Raising a racket: invasive species compete acoustically with native treefrogs

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Environmental noise is increasing worldwide, limiting the space available for species to send and receive important acoustic information. Many invasive species produce acoustic signals that alter the spectral-temporal characteristics of available signalling space. This provides an opportunity to test ideas about competitive exclusion by quantifying whether species with shared requirements for acoustic resources will become excluded or partition resource use to permit coexistence. We conducted a field playback experiment to test whether native *Hyla cinerea* and *H. femoralis* (green and pine woods treefrogs) modify their acoustic behaviour to minimise acoustic competition from invasive *Osteopilus septentrionalis* (Cuban treefrog) chorus noise. We demonstrate that noise from an invasive species differentially affects the vocal behaviour of native species. Those with similar calls (*H. cinerea*) shortened calls, called louder and persisted calling in response to masking stimuli while those with different calls (*H. femoralis*) did not modify behaviour. This evidence suggests that acoustic competition by invasive *O. septentrionalis* has altered the acoustic community structure, identifying acoustic competition as a mechanism by which invasive species can impact communities. Furthermore, these results broaden the concept of noise pollution, demonstrating fitness-relevant consequences of noise produced by invasive species.

KEYWORDS

Noise, invasive species, acoustic niche, acoustic competition, vocal compensation, vocal behaviour, *Hyla cinerea*, *Hyla femoralis*, *Osteopilus septentrionalis*

24 Many species depend on acoustic advertisement displays to communicate in
25 mixed species breeding aggregations, including chorusing species of insects, birds and
26 frogs (Amezquita *et al.* 2011; Sueur *et al.* 2010; Brumm & Slabbekoorn 2005; Gerhardt
27 & Huber 2002). Ambient noise has increased in recent decades (Barber *et al.* 2010),
28 creating a variety of new pressures for species that rely on acoustic cues for important life
29 functions (see reviews in Francis & Barber 2013; Kight & Swaddle 2011; Barber *et al.*
30 2010). Invasive species can be a source of noise, since many produce acoustic signals to
31 attract mates and defend territories (e.g. Vargas-Salinas 2006), and these sounds may
32 dominate particular frequency or temporal patterns, thereby altering available signalling
33 space (Bleach *et al.* 2015; Both & Grant 2012). It is well established that invaders can
34 modify native species' survival, development, distribution and behaviour (e.g. Langkilde
35 2009; Suarez & Case 2002; Wauters *et al.* 2002; Case & Bolger 1991). The consequences
36 of the novel noise produced by invasive species for native species acoustic
37 communication have rarely been explored (but see Bleach *et al.* 2015; Both & Grant
38 2012), yet are fundamental to understanding the novel pressures that invasive species
39 exert on native communities.

40 The dynamic nature of the sounds within ecological communities can lead to
41 intense competition for acoustic space, the multi-dimensional channel through which
42 species send and receive acoustic signals (Foote *et al.* 2011; Luther 2009; Brumm 2006;
43 Marler 1960). Sounds that overlap calling species in time, frequency and space can
44 interfere with their signalling networks by reducing the signal's active space, thereby
45 compromising communication ranges (Barber *et al.* 2010; Patricelli & Blickley 2006).
46 Acoustic space is, therefore, a limited resource (Bradbury & Vehrencamp 2012).

47 Competitive exclusion theory predicts that, when faced with such limited resources,
48 species with shared requirements for resources will either (1) become competitively
49 excluded, or (2) partition their resource use to permit coexistence (Gause 1934).
50 Therefore, given limited acoustic space, species whose acoustic signals are similar to
51 competitors' signals may either become excluded from successfully signalling at a
52 particular time or frequency, or may employ vocal compensation to partition their
53 acoustic space to minimise competition (Foote *et al.* 2011; Luther 2009; Brumm 2006).
54 Evidence of acoustic resource partitioning is provided by the fact that vocalisations in
55 South American frog communities are more widely and regularly spaced in use of
56 acoustic frequency and call timing than expected by chance (Chek *et al.* 2003), and birds
57 singing in dawn choruses in the Amazonian rainforest partition their signalling space
58 through spatio-temporal changes in singing behaviour or evolved differences in songs
59 (Luther 2009). Furthermore, when acoustic space is limited by ambient anthropogenic
60 noise, such as by mechanised transportation (e.g. Tennessen *et al.* 2014; Barber *et al.*
61 2010), species across many taxa including birds, mammals, anurans, and fish partition
62 their signalling space by employing vocal compensation through short-term vocal
63 behavioural modifications such as altering duration, amplitude, frequency, or rate of their
64 calls (for reviews, see Hotchkin & Parks 2013; Warren *et al.* 2006; Brumm &
65 Slabbekoorn 2005). Frogs in particular have demonstrated vocal plasticity to increased
66 anthropogenic noise (e.g. Cunnington & Fahrig 2010; Parris *et al.* 2009; Lengagne 2008;
67 Schwartz *et al.* 2008; Penna & Hamilton-West 2007). Thus, while few studies have
68 explored the role of competition for acoustic space in structuring acoustic communities,

the documented behavioural responses of species to changes in their acoustic environments suggest that acoustic competition may be an underlying mechanism.

