Continental-scale citizen science data reveal no changes in acoustic responses of a widespread tree frog to an urbanisation gradient

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Submitted: 19 July 2019; Received (in revised form): 21 December 2019; Accepted: 1 January 2020

Abstract

One of the major drivers of global biodiversity declines is habitat loss and modification, often associated with anthropogenic environments. To mitigate biodiversity declines, a comprehensive understanding of how species respond to novel anthropogenic environments is required. Compared to natural habitats, human-modified environments often have increased noise and light pollution, potentially affecting acoustically communicating species, such as frogs. These areas may force animals to modulate or alter their calls to communicate with potential mates, as they compete with anthropogenic noise. Using large-scale citizen science data, coupled with remotely sensed data, we examined how the advertisement calls of the Australian red tree frog (Litoria rubella) varied in response to a gradient consistent with anthropogenic disturbance. After measuring a suite of acoustic properties of L. rubella across its range, we discovered that their advertisement calls showed no response to a disturbance urbanisation gradient. The advertisement calls of the species were highly variable, both at continental and local scales. Our results indicate that acoustic communication in male L. rubella may not be impeded in human-modified habitats as (1) they are a loud species typically heard over background noise and multi-species choruses and (2) their calls are highly variable—potentially serving as a buffer to any acoustic disturbances. Overall, our results provide evidence that some frog species may be acoustically urban tolerant and provide a greater understanding of the responses frogs exhibit to human-mediated environmental change.

Key words: bioacoustics, frogs, urbanisation, citizen science, advertisement call

Introduction

The greatest threat to biodiversity is habitat loss and modification (McKinney 2006; Dirzo et al. 2014; Maxwell et al. 2016). Not all species are adapted to the challenges associated with highly human-modified ecosystems (Blair and Launer 1997; Tait, Daniels, and Hill 2005; McKinney 2006; McKinney 2008; Urban et al. 2006), resulting in significant biodiversity declines. Human-modified ecosystems are characteristic of extensive habitat degradation from pollution and changes in land use (McKinney 2006; McKinney 2008; Grimm et al. 2008) and as a result, species in these environments are presented with novel ecosystems of vastly different characteristics and selection pressures to those they evolved in, which may lead to changes in behaviour (Lowry, Lill, and Wong 2013). However, some species can be relatively unaffected and thus survive and sometimes thrive in human-modified habitats (Fischer et al. 2015;
Lowe, Wilder, and Hochuli 2017). For example, species may change breeding phenology, shift vocalisation frequencies or alter their foraging activity in response to disruptions in diurnal periods or from noise and light pollution (Katti and Warren 2004; Hage et al. 2013; Newport, Shorthouse, and Manning 2014; Peck et al. 2014; Chejanovski et al. 2017). Characterising how species respond to urbanised and highly human-modified environments is important in understanding which species are likely to succeed in anthropogenically disturbed areas and which are more likely to be under threat.

Acoustic signals are paramount for species survival and reproductive success, and human-modified environments may threaten this (Goutte et al. 2018). Higher levels of light and noise pollution are associated with anthropogenic environments and this can greatly impair animal communication as a greater range of sensory disturbances are encountered (Lowry, Lill, and Wong 2013; Halfwerk and Slabberkoorn 2015). This is particularly concerning as acoustic communication is used by a variety of animals, including insects, birds, mammals and frogs to transmit information regarding territory ownership, predators and the attraction of potential mates (Roca et al. 2016). Anthropogenic noise is generally of low frequency and overlaps with the acoustic frequencies used by many species (Barber, Crooks, and Fristrup 2010). Thus, animals may be required to modify their acoustic signals to be heard, relying on various strategies to compete with chronic anthropogenic noise (Slabberkoorn and Peet 2003; Cunnington and Fahrig 2016; Lampe, Reinhold, and Schmoll 2014, Marley et al. 2017, Parris, Velik-Lord, and North 2009). Examples of such strategies include shifts in frequency above that of anthropogenic noise (out of the range of 0–3 kHz), adjusting the amplitude of their vocalisation to sing/call louder (i.e. the Lombard effect), and increasing their call rate or producing shorter yet faster songs/calls (Slabberkoorn and Peet 2003; Katti and Warren 2004; Slabberkoorn and den Boer-Visser 2006; Lampe, Reinhold, and Schmoll 2014). However, these modifications can be energetically costly (Zollinger, Goller, and Brumm 2011) and some species appear unable to modulate their vocalisations or reception (Barber, Crooks, and Fristrup 2010; Siemers and Schaub 2011).

