Quantitative description of the vocal repertoire of the territorial olive frog *Babina adenopleura* from Taiwan

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(Received 22 May 2015; accepted 20 July 2015)

Anurans communicate using a repertoire of acoustic signals that can be classified as different call types based on differences in both their acoustic properties and the contexts in which they are produced. Descriptions of these repertoires represent a key first step towards understanding the vocal behaviour of any species and provide a critical foundation for comparative bioacoustic studies. In this study of the olive frog, *Babina* (formerly *Rana*) *adenopleura*, a territorial species from subtropical Taiwan, we used focal observations, acoustic recordings and statistical analyses of temporal, spectral and amplitude-related call properties to describe six different call types in the species’ vocal repertoire. These call types included a putative advertisement call, territorial call, encounter call, amplexant call, distress call and alarm call. Advertisement calls consisted of four to five discrete notes, and they were the most commonly produced call. Territorial and encounter calls were delivered during interactions with other males; the former is a two-note call, whereas the latter is a distinctly pulsatile signal. Amplexant calls were acoustically similar to territorial calls but were delivered while males were in amplexus. Distress calls were given while the animal was being consumed by a snake, and alarm calls were recorded in response to startling stimuli. With basic descriptions of these call types in hand, we discuss several testable hypotheses for their putative adaptive functions.

**Keywords:** amphibian; behaviour; communication; Lien-Hua-Chih research center

Introduction

Most anuran amphibians use vocal signals to convey information during social and reproductive interactions (Gerhardt and Huber 2002; Wells and Schwartz 2006; Wells 2007). Although male frogs are more often the signalers, both male and female frogs benefit from receiving and responding to signals. Frog calls can inform potential receivers about a signaler’s species identity (Blair 1955; Littlejohn 1965), body size and fighting ability (Davies and Halliday 1978; Wagner 1992; Bee et al. 1999), individual identity (Bee and Gerhardt 2001a, 2001b; Bee et al. 2001; Gasser et al. 2009), genetic quality (Welch et al. 1998), distance (Wilczynski and Brenowitz 1988; Marshall et al. 2003), and its position in azimuth (Klump and Gerhardt 1989, Caldwell and Bee 2014) and elevation (Gerhardt and Rheinlaender 1982).

Quite commonly, male frogs produce a repertoire of vocalizations consisting of two or more acoustically distinct call types used in different contexts (Bogert 1960; Duellman and Trueb 1986; Wells 2007; Toledo et al. 2014). ‘Advertisement calls’ are the vocalization most often produced by male frogs (and most often studied by biologists).
They are used both to announce possession of a territory or calling site to rival males and to attract sexually responsive females (Wells 1977b). Males, and sometimes females, also use ‘courtship calls’ during close-range reproductive interactions (Wells 1980, 2007). Calls that males give while in amplexus have been termed ‘amplectant calls’ and may function to stimulate oviposition (Odendaal et al. 1983). Males may also produce one or more distinct call types used primarily or exclusively in aggressive contexts (Hutter et al. 2013). For example, ‘territorial calls’ may be relatively long-range signals to other males that function in maintaining minimum inter-male spacing. ‘Encounter calls’ are also directed towards rival males, usually in more close-range interactions that may also involve physical aggression. Both sexes of frogs also commonly produce vocalizations during encounters with predators or in response to startling stimuli that have variously been termed ‘distress’, ‘alarm’ or ‘warning’ calls (Toledo et al. 2014).

Although acoustic communication in frogs has been a topic of intensive research for several decades, we still know relatively little about the vocal repertoires used by most of the more than 6500 described species of frogs (Frost 2015). A better understanding of vocal repertoires across a greater range of species would facilitate further comparative studies of both the mechanisms and evolution of vocal behaviour in this group. Our objective in this study was to describe the vocal repertoire of males of the olive frog, Babina adenopleura (Ranidae). Common throughout China and Taiwan (Chuaynkern et al. 2010), the olive frog is a territorial species with a mating system best characterized as resource-defence polygyny. Territories function as oviposition sites, and individual males have been observed to defend the same territory for up to 43 consecutive nights (Chuang et al. 2013). Here, we report results from in-depth, within-individual quantitative analyses of putative advertisement calls, territorial calls and encounter calls. We also describe three additional call types – a putative amplectant call, distress call and alarm call – recorded opportunistically during the course of the study.