The invasion of *O. septentrionalis* in Florida provides an excellent opportunity to study how novel sound produced by an invasive species affects native acoustic communities. *Osteopilus septentrionalis* is an anuran hylid native to Cuba, the Cayman Islands and the Bahamas, and has been introduced to many tropical and sub-tropical locations, including Florida (Lindsay & Cooper 2008; Owen *et al.* 2005; Meshaka 2001). *O. septentrionalis* was observed in southern Florida by 1931 (Barbour 1931), having likely arrived through materials shipped for the garden nursery industry (Meshaka 2001), and subsequently spread rapidly northward, facilitated by the ornamental plants trade and by hitchhiking on vehicles and in cargo (Meshaka 2001). Presently, *O. septentrionalis* is established throughout most of the Florida peninsula, with isolated observations from the Florida panhandle (S. Johnson, personal communication, 15 January 2015). In addition to depredating native species (Wyatt & Forsy 2004), *O. septentrionalis* produces a nocturnal breeding call that has been described as a “grating squawk” (Meshaka 2001). These calls represent pressure on acoustic space within native anuran communities throughout Florida.

We predicted that novel sounds created by invasive *O. septentrionalis* would cause native treefrogs whose calls overlap in frequency and timing with those of *O. septentrionalis* to partition acoustic space through vocal modification in order to minimise acoustic competition, but have no effect on native treefrogs with acoustically different calls. To test this hypothesis, we conducted a field playback experiment with two native species, one whose breeding calls are similar in frequency, structure and

timing to *O. septentrionalis* and one whose breeding calls are not. Our findings reveal a poorly understood pathway through which invasive species may alter the behaviour of native species, and suggest that acoustic competition for limited acoustic space is driving native species responses.

METHODS

Study Sites and Species

H. cinerea and *H. femoralis* (green and pine woods treefrogs, respectively) are native hylid species found throughout the southeastern United States. *H. cinerea* inhabits wetlands between central Texas and Florida, extending north to eastern Maryland and Delaware. *H. femoralis* is found between Louisiana and Florida, extending north to Virginia (Dorcas & Gibbons 2008). Breeding occurs in permanent (*H. cinerea*) and temporary (*H. femoralis*) bodies of water at approximately the same time of year, between March and October, overlapping *O. septentrionalis* breeding activities in space and time (Dorcas & Gibbons 2008; Meshaka 2001). All species call from perches or within clumps of emergent vegetation (Dorcas & Gibbons 2008). While closely related, the breeding calls produced by male *H. cinerea* and *H. femoralis* differ substantially in dominant frequency, duration, rate, inter-call interval, and structure (Fig. 1, Table 1). *H. cinerea* calls are composed of a series of harmonics, with two emphasised at approximately 1000 and 3200 Hz. *H. femoralis* calls are pulsed with peak energy at approximately 2100 and 4500 Hz. *O. septentrionalis* calls are harmonic in structure, like green treefrogs, and emphasised at approximately 1100 and 2500 Hz.

Figure 1: Waveforms (a) and spectrograms (b) of the breeding calls of *O. septentrionalis*, *H. cinerea* and *H. femoralis*. Lighter shading (white) in the spectrograms indicates greater acoustic energy. *O. septentrionalis* calls are similar to those of *H. cinerea* in dominant frequency, duration, rate, harmonic structure, and inter-call interval, but have only dominant frequency in common with *H. femoralis*.

Table 1: Summary of measurements of baseline call parameters for *H. cinerea* and *H. femoralis*. Values are reported as mean $\pm$ standard error of the mean (sample size). Measurements were made on calls from the “before” period of each trial, recorded as described under *Playback Experiment* and measured and analysed as described under *Acoustical and Statistical Analysis*.

Parameter	<i>H. cinerea</i>	<i>H. femoralis</i>
Duration (s)	0.15 $\pm$ < 0.01 (580)	0.05 $\pm$ < 0.01 (300)
Received level (dB rms re 20μPa)	87.7 $\pm$ 0.1 (580)	91.4 $\pm$ 0.2 (300)
Inter-call interval (s)	0.53 $\pm$ 0.02 (561)	0.07 $\pm$ 0.01 (300)
Dominant freq. (Hz)	966 $\pm$ 4 (580)	2,086 $\pm$ 5 (300)
Rate (calls/min)	56.3 $\pm$ 5.1 (29)	504.7 $\pm$ 17.8 (15)

