog appeared to respond to the playback or to the subject, or when the subject appeared to respond to or interact with a neighbouring frog. After placing the speaker, we retreated 2–3 m and waited several minutes for subjects to resume normal calling behaviour before beginning the test. One observer used either a portable Sony TC-D5M cassette recorder or Marantz PMD670 solid-state recorder (D&M Professional, Itasca, IL, U.S.A.) connected to the microphone to record each subject's vocalizations during a playback test. A second observer monitored the subject's vocalizations through headphones and simultaneously ran the experiment from the computer. Immediately following each playback test, we captured the subject and gave it a cohort toe clip to avoid retesting individuals. We measured the substrate temperature at the subject's calling site to the nearest 0.1 °C using a quick-reading thermometer. Temperatures ranged from 7.5 °C to 22.2 °C, and most fell between 9 °C and 18 °C.

#### *Experimental Design*

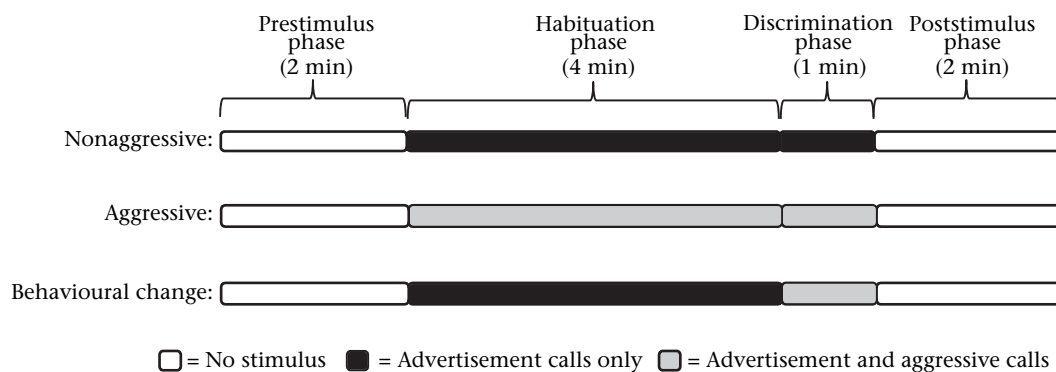
A playback test lasted 9 min and consisted of four phases (Fig. 2). During an initial 2 min prestimulus phase, we recorded a male's spontaneous calling prior to broadcasts of any stimulus. The prestimulus phase was followed immediately by a 4 min habituation phase, which was then followed immediately by a 1 min discrimination phase. During the habituation and discrimination phases, we broadcast acoustic stimuli and recorded all vocalizations made by the subject. The discrimination phase was followed immediately by a 2 min poststimulus phase during which we recorded all of a male's vocalizations without broadcasting any stimuli. Subjects were randomly assigned to one of three treatments differing in the type of stimulus broadcast during the habituation and discrimination phases (Fig. 2) as follows.

#### *Nonaggressive treatment*

Playbacks in this treatment simulated a nonaggressive neighbour that persistently produced only advertisement calls throughout the playback phases of the experiment. We broadcast a sequence of advertisement calls that repeated at a rate of 1 call/s for 5 min over the course of the habituation phase (4 min) and the discrimination phase (1 min). Hence, there was no change in the behaviour of the simulated neighbour during the transition from the habituation phase to the discrimination phase (Fig. 2).

#### *Aggressive treatment*

In this condition, both the habituation and discrimination phases consisted of mixtures of advertisement calls and aggressive calls, thereby simulating a neighbour that behaved aggressively during both of these phases of the experiment (Fig. 2). During the habituation phase, advertisement calls were repeated at a rate of 1 call/s. When the subject produced an aggressive call during the habituation phase, we momentarily interrupted the sequence of advertisement calls, 'interactively' responded by broadcasting a single aggressive call within 1–2 s, and then immediately resumed the regularly repeating advertisement calls. Our interactive playback of aggressive calls mimicked the aggressive counter-calling that often occurs between neighbouring males within choruses (Schwartz 1989). This interactive habituation phase was then followed by a discrimination phase in which we alternated



**Figure 2.** Schematic of experimental design. Playback experiments were divided into four phases totalling 9 min in duration and subjects were assigned to one of three treatments that differed in the type of acoustic stimuli broadcast during the habituation and discrimination phases of the experiment.

advertisement calls and aggressive calls at a composite rate of 1 call/s. By necessity, we could not continue using interactive aggressive calls to simulate aggression during the discrimination phase because males habituate to the playback stimulus. We recognize that the acoustic stimuli differed between the habituation and discrimination phases in ways that could potentially influence subject responsiveness. For our purposes, however, we considered the simulated neighbour to be 'aggressive' in both the habituation and discrimination phases; therefore, we do not regard this treatment as introducing any contextual change during the transition between these two phases of the experiment.