Despite the clear evidence that anthropogenic disturbance can hinder communication in a range of taxa, with implications for reproductive success (Beck and Heinsohn 2006), foraging ability (Siemers and Schaub 2011) and social processes (Marley et al. 2017), most studies to date have focused on birds (Slabberkoorn and Peet 2003; Katti and Warren 2004; Beck and Heinsohn 2006; Slabberkoorn and den Boer-Visser 2006; Zollinger et al. 2011; Peck et al. 2014; LaZerte, Otter, and Slabberkoorn 2015; Potvin and MacDougall-Shackleton 2015; Cavalli et al. 2016; Ciądałaśki et al. 2016; Machovsky-Capuskas et al. 2016; Roca et al. 2016; Hardman and Dalesman 2018). There exists relatively little literature on other key taxa, such as mammals, insects and amphibians; limiting our general understanding of the influences of urbanisation processes on biodiversity. Frogs are part of the fastest declining vertebrate group globally, with ~42% of all amphibians listed as threatened (Dirzo et al. 2014; IUCN 2017), yet, alarmingly, we are unsure how they respond to increasingly urbanised landscapes.

If anthropogenic noise interferes with the integrity of male frogs’ advertisement calls, it may inhibit breeding success as females recognise and assess males by the frequency, rate and loudness of their calls (Czarnowski and Forester 1985; Sun and Narins 2005; Kaiser et al. 2011). Male frogs, however, can employ a suite of strategies in response to anthropogenic noise, including shifting the frequency of their call above that of anthropogenic noise (0–3 kHz) (Hoskin and Goosen 2010; Caorsi et al. 2017), increasing vocalisation amplitude to call louder (Bee and Swanson 2007), changing call durations or rates (Sun and Narins 2005; Kaiser and Hammers 2009; Caorsi et al. 2017) and avoiding noise disturbances (Caorsi et al. 2017; Grace and Noss 2018). But a frog species’ ability to cope with anthropogenic noise appears to be species-specific (Nelson et al. 2017): some frogs only modulate call parameters when their call overlaps with anthropogenic noise (Cunnington and Fahrig 2010; Vargas-Salinas et al. 2014) or when the noise is above a certain threshold (Yeo and Sheridan 2019). Some frog species appear to be facilitated by urban noise as they exploit the reduction in calling of other frog species (Sun and Narins 2005), or have the ability to modify their calls to be more attractive to females (Halfwerk et al. 2018).

Frog call characteristics can also be influenced by other anthropogenic factors including nearby human activity (Rodríguez-Prieto and Fernández-Juricic 2005) and light pollution (Baker and Richardson 2006; Hall 2016). Even where traffic noise may not be apparent, buildings can act as ‘canyons’ and barriers for signal transmission (see review by Warren et al. 2006). No studies to date have investigated the collective impact of multiple anthropogenic factors on frog calls. Our current understanding of frog acoustic responses to anthropogenic disturbance is generally derived from single studies that are experimental in nature, have low sample sizes (one frog per site; Cunnington and Fahrig 2010), limited in spatial scale (Hoskin and Goosen 2010; Kaiser et al. 2011) or tested only one or few call parameters (e.g. examined only frequency or only call rate; Sun and Narins 2005; Parris, Velik-Lord, and North 2009). Data from the entire range of widely distributed species should provide generalisable patterns of the effects of anthropogenic disturbance.

Obtaining data from across the entire range of a widely distributed species is logistically challenging. However, the recent rise of citizen science projects has allowed scientists to expand the spatial and temporal scale of data collection (Kobori et al. 2016). Through continental-scale citizen science data, we examined acoustic responses of a widespread frog species, the Australian red tree frog (Litoria rubella), to anthropogenic disturbance. We hypothesised that the call structure of L. rubella would vary in temporal and spectral properties in response to levels of anthropogenic disturbance. We first assessed intra-specific variation in call structure of L. rubella by measuring call properties throughout its geographic range. Second, we assessed call variation in L. rubella across an urban gradient (from rural areas to high-density cities) after accounting for temperature, potential taxonomic differences and time of year. Higher human population densities are associated with higher levels of light and noise pollution, as well as a greater increase in human activity. As light and noise pollution, in addition to increased human activity are known to impact frog calling behaviour (Rodríguez-Prieto and Fernández-Juricic 2005, Baker and Richardson 2006; Hall 2016), we expect that properties of frog advertisement calls are likely to change with higher human population density (i.e. from rural population densities of <100 person/km² to high-density cities with greater than 1000 persons/km²). We predicted there to be an upward shift in the dominant frequency (kHz) of their calls (out of the anthropogenic noise range), changes to call/intercall durations (increased call rate) and an altered number of notes (call complexity) as habitat becomes more urbanised or human modified. This is the first study to investigate the impacts of anthropogenic disturbance at a
continental scale and aids in a better understanding of permanent acoustic changes in populations.