We conducted night field playback experiments with *H. cinerea* and *H. femoralis* in the southeastern United States over two seasons. Playback trials with *H. cinerea* were conducted at artificial ponds at the Harrison Lake Fish Hatchery (HLH) in Charles City, VA (coordinates: 37.34, -77.19) between 16-18 July 2013. These ponds were located within an area of mixed hardwood and southern pine forest, and contained emergent vegetation (primarily *Juncus sp.*, *Polygonum sp.*, and grasses). Trials with *H. femoralis* were conducted at an ephemeral pond at the Archbold Biological Station (ABS) in Venus, FL (coordinates: 27.1815, -81.3571) between 16-17 July 2012. These ponds were located within scrubby flatwoods and contained emergent vegetation (primarily *Panicum*

sp.). HLH is located north of the northernmost part of the *O. septentrionalis* invasion front. While *O. septentrionalis* has been present at ABS for at least 20 years (since the early 1990s), the population remains restricted to the immediate vicinity of buildings at the Station headquarters (Rothermel *et al.* 2013). Therefore, both pond sites represent areas that have not experienced the invasion, and thus playback subjects were naïve to *O. septentrionalis* breeding calls. Temperatures during the playback experiments ranged from 21.4-28.8 °C, and occurred between 20:50-01:15 h.

Playback Experiment

To conduct the acoustic playback trials, we searched for calling male treefrogs located on vegetation within or adjacent to ponds. Once a focal male was located, we placed an AN Mini speaker (Anchor Audio Portable Sound Systems, Carlsbad, CA, USA) on a stand, approximately 1 m away from the caller and at approximately the same height. This speaker was connected to an iPod Nano (Apple, Inc., Cupertino, CA, USA) [system frequency response: 100-15 000 Hz flat] that played the acoustic stimulus – either an *O. septentrionalis* chorus, masking white noise, or upshifted white noise (Fig. 2; see *Playback Stimuli Design*) which we selected using a stratified-random procedure. The playback trial consisted of a five-minute before-stimulus phase, followed by a two-minute stimulus period. The received level of the stimuli during a trial was calibrated using a handheld digital sound level meter (RadioShack 33-2055, RadioShack Corporation, Fort Worth, Texas, USA) with C-weighting and fast response (received level 87 dBC $\pm$ 1 dB) with no focal caller present (but with the same set up and equipment) to avoid disturbing the calling frog's behaviour. We did not include a post-

stimulus phase because the cessation of a signal and silence itself can alter behaviour and elevate stress hormone levels (Tennessen *et al.* 2014). During a trial, we recorded calls of focal males (*H. cinerea*: *O. septentrionalis* chorus, $N = 12$; masking white noise, $N = 9$; upshifted white noise, $N = 10$; *H. femoralis*: *O. septentrionalis* chorus, $N = 7$; masking white noise, $N = 7$; upshifted white noise, $N = 4$) using a Sennheiser ME66 directional microphone with a 44 100 Hz sampling rate (Sennheiser, Wedemark, Germany) mounted on a microphone stand approximately 1 m away from and approximately 0.5 m above the calling male and oriented on-axis when possible, and approximately 0.5 m directly above the playback speaker (to reduce interference). Frogs were typically facing the same direction at the beginning and conclusion of each trial, and thus it is unlikely that changes in body orientation account for the observed changes in call parameters. The microphone was connected to a Marantz PMD 661 digital audio recorder (Marantz America, LLC, Mahwah, NJ, USA) [system frequency response: 40 – 20 000 Hz]. Immediately after beginning the trial we retreated to a location away from and out of sight of the focal male, and returned at the end of the trial. To reduce the chance of re-sampling individuals we never conducted playbacks in the same area within a night, and when possible we conducted the playbacks in different areas on subsequent nights. Since temperature affects calling behaviour in frogs (Gerhardt & Hüber 2002), we collected ambient temperature data to use as a covariate in the analyses. Temperature data were collected at least every 15 minutes during the playback trials by a Kestrel Meter 4000 (Nielsen-Kellerman, Boothwyn, PA, USA) and provided by the Virginia Commonwealth University Rice Rivers Center's autonomous weather station (coordinates: 37.33, -77.21) located less than 2 km from Harrison Lake Fish Hatchery.

Figure 2: Power spectra of the three playback stimuli, an *O. septentrionalis* chorus (dark grey), masking white noise (light grey), and upshifted white noise (black). Spectra were generated using a 1024-point DFT, 61.9 Hz 3-dB bandwidth, 50% overlap and 43.1 Hz grid spacing.