#### Behavioural change treatment

The habituation phase of this treatment consisted of 4 min of advertisement calls repeated at a rate of 1 call/s and was, thus, the same as that for the nonaggressive treatment in simulating an initially nonaggressive neighbour (Fig. 2). In the transition from the habituation phase to the discrimination phase, we simulated a change in the neighbour's behaviour from nonaggressive to aggressive. Hence, this treatment mimicked an escalation on the part of the frog simulated by our playbacks; we did not investigate other forms of behavioural change in the present study. During the discrimination phase, we broadcast alternating advertisement calls and aggressive calls at a composite rate of 1 call/s (Fig. 2). The only difference between the behavioural change and nonaggressive treatments was that in the former, every other advertisement call in the discrimination phase was replaced with an aggressive call. The only difference between the behavioural change and aggressive treatments was that the former lacked broadcasts of aggressive calls during the habituation phase.

#### Data Analyses

We tested 12–15 subjects in each of the three treatments. We considered valid playback tests to be those in which the subject gave at least one aggressive call during the first minute of the habituation phase and remained at his original calling site until the end of the test. These two criteria yielded final sample sizes of  $N = 11$  for the nonaggressive and behavioural change treatments and  $N = 10$  for the aggressive treatment (total  $N = 32$ ).

To examine treatment-dependent changes in a subject's calling behaviour, we determined the rates of advertisement calls and aggressive calls produced during each minute (calls/min) of the 9 min playback test comprising the prestimulus, habituation, discrimination and poststimulus phases. Because the rates of advertisement call and aggressive call production are not independent in playback tests (Schwartz 1989; Marshall et al. 2003), we

treated both as related dependent variables. We used a combination of parametric statistics that included multivariate and univariate analyses of variance (MANOVA and ANOVA) and two-tailed  $t$  tests to analyse differences in call rates both within a single phase and between two temporally adjacent phases. We used Spearman rank correlations to investigate the relationships between call rates at the beginning of the habituation phase and during the discrimination phase; these analyses were performed separately for each playback treatment.

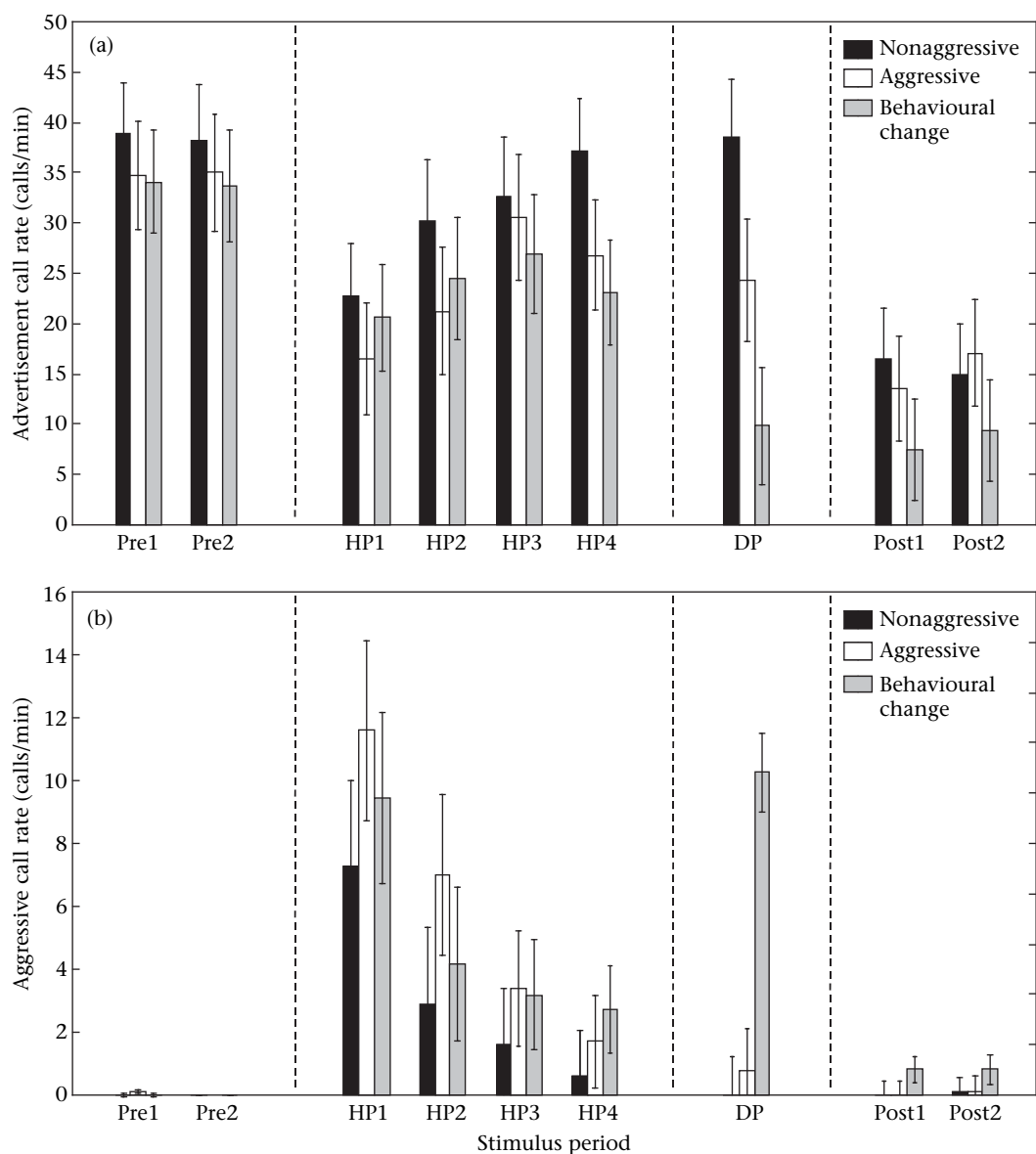
For the habituation phases of the nonaggressive and behavioural change treatments, which comprised a stimulus of regularly repeating advertisement calls, we determined the delay of each subject's advertisement calls relative to the onset of the immediately preceding stimulus call. After converting delay times (in ms) to phase angles (in degrees), we computed circular statistics and used Rayleigh tests (Zar 1999) to evaluate the null hypothesis that males called at random with respect to the stimulus. These analyses were designed to investigate stimulus entrainment during the habituation phase (Rosen & Lemon 1974; Gerhardt et al. 1989).