**Methods**

**Study species**

The red tree frog (*L. rubella*) is a small tree frog native to Australia, New Guinea, Timor Leste and Indonesia; with a body size range between 3.0 and 4.5 cm. Within Australia, it has a widespread distribution of approximately five million square kilometres, inhabits a wide variety of habitat types and is known to breed in ponds (Tyler and Knight 2011). This species is ideal for studying the effects of human-modified habitats as it is commonly found in both urban and relatively unmodified areas, has a high volume of calls available through the FrogID project (Rowley et al. 2019) and has a dominant call frequency between 1.1 and 3.6 kHz, within the anthropogenic noise range of 0–3 kHz (Barber, Crooks, and Fristrup 2010; Xie 2017).

**FrogID call data**

Frog call data were derived from the FrogID project, whereby a user submits a 20–60-s audio recording [MPEG AAC audio file (mp4a), standard sampling rate of 44.1 kHz] of a frog advertisement call through the FrogID smartphone app (Rowley et al. 2019). No special recommendations or instructions were requested of users regarding recording distance to frogs or device configuration when recording. The app automatically includes the time and date the recording was made in addition to the geographic location (latitude, longitude and an estimate of location accuracy). Once the submission is received, FrogID validators at the Australian Museum manually review the frog call audio and associated data to identify the frog species calling (Rowley et al. 2019). We examined 380 calls of *L. rubella* from 348 unique coordinates throughout its geographic range (Fig. 1).

**Effect of urbanisation**

**Spatial data**

The definition of an urban environment is highly variable among studies (see Hahs and McDonnell 2006; Hamer and McDonnell 2008; Fischer et al. 2015). Furthermore, not all human-modified habitats are classified as ‘urban’ (e.g. a mine site in a rural region). As such, a range of anthropogenic disturbance measures relevant to the study species should be examined when choosing a proxy for human-mediated disturbance. In Google Earth Engine (Gorelick et al. 2017), we spatially overlaid layers that were representative of anthropogenic disturbance over the frog call submissions. Specifically, we investigated the following anthropogenic metrics: (1) population density as human disturbance can affect frog call parameters (Rodríguez-Prieto and Fernández-Juricic 2005; Garner et al. 2008; Hamer and McDonnell 2008); (2) night-time light values as human-modified habitats are commonly well lit and frogs can exhibit altered calls under artificial light (Baker and Richardson 2006; Hall 2016); and (3) vegetation cover—a common proxy used for human-modified environments, with these environments exhibiting higher impervious surfaces and reduced vegetation (Cadenasso, Pickett, and Schwarz 2007; Lowe, Wilder, and Hochuli 2017). Of these three potential metrics for anthropogenic disturbance, we selected human population density (instead of night-time light values or vegetation cover) as the

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*Figure 1: Distribution of analysed FrogID call submissions from across the range of *L. rubella*. Blue area represents the current distribution of *L. rubella*. Grey dots indicate locations of the 380 call submissions. Map produced in Tableau.*
proxy for anthropogenic disturbance for three reasons. First, because it correlated with both night-time light values and vegetation cover classified as ‘urban or built up’. Second, night-time lights occasionally had extremely high values due to proximity to mining sites, despite a lack of an urban centre (producing outliers in the data). Lastly, as we wished to assess responses to an urbanisation gradient—as opposed to categorical classifications of urbanisation—given its importance in understanding how organisms adapt to anthropogenic change (Callaghan et al. 2019).

**Call selection**

To ensure representative sample sizes across the range and prevent oversampling calls from a single geographic location (i.e. potentially the same frog), we visualised call submissions in Tableau data analytics software (version 3.2) and selected calls based on the following factors: geographic location, urban classification, location accuracy and number of other frogs calling in recording. We discarded calls from further analysis where acoustic parameters could not be accurately measured. Thus, submissions which had more than one frog species calling at the same time and in the same frequency range as *L. rubella*, contained large choruses of *L. rubella*, were too faint, or where the frog called <6 times in a particular recording (n = 434) were omitted from analyses (except for 13 high-quality calls with 5 calls and 6 with 4 calls). Furthermore, we used a maximum of three calls from the same location (i.e. exact coordinates). Subsequent calls were deemed ‘location repeats’ and excluded from analysis (n = 526). Of a total of 1340 calls examined, 380 calls were deemed of appropriate quality for analysis—still representing a dramatic improvement in sample size of papers which have looked at acoustic signalling in frogs.