Playback Stimuli Design

We collected acoustic recordings of a large *O. septentrionalis* chorus in the early afternoon in Venus, FL (coordinates: 27.06, -81.43) following a heavy rain on 27 June 2012, using a Sennheiser ME66 directional microphone with a 44 100 Hz sampling rate, connected to a Marantz PMD 661 digital audio recorder. Some hylids could be detected in the background, but the chorus was predominantly composed of continuous, overlapping mating calls from *O. septentrionalis* males, representative of a typical large chorus for this species. From these recordings, we created three different exemplars of the *O. septentrionalis* chorus stimulus in order to reduce the likelihood that any response to the chorus stimulus was simply due to a stochastic event within one stimulus, and to improve external validity by using multiple exemplars (e.g. Wiley 2003). We visualized

207 spectrograms and power spectral densities and measured signals using Raven Pro v.1.4
208 (Bioacoustics Research Program, The Cornell Lab of Ornithology, Ithaca, NY, USA). We
209 selected three, one-minute segments of the recordings that contained an *O.*
210 *septentrionalis* chorus with few heterospecific calls to use as the three *O. septentrionalis*
211 exemplars. For each exemplar, we determined lower, upper and dominant frequency,
212 respectively (exemplar 1: 499.5 Hz, 3719.3 Hz, 2240 Hz; exemplar 2: 642.2 Hz, 3734.5
213 Hz, 2411 Hz; exemplar 3: 523.3 Hz, 3615.5 Hz, 2,411 Hz) and centre frequency (2240
214 Hz for all exemplars) (Hann window, 256 sample length DFT, 248 Hz 3-dB filter
215 bandwidth, 50% overlap). We computed means for lower, upper and dominant frequency,
216 respectively (555 Hz, 3690 Hz, 3135 Hz). We looped each one-minute segment to create
217 three, two-minute exemplars of the *O. septentrionalis* stimuli. Additionally, we created
218 two control stimuli: a continuous white noise stimulus of approximately the same
219 bandwidth and frequency range as the mean bandwidth and mean frequencies of the *O.*
220 *septentrionalis* exemplars (“masking white noise”), and a continuous white noise
221 stimulus of approximately the same bandwidth but shifted approximately 5.5 kHz higher,
222 out of the frequency range of the *O. septentrionalis* chorus (“upshifted white noise”) (Fig.
223 2). The masking white noise stimulus was designed to tease apart whether responses are
224 specific to *O. septentrionalis* calls, for example an anti-predator response to this novel
225 sound, or to masking noise within the same frequency range, for example a response to
226 limited acoustic space. The upshifted white noise stimulus was designed to determine
227 whether a response is unrelated to acoustic masking but rather due to general acoustic
228 disturbance; for example, a general territory defence response to a potential
229 heterospecific competitor (regardless of acoustic frequency of its call), or a behavioural

change due to distraction. To make the masking and upshifted white noise controls, we generated a white noise signal with a 48 kHz sampling rate using a custom script in Matlab R2007a (The Mathworks, Inc., Natick, MA, USA). We then applied a custom bandpass filter to each control, as follows: (1) we created a 10-point filter envelope designed based on the shape of the power spectral density of the *O. septentrionalis* chorus (Hann window, 256 sample length DFT, 248 Hz 3-dB filter bandwidth, 50% overlap) using Adobe Audition CS5.5 (Adobe Systems, Inc., San Jose, CA, USA). (2) We averaged the three minimum and three maximum frequencies from the power spectral densities of the three separate one-minute selections of the *O. septentrionalis* chorus to determine the average frequency range of the chorus. (3) We applied this envelope to the white noise signals in the same frequency range (550-3695 Hz) or shifted 5.5 kHz higher (6000-9145 Hz) to make the masking and upshifted white noise controls, respectively, with equal bandwidths to the *O. septentrionalis* stimulus. (4) We silenced the two controls above and below the desired frequency ranges, applied a dB-equalising function (with Adobe Audition) to all stimuli, and used a Matlab custom script to taper the first and last 5% of each stimulus so that they faded in and out. (5) Finally, we added five minutes of silence before and after each stimulus, so that we would not interrupt the playback trial to turn on the stimulus.

Acoustical and Statistical Analysis

We examined all playback trial recordings using Raven Pro v.1.4 (The Cornell Lab of Ornithology), to measure five response variables: call rate, duration, dominant frequency, inter-call interval, and call amplitude. For each recording we constructed

253 spectrograms and power spectral densities (Hann window, 1024 sample length DFT, 61.9
254 Hz 3-dB filter bandwidth, 50% overlap), counted calls in each of the two playback
255 periods (in two-minute and one-minute scans for *H. cinerea* and *H. femoralis*,
256 respectively, immediately before and during the stimulus; since *H. femoralis* calls are
257 spaced at least one order of magnitude closer together than *H. cinerea*, it was impractical
258 to scan two-minute sections for *H. femoralis*), and determined call rate per minute. This
259 approach allowed us to minimise variability in calling behaviour between calling periods
260 due to extraneous factors (i.e. time effects) by measuring calling activity spaced closely
261 in time. To measure call parameters we randomly selected 20 calls from each call period
262 (40 calls per individual) using a random number generator and quantified duration (s;
263 length of call), dominant frequency (Hz; for consistency, the lower of the two
264 emphasized frequencies, though there was individual variation in which of the two
265 frequencies had the greatest amplitude), and inter-call interval (s; the duration between
266 the end of one call and the beginning of the next). In the rare event that one call ended
267 and vocalisation ceased for more than five seconds, we omitted the inter-call interval
268 measurement for that particular call. Additionally, we measured call amplitude (in dBC
269 rms re 20 μ Pa) following procedures similar to those in other animal studies on amplitude
270 compensation in noise (e.g. Parks *et al.* 2011; Love & Bee 2010; Holt *et al.* 2009; Brumm
271 & Todt 2002). First, we measured the relative sound pressure level of the dominant
272 frequency band of the call (in dBC, 43.1 Hz bandwidth, averaged over the duration of the
273 call), and the relative sound pressure level of background noise in the same peak
274 frequency band within one second of the call onset (averaged over an interval ranging
275 from 0.05-0.5 s). Next, we logarithmically subtracted the noise portion of the recording

