



Unravelling male advertisement call variability in the brown tree frog (*Litoria ewingii*) complex by using citizen science

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ABSTRACT

In the face of global biodiversity declines, conservation measures are urgently needed. However, our lack of knowledge on species' diversity, distributions and population trends presents a major obstacle, particularly for morphologically cryptic species. Field surveys to gather this information present a logistical challenge, but the rapid rise of citizen science presents a new opportunity, generating huge volumes of data rapidly across a species' range. We use data from a national citizen-science project to document the distribution and advertisement call variability in a group of five morphologically conserved treefrogs. Using 542 male advertisement calls across the entire range of five species, we found considerable levels of acoustic variation both among and within species. Note shape, notes per call and positions of the longest and shortest notes were most useful to delineate species. Our research will assist in species delineation and identification in the field and form the basis for a more accurate understanding of species diversity and distributions in the brown tree frog complex. We also demonstrated the utility of citizen science in documenting species distributions and diversity.

Keywords: advertisement call, citizen science, geographic variation, *Litoria calliscelis*, *Litoria ewingii*, *Litoria paraewingi*, *Litoria sibilus*, *Litoria verreauxii*.

Introduction

The earth is currently in the midst of its sixth mass extinction event (Kim and Byrne 2006; Wake and Vredenburg 2008; Gillespie *et al.* 2020). There is an urgent need to stem this biodiversity loss, but there are major obstacles (Krauss *et al.* 2010; Whiles *et al.* 2013; Gillespie *et al.* 2020), including a lack of information on species richness, distribution, and population trends, which can culminate in ineffective conservation strategies (O'Connor and Crowe 2005; Kim and Byrne 2006; Souza *et al.* 2015; Bull *et al.* 2020). To address these gaps in knowledge, more comprehensive biodiversity surveys are required; however, traditional surveys are often expensive and time-consuming (Kim and Byrne 2006; Theodoridis *et al.* 2019; Gillespie *et al.* 2020). With the recent rise of citizen science, species occurrence data is being collected at greater temporal and spatial scales than ever before, helping overcome these surveying difficulties and fill the gaps present in our biodiversity knowledge base (Amano *et al.* 2016; Bela *et al.* 2016; Theodoridis *et al.* 2019).

One of the most threatened yet poorly known groups of animal is amphibians. Currently, 41% of all amphibian species are threatened, up from 33% in 2004 (IUCN 2021). Additionally, over 16% of amphibian species are listed as Data Deficient (IUCN 2021), indicating such a paucity of data that the conservation status of a species cannot be determined. One of the biggest gaps in knowledge for amphibian species is our lack of understanding of their true species diversity. At present, more than 7500 frog species are known but new species are consistently being scientifically described (Frost 2023). The continued rate of species discovery in such a threatened taxon highlights the need for accurate data on frog biodiversity (Hero and Morrison 2004).

The relative lack of data on frogs is in large part due to difficulties in detecting and identifying frog species. Frogs are often small in size, nocturnal, camouflaged, morphologically similar and reliably detectable only under certain environmental conditions (Oseen and Wassersug 2002; Renan *et al.* 2017). As a result, one of the most effective methods of

surveying is via acoustic surveys (Rocha *et al.* 2004; Brown *et al.* 2007). As the male advertisement call of frogs is used to attract mates, it is often species-specific and can be an accurate means by which we can identify frog species (Watson *et al.* 1971; Smith *et al.* 2003).

Despite the species-specific nature of frog calls, it is being increasingly recognised that there is a large degree of intraspecific variation across both temporal and spatial scales (Mitchell *et al.* 2020; Weaver *et al.* 2020). However, existing guides to identify frogs via male advertisement calls are typically based on calls from few individuals from a small geographic area, because of surveying constraints, and are therefore unlikely to be useful across the whole range of a species (Gerhardt and Davis 1988; Oseen and Wassersug 2002; Weaver *et al.* 2020). In addition, because of the high degree of both undiagnosed species diversity and morphological conservatism ('cryptic species'; Lötters *et al.* 2009) in frogs, an examination of the variability of frog advertisement calls across the range of a taxon may be useful in identifying potentially unrecognisable species (Watson *et al.* 1971; Lötters *et al.* 2009; Darwell and Cook 2017). Despite the utility of advertisement calls, obtaining a sufficient sample size to assess geographic variability, map species distributions and identify areas of call divergence presents a logistical challenge (Theodoridis *et al.* 2019; Weaver *et al.* 2020).

Obtaining data rapidly and from large spatial scales is increasingly possible as a result of citizen science (Van Sluys *et al.* 2012; Narins and Meenderink 2014; Heard *et al.* 2015; Forti *et al.* 2016). In recent years, acoustic data collected via citizen-science projects has successfully been used in research projects examining the variability of advertisement calls (Mitchell *et al.* 2020; Weaver *et al.* 2020); however, there remains a considerable lack of research on the variability of advertisement calls in species over geographic space and how this might affect our ability to delineate species.

We used a database of frog call recordings gathered as part of the national citizen-science project FrogID (Rowley *et al.* 2019) to document advertisement-call variability across the range of five morphologically conserved tree frogs in Australia, (*Litoria ewingii*, *Litoria verreauxii*, *Litoria paraewingii*, *Litoria sibilus* and *Litoria calliscelis*) and develop a key for acoustically identifying each species across its entire range. By examining the geographic variability of advertisement calls, we ascertained whether there is bioacoustic support for the subspecies division of *Litoria verreauxii*.

Methods

Study species

The brown tree-frog group encompasses the following nine species: *Litoria ewingii*, *Litoria verreauxii*, *Litoria paraewingii*, *Litoria littlejohni*, *Litoria jervisiensis*, *Litoria watsoni*, *Litoria revelata* and the newly described *Litoria sibilus* and *Litoria*

calliscelis (reviewed in Mahony *et al.* 2020; Sanders 2021; Parkin *et al.* 2024). Three species within the group represent a particular challenge for identification, namely *L. ewingii*, *L. paraewingii* and *L. verreauxii*. These species are highly similar in appearance and male advertisement call, and have overlapping ranges (Fig. 1; Littlejohn 1965; Watson *et al.* 1971; Gerhardt and Davis 1988). In addition, existing male advertisement descriptions have been prepared using few recordings from geographically restricted areas, failing to capture the variation in the calls across the ranges of the species, therefore limiting their utility (Littlejohn 1976; Smith *et al.* 2003, 2013a). We also include the newly described *L. sibilus* and *L. calliscelis* (Parkin *et al.* 2024), formerly considered to be populations of *L. ewingii*, because their advertisement calls have not been compared with other members of the group outside of *L. ewingii*. *Litoria verreauxii* is currently considered to be two subspecies, *Litoria verreauxii alpina* and *Litoria verreauxii verreauxii*. The former is restricted to high-elevation sites in the Australian Alps bioregion, has undergone extensive population declines, is considered Vulnerable and is, therefore, the focus of conservation efforts (Brown *et al.* 2007; Department of Agriculture, Water and the Environment 2014). The ability to correctly identify and map taxon distribution is essential, given it is critical for any successful conservation strategies. However, the validity of the subspecies has been questioned. The subspecies status was assigned largely on the basis of the difference in skin