Materials and methods

Study site and subjects

We recorded males of B. adenopleura between June and September of 2008 and 2009 at the Lien-Hua-Chih Research Center in central Taiwan. Our recordings were made in one of two locations approximately 600 m apart. One was a permanent pond (5 m × 10 m, maximum depth of approximately 1 m) located in a natural forest (120°52'59.5"E, 23°55'8.9"N), and the second was in a flooded (<30 cm deep) palm farm where areca nuts (Areca catechu; also known as betel nuts) are grown and harvested (120°53'12"E, 23°55'01"N). Territorial males were abundant at both sites. After each recording, we captured unmarked males, measured their body size (SVL, snout-vent length, to the nearest 0.01 mm and mass to the nearest 0.05 g) and individually marked them using a numbered waistband. We released all animals at their site of capture within 10 to 30 min of capture. In total, we recorded 17 males having a mean (±SD here and throughout) SVL of 51.3 ± 2.5 mm and mass of 12.8 ± 1.9 g.

Acoustic recordings and analyses

All of our acoustic recordings were obtained using a Marantz PMD670 digital recorder (D&M Holdings Inc, Kanagawa, Japan) and a Sennheiser ME67/K6 directional microphone (Sennheiser Electronic GmbH & Co. KG, Hanover, Germany), which was mounted on a tripod and placed with its recording tip approximately 0.5 m in front of a focal male. Sounds
were recorded at a sampling rate of 44.1 kHz with 16-bit resolution. At the completion of recordings, we measured the water temperature at the focal male’s calling site using a Tecpel DTM-3108 digital thermometer (0.1°C accuracy). We measured water temperature because males called from the surface of the water. Across our recordings, water temperature varied over a narrow range between 22.4°C and 24.8°C (\(X = 23.8°C; N = 24\)). We used Raven Pro v1.5 (Bioacoustics Research Program 2014) to analyse our recordings. In total, we analysed 420 vocalizations, including 280 advertisement calls (20 from each of 14 males), 50 territorial calls (1 to 8 calls from each of 14 males), 32 encounter calls (1 to 6 calls from each of 14 males), 45 amplexant calls (22 and 23 calls, respectively, from 2 males), 9 distress calls (from 1 male) and 4 alarm calls (1 call from each of 4 males). Waveforms and spectrogram of these different call types are presented in Figure 1.

We focus most of our analyses on advertisement calls, territorial calls and encounter calls, because all three call types were recorded from the same sample of 14 individuals. Table 1 describes the 15 acoustic properties we measured for these 3 call types. In total, nine properties were measured using the entire call as the unit of measurement (Figure 2(A)). Three temporal properties were measured for each call, including call duration, the number of sound elements (i.e. notes or pulses) per call and the element rate within a call. Preliminary analyses indicated that calls have a bimodal frequency spectrum with a prominent low-frequency peak and high-frequency peak (Figure 2(B)). Five spectral properties were measured for each call to characterize this spectrum, including low peak frequency, low peak harmonic number, high peak frequency, high peak harmonic number and the relative amplitude of the low and high peaks (Table 1; Figure 2(B)). In all advertisement, territorial and encounter calls, the low peak frequency was the second harmonic of a fundamental frequency that was either missing or significantly attenuated relative to the low and high peaks. This was confirmed by determining the fundamental frequency for a subset of calls as the reciprocal of the average period of the quasi-periodic fine-temporal waveform. All frequency measurements were based on 1024-point fast Fourier transforms and Hann windows and were made from the average power spectrum computed over the duration of a call (unless indicated otherwise below). We also measured the peak power of each call; however, we note that these values are only used for comparisons across call types from the same recording of the same individual because they do not represent independently calibrated measures of sound pressure level. As illustrated in Figure 2(A), three additional temporal properties were measured for each of two consecutive sound elements in each call (duration, rise time and fall time). For convenience, we designate these two elements as E1 and E2, noting that they could correspond to what are commonly referred to as ‘notes’ or ‘pulses’ in the literature, depending on the acoustic structure of the call itself. For calls with more than two elements, we selected the two middle elements as E1 and E2. If there was an odd of number of elements, we always selected the earlier two (e.g. elements two and three in a five-element call). Further details of these 15 acoustic properties are provided in Table 1, and their measurement is illustrated in Figure 2. We also measured a small number of additional acoustic properties for a subset of call types (e.g. range of frequency modulation), which we describe in more detail in the Results.