from the call. We only subtracted the noise from those calls in the “during” period of the playback, as the signal to noise ratio was sufficiently high (e.g. > 10 dB) for all calls in the “before” period such that noise had a negligible effect on signal amplitude (affected overall signal level by < 1 dB). Finally, we applied a scaling factor, determined for the recording system, to each call amplitude to calculate received level. This scaling factor was determined by generating test signals of known sound pressure level (SPL), recording the signals with our recording system, measuring the level of the recorded signals, and computing the scaling factor. Specifically, we played four separate tones (100 Hz, 1 kHz, 3 kHz, 10 kHz), generated using Matlab R2014a (The Mathworks, Inc., Natick, MA, USA), and measured the level using a RadioShack 33-2055 sound level meter. Simultaneously, we recorded the tones with our recording system (Sennheiser ME66/K6 connected to a Marantz PMD 661 digital recorder), with the microphone in the same position as the sound level meter. We measured the levels of the recorded signals using Raven Pro v.1.4, and determined the scaling factor necessary to correct for the difference between the levels.

We analysed the resulting data using a mixed model approach in JMP 12 (SAS Institute Inc., Cary, NC, USA). To determine the effect of the stimuli (*O. septentrionalis* chorus, masking white noise and upshifted white noise) on species’ responses, we constructed individual mixed models for each response variable (duration, amplitude, dominant frequency, inter-call interval, call rate) with species, treatment, playback period and their interactions and exemplar nested within treatment as fixed effects, individual as a random effect, and temperature as a covariate. To test for within-species differences in the responses to the stimuli, we constructed mixed models for each species, with

treatment, playback period, their interaction and exemplar nested within treatment as fixed effects, individual as a random effect, and temperature as a covariate. We used Tukey HSD *post hoc* tests to compare means between groups, and additionally linear pairwise contrasts to further resolve differences between groups for the within-species inter-call interval model. For all analyses we used a significance level of $\alpha = 0.05$. Call amplitude values were converted to μPa (linear scale) for statistical analyses, but are reported in dBC re 20 μPa for comparison with other studies. We omitted five playback trial recordings from the analyses (two upshifted white noise trials from *H. cinerea*, and one chorus and two masking white noise trials from *H. femoralis*), in which the focal frogs either failed to vocalise or the signal to noise ratio of the vocalisations was insufficient to accurately measure the calls. The call was the unit of analysis for the parameters duration, amplitude, inter-call interval and dominant frequency, and the recording was the unit of analysis for the parameter call rate. Sample sizes for each period for the different treatments (*O. septentrionalis*, masking, and upshifted, respectively) for *H. cinerea* and *H. femoralis* are as follows: *H. cinerea*, before: duration = 240, 180, 160; amplitude = 240, 180, 160; inter-call interval = 232, 175, 154; dominant frequency = 240, 180, 160; rate = 12, 9, 8; *H. cinerea*, during: duration = 240, 180, 160; amplitude = 240, 180, 160; inter-call interval = 236, 179, 158; frequency = 240, 180, 160; rate = rate = 12, 9, 8. *H. femoralis*, before: duration = 120, 100, 80; amplitude = 120, 100, 80, inter-call interval = 120, 100, 80; dominant frequency = 120, 100, 80; rate = 6, 5, 4; *H. femoralis*, during: duration = 120, 100, 80; amplitude = 119, 100, 80, inter-call interval = 120, 100, 80; dominant frequency = 120, 100, 80; rate = 6, 5, 4.