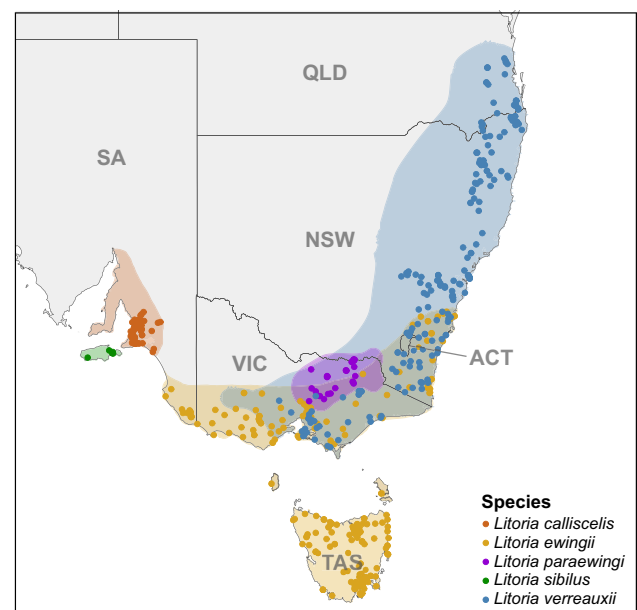


Fig. 1. A map of south-eastern Australia, showing the distribution of frogs in the brown tree-frog complex. Shaded areas represent species distributions created from the locality data from all *Litoria ewingii*, *Litoria verreauxii*, *Litoria paraewingii*, *Litoria sibilus* and *Litoria calliscelis* recordings in FrogID. Dots represent locality of each advertisement call analysed within this study.

colour, but more recent research has indicated a lack of genetic support (Banks *et al.* 2020) and little bioacoustic support (Smith *et al.* 2003) for the subspecies status. However, these studies had smaller sample sizes and were geographically limited, and therefore unable to consider the breath of variation within the range of *L. verreauxii*.

FrogID data

FrogID recordings were uploaded as an MPEG AAC audio file with a sampling rate of 44.1 kHz. The time, date and location data (latitude, longitude and an estimate of location accuracy) were automatically uploaded with each recording. Any calling frogs in a submission were identified to a species by an expert (Rowley *et al.* 2019). FrogID submission quality varied substantially depending on the model of phone, the proximity from phone to frog and background noise (Rowley *et al.* 2019; Rowley and Callaghan 2020).

Audio selection

We obtained 33,137 audio recordings of *L. ewingii* (17,309), *L. paraewingii* (1303), *L. verreauxii* (13,471), *L. sibilus* (50) and *L. calliscelis* (1001) from the FrogID database (exported 11 May 2022). To avoid resampling the same individual, we removed duplicate recordings from single latitude and longitude locations. We then ranked submissions according to the number of frog species detected, selecting recordings with a single species calling and those marked in the FrogID database as 'quality'. To ensure submissions from across the range of each species, we then mapped locations of all submissions and filled spatial gaps by selecting submissions with more than one species calling (i.e. including species other than the target species). This resulted in 1758 recordings (606 *L. ewingii*, 159 *L. paraewingii*, 767 *L. verreauxii*, 27 *L. verreauxii alpina*, 13 *L. sibilus* and 186 *L. calliscelis*). Some submissions were not of sufficient quality for accurate bioacoustic analyses, containing only incomplete calls, too much background noise or too many overlapping calls, bringing the number of analysed submissions down to 469 (28% of those originally selected). As FrogID recordings are

identified only to species level, we identified recordings of *L. verreauxii* above 1100 m elevation in the Australian Alps bioregion to *L. v. alpina* (Gartside 1982; Watson *et al.* 1985; Brown *et al.* 2007; Banks *et al.* 2020) and those in all other areas to *L. v. verreauxii*.

Call analysis

We converted FrogID recordings from MPEG AAC audio files into a WAV format (sampling rate of 48 kHz, 16 bits/sample) using the R vers. 4.1.0 (R Core Team 2021) package 'av' (Ooms 2023) prior to analysis in Raven Pro 1.6.3 (K Lisa Yang Center for Conservation Bioacoustics 2022) by using fast-Fourier transformation of 512 points and 50% overlap.

For one call per individual in each recording, we measured call duration (s), note duration (s), dominant frequency (Hz), notes per call, pulses per note, note rate (notes/s), pulse rate (pulses/s), note shape (Fig. 2), frequency modulation, pulse pattern, pulse-rate pattern, frequency pattern and the positions of the longest and shortest notes (for definitions, see Table 1). We selected these call parameters on the basis of their utility in distinguishing the male advertisement calls of different frog species (Lötters *et al.* 2009; Forti *et al.* 2016; Köhler *et al.* 2017), including the brown tree-frog group (Littlejohn 1976; Littlejohn and Watson 1983; Smith *et al.* 2013b). To our knowledge, several of these parameters (see Table 1) were not commonly used in previous assessments of bioacoustic variation in the study species. We calculated dominant frequency, pulse rate and pulses per note for each note in a call and then calculated an average (versus calculating from a single note in each call as per previous studies on the species Watson *et al.* 1971, 1985; Watson 1972; Watson and Littlejohn 1978), allowing us to examine changes over the course of a call.

Data analysis

We analysed recordings from allopatric populations first, before analysing from areas where the species exist in sympatry. After determining the range in parameters of each species, we identified calls in areas of sympatry that had call parameters of another species in the group, and after re-analyses of these

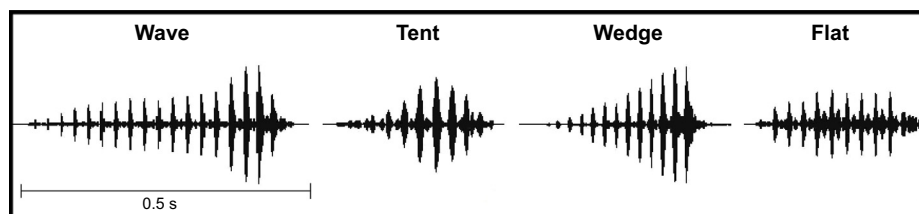


Fig. 2. Visual representations of four of the note shapes (wave, tent, wedge, flat) we identified during call analysis of male advertisement calls of five species of the *Litoria ewingii* complex. The note shape 'other' (not shown) refers to a shape other than those shown or a call without a dominant note shape. Note shape is based on the waveform view of an advertisement call, which shows the changing amplitude throughout a note.