A criterion of  $\alpha = 0.05$  was used to determine statistical significance. Parametric and nonparametric tests were computed using Statistica 7.1 (StatSoft 2006); Oriana 2.0 (Kovach Computing Services, Anglesey, Wales, U.K.) was used to compute circular statistics. We report partial  $\eta^2$  and the length of the mean vector ( $r$ ) as measures of effect size in analyses of variance and circular statistics, respectively. Both of these measures range between 0 and 1 and reflect the strength of association between independent and dependent variables. Partial  $\eta^2$  is the proportion of the combined effect plus error variance that can be attributed to the effect and thus represents the proportion of variance explained by the effect. The length of the mean vector ( $r$ ) is a measure of dispersion, with greater values representing greater clustering around the mean vector.

## RESULTS

### Behaviour during the Prestimulus Phase

Males assigned to different treatment groups behaved similarly during the 2 min prestimulus phase (Fig. 3). On average, males in all three treatment groups produced advertisement calls at rates of about 36 calls/min during the prestimulus phase. There were no significant differences in the rates of advertisement calls produced by males assigned to the three treatment groups ( $F_{2,29} = 0.21$ ,  $P = 0.8082$ , partial  $\eta^2 = 0.01$ ) or between the first and second minutes of the prestimulus phase ( $F_{1,29} = 0.02$ ,  $P = 0.8719$ , partial  $\eta^2 < 0.01$ ), nor was there an interaction between these two effects



**Figure 3.** Calling behaviour before, during and after playbacks. Mean  $\pm$  SE rates of (a) advertisement calls and (b) aggressive calls during the 2 min prestimulus phase (Pre1–Pre2), the 4 min habituation phase (HP1–HP4), the 1 min discrimination phase (DP), and the 2 min poststimulus phase (Post1–Post2).

( $F_{2,29} = 0.04$ ,  $P = 0.9641$ , partial  $\eta^2 < 0.01$ ). We did not statistically analyse the rates of aggressive calls produced during the prestimulus phase because of a lack of variance; a single aggressive call was produced during the first minute of the prestimulus phase by a male assigned to the aggressive treatment.