RESULTS

The effect of noise treatment on vocal modification significantly differed between species for both call duration and amplitude (mixed model: *duration*: species x treatment x period interaction: $F_{2,1726} = 16.58$, $P < 0.0001$; exemplar[treatment]: $F_{2,74} = 0.64$, $P = 0.531$; temperature: $F_{1,40} = 0.45$, $P = 0.507$; *amplitude*: species x treatment x period interaction: $F_{2,1709} = 4.04$, $P = 0.018$; exemplar[treatment]: $F_{2,77} = 1.74$, $P = 0.182$; temperature: $F_{1,62} = 14.58$, $P = 0.0003$). We thus examined the effect of noise treatment on vocal modification by each species separately. *H. cinerea* modified vocal behaviour in response to the noise treatment (mixed model: *duration*: treatment x period: $F_{2,1127} = 31.78$, $P < 0.0001$, Fig. 3a; exemplar[treatment]: $F_{2,22} = 1.48$, $P = 0.25$; temperature: $F_{1,26} = 0.46$, $P = 0.504$; *amplitude*: treatment x period: $F_{2,1107} = 34.75$, $P < 0.0001$, Fig. 3c; exemplar[treatment]: $F_{2,12} = 1.47$, $P = 0.268$; temperature: $F_{1,46} = 36.42$, $P < 0.0001$), but *H. femoralis* did not (mixed model: *duration*: treatment x period: $F_{2,588} = 1.83$, $P = 0.162$, Fig. 3b; exemplar[treatment]: $F_{1,51} = 0.01$, $P = 0.933$; temperature: $F_{1,11} = 3.85$, $P = 0.076$; *amplitude*: treatment x period: $F_{2,582} = 0.55$, $P = 0.578$, Fig. 3d; exemplar[treatment]: $F_{1,590} = 0.92$, $P = 0.337$; temperature: $F_{1,11} = 0.09$, $P = 0.766$). Tukey HSD *post hoc* tests showed that *H. cinerea* significantly shortened their calls and increased call amplitude (made them louder) in the presence of *O. septentrionalis* noise (effect size percentage of mean $\pm$ standard error of the mean = 11.6 ± 2.6 % and 16.2 ± 5.6 %, respectively) and masking white noise in the same frequency range as the *O. septentrionalis* calls (effect size percentage of mean $\pm$ standard error of the mean = 5.9 ± 2.6 % and 14.9 ± 5.5 %, respectively), but not in response to the upshifted white noise that was above the frequency range of *O. septentrionalis* calls.

While species did not statistically differ in the effect of noise treatment on modification of inter-call interval (mixed model: species x treatment x period: $F_{2,1697} = 2.66$, $P = 0.07$; exemplar[treatment]: $F_{2,41} = 2.86$, $P = 0.069$; temperature: $F_{1,36} = 0.002$, $P = 0.962$), within-species models suggested a differential effect of noise treatment on *H. cinerea* modification of inter-call interval (mixed model: treatment x period: $F_{2,1103} = 4.63$, $P = 0.01$, Fig. 3e; exemplar[treatment]: $F_{2,23} = 1.91$, $P = 0.171$; temperature: $F_{1,24} = 0.01$, $P = 0.925$) but not *H. femoralis* (mixed model: treatment x period: $F_{2,587} = 0.50$, $P = 0.606$, Fig. 3f; exemplar[treatment]: $F_{1,13} = 0.02$, $P = 0.894$; temperature: $F_{1,10} = 4.59$, $P = 0.057$). While Tukey HSD *post hoc* tests suggested no difference between treatment groups for *H. cinerea*, linear pairwise contrasts indicated *H. cinerea* significantly increased the inter-call interval (the time gap between calls) in the upshifted white noise (effect size percentage of the mean $\pm$ standard error of the mean = 19.6 ± 7.2 %) but not in either of the masking noise treatments (*O. septentrionalis* call or white noise in the same frequency range).

There was no difference between species in the effect of noise treatment on vocal modification of dominant frequency or call rate (mixed model: *dominant frequency*: species x treatment x period: $F_{2,1718} = 0.87$, $P = 0.42$; exemplar[treatment]: $F_{2,79} = 3.42$, $P = 0.038$; temperature: $F_{1,48} = 6.46$, $P = 0.014$; *call rate*: species x treatment x period: $F_{2,38} = 1.46$, $P = 0.245$; exemplar[treatment]: $F_{2,35} = 0.42$, $P = 0.663$; temperature: $F_{1,35} = 3.96$, $P = 0.055$). Neither *H. cinerea* nor *H. femoralis* significantly modified dominant frequency (mixed model: *H. cinerea*: treatment x period: $F_{2,1122} = 0.12$, $P = 0.88$, Fig. 3g; exemplar[treatment]: $F_{2,19} = 2.11$, $P = 0.150$; temperature: $F_{1,32} = 7.62$, $P = 0.010$; *H. femoralis*: treatment x period: $F_{2,585} = 0.66$, $P = 0.518$, Fig. 3h; exemplar[treatment]:

368 $F_{1,520} = 2.82, P = 0.094$; temperature: $F_{1,11} = 1.45, P = 0.253$) or call rate (mixed model:
369 *H. cinerea*: treatment x period: $F_{2,26} = 2.14, P = 0.138$, Fig. 3i; exemplar[treatment]: $F_{2,23}$
370 $= 0.76, P = 0.480$; temperature: $F_{1,23} = 2.66, P = 0.116$; *H. femoralis*: treatment x period:
371 $F_{2,12} = 0.39, P = 0.686$, Fig. 3j; exemplar[treatment]: $F_{1,10} = 1.00, P = 0.340$;
372 temperature: $F_{1,10} = 17.11, P = 0.002$) in response to any of the noise treatments.