**Call analysis**

We converted calls from MPEG AAC audio file (mp4a) to a WAV format (sampling rate of 48 kHz with 16 bits per sample) via MediaHuman Audio Converter (www.medialhuman.com/audiocconverter). We conducted call analysis in RavenPro 1.5 software (http://www.birds.cornell.edu/bpr/raven) under a fast-Fourier transformation of 512 points with 50% overlap, and measured key call parameters, including call duration (s), intercall duration (s), dominant frequency (kHz), number of pulses, call rate (calls/min) and pulse rate (pulses/s) (Table 1). These are standard characteristics used for frog bioacoustic analysis and are useful measures to show call variation in response to environmental and social conditions, as well as among species (see review by Köhler et al. 2017). Further variability within the call structure of *L. rubella* was identified through oscillograms, where we classified variation in call amplitude over time either as symmetrical, increasing (amplitude lower at the beginning of a call), or decreasing (amplitude was lower at the end of a call). Not all parameters were able to be obtained for every call submission. For example, number of pulses were only available on high-quality submissions (such as a high amplitude call recording of a single *L. rubella*). A minimum of six calls per submission were measured (except for 13 high-quality calls with 5 calls and 6 with 4 calls as mentioned above) and average parameters for each individual used for subsequent analysis. We noted the number of other frog species calling and the identity of background noise (e.g. motor vehicles) for examination of any short-term and immediate effects on *L. rubella* call modulation. Some individuals had co-dominant frequency bands in their calls. In these cases, we used the lower frequency band, which corresponded with the typical dominant frequency range and is most likely to be affected by low-frequency anthropogenic noise (Parris, Velik-Lord, and North 2009). We also recorded the number of all co-dominant bands.

**Accounting for co-variates**

**Temperature-induced call variation**

Temperature influences the temporal aspects of frog calls, with higher temperatures typically increasing temporal call parameters (call rate, call and intercall durations, and pulse rate) (Köhler et al. 2017). As no temperature data are submitted with FrogID calls, we estimated ambient temperature by first sourcing temperature data for each call submission location from the Australian Bureau of Meteorology via the ‘bomrang’ R package (Sparks et al. 2017; Sparks et al. 2019). Historical minimum and maximum temperature data from the nearest weather station from 15 days prior, the day of, and 15 days after the FrogID submission were extracted through this package. Second, we estimated the temperature at the time of calling (to the nearest hour of the call submission time stamp) via the R package, ‘chillR’ (Luedeling 2019). This package interpolated any missing daily temperatures and then estimated the hourly temperature for each FrogID call submission.

**Geographic and/or taxonomic differences**

Call frequency typically decreases inversely with body size in frogs (Czarnowsky and Forester 1985). To investigate the variation in body size of *L. rubella* (as a result of potential taxonomic differences or size–latitude relationships—whereby body size increases with latitude; Ashton 2002; Ashton 2004), we measured the snout-vent length (mm) of Australian Museum *L. rubella* specimens. Adult male *L. rubella* specimens were selected from geographic locations across the species range in Australia; n = 12 from Western Australia (WA), n = 31 from the Northern Territory (NT), n = 85 from Queensland (QLD) and n = 108 from New South Wales (NSW) (see Supplementary Table S1). Males were identified by the presence of dark vocal sacs and/or nuptial pads. We then investigated the relationship between body size and geographic variability and accounted for geographic variability in our models, given this relationship (see below).

As an additional control for call variation due to any cryptic speciation diversity or geographic variation in call variation across geographical regions can act as an indicator of speciation (Vences and Wake, 2007; Littlejohn, 2008), and to allow for better comparison to small-scale studies that dominate the literature, we investigated three small-scale exploratory analyses of call variation for a 100-km radius around three geographically discrete urban centres: Brisbane, Darwin and Townsville. These sites were chosen as they had a large volume of calls across an urban gradient and because these sites would have a high probability of consisting of single species of *L. rubella* within each of the respective regions. We classified call submissions by a categorical classification of urbanisation level, thus making comparisons among the three urban centres permissible. Calls were classified into low, medium and high disturbance categories by splitting the population density (person/km²) values for call submissions into quartiles separately for each population centre. All calls which were submitted below the lower quartile were considered low disturbance, and conversely, all calls above the upper quartile were considered high disturbance, respective for each urban centre. Any remaining calls were classified as medium disturbance. For Brisbane, low disturbance was <64 persons/km² and high disturbance was >366 persons/km². For Darwin, low disturbance was <44 persons/km² and high disturbance was >505 persons/km².
For Townsville, low disturbance was <535 persons/km² and high disturbance was >1434 persons/km². We then measured the variability of call parameters across these three disturbance levels by calculating the standard deviation of each call parameter, stratified by disturbance level and urban centre.