#### Behaviour during the Habituation Phase

At the beginning of the habituation phase, most males (31/32) began producing aggressive calls within seconds (<15 s) of the stimulus onset. The increase in the rate of aggressive calls between the final minute of the prestimulus phase, during which no aggressive calls were produced, and the first minute of the habituation phase (Fig. 3) was significant for all three treatment groups ( $t$  tests of differences from zero: nonaggressive:  $t_{10} = 3.01$ ,  $P = 0.0132$ ; aggressive:  $t_9 = 3.72$ ,  $P = 0.0048$ ; behavioural change:  $t_{10} = 3.43$ ,  $P = 0.0065$ ). Concomitant with the onset of aggressive calling, males continued to produce advertisement calls during the

first minute of the habituation phase (Fig. 3), but at a slower rate compared to the last minute of the prestimulus phase ( $F_{1,29} = 16.80$ ,  $P = 0.0003$ , partial  $\eta^2 = 0.37$ ). A nonsignificant effect of treatment ( $F_{2,29} = 0.30$ ,  $P = 0.7447$ , partial  $\eta^2 = 0.02$ ) and treatment  $\times$  minute interaction ( $F_{2,29} = 0.16$ ,  $P = 0.8493$ , partial  $\eta^2 = 0.01$ ) confirmed that this initial decrease in the rate of advertisement calls was consistent across treatment groups.

The rates of advertisement calls and aggressive calls were not constant across the 4 min of the habituation phase, but there were no differences in calling behaviour during the habituation phase due to the main effect of treatment or an interaction between treatment and minute (Table 1, Fig. 3). The rate of aggressive calls decreased steadily in all three treatment groups during the habituation phase (Fig. 3). By the end of the 4 min habituation phase, 29 of 32 subjects had stopped producing aggressive calls. At the same time, the rate of advertisement calls increased over the course of the habituation phase back towards the levels observed during the prestimulus phase (Table 1, Fig. 3). Additional analyses of call

**Table 1**

Multivariate and univariate repeated measures ANOVAs comparing the rates of advertisement calls and aggressive calls during the 4 min habituation phase as a function of treatment

| Analysis Source                    | Wilks' $\lambda$ | F     | df     | P       | Partial $\eta^2$ |
|------------------------------------|------------------|-------|--------|---------|------------------|
| <b>MANOVA</b>                      |                  |       |        |         |                  |
| Minute                             | 0.27             | 10.70 | 6, 24  | <0.0001 | 0.73             |
| Treatment                          | 0.94             | 0.41  | 4, 56  | 0.8034  | 0.03             |
| Treatment $\times$ minute          | 0.65             | 0.96  | 12, 48 | 0.4942  | 0.19             |
| <b>ANOVA (Aggressive calls)</b>    |                  |       |        |         |                  |
| Minute                             | —                | 31.60 | 3, 87  | <0.0001 | 0.52             |
| Treatment                          | —                | 0.52  | 2, 29  | 0.6024  | 0.03             |
| Treatment $\times$ minute          | —                | 0.87  | 6, 87  | 0.4658  | 0.06             |
| <b>ANOVA (Advertisement calls)</b> |                  |       |        |         |                  |
| Minute                             | —                | 5.98  | 3, 87  | 0.0009  | 0.17             |
| Treatment                          | —                | 0.66  | 2, 29  | 0.5265  | 0.04             |
| Treatment $\times$ minute          | —                | 0.93  | 6, 87  | 0.4627  | 0.06             |

Results are from factorial analyses showing the main effects of minute (within subjects) and treatment (between subjects) and their interaction. ANOVAs were performed separately for each variable following the preliminary MANOVA.

timing in the nonaggressive and behavioural change treatments indicated that advertisement calls were not produced randomly with respect to the regularly repeated advertisement calls in the stimulus (Fig. 4). Rather, across all 4 min of the habituation phase, subjects tended to place most of their own advertisement calls in a temporal window between about 150 ms and 350 ms after the start of the stimulus advertisement calls, which had durations between 180 ms and 196 ms across the five stimulus exemplars (Fig. 4).

Taken together, changes in subjects' calling patterns during the habituation phase were consistent with an initial shift from advertisement calling to a mixture of advertisement and aggressive calling, followed by a gradual return to the production of solely advertisement calls in an interactive manner with the simulated neighbour. Importantly, these changes were independent of the type of social experience simulated by our treatment conditions.

#### *Behaviour during the Discrimination Phase*

MANOVA revealed a significant difference in the rate of calls between the last minute of the habituation phase and the 1 min discrimination phase (Wilks'  $\lambda = 0.75$ ,  $F_{2,28} = 4.51$ ,  $P = 0.0200$ , partial  $\eta^2 = 0.24$ ) that depended on an interaction with the main effect of treatment (Wilks'  $\lambda = 0.50$ ,  $F_{4,56} = 5.77$ ,  $P = 0.0006$ , partial  $\eta^2 = 0.29$ ); the effect of treatment was also significant (Wilks'  $\lambda = 0.56$ ,  $F_{4,56} = 4.74$ ,  $P = 0.0023$ , partial  $\eta^2 = 0.25$ ). In subsequent univariate analyses of advertisement calls and aggressive calls, there were significant effects of treatment, minute, and a treatment  $\times$  minute interaction (Table 2).