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Figure 3: *H. cinerea* and *H. femoralis* differed in their vocal modification in response to noise stimuli. Bars represent mean + one standard error for call duration (a-b), amplitude (c-d), inter-call interval (e-f), dominant frequency (g-h), and rate (i-j) before (black) and during (grey) three noise playback treatments: *O. septentrionalis* chorus, masking white noise, or upshifted white noise.

381

382 **DISCUSSION**

383 Masking noise from both the *O. septentrionalis* chorus and white noise of a
384 similar frequency affected the calling behaviour of *H. cinerea* but not *H. femoralis*. *H.*
385 *cinerea*, whose calls are similar in structure and timing to *O. septentrionalis* calls,
386 produced shorter-duration calls and increased call amplitude in response to both noise
387 stimuli in the masking frequency range. Additionally, by maintaining call spacing *H.*
388 *cinerea* made more frequent calls during both masking noise stimuli compared to
389 upshifted white noise. *H. femoralis*, whose acoustic behaviour differs from that of *O.*
390 *septentrionalis*, did not modify any of the components of acoustic signalling that we
391 investigated.

392 The species differences in the effect of noise playback on vocal modification may
393 be due to differences in acoustic niche. *H. femoralis* breeding calls differ from *O.*
394 *septentrionalis* calls in several acoustic parameters that determine acoustic niche (Fig 1).
395 Therefore, male *H. femoralis* may not need to modify calls to ensure that females receive
396 them in the presence of *O. septentrionalis* calls. Indeed, this species may not compete
397 acoustically with *O. septentrionalis*. It would be interesting to explore whether *H.*
398 *femoralis* modify their vocal behaviour in response to stimuli that more closely overlap
399 their calls. This would help determine whether the lack of vocal compensation is due to a
400 constraint (e.g. behavioural or physiological, such as fixed call structure) or to an absence
401 of acoustic competition. *H. cinerea* breeding calls are similar to those of *O.*
402 *septentrionalis* in several acoustic parameters including duration, inter-call interval, rate
403 and frequency structure (Fig 1). We found that *H. cinerea* modified call duration and

amplitude and maintained inter-call interval in response to both *O. septentrionalis* chorus and masking white noise in the same frequency range. In response to upshifted white noise at a higher frequency, they did not modify call duration and amplitude, and they increased call spacing by lengthening their inter-call interval (slowing down calling activity). This suggests a general response to masking noise interference within the *O. septentrionalis* and, importantly, *H. cinerea* frequency range, and not simply anti-predator or aggressive behaviour to a novel predator sound.

Modifying call duration is a common noise compensation strategy across animal taxa (e.g. Patricelli & Blickley 2006). Typically, however, species lengthen calls, presumably to enhance their ability to be detected by conspecifics (Schwartz *et al.* 2008; Brumm & Slabbekoorn 2005). Few studies have shown the opposite response – a shortening of call duration (but see Lengagne 2008; Francis *et al.* 2011) – as we found for *H. cinerea*. Because of energetic trade-offs of acoustic signalling (Podos & Patek 2014), shortening calls may conserve calling effort, which could allow a frog to persist calling for longer, or could enable a frog to increase call amplitude, which we also observed. In species with prolonged chorus tenure (several months for *H. cinerea*; Dorcas & Gibbons 2008), this scaling back of calling effort may help conserve energy. *H. cinerea* is notable in its ability to insert calls into gaps in conspecific choruses in order to minimize acoustic interference (Höbel 2014). In order for a call to effectively be inserted into a gap, it cannot overlap the end of the previous or beginning of the following call. Indeed, shorter calls may be more effective for gap insertions. Therefore, even amidst continuous signals, duration-shortening, as opposed to duration-lengthening, may be a useful compensation strategy for *H. cinerea*. However, shortening calls also carries the trade-off that these

calls are likely harder for females to detect against the continuous background noise, or may be perceived as less attractive. Indeed, *H. versicolor* (gray treefrog) offspring performance is predicted by call duration, with offspring of fathers with longer duration calls performing better across several fitness-relevant factors (Welch *et al.* 1998). Indeed, call duration may be an indicator of heritable male genetic quality (Welch *et al.* 1998). Therefore, it would be interesting to test whether female *H. cinerea* exhibit preference for longer or shorter duration male calls.