Table 1. List of call parameters and their definitions measured in this study.

| Call parameter | Definition |
|--|--|
| Call duration | Length of one call, measured from the beginning of the first note to end of the last note (s). |
| Average note duration | The average length of each note, measured beginning to end (s). |
| Average dominant frequency | The average peak frequency of each note (Hz). |
| Notes per call | The average number of notes present in one call. |
| Average pulses per note | The average number of pulses present in one note. |
| Average pulse rate | The average number of pulses in 1 s (pulses/s). Calculated using: (number of pulses – 1)/note duration. |
| Note rate | The number of notes in 1 s (notes/s). Calculated using: (number of notes – 1)/call duration. |
| Frequency modulation | The difference in the dominant frequency between the last and the first notes of the call (Hz). |
| Frequency pattern ^A | The pattern of dominant frequency within a call. Six categories: increasing, decreasing, stable, up and down, up then down, stable with dips. |
| Note shape ^A | The most prevalent perceived shape of notes within a call based on waveform view. Five categories: wedge, wave, flat, tent, other (Fig. 2). |
| Position of longest note ^A | The position of the longest note (s) in the call, (beginning, middle, end, other). |
| Position of shortest note ^A | The position of the shortest note (s) in the call, (beginning, middle, end, other). |
| Pulse pattern ^A | The pattern in the number of pulses per note over the length of the call. (increasing, decreasing, stable, up and down, up then down). |
| Pulse-rate pattern ^A | The pattern of the pulse rate calculated for each note over the length of the call. (increasing, decreasing, stable, up and down, up then down). |

^AParameter has not previously been used in call analysis for this species group. See Methods - Call analysis for further details.

recordings, they were re-identified. These were corrected in our analyses and in the FrogID database before further analyses.

There was a large amount of variation in the spatial and temporal parameters of advertisement calls. To characterise a typical advertisement call and create a more useful key for species delineation, we removed outliers (above the 3rd quartile + 1.5× interquartile range or below the 1st quartile – 1.5× interquartile range) from Table 2 and the advertisement call key.

Temperature data were not submitted with FrogID recordings; therefore, to examine the effect of temperature on call parameters, we followed the methods outlined in Mitchell *et al.* (2020), except that temperature data were retrieved from the Australian Bureau of Meteorology directly rather than by using Bomrang (Sparks *et al.* 2019). We then used the R package ‘chillR’ vers. 0.7.5 (Luedeling 2019) to

estimate the ambient temperature for each FrogID recording at the time of recording. The temperature data extracted included the daily minimum and maximum and an estimation of the temperature at the time of calling (the nearest hour of the FrogID-call submission timestamp) collected from the nearest weather station. Temperature data were not able to be retrieved for 23 recordings. To assess the effect of temperature on call parameters, we created scatterplots with regression lines (Supplementary Fig. S1) and completed a Pearson's correlation coefficient to determine the strength and direction of the relationship (Supplementary Table S1). Performing multiple analyses raises the chance of encountering Type 1 errors. To mitigate this risk, we applied the Bonferroni correction to adjust *P*-values and minimise the likelihood of Type 1 errors (Table S1). We created variation maps using R to visually document the geographic spread of call variability, and these, along with the data, were used to assess the validity of the taxonomic status of *L. v. alpina*. Packages ‘readr’ (vers. 2.1.4, Wickham *et al.* 2022b, <https://CRAN.R-project.org/package=readr>), ‘dplyr’ (vers. 1.1.3, Wickham *et al.* 2022a, <https://CRAN.R-project.org/package=dplyr>), ‘tidyr’ (vers. 1.3.0, Wickham and Girlich 2022, <https://CRAN.R-project.org/package=tidyr>), ‘ggplot2’ (vers. 3.5.0, Wickham 2016, <https://CRAN.R-project.org/package=ggplot2>), ‘scales’ (vers. 1.3.0, Wickham and Seidel 2020, <https://CRAN.R-project.org/package=scales>), ‘ggpubr’ (vers. 0.6.0, Kassambara 2020, <https://CRAN.R-project.org/package=ggpubr>), ‘sf’ (vers. 1.0-14, Pebesma 2018, <https://CRAN.R-project.org/package=sf>), ‘rnatuarearthdata’ (vers. 1.0.0, South 2017b, <https://CRAN.R-project.org/package=rnatuarearthdata>), ‘rnatuarearth’ (vers.1.0.1, South 2017a, <https://CRAN.R-project.org/package=rnatuarearth>), ‘ozmaps’ (vers. 0.4.5, Sumner 2021, <https://CRAN.R-project.org/package=ozmaps>), ‘raster’ (vers. 3.5-29, Hijmans 2022, <https://CRAN.R-project.org/package=raster>), ‘rasterVis’ (vers. 0.51.5, Perpignan Lamigueiro and Hijmans 2022, <https://CRAN.R-project.org/package=rasterVis>), ‘rgdal’ (vers. 1.6-7, Bivand *et al.* 2022, <https://CRAN.R-project.org/package=rgdal>) were used in data analysis.

Results

Acoustic variation among and within species

Of the 469 recordings used, 61 had multiple individuals of the target species calling, resulting in a total of 542 calls (=individuals) (236 *L. ewingii*, 31 *L. paraewingii*, 197 *L. v. verreauxii*, 13 *L. v. alpina*, 6 *L. sibilus* and 59 *L. calliscelis*) being analysed (Fig 1.). Each species had a high degree of variation in the acoustic parameters of their advertisement calls, particularly the more widely distributed *L. ewingii* and *L. verreauxii* (Fig. 3).