The significant interactions between minute and treatment were of particular interest, because they specifically examined whether the subjects' behaviour during the transition from habituation to discrimination phase differed between-treatment groups. In the last minute of the habituation phase, there were no between-treatment differences in the rates of advertisement calls ( $F_{2,29} = 1.98$ ,  $P = 0.1565$ ,  $\eta^2 = 0.12$ ) or aggressive calls ( $F_{2,29} = 0.55$ ,  $P = 0.5813$ ,  $\eta^2 = 0.04$ ). There were, however, significant between-treatment differences in the rates of both call types in the discrimination phase (advertisement calls:  $F_{2,29} = 6.12$ ,  $P = 0.0061$ ,  $\eta^2 = 0.25$ ; aggressive calls:  $F_{2,29} = 20.84$ ,  $P < 0.0001$ ,  $\eta^2 = 0.59$ ). Planned contrasts revealed that males in the behavioural change treatment produced advertisement calls and aggressive calls at significantly lower and higher rates, respectively, in the

discrimination phase compared with their behaviour at the end of the preceding habituation phase (Table 2, Fig. 3). The calling behaviour of males in both the nonaggressive and aggressive treatment groups was similar during the discrimination phase and the preceding final minute of the habituation phase (Table 2, Fig. 3). Hence, the change in signalling context simulated by the onset of the discrimination phase in the behavioural change treatment (from nonaggressive to aggressive) elicited recovery of habituated aggressive responses. The lack of any contextual changes in the simulated neighbour's signalling behaviour in both the nonaggressive and aggressive treatments was associated with a lack of renewed aggression in the discrimination phase.

Calling behaviour during the discrimination phase was not predicted by subjects' initial responses during the first minute of the habituation phase in any of the three treatment conditions when assessed in terms of both the rates of advertisement calls (Spearman rank correlation:  $0.23 < r_s < 0.42$ ,  $N = 10$ –11 per treatment,  $0.2351 < P < 0.4841$ ) and the rates of aggressive calls (Spearman rank correlation:  $0.29 < r_s < 0.35$ ,  $N = 10$ –11 per treatment,  $0.3226 < P < 0.3943$ ).

#### *Behaviour during the Poststimulus Phase*

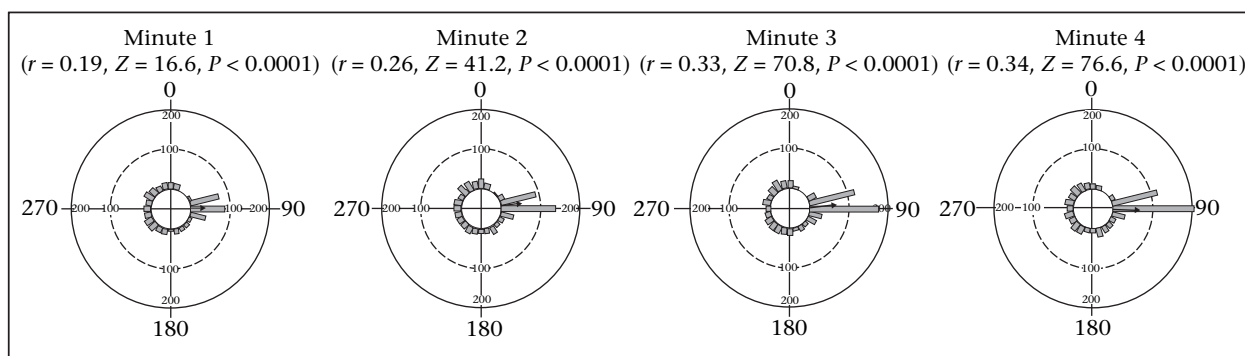
Males generally gave fewer advertisement calls in the post-stimulus phase relative to their calling activity during the other three phases (Fig. 3). Males also gave fewer aggressive calls during the poststimulus phase compared with their aggressive responses during the habituation phase (Fig. 3). We found no significant differences in calling activity between the first and second minute of the poststimulus phase (MANOVA: Wilks'  $\lambda = 0.98$ ,  $F_{2,28} = 0.31$ ,  $P = 0.7394$ , partial  $\eta^2 = 0.02$ ), nor were there any differences according to treatment (Wilks'  $\lambda = 0.85$ ,  $F_{2,28} = 1.18$ ,  $P = 0.3306$ , partial  $\eta^2 = 0.08$ ) or a minute  $\times$  treatment interaction (Wilks'  $\lambda = 0.95$ ,  $F_{4,56} = 0.40$ ,  $P = 0.8067$ , partial  $\eta^2 = 0.03$ ).