 In addition to shortening the duration of their calls, *H. cinerea* increased call amplitude in response to the two masking noise stimuli (*O. septentrionalis* chorus and white noise of the same frequency), again suggesting a general response to masking rather than anti-predator or aggressive behaviour. It is possible that the apparent increase in amplitude during the masking stimuli could be due to the focal caller changing orientation during the stimuli (Bernal *et al.* 2009). However, focal frogs were typically found facing the same direction at the end of the trial, making phonotaxis an unlikely explanation but one worthy of further investigation. Alternatively, increasing call amplitude in response to noise, widely established across taxa, is known as the Lombard effect, and allows an organism to increase the active space (range of detection) of its calls in noise (Hotchkin & Parks 2013). The Lombard effect, however, is uncommon in anurans (Love & Bee 2010; but see Penna & Hamilton-West 2007; Lopez 1988). Love and Bee (2010) suggest that this is because anurans have evolved to chorus at or near their physiological or morphological maxima. They additionally suggest that due to the temporal and seasonal constraints of breeding and the linkage between acoustic behaviour, reproduction and fitness, selection may favour individuals that call at

maximum amplitude. In *H. cinerea*, however, the breeding season lasts for up to seven months (Dorcas & Gibbons 2008). Therefore, male *H. cinerea* may not be temporally or seasonally constrained in their breeding. Since calling requires substantial energy expenditure (Gerhardt & Huber 2002), we would expect selection to favour individuals that strike an optimal balance between energy expenditure and mate attraction. Indeed, plastically increasing amplitude on an as-needed basis may enhance active space for *H. cinerea* calls while minimising unnecessary energy expenditure. Because of the trade-offs in energy usage in acoustic signalling (Podos & Patek 2014), the observed shortening of call duration may have simultaneously freed energy to increase call amplitude.

H. cinerea increased the interval between calls (e.g. individual calls became farther apart), but only during the upshifted white noise control which did not overlap with their own call frequency. This gap-lengthening response in only the upshifted white noise may reflect a general “relaxing” response that may conserve energy by reducing effort in the absence of nearby acoustic competitors (e.g. no masking noise). In contrast, *H. cinerea* maintained intense call effort during the masking stimuli, and this persistence in calling may be a strategy to compete with noise. Two invasive species similarly impacted other native species’ calling behaviour. Calls from invasive *Lithobates catesbeianus* (American bullfrog) and invasive *Rhinella marina* (cane toad) caused increases in the call rates of native frogs (Both & Grant 2012; Bleach *et al.* 2015). Interestingly, while *H. cinerea* exhibited a similar trend of increasing call rate in the presence of the *O. septentrionalis* stimulus but not the other noise treatments, which was significant at the within-treatment level (individual pairwise contrasts of playback periods, *O. septentrionalis* noise: $F_{1,26} = 6.50$, $P = 0.02$; masking white noise: $F_{1,26} =$

0.22, $P = 0.64$; upshifted white noise: $F_{1,26} = 0.24$, $P = 0.63$, Fig. 3g), this increase was not significantly different across noise treatments in the larger within- or between-species models.

Despite their similarity in call structure to that of *O. septentrionalis*, *H. cinerea* did not modify dominant frequency in response to either of the noise stimuli in the masking frequency range. We can identify three possible explanations for these results. First, subtle changes in dominant frequency of *H. cinerea* calls would still render these calls within the wide bandwidth of masking noise, and thus expending energy on frequency modification may not be effective. This explanation is also supported by the generally subtle (if any) frequency modifications to ambient noise made by species across diverse taxa (reviewed in Hotchkiss & Parks 2013). Second, females may find modified-frequency calls less attractive, selecting against changes in this call parameter. Indeed, *H. cinerea* calls in which an extra frequency band had been added were less attractive to females (Gerhardt 1974). There can be a trade-off between preferred call frequency (for example, low frequencies for some bird species; Cardoso 2012) and effective communication in ambient anthropogenic noise (high frequencies) (Huet des Aunay *et al.* 2013; Halfwerk *et al.* 2011). Finally, frequency plasticity in *H. cinerea* may be constrained by fixed traits such as body size, or by external factors such as temperature, as in other frog species (Gerhardt 1994). While we studied vocal responses to novel sound by an invasive species in uninvaded sites, it would be interesting to compare vocal plasticity in *H. cinerea* populations at different points along the invasion, to determine whether certain vocal modification responses, as well as female preferences, have been favoured by selection. Since selective pressure from invaders can result in fixed changes

in population traits (Phillips & Shine 2004), shorter or louder calls or the tendency to plastically modify them may become fixed in populations with long-term exposure to *O. septentrionalis*.

This study adds to the recently growing evidence that sounds from an invasive species affect the vocal behaviour of some native species (Both & Grant 2012; Bleach *et al.* 2015). Our results extend these findings by providing evidence that acoustic competition with the novel sounds of an invasive species is driving these behavioural changes. We showed that masking noise by an invasive species differentially affects native species' vocal behaviour, with a species with more similar calls modifying communication signals to minimise acoustic competition. We suggest that vocal compensation may reflect acoustic niche partitioning to prevent competitive exclusion. Future research should examine whether the documented changes in vocal behaviour do in fact reduce masking. These results provide insight into a novel pathway through which invasive species may alter native communities. Our findings contribute to the growing evidence of the widespread impacts of acoustic habitat loss by revealing an additional source of environmental noise that interferes with acoustic communication.

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