Most measured call parameters overlapped among species (Fig. 3, Tables 2, S2), but the calls of each species could most reliably be distinguished via a combination of note shape,

Table 2. Results table showing the maximum, minimum and mean (exclusive of outliers) of each measured call parameter for *Litoria ewingii*, *Litoria verreauxii*, *Litoria paraewingi*, *Litoria sibilus* and *Litoria calliscelis*.

| Species | Number of calls (n) | Call duration (s) | Note duration (s) | Dominant frequency (Hz) | Notes/call | Pulses/note | Note rate (notes/s) | Pulse rate (pulses/s) | Note shape ^A | Longest note ^A | Shortest note ^A |
|-----------------------|---------------------|-------------------------|-------------------------|-------------------------|------------------|-------------------|-------------------------|-----------------------------|-------------------------|---------------------------|----------------------------|
| <i>L. ewingii</i> | 223 | 1.05–4.31 (2.33) | 0.10–0.43 (0.21) | 1875–3167 (2475) | 4–18 (9) | 10–46 (23) | 1.6–5.79 (3.37) | 33.59–205.69 (108.55) | Wedge | Beginning | Other |
| <i>L. verreauxii</i> | 197 | 1.2–6.08 (3.02) | 0.1–0.43 (0.23) | 1781–3153 (2499) | 5–19 (11) | 13–61 (30) | 1.38–6.56 (3.46) | 60.55–221.22 (135.07) | Wave | End | Beginning |
| <i>L. paraewingi</i> | 26 | 1.4–4.11 (2.64) | 0.23–0.62 (0.36) | 2156–3375 (2741) | 4–9 (6) | 18–43 (28) | 1.02–2.90 (1.88) | 42.52–166.46 (78.12) | Wave | Beginning | Middle |
| <i>L. sibilus</i> | 5 | 1.48–1.87 (1.67) | 0.11–0.18 (0.14) | 2438–2541 (2510) | 9–11 (10) | 15–31 (22) | 4.29–6.45 (5.19) | 98.91–175.17 (144.39) | Other/Tent | End | Beginning |
| <i>L. calliscelis</i> | 53 | 1.57–5.24 (3.23) | 0.09–0.23 (0.16) | 2063–2809 (2438) | 7–25 (15) | 10–33 (19) | 2.72–6.43 (4.45) | 43.02–192.68 (111.43) | Other | End | Beginning |

^AResult is shown as a mode (as opposed to a range with the mean) Bolded cells indicate parameters that can be used to distinguish one species from another, e.g. *Litoria ewingii* having a wedge not shape is different from *Litoria paraewingi* and *Litoria verreauxii* and, therefore, a distinguishing parameter. See Table 1 in Methods for parameter definitions.

notes per call and positions of the longest and shortest notes (Table 2, Figs 4, 5). These structural parameters were largely unaffected by temperature (Fig. S1, Table S1), whereas the temporal call parameters (call duration and note duration) were affected by temperature (Fig. S1, Table S1). Call duration, note duration, dominant frequency and pulses per note were also useful in delineating some species pairs (Table 2). Pulse-rate pattern, frequency modulation and pulse pattern were not useful in species delineation (Tables 2, S2). Fifty outliers were identified across the five species and were excluded from the call key (*L. ewingii* – 23, *L. verreauxii* – 13, *L. paraewingi* – 5, *L. sibilus* – 1 and *L. calliscelis* – 6) (Table S3).

To the human ear, the advertisement call of *L. ewingii* sounds more short, sharp and harsh than those of the other study species. The advertisement call of *L. paraewingi* sounded higher pitched and drawn out owing to its higher dominant frequency and long note durations (Table 2, Fig. 4). Individual pulses in the call of *L. paraewingi* are also more audible than those of both *L. ewingii* and *L. verreauxii*. In contrast, the advertisement call of *L. verreauxii* call commonly starts with short, low-amplitude notes that increase in duration and amplitude over the course of the call. Although *L. verreauxii* typically has longer note durations and a higher number of pulses, similar to *L. paraewingi*, they do not sound as pulsed to the human ear. Instead, the amplitude modulation of the ‘wave’-shaped (Fig. 2) notes could be heard. The advertisement calls of the newly described *L. sibilus* and *L. calliscelis* are more difficult to discern by ear, but both species are geographically isolated from all other members of the group. The advertisement call of *L. sibilus* sounds short and fast compared with those of the other study species, because of its typically short note and call durations (Table 2, Fig. 4), whereas the advertisement call of *L. calliscelis* is similarly fast but has a longer call duration owing to the higher number of notes.

The call key created from analysis of call data to assist with identification of male advertisement calls of *L. ewingii*, *L. paraewingi* and *L. verreauxii* is shown below. Because *L. sibilus* and *L. calliscelis* are geographically isolated from the other study species and thus can be identified via their location, we have excluded them from the call key.

1. Long introductory note? 2
Short, low-amplitude (quiet) introductory note? 3
2. Introductory note followed by a series of shorter wedge-shaped notes? *Litoria ewingii* (Fig. 4a)
Introductory note followed by a small number (fewer than five) of long wave-shaped notes? *Litoria paraewingi* (Fig. 4c)
3. Wave-shaped notes that increase in amplitude (gets louder)? ...
..... *Litoria verreauxii* (Fig. 4b)

Geographic variation in call parameters

We found evidence of geographic patterns within the advertisement calls of *L. ewingii* and *L. verreauxii*. In particular, the advertisement calls of *L. ewingii* in the New South Wales coast region and Tasmania (Fig. 6) appeared to diverge somewhat from those of *L. ewingii* from other areas in terms of the positions of the shortest and longest notes, pulse rate, note rate and notes per call.

Although there was variation in advertisement calls within the relatively small ranges of *L. paraewingi*, *L. sibilus* and *L. calliscelis*, we found no distinct geographic patterns (Figs S2, S3, S4). *Litoria verreauxii* showed geographic patterns in several call parameters, most notably between Queensland and northern New South Wales and the rest of the *L. verreauxii* range (Fig. 7). Call parameters that showed differentiation between these two geographic groups included note duration, notes per call, note rate, pulses per note and dominant frequency. Divergence in *L. verreauxii* call parameters in the range of *L. v. alpina* was not evident (Figs 7, S5). The range of all parameters of *L. v. alpina* (except for dominant frequency) fell within the range of