### Statistical analysis

All statistical analyses were conducted in R ver. 3.5 (R Core Team 2019) relying heavily on the tidyverse workflow (Wickham 2017).

To test whether the advertisement call of *L.rubella* differs in response to corresponding levels of urbanisation, we first employed a univariate approach through a suite of seven generalised additive models (GAMs), with log transformed human population density (as a proxy for anthropogenic disturbance) as the independent variable for each of the following dependent variables (Table 1): call duration (s), intercall duration (s), call rate (calls/min), dominant frequency (kHz), number of co-dominant frequency bands, number of pulses and pulse rate (notes/s). Smoother co-variates added into the analyses included geographic location (latitude and longitude) accounting for a latitude–body size relationship (see Results section), temperature at hour of calling and time of year (month of breeding season). Correlation between variables was checked prior to performing the GAM (Supplementary Fig. S1) and there was no substantial correlation among model variables. Data were normally distributed except for frequency bands, intercall duration, call rate and pulse rates, which were log transformed. A Benjamini and Hochberg correction was applied to account for increased type I error from the multiple analyses (Thissen, Steinberg, and Kuang 2002).

Second, we used a principal component analysis (PCA) to collapse the seven call measurements into a single measure of response. Any missing data values were imputed using the R package ‘missForest’ (Stekhoven and Buehlmann 2012). Similar to above, we then ran a GAM accounting for the aforementioned co-variates, but with PC1 as the response variable. We repeated this model for PC2 and PC3, ensuring robustness of the results.

To identify whether call parameters in urban centres had differing levels of variability at different anthropogenic disturbance levels (low, mid and high), we used a linear mixed effects model (Bates et al. 2015). Standard deviation (log transformed) was the dependent variable, and disturbance level (low, mid and high) and call parameter were fixed effects while urban centre (Brisbane, Darwin and Townsville) was a random effect.

### Geographic variation in *L. rubella*

*Litoria rubella* calls were highly variable across the geographic range of the species, with geographic location (latitude and longitude) having a significant effect on all call parameters (Table 3; Fig. 4). For example, calls from WA and NSW typically had lower frequency (~1.5 kHz) compared to other regions (usually ~2.2 kHz). Although some location trends appear to exist in call parameters (dominant frequency and pulse rate increased with increasing latitude, call rate decreased with increasing latitude), call parameters were highly variable (Figs 2 and 4). There were no obvious geographic trends in amplitude variation over time. Co-variates also varied with geography. Body size in the specimens measured ranged from 24.1 to 41.3 mm, and was in part explained by latitude, with larger specimens of *L. rubella* found at greater latitudes ($R^2 = 0.41, F(1, 235) = 162.9, P < 0.001$; Supplementary Fig. S2).

### Response to anthropogenic disturbance

Calls of sufficient quality fell across an urbanisation gradient from rural land (approximately <100 persons/km²), through to suburbia/urban edge (approximately 400–1000 persons/km²), to high-density cities and central business districts (over 1000 persons/km²). However, submissions were more concentrated in areas with human population densities of <50 persons/km² (Supplementary Fig. S3). After controlling for geographic location, temperature and time of year, *L. rubella* did not significantly modify any call parameters in more urbanised areas compared to less urbanised areas except for intercall duration (Table 3). Interestingly, however, one call submission with a single vehicle in the background did elicit a temporary modification in call rate by reducing intercall duration. Call rate increased to 70 calls/min during the disturbance from an initial 58 calls/min, before decreasing to 60 calls/min as the vehicle drove further away.