## DISCUSSION

#### *Plasticity of Aggressive Signalling in Frogs*

There is a growing appreciation for the role of experience-based behavioural plasticity in allowing signalling male frogs to balance the costs and benefits of aggressive interactions with other nearby participants in the dynamic social environment of a chorus (Brenowitz 1989; Rose & Brenowitz 1991, 1997; Brenowitz & Rose 1994, 1999; Burmeister et al. 1999b; Bee 2003; Marshall et al. 2003). Thus far, however, relatively few studies have investigated how variation in a signaller's social experiences or local social environment influences patterns of plasticity in aggressive behaviour. Our aim in this study was to investigate how features of social context related to the calling behaviour of a simulated neighbour might influence the plasticity of aggressive signalling.

In contrast to our expectations, neither the initial magnitude of acoustic responses nor changes in those responses with repeated stimulation depended on differences in the type of signalling interaction that males experienced during the habituation phase. Subjects in all three treatment groups initially responded to the stimulus by trading off advertisement calls with aggressive calls. With repeated stimulation during the habituation phase, the rates of advertisement calls and aggressive calls increased and decreased, respectively, in all three treatment groups. We had expected that males might behave more aggressively during the habituation phase in response to the aggressive treatment compared with the other two treatments. While such a trend was evident during the first 2 min of the habituation phase (Fig. 3), we were unable to detect any statistically significant differences in



**Figure 4.** Entrainment of advertisement calling during the habituation phase. Circular histograms (15° bin width) show the timing (in phase angles; 1 s = 360°) of advertisement calls produced by males in the nonaggressive and behavioural change treatments during each minute of the habituation phase relative to the onset of the preceding stimulus call (0°), which repeated once per second. Arrows depict the mean vector. Statistical results are from Rayleigh tests with sample sizes ranging from 475 calls (Minute 1) to 661 calls (Minute 4).

calling behaviour during the habituation phase related to the three treatment conditions (Table 1). Clearly, some caution is appropriate in interpreting this null result because of our somewhat limited sample sizes of 10–11 subjects in each treatment condition; however, we would also point out that the effect sizes associated with the main effects of treatment and the treatment × minute interactions were generally quite small (e.g.  $\eta^2 < 0.07$ ) compared with those for the within subjects main effect of minute during the habituation phase ( $0.17 < \eta^2 < 0.73$ ).

Our results provide evidence that the maintenance of reduced aggressiveness towards a persistently calling neighbour is specific to the type of experiential interactions that males have with that neighbour. In the discrimination phase, a mixture of advertisement calls and aggressive calls in the stimulus elicited significant recovery of the habituated aggressive response only for subjects in the behavioural change treatment, which had not been exposed previously to aggressive call stimuli in our playback. For this treatment, the transition between the habituation and discrimination phases simulated an escalatory shift in the aggressiveness of our simulated neighbour, from that of a nonaggressive male producing only advertisement calls to that of an aggressive male for which every other call produced was an aggressive call. This change in social context from nonaggressive to aggressive neighbour was sufficient to elicit a renewed aggressive response, thereby

demonstrating the contextual specificity of behavioural plasticity in aggression. There was no significant recovery of aggression in the discrimination phase when only additional advertisement calls were broadcast (the nonaggressive treatment), nor did a mixture of advertisement and aggressive calls elicit aggressive responses towards a simulated intruder that had previously behaved aggressively towards the subject (the aggressive treatment). Together, these results suggest that plasticity in aggressive signalling depends on the type of social interaction involved.

That plasticity in aggressive signalling by male frogs is specific to features of the social context has also been described in other species. For example, in North American bullfrogs, *Rana catesbeiana*, territorial males show lower levels of aggression towards their nearby neighbours compared with unfamiliar strangers (Davis 1987). With repeated exposure to the calls of a new neighbour, the magnitude of a male's aggressive response decreases (Bee 2003), but can be reinstated by changes in social context effected by changes in either the neighbour's position or acoustic cues to the neighbour's individual identity (Bee & Gerhardt 2001a, b, 2002). In bullfrogs, the stimulus-specific nature of the plastic aggressive response probably contributes to the ability of males to recognize their nearby neighbours as 'dear enemies' and perhaps as specific individuals (Davis 1987; Bee & Gerhardt 2002). Owen & Perrill (1998) also reported similar patterns of plasticity in the aggressive responses of the closely related North American green frog, *Rana clamitans*.