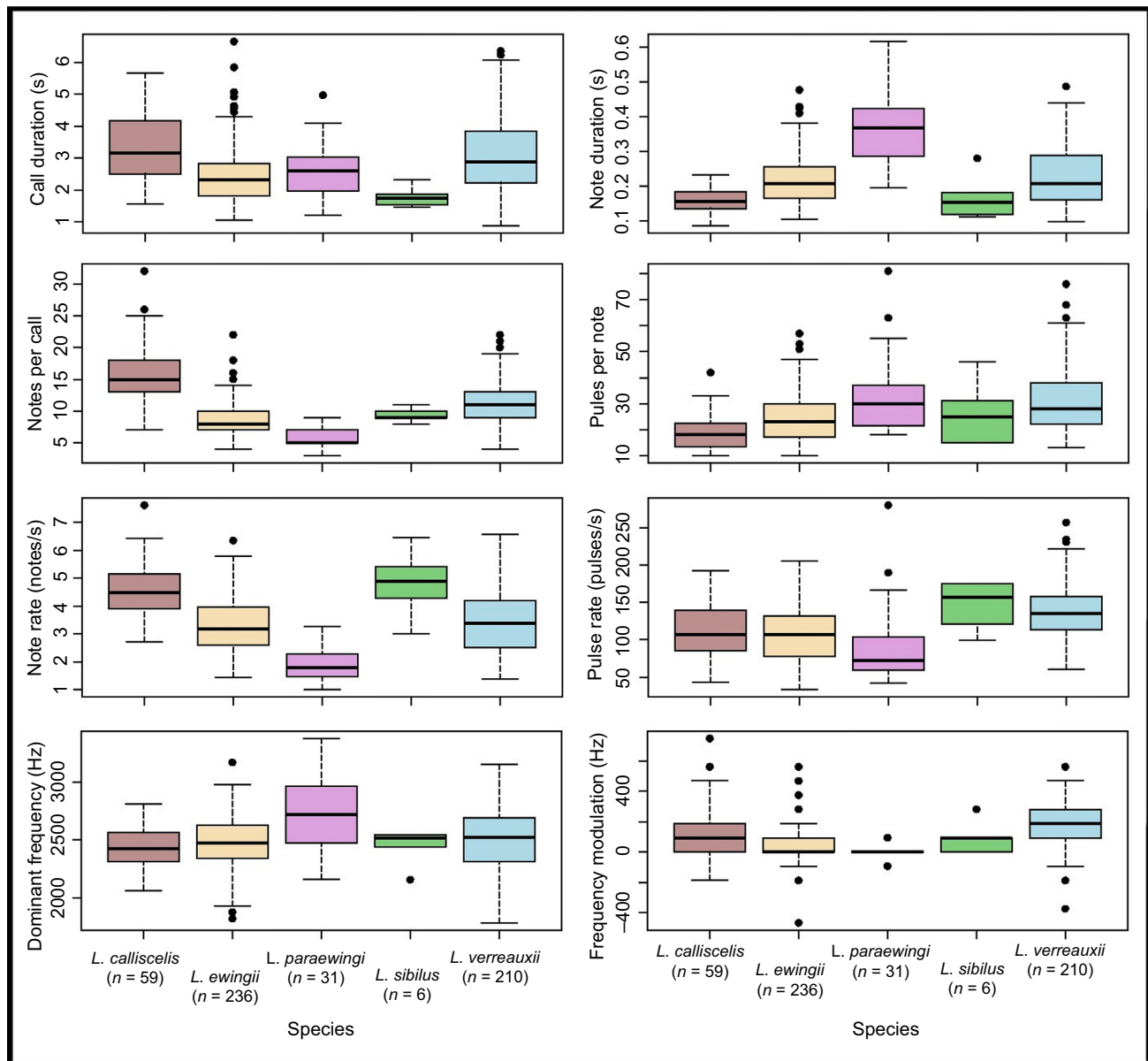


Fig. 3. Variation in measured temporal advertisement-call parameters for *Litoria ewingii*, *Litoria verreauxii*, *Litoria paraewingii*, *Litoria sibilus* and *Litoria calliscelis*. Medians are represented by the bold line and outliers indicated by solid circles. The box represents the interquartile range (~50%) of the data set. *n* indicates the sample size for each species.

L. v. verreauxii. This pattern held true with (Table S4) and without (Table 3) outliers.

Discussion

We characterised the male advertisement call of five widely distributed Australian frog species, by using >500 advertisement calls across the range of each species. Our study showed a high degree of variation in call parameters within each frog species, reinforcing the findings of several recent studies using

large acoustic datasets via the FrogID project (Mitchell *et al.* 2020; Weaver *et al.* 2020). Within the currently accepted taxonomy, there is a higher degree of intra-specific variations within a species' advertisement call than was previously thought within a single species (Lötters *et al.* 2009).

In part because of the high degree of variation in acoustic parameters, there was a substantial amount of overlap in individual call parameters among the species. We found that a combination of call parameters, in particular the structural parameters, was needed to successfully delineate the species. The most effective call parameters for delineation were a