From the seven input call parameters in the PCA, we retained three principal components (eigenvalues greater than one; see Supplementary Table S2). The first axis (PC1; explaining 36.7% of variance) was linked to temporal call characteristics...
Table 2: Mean, minimum, and maximum values for all call parameters, alongside associated standard deviation (SD) and number of calls able to be used for each call parameter (N)

| Call parameter                        | Mean value | Minimum value | Maximum value | SD  | N  |
|---------------------------------------|------------|---------------|---------------|-----|----|
| Call duration (s)                     | 0.58       | 0.26          | 0.99          | 0.12| 350|
| Intercall duration (s)                | 0.43       | 0.18          | 1.69          | 0.18| 349|
| Call rate (calls/min)                 | 61         | 26            | 125           | 13  | 284|
| Dominant frequency (kHz)              | 2.1        | 1.1           | 3.6           | 0.4 | 380|
| Number of co-dominant bands           | 2          | 1             | 5             | 1   | 380|
| Pulses                                | 33         | 19            | 62            | 7   | 140|
| Pulse rate (pulses/s)                 | 59         | 31            | 88            | 11  | 140|

Figure 2: Boxplots demonstrating the variable nature of all call parameters in L.rubella calls across its geographical range

Figure 3: Oscillograms and spectrograms of three characteristic calls of L.rubella demonstrating their variable structure. Calls are from the following geographic locations: (A) 'increasing' call shape—Kurratha, Western Australia (−20.73, 116.88), (B) 'symmetrical' call shape—Sunshine Coast, Queensland (−26.69, 152.94) and (C) ‘decreasing’ call shape—Mackay, Queensland (−24.52, 151.97), however, are not exclusive to these locations
The second axis (PC2; explaining 24.1% of total variance) was linked to call parameters relating to call complexity (pulses, call duration). The third axis (PC3; explaining 17.4% of total variance) was linked to frequency (dominant frequency, number of co-dominant frequency bands) (see Supplementary Tables S2 and S3). None of the principal components were significantly affected by the level of human population density ($P > 0.05$), however, they were influenced by location, temperature and time of year (Table 3).

Even after partially removing the effect of geographic location, through spatially focused analyses around the urban centres of Brisbane, Darwin and Townsville, there was still relatively large variation in all call parameters (Table 4; Fig. 5). Additionally, variation in call parameters did not differ with level of anthropogenic disturbance [$F_{(2,52)} = 1$, $P > 0.05$; Fig. 5].

**Discussion**

Despite the global declines in amphibian populations, we still lack key knowledge regarding the full impacts of urbanisation processes on this taxa (Magle et al. 2012). Through the first large-scale analysis of bioacoustic data across five million square kilometres, and across various urban gradients, we found that anthropogenic disturbance from human-modified habitats had no discernible effect on the advertisement calls of *L.rubella* in comparison to less human-modified habitats (except...
for intercall duration; see below). We also found immense variability in the calls of the species throughout its geographic range and this pattern was confirmed when investigating targeted analyses around Brisbane, Darwin and Townsville. This variability in calls may act as a buffer allowing *L.rubella* to be tolerant to acoustic disturbance in anthropogenic environments, thus explaining why advertisement calls of *L.rubella* did not differ along an anthropogenic gradient.

Table 4: Mean, minimum and maximum values for all call parameters in targeted analyses around Brisbane, Darwin and Townsville

| Call parameter          | Urban centre | Mean value | Minimum value | Maximum value | SD  | N  |
|-------------------------|--------------|------------|---------------|---------------|-----|----|
| Call duration (s)       | Brisbane     | 0.56       | 0.33          | 0.78          | 0.11| 27 |
|                         | Darwin       | 0.49       | 0.31          | 0.67          | 0.07| 56 |
|                         | Townsville   | 0.59       | 0.41          | 0.81          | 0.08| 42 |
| Intercall duration (s)  | Brisbane     | 0.45       | 0.25          | 1.01          | 0.18| 27 |
|                         | Darwin       | 0.40       | 0.18          | 0.94          | 0.15| 56 |
|                         | Townsville   | 0.38       | 0.22          | 0.85          | 0.12| 42 |
| Call rate (calls/min)   | Brisbane     | 61         | 36            | 89            | 14  | 22 |
|                         | Darwin       | 69         | 45            | 101           | 12  | 44 |
|                         | Townsville   | 64         | 46            | 95            | 10  | 32 |
| Dominant frequency      | Brisbane     | 2.2        | 1.1           | 3             | 0.4 | 29 |
|                         | Darwin       | 2.3        | 1.7           | 3.1           | 0.3 | 59 |
|                         | Townsville   | 2.4        | 1.9           | 3.3           | 0.3 | 42 |
| Number of co-dominant bands | Brisbane   | 2          | 1             | 4             | 1   | 29 |
|                         | Darwin       | 2          | 1             | 5             | 1   | 59 |
|                         | Townsville   | 2          | 1             | 4             | 1   | 42 |
| Pulses                  | Brisbane     | 26         | 20            | 34            | 5   | 16 |
|                         | Darwin       | 34         | 29            | 40            | 3   | 17 |
|                         | Townsville   | 35         | 30            | 41            | 4   | 8  |
| Pulse rate (pulses/s)   | Brisbane     | 48         | 34            | 63            | 9   | 16 |
|                         | Darwin       | 70         | 62            | 81            | 5   | 17 |
|                         | Townsville   | 62         | 51            | 69            | 6   | 8  |

Associated standard deviation (SD) and number of calls able to be used for each call parameter (N) are also presented.