**Statistical analyses**

All statistical analyses were performed using Statistica, version 10 (StatSoft 2011). Descriptive statistics, including mean values and standard deviations (or the median and interquartile range) and minimum and maximum values, are reported based on first
determining the median value of each acoustic property for each individual. Our sample of individual males \((N = 14)\) that produced advertisement, territorial and encounter calls during a recording session was large enough for further statistical analyses. This was not the case for opportunistically recorded amplectant, distress and alarm calls \((N = 2, 1\) and 4 males for each call type, respectively). Pearson product-moment correlations between 13 of 15 call properties (excluding low peak harmonic and peak power) for advertisement, territorial and encounter calls are reported in the supplemental online material (Tables S1–S3, respectively). We used linear regression to investigate the well-known effects of temperature on the properties of anuran vocalizations (Gerhardt and Huber 2002). However, none of the
properties we analysed were significantly related to water temperature (Table S4). This is perhaps not too surprising given that water temperature varied over a range of just 2.4°C across our recordings. We also used linear regression to explore the relationships between the properties of each call type and SVL, mass and an index of body condition (after Baker 1992). We did not correct for multiple comparisons because these analyses were exploratory in nature and were conducted simply to identify potential relationships of interest for future study.

We used repeated-measures analysis of variance (rmANOVA) to compare 13 of the 15 acoustic properties across advertisement, territorial and encounter calls within individuals. These analyses were based on using each individual’s median value computed separately over the calls of each call type. A separate analysis was performed for each property, although we emphasize that these cannot be considered independent tests considering the correlations between some call properties (Tables S1–S3). Partial $\eta^2$ is reported as a measure of effect size for the effect of call type, and the reported $P$-values are corrected for violations of the sphericity assumption using the Greenhouse and Geisser (1959) method. For significant main effects, we conducted pairwise Tukey HSD post hoc tests comparing

<table>
<thead>
<tr>
<th>Acoustic property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temporal call properties</strong></td>
<td></td>
</tr>
<tr>
<td>1. Call duration</td>
<td>Time between onset of the first element of a call (note or pulse) and the offset of last element.</td>
</tr>
<tr>
<td>2. Elements per call</td>
<td>Count of the number of elements (notes or pulses) in a call</td>
</tr>
<tr>
<td>3. Element rate (notes or pulses / s)</td>
<td>Number of elements (notes or pulses) divided by call duration</td>
</tr>
<tr>
<td><strong>Temporal element properties</strong></td>
<td></td>
</tr>
<tr>
<td>4. E1 duration</td>
<td>Time between the onset and offset of element 1</td>
</tr>
<tr>
<td>5. E1 rise time</td>
<td>Time between the onset of element 1 and the earliest local maximum in the element 1 waveform</td>
</tr>
<tr>
<td>6. E1 fall time</td>
<td>Time between the last local maximum in the element 1 waveform and the offset of element 1</td>
</tr>
<tr>
<td>7. E2 duration</td>
<td>Time between the onset and offset of element 2</td>
</tr>
<tr>
<td>8. E2 rise time</td>
<td>Time between the onset of element 2 and the earliest local maximum in the element 2 waveform</td>
</tr>
<tr>
<td>9. E2 fall time</td>
<td>Time between the last local maximum in the element 2 waveform and the offset of element 2</td>
</tr>
<tr>
<td><strong>Spectral call properties</strong></td>
<td></td>
</tr>
<tr>
<td>10. Low peak frequency</td>
<td>Maximum frequency in the range of 0.3–0.8 kHz (low peak) determined over the duration of a call</td>
</tr>
<tr>
<td>11. High peak frequency</td>
<td>Maximum frequency in the range of 0.8–3.0 kHz (high peak) determined over the duration of a call</td>
</tr>
<tr>
<td>12. Low peak harmonic number</td>
<td>The harmonic number of the low peak frequency (computed based on the fundamental frequency as the first harmonic)</td>
</tr>
<tr>
<td>13. High peak harmonic number</td>
<td>The harmonic number of the high peak frequency (computed based on the fundamental frequency as the first harmonic)</td>
</tr>
<tr>
<td><strong>Amplitude properties</strong></td>
<td></td>
</tr>
<tr>
<td>14. Relative amplitude</td>
<td>The difference in amplitude between the high and low peak frequencies (high – low)</td>
</tr>
<tr>
<td>15. Peak power</td>
<td>The maximum power in a call</td>
</tr>
</tbody>
</table>
A) Temporal properties