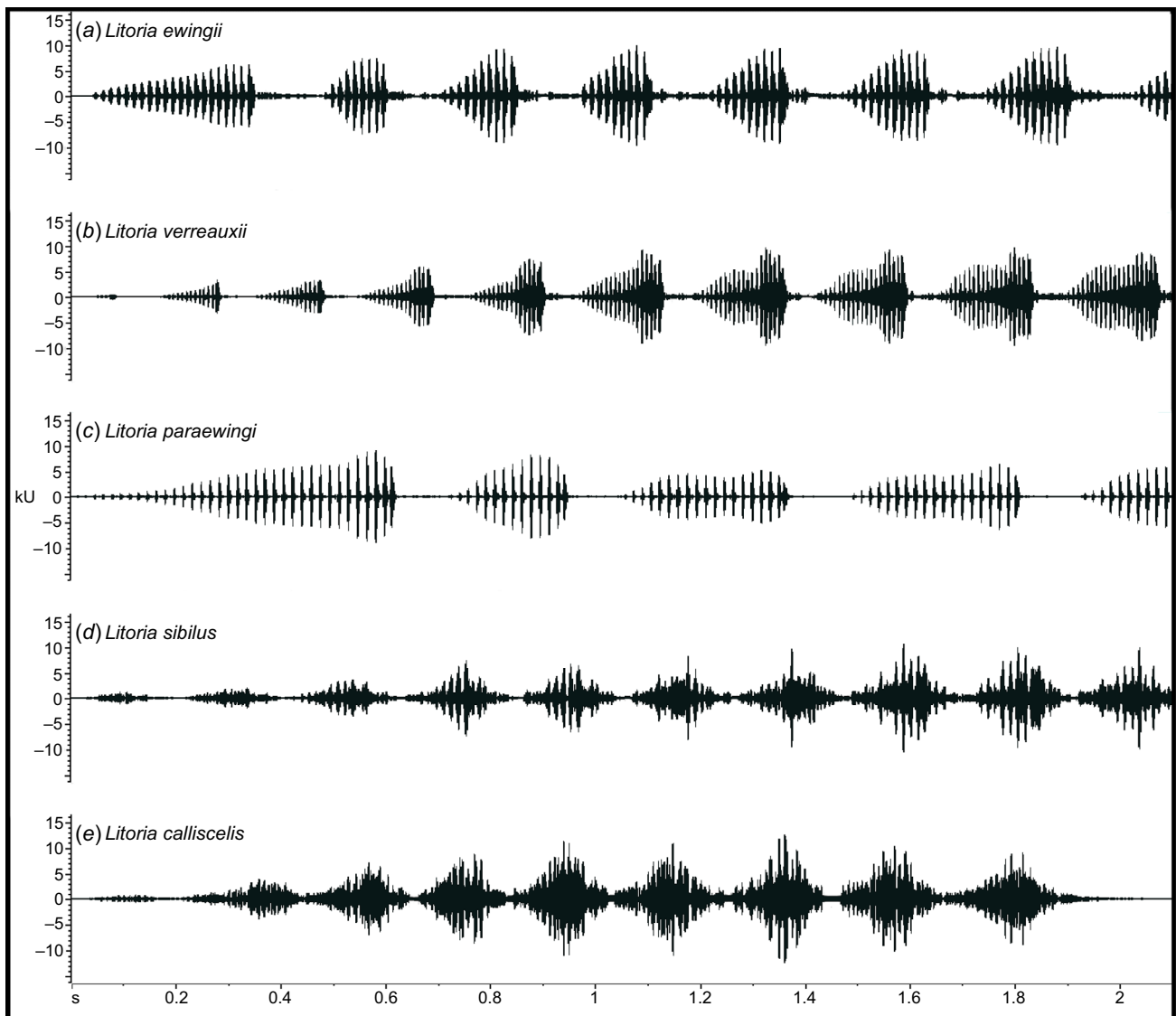


Fig. 4. Representative male advertisement calls of (a) *Litoria ewingii*, (b) *Litoria verreauxii*, (c) *Litoria paraewingii*, (d) *Litoria sibilus* and (e) *Litoria calliscelis*. Shown in waveform view (amplitude over time) to visually examine the differences among the species.

combination of notes per call, note shape and the positions of the longest and shortest notes. This is not only because there was less overlap in these call parameters, but because each parameter can rule out a species when used for identification. Importantly, the most useful call parameters were largely unaffected by temperature (Fig. S1, Table S1).

Previous research specified pulse rate and notes per call as being the main delineating factors among species in the brown tree-frog group (Littlejohn 1965; Watson *et al.* 1971; Littlejohn 1976; Littlejohn and Watson 1983). However, as most previous research on these species was conducted in northern Victoria (Gartside 1972; Watson and Littlejohn 1978; Gartside 1982) with limited sample sizes, the true extent of acoustic variation in the species was not previously captured. Indeed, sample sizes of fewer than 20 individuals and those

that do not cover the species range are unlikely to capture the true extent of variation present within a species advertisement call (Weaver *et al.* 2020). In addition, note shape and note positions proved to be more useful than were other call parameters in delineating species.

Variation in advertisement calls both within and among species can be due to a variety of reasons. Body size is known to influence dominant frequency (Sullivan and Hinshaw 1990) and temperature is known to affect the temporal parameters such as call duration and pulse rate (Narins and Meenderink 2014; Köhler *et al.* 2017). In addition, habitat structure also affects call parameters (Ryan *et al.* 1990; Forti *et al.* 2016). Advertisement call variation within a species can also depend on social contexts, such as female-led preferences, for example, increasing call rate while decreasing

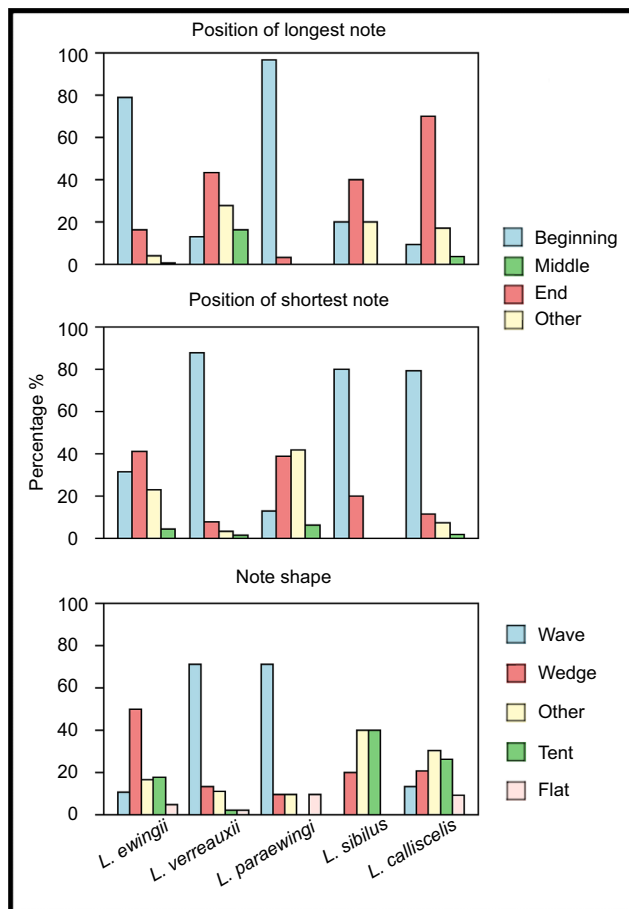


Fig. 5. Bar graphs of the percentage for each note shape (wedge, wave, flat, other) and the positions of the longest and shortest notes (beginning, middle, end, other) present in the male advertisement calls of *Litoria ewingii*, *Litoria verreauxii*, *Litoria paraewingii*, *Litoria sibilus* and *Litoria calliscelis*.

call duration and *vice versa* in the presence of females and male competitors (Wells and Schwartz 1984; Lesbarreres and Lodé 2002; Morais *et al.* 2012) and male conspecific competition, such as, for example, shifting dominant frequency to avoid overlap and increases in call duration when males are chorusing versus isolated calling (Wells and Taigen 1986; Gerhardt 1991; Bee and Perrill 1996; Howard and Young 1998; Bee *et al.* 2000; Kime *et al.* 2004; Martínez-Rivera and Gerhardt 2008; Fang *et al.* 2014; Toledo *et al.* 2014). Weaver *et al.* (2020) found that as the geographic area increased, so too did the level of variability; consequently, a large degree of variation was expected. The geographical patterns of advertisement call variation in *L. ewingii* and *L. verreauxii* may be related to various factors, including differences in temperature and habitat, isolation by distance, reproductive character displacement (RCD) (Littlejohn 1965; Littlejohn 1976; Gerhardt and Davis 1988) or may be indicative of undiagnosed species diversity within the group. Additional fieldwork, morphological, acoustic and

genetic analysis will be required to elucidate the drivers of this variation. Our findings confirmed the persistence of a stable relationship described almost six decades ago by Littlejohn (1965), a pattern of call divergence in regions of sympatry between *L. ewingii* and *L. verreauxii*. It is not surprising that no patterns of geographic variation were found for *L. paraewingii*, *L. sibilus* and *L. calliscelis*, given their small distributions and few advertisement calls collected (*L. paraewingii* – 26, *L. sibilus* – 5, *L. calliscelis* – 57).