In an elegant series of field playback experiments, Brenowitz & Rose (1994; Rose & Brenowitz 1997) demonstrated that plasticity in the aggressive thresholds of male Pacific treefrogs, *Pseudacris regilla*, was specific to the type of signal (advertisement call versus aggressive call) to which males were exposed. When calling males were repeatedly exposed to above-threshold diphasic advertisement calls, their thresholds for responding aggressively to this signal increased by a magnitude of 7 dB, whereas those for responding to the acoustically distinct aggressive call increased less than 2 dB (Brenowitz & Rose 1994). In a follow-up study, Rose & Brenowitz (1997) showed that repeated exposure to the aggressive call significantly elevated the thresholds for responding aggressively to this signal by a magnitude of 15 dB, but those for responding to the advertisement call increased by only 2 dB.

#### Context Specificity and Call Type Discrimination

Our results with spring peepers and those of Brenowitz & Rose (1994; Rose & Brenowitz 1997) with Pacific treefrogs suggest that plasticity in aggression is specific to a context that depends on the type of signalling behaviours shown by neighbouring males. This context specificity, in turn, depends on a receiver's ability to

**Table 2**  
ANOVAs comparing the number of calls during the final minute of the habituation phase and the discrimination phase as a function of treatment

| Variable Source              | F     | df    | P       | Partial $\eta^2$ |
|------------------------------|-------|-------|---------|------------------|
| <b>Advertisement calls</b>   |       |       |         |                  |
| Minute                       | 6.25  | 1, 29 | 0.0183  | 0.18             |
| Treatment                    | 4.18  | 2, 29 | 0.0254  | 0.22             |
| Treatment × minute           | 5.35  | 2, 29 | 0.0105  | 0.27             |
| Nonaggressive treatment      | 0.17  | 1, 29 | 0.6800  | <0.01            |
| Aggressive treatment         | 0.53  | 1, 29 | 0.4723  | 0.02             |
| Behavioural change treatment | 16.45 | 1, 29 | 0.0003  | 0.36             |
| <b>Aggressive calls</b>      |       |       |         |                  |
| Minute                       | 6.40  | 1, 29 | 0.0171  | 0.18             |
| Treatment                    | 8.42  | 2, 29 | 0.0013  | 0.37             |
| Treatment × minute           | 12.46 | 2, 29 | 0.0001  | 0.46             |
| Nonaggressive treatment      | 0.22  | 1, 29 | 0.6406  | <0.01            |
| Aggressive treatment         | 0.40  | 1, 29 | 0.5296  | 0.01             |
| Behavioural change treatment | 31.30 | 1, 29 | <0.0001 | 0.52             |

Results are from univariate repeated measures ANOVAs showing the main effects of minute (within subjects) and treatment (between subjects) and their interaction. ANOVAs were performed separately for each variable following a preliminary MANOVA (see text). The results of planned contrasts comparing the main effect of minute separately for each treatment are included under the interaction terms.

discriminate between aggressive and nonaggressive vocalizations (e.g. Burmeister et al. 1999b). Many frogs use distinct aggressive vocalizations to defend their calling sites or territories (Gerhardt & Huber 2002; Wells 2007; Wells & Schwartz 2007). These aggressive calls can either be acoustically quite distinct from advertisement calls (Wiewandt 1969; Wells 1978; Fellers 1979) or show graded departures in spectro-temporal structure from advertisement calls (Schwartz 1989; Wagner 1989; Wells 1989; Grafe 1995; Burmeister et al. 1999b; Owen & Gordon 2005). Surprisingly few anuran studies, however, have investigated the mechanisms by which receivers discriminate between different call types. Experimental studies that exploit the plasticity and specificity of aggressive signalling have considerable potential to provide insight into such mechanisms.

In their work on Pacific treefrogs, Rose & Brenowitz (1997, 2002) used an experimental paradigm similar to the habituation–discrimination paradigm to investigate the mechanisms by which males discriminated between advertisement calls and aggressive calls, which have similar frequency spectra but differ in pulse rate. They discovered that temporal integration over a number of pulses with the correct interpulse intervals gave rise to temporally selective filters that functioned in call type discrimination based on differences in pulse-timing information. Their results were consistent with neural recordings from a subset of cells in the midbrain (torus semicircularis) that fired only after integrating over several consecutive pulses with call-specific timing (Alder & Rose 1998). Together, results from these studies of Pacific treefrogs support the hypothesis that advertisement calls and aggressive calls are processed by different ‘channels’ in the central auditory system that can be differentiated based on their selectivity for the pulse-timing information conveyed by the two different call types.