Figure 5: Variability within *L.rubella* call parameters across three disturbance levels (low, mid and high) within a 100-km radius of three urban centres: (A) Brisbane (N = 29), (B) Darwin (N = 59) and (C) Townsville (N = 42)
Species respond differentially to anthropogenic disturbances. Some species are detrimentally affected by high levels of anthropogenic disturbance (Lengagne 2008; Parris, Velik-Lord, and North 2009; Hoskin and Goosm 2010; Bailly et al. 2016; Nelson et al. 2017) while others show no detrimental effects (Cunnington and Fahrig 2013; Vargas-Salinas et al. 2014; Melliger et al. 2018), and some even thrive within high levels of anthropogenic disturbance (Murray and Shaw 2009; Lampe, Reinhold, and Schmoll 2014; Kaluza et al. 2016; Lowe, Wilder, and Hochuli 2017). One explanation of species’ persistence in anthropogenic environments is plasticity in behavioural signalling (Cunnington and Fahrig 2010; Slabbeokoom 2013), where species are able to behaviourally adapt their signalling patterns based on their surroundings, maintaining fitness levels (Sih 2013). Even though L.rubella showed no discernible difference in variability of call parameters among low, medium and high levels of anthropogenic disturbance, we still found substantial variability within call parameters irrespective of disturbance level. This variability in vocalisations may be evidence for behavioural flexibility, and could provide L.rubella with the ability to adjust their call, maintaining fitness in acoustically variable environments (Martinez-Rivera and Gerhardt 2008), including anthropogenic environments dominated by increased acoustic disturbances (e.g. noise and light pollution, canyon effects) (Warren et al. 2006). As call variability was observed both across the continent and in targeted analyses around discrete urban centres, this may be an adaptive trait enabling L.rubella to compete with large multi-species choruses (Littlejohn and Martin 1969; Martinez-Rivera and Gerhardt 2008; Bleach et al. 2015; Tennesen et al. 2016). Future work to confirm this should examine whether high variability in call parameters occurs in other frog species commonly found in urbanised landscapes by measuring the variability in call parameters across frog species common and uncommon in urban areas. Future studies should also include female choice experiments in both anthropogenic habitats and less modified habitats to determine whether variability confers similar fitness in both habitat types (Wollerman and Wiley 2002; Underhill and Höbel 2018). Moreover, this hypothesised behavioural flexibility could explain why one individual in this study, during a calling bout, elicited a shift in call rate in response to a vehicle passing. This finding was similar to Amazonian tree frogs (Dendropsophus triangulum), whereby exposure to continuous motorcycle noise induced individuals to increase their call rate, before returning to a baseline call rate once the noise disturbance ceased (Kaiser and Hammers 2009).

It is possible that L.rubella also increase the amplitude of their calls (Lombard effect) to be heard over urban noise in conjunction with eliciting a highly variable advertisement call. Litoria rubella do exhibit a loud call and can be heard clearly over other frog species during choruses (pers. obs.). Unfortunately, amplitude could not be measured in this study due to non-standardised recording distances between a calling frog and the recorder (Rowley et al. 2019), and this should be confirmed in future work. However, this phenomenon has been documented in other frog species. For example, the Southeast Asian rhacophorid tree frog (Kurixalus chasmani) did not change any call parameters during choruses (pers. obs.). Unfortuntately, amplitude could not be measured in this study due to non-standardised recording distances between a calling frog and the recorder (Rowley et al. 2019), and this should be confirmed in future work. However, this phenomenon has been documented in other frog species. For example, the Southeast Asian rhacophorid tree frog (Kurixalus chasmani) did not change any call parameters in response to anthropogenic noise, except for amplitude (Yeo and Sheridan 2019). Only high-level traffic noise elicited this response, and low-level noise elicited no change in any call parameter, including amplitude. This is not just common to frog species, however, and has been documented in a variety of taxa (Pytte, Rusch, and Ficken 2003; Brumm et al. 2004; Holt et al. 2009; Hage et al. 2013). Furthermore, it should be noted, we did not explicitly test background noise along this gradient and future work should correlate citizen science recordings with measurements of background noise. We also found some evidence of urbanisation impacting intercall duration. However, as intercall duration is greatly impacted by social conditions, for example the number of conspecific males and females at the time of calling (Grafe 1996; Tarano 2001) and we were unable to quantify this, intercall duration may be a less reliable trait in considering the effects of urbanisation compared to the other, more static traits examined. Further, given our lack of impact on call rate—which should be correlated with intercall duration—future research should investigate the effect of urbanisation gradients on these specific traits.