Although we found slight divergence in three call parameters (Table 3), we found no distinct difference between the advertisement calls of *L. v. alpina* and *L. v. verreauxii*. These results support those of previous acoustic research (Smith *et al.* 2003). However, we analysed only a small number ($n = 14$) of calls within the most likely range of *L. v. alpina* at above 1100 m. This may not reflect the true geographic distribution of the species, because additional research is required to understand the current range of the species. In addition, there were no FrogID recordings of the species available within 23.5 km of the type locality of the subspecies, Mount Kosciuszko. The FrogID submissions we used were 23.5–171.9 km from the type locality (mean 103.4 km). The relatively low numbers of recordings in the FrogID database attributed to this species are likely to reflect the true abundance of the subspecies, with the species having disappeared from much of its former range (Osborne *et al.* 1999; Brown *et al.* 2007), but also its remote nature, rendering it less likely to be sampled via citizen science. A detailed investigation incorporating morphological, genetic and bioacoustic data is therefore required to resolve the taxonomic status of *L. v. alpina*.

While citizen-science data provided an unprecedented volume of data across a wide spatial scale to address our study aims, there were important considerations. As with most citizen-science data, there was a geographic bias in sampling (Dickinson *et al.* 2010; Callaghan *et al.* 2020), with more remote or sparsely populated areas less well-sampled. Furthermore, many recordings (72%) were of sufficient quality to identify species, and thus provided occurrence records for the species, but were of insufficient audio quality to allow bioacoustic analysis. Previous bioacoustic studies using FrogID data have found a relatively high proportion of submissions of insufficient acoustic quality (Weaver *et al.* 2020; Mitchell *et al.* 2020). Because FrogID data do not include temperature, we extrapolated ambient temperature (Mitchell *et al.* 2020; Gillard and Rowley 2023). Although frog body temperature may differ from ambient temperature as a result of microhabitat use (Brattstrom 1963; Lillywhite 1970; Rowley and Alford 2013), all five study species are known to call from water or adjacent overhanging vegetation or pond or stream banks (Littlejohn 1965; Watson *et al.* 1971, 1985; reviewed by Hunter and Waudby 2017). As such, frog body temperatures are likely to differ from ambient temperatures in a similar manner across taxa and geographic space.

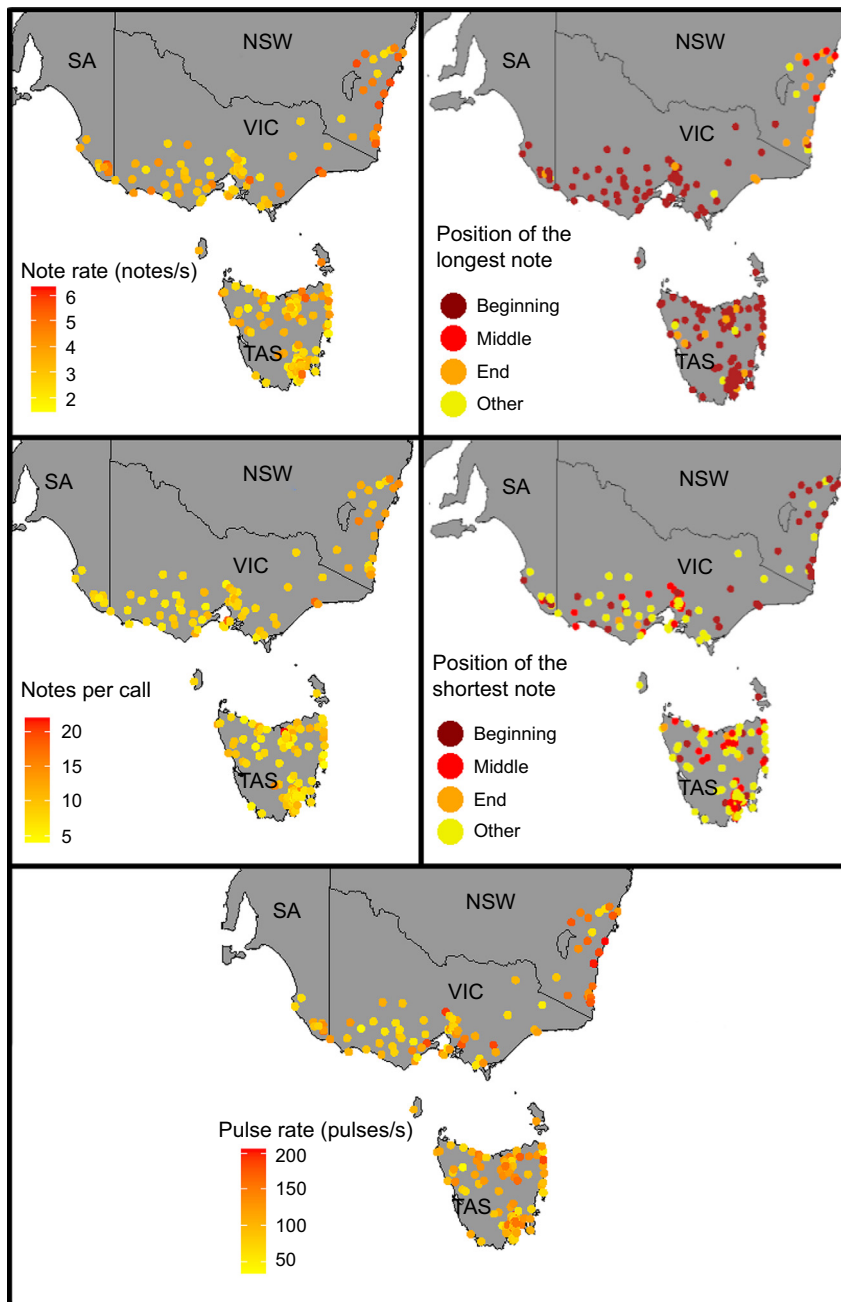


Fig. 6. Geographic variation in note duration, notes per call, note rate, note shape and positions of the longest and shortest notes present within the male advertisement call of *Litoria ewingii*.

Our analyses of a large volume of advertisement calls across the entire ranges of three tree frog species demonstrated the usefulness of citizen-science data in documenting within- and among-species variation in advertisement calls. Understanding the geographic spread of variation is crucial for several reasons. First, it aids in more accurate, location-specific species identification, providing insights into species diversity, which is pivotal for conservation prioritisation (Caughley and Gunn 1996; Scheele *et al.* 2018). Additionally, as frogs are indicator species (Blaustein and Wake 1995; Wake and Vredenburg 2008), monitoring frog populations over time can allow a greater understanding of broader environmental

health (Blaustein and Wake 1995; Woodford and Meyer 2003; Hopkins 2007). Understanding variation in frog calls also contributes to our understanding of the evolutionary history of frog species, speciation and adaptation (Cocroft and Ryan 1995; Gerhardt and Huber 2002; Wells 2007; Tessarolo *et al.* 2016). The large amount of variation within a single species, something that has been shown only recently (Lötters *et al.* 2009; Mitchell *et al.* 2020; Weaver *et al.* 2020), is also an important consideration when using frog advertisement calls in delineating species.