In spring peepers, the significant recovery of aggressive responses in the discrimination phase shown by males in the behavioural change treatment, and the lack thereof in the nonaggressive group, confirms that males in our study were able to discriminate between advertisement calls and aggressive calls. Given the spectral similarity between the two call types (Fig. 1), the most likely acoustic cues for discrimination are in the temporal domain. Compared to advertisement calls, aggressive calls are longer and have a pulsed structure (Fig. 1; Schwartz 1989). Thus, it seems likely that the neural filters that select for the two different call types are related to neurons that show duration selectivity, interval selectivity, or both. For example, advertisement calls may activate neurons with long-pass duration selectivity, whereas the pulsed structure of the aggressive call may activate neurons with particular interval selectivity. Neurons showing these general response properties have been described recently in the anuran auditory midbrain (Edwards et al. 2008; Leary et al. 2008). It will be important for future behavioural and neurophysiological studies to investigate further how differences in duration and the presence of amplitude modulation contribute to discriminating between advertisement calls and aggressive calls.

#### *Short-term Habituation of Aggression*

At present, we know little about the precise neural mechanisms underlying the plasticity of aggressive signalling thus far demonstrated in spring peepers (Marshall et al. 2003; this study), Pacific treefrogs (Brenowitz & Rose 1994; Rose & Brenowitz 1997, 2002), bullfrogs (Bee & Gerhardt 2001a, b, 2002; Bee 2003) and green frogs (Owen & Perrill 1998). Behavioural evidence from all four species suggests, however, that such mechanisms are probably involved in mediating a form of learning known as habituation. As a form of learning, habituation refers to a stimulus-specific decrease in responsiveness that results from repeated or continuous presentations of a salient stimulus (Thompson & Spencer

1966; Groves & Thompson 1970). Important distinctions have been made between short-term and long-term forms of habituation that may ultimately stem from different underlying processes (Sharpless & Jasper 1956; Hinde 1970; Wagner 1976, 1979, 1981; Petrinovich 1984; Whitlow & Wagner 1984). Short-term habituation typically refers to response decrements that occur with a relatively fast time course during a single habituation training session and that have relatively short-lived effects (e.g. <24 h). In contrast, the effects of long-term habituation can be retained on the order of days or weeks.

There is a well-developed literature on the role of habituation in socially mediated plasticity of aggression in fish and birds (reviewed in: Peeke & Peeke 1973; Peeke 1984; Petrinovich 1984). As in these other taxa, plasticity of aggressive signalling in frogs shows a number of the hallmark features of habituation (sensu Thompson & Spencer 1966). For example, in all frogs in which it has been studied, aggressive responses show characteristic decrements with repeated stimulation. Effector fatigue can be ruled out as an explanation for these decrements because aggressive responses can be reinstated upon presentation of a sufficiently novel stimulus (e.g. Rose & Brenowitz 1997; Owen & Perrill 1998; Bee & Gerhardt 2001a; this study). The present study also rules out sensory adaptation as a mechanistic explanation because aggressive calls elicited response recovery in the behavioural change treatment even though they have the same frequency spectrum as advertisement calls and thus stimulated the same auditory nerve fibres. Our results on male entrainment behaviour also indicate that subjects continued to perceive many calls to which they did not aggressively respond. Sensory adaptation has also been ruled out as a general mechanism for plasticity of aggression in other frogs (e.g. Rose & Brenowitz 1997; Bee & Gerhardt 2001a). Importantly, response decrements in frogs also show ‘spontaneous recovery,’ which is another feature of response habituation (Thompson & Spencer 1966). A cessation of stimulation results in the spontaneous recovery of responses or restoration of response thresholds to their prehabituation levels (Brenowitz & Rose 1994; Bee & Gerhardt 2001a; Marshall et al. 2003).

Collectively, many of the patterns of plasticity in aggression in frogs resemble those expected for short-term forms of habituation. In spring peepers (Marshall et al. 2003; this study) and Pacific treefrogs (Brenowitz & Rose 1994), for example, response decrements occur rapidly over the course of a few minutes, responses show recovery upon presentation of a novel stimulus, and spontaneous recovery can also occur relatively rapidly (on the order of 10–20 min) upon the cessation of stimulation. We postulate that in chorusing species like spring peepers and Pacific treefrogs, neural mechanisms related to short-term habituation may function to allow males to fine-tune their aggressive interactions to local conditions in adaptive ways. Rapid response decrements would allow calling males to avoid paying costs associated with aggressive signalling by accommodating new and persistently calling neighbours as the local density of males increased. A rapid recovery of aggression upon contextual changes in the local signalling environment could allow males to maximize the benefits of defending a calling site through a reinstatement of aggression in response to new calling neighbours or to changes in signalling behaviour in an established neighbour. Likewise, relatively rapid spontaneous recovery would allow males to ‘reset’ their threshold for aggression if local density decreased. Hence, processes akin to short-term habituation might allow a male frog’s propensity to interact aggressively with neighbours to optimally track the temporal and spatial variability present in the dynamic social environment of a chorus. Future behavioural and neurophysiological studies should test this short-term habituation hypothesis in greater detail and in a greater number of species.

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