Although our results suggest that L.rubella was largely unaffected by acoustic disturbances in urban environments (Sun and Narins 2005; Kaluza et al. 2016; Lowe, Wilder, and Hochuli 2017; Halfwerk et al. 2018), this does not exclude other negative impacts anthropogenic environments pose. Interestingly, we found a reduced number of L.rubella recordings in areas of higher human population despite most FrogID submissions being received from urban and suburban areas (Rowley et al. 2019). This is suggestive of L.rubella likely still being affected by other anthropogenic mediators, such as loss of key habitat and breeding pools. Additionally, anthropogenic environments can act as ecological traps; whereby an individual is deceived into selecting a poor-quality habitat over a good quality one based on cues they receive (Battin 2004). These cues can become uninformative and detrimental to fitness in habitats that have undergone rapid environmental change (Haile and Swearer 2016). For example, indigo buntings (Passerina cyanea) were attracted to more dangerous habitat types, despite increased nest predation and lower fledging success in these habitats (Weldon and Haddad 2005). Ecological traps, in conjunction with general habitat loss likely still affect L.rubella populations, something that we did not explicitly test in our study.

Citizen science enables an ability to answer broad-scale questions and move beyond small-scale studies which can be confounded by site-specific effects (Dickinson, Zuckerberg, and Bonter 2010; Dickinson et al. 2012; Kobori et al. 2016). As a result, we provided a greater understanding of species-level responses to environmental change, allowing comparison to broader ecological theory. The FrogID citizen science data allowed, for the first time, an examination of male advertisement calls from across an entire frog species’ range. The large and ever-increasing continent-wide dataset will allow for an evaluation of (1) the variation in male advertisement calls within and among species, (2) how these call parameters vary in space and time and (3) the degree to which differences in advertisement calls can be used in frog species delineation. For example, the high inherent variability in the call of L.rubella, even within a single city, casts doubt on the current practice of using a small number of calls with relatively small differences in parameters to support species delineation (Köhler et al. 2017). As these citizen science data continue to increase, then increasingly larger sample sizes can be used to understand the pattern of response to urbanisation at increasingly finer scales.

Behavioural signals are integral for exchanging significant biological information and maintaining fitness. In the case of frogs, maladaptive behavioural signalling (e.g. acoustic signalling) can have critical consequences for individual and population-level population success (Simmons and Narins 2018). As such, a cohesive understanding of the effects of human-modified habitats on acoustic communication is required for adequate conservation decisions. For L.rubella, citizen science data revealed that L.rubella is acoustically unaffected by
anthropogenic disturbance, and this may in part be due to their loud, highly variable calls. However, calling is an indication of breeding effort and not breeding success or recruitment, and it is possible that other anthropogenic stressors, such as ecological traps, still have a negative impact on *L. rubella*. By understanding how species are adapting their signalling behaviours to the pressures imposed by novel urban ecosystems, we are better suited to understand the broad-scale ecological consequences of these pressures and prioritise the conservation of species most at risk (Sol, Lapiedra, and González-lagos 2013).

**Supplementary data**

*Supplementary data* are available at JUECOL online.

**Acknowledgements**

We wish to acknowledge the efforts of the FrogID team at the Australian Museum for reviewing and identifying all *L. rubella* calls, and the FrogID community who recorded and submitted all the frog calls used in this research. We also acknowledge the invaluable contributions of J. Wilshire for providing advice regarding Google Earth Engine and L. Crawford for assistance in data management.

**Data availability**

Some data cannot be made Open Access due to data sensitivity/privacy but can be requested from the Australian Museum or the Corresponding Author.

**Funding**

The authors received no specific funding for this research; however, we express thanks to the Citizen Science Grants of the Australian Government for providing funding for the FrogID project; the Impact Grants programme of IBM Australia for providing the resources to build the FrogID App; as well as Bunnings and Fyna Foods for supporting FrogID as project partners.

**Conflict of interest statement.** None declared.

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