Using these data, we produced the only description of the male advertisement call across the entire range of any species

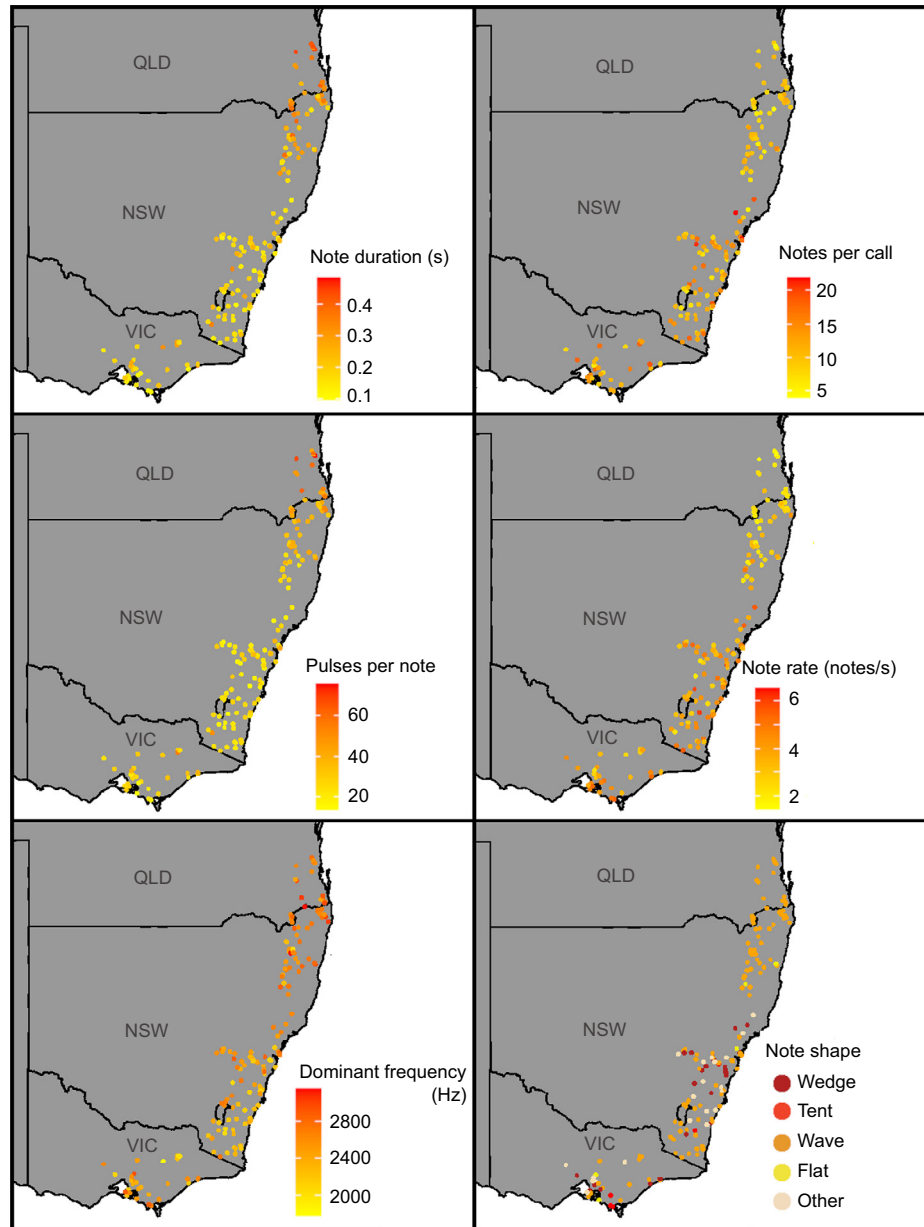


Fig. 7. Geographic variation in note duration, note rate, note shape, notes per call, pulses per note and dominant frequency present within the male advertisement call of *Litoria verreauxii*.

Table 3. Male advertisement-call parameters for subspecies of *Litoria verreauxii*. The data table shows the maximum, minimum and mean of each temporal call parameter, exclusive of outliers.

| Species | Number of calls (n) | Call duration (s) | Note duration (s) | Dominant frequency (Hz) | Notes/call | Pulses/note | Note rate (notes/s) | Pulse rate (pulses/s) | Frequency pattern ^A | Note shape ^A | Longest note ^A | Shortest note ^A |
|-------------------------|---------------------|-------------------|-------------------|-------------------------|------------|-------------|---------------------|-----------------------|--------------------------------|-------------------------|---------------------------|----------------------------|
| <i>L. v. alpina</i> | 12 | 1.46–4.35 (2.98) | 0.15–0.32 (0.22) | 1781–2560 (2161) | 7–15 (11) | 20–37 (26) | 2.41–4.11 (3.32) | 90.07–161.89 (120.39) | Stable (33.33%) | Wave (83.33%) | End (41.67%) | Beginning (75%) |
| <i>L. v. verreauxii</i> | 185 | 1.20–6.08 (3.03) | 0.09–0.43 (0.23) | 2031–3153 (2521) | 5–19 (11) | 13–61 (31) | 1.38–6.56 (3.46) | 60.55–221.22 (136.03) | Increasing (42.93%) | Wave (70.11%) | End (43.48%) | Beginning (88.59%) |

^AResult is shown as a mode (as opposed to a range with the mean), with the percentage of how many individual calls of that species reported that result in parentheses. See Table 1 in Methods for parameter definitions.

of Australian frog (but see wide spatial-scale studies of Littlejohn and Roberts 1975; Roberts 1993) and provided a key to assist identification of the five species in the field, allowing the production of more precise maps, such as Australian Frog Atlas (Cutajar *et al.* 2022). We also identified geographic patterns in call variability for each species, and drivers of this variation should be the focus of future research.

Our research has demonstrated the utility of citizen-science data in overcoming challenges of traditional surveying, obtaining large volumes of data across a wide spatial scale.

Supplementary material

Supplementary material is available [online](#).

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Data availability. The complete raw dataset is not fully available due to sensitivities in relation to locations of rare or threatened species and citizen-scientist information (Rowley and Callaghan 2020). However, the data, with sensitive species' localities removed or buffered, are made available annually (Rowley and Callaghan 2020; data available through GBIF: <https://doi.org/10.15468/wazqft> and FrogID: <https://www.frogid.net.au/explore>) and audio can be requested from the FrogID project. Maps of the current ranges of *Litoria ewingii*, *Litoria paraewingi* and *Litoria verreauxii* in Australia are available as part of the Australian Frog Atlas (<https://zenodo.org/record/6544829>).

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