![Waveform Illustration]

- **Call duration**
- **Element duration**
- **Peak**
- **Rise time**
- **Fall time**

![Power Spectrum Illustration]

B) Spectral properties

- **Relative amplitude**
- **High peak frequency**
- **Low peak frequency**

Figure 2. Acoustic measurements. Depicted here are schematic illustrations of measurements of several (A) temporal call properties from Raven’s waveform display and (B) spectral properties computed from Raven’s power spectrum display (1024 pt. FFT). Temporal scales in (A) are indicated by the separate scale bars. Table 1 provides additional descriptions of the measured call properties illustrated here.
the three call types. The number of elements per call and the low peak harmonic number could not be included in these rmANOVAs because of limited variation within and between individuals in these two properties.

We used cluster analyses based on standardized values (i.e. Z-scores) (Milligan and Cooper 1988) to explore the tendency of call types to group together based on similarities and differences in their acoustic properties. We used a joining algorithm to create hierarchical trees computed using a complete linkage rule and Euclidean distance measure. Four separate cluster analyses focused on advertisement, territorial and encounter calls. The first was based on 13 acoustic properties (excluding peak power and low peak harmonic number). The second was based on 10 of the 11 properties (excluding peak power) that differed significantly in the rmANOVAs. The third was based only on the nine temporal properties analysed for calls and sound elements. The last was based only on four spectral properties of calls (excluding low peak harmonic number). We used results from these separate analyses to explore how different call properties contribute to the distinctiveness of separate call types. We performed one additional cluster analysis based on the nine temporal properties that included amplexant calls along with advertisement, encounter and territorial calls.

Results

Comparison of three common call types

There were distinct and statistically significant acoustic differences in the spectral and temporal properties of advertisement, territorial and encounter calls. Figure 1 illustrates spectrograms and waveforms for all three call types, and Table 2 reports descriptive statistics and the results from the rmANOVAs comparing separate call properties within subjects. Of the 13 properties included in these analyses, 11 differed significantly across call types (Table 2). The properties of low peak frequency and high peak harmonic number were not significantly different across the three call types (Table 2). Post hoc analyses revealed several significant differences in pairwise comparisons of call types, but there was no consistent pattern of significant differences between the three call types (Table 2). Based on comparisons of effect sizes, the temporal properties of calls (0.95 \( \leq \eta^2 \leq 0.98 \)) and individual sound elements (0.41 \( \leq \eta^2 \leq 0.99 \)) tended to differ more between the three call types than did the spectral properties (0.06 \( \leq \eta^2 \leq 0.45 \)) and peak power (\( \eta^2 = 0.72 \)).

The cluster analysis based on using only the nine temporal properties revealed a clustering perfectly consistent with the separation of advertisement, territorial and encounter calls as distinct call types (Figure 3). Separate cluster analyses based on using 13 properties (Figure S1) and only the 10 properties that were significantly different across call types (Figure S2) also did a reasonably good job of grouping advertisement, territorial and encounter calls into separate clusters. Territorial calls from two males (19 and 46) formed a distinct cluster in these two analyses because they had values of relative amplitude that were markedly different from other males. The cluster analysis based on only five spectral properties did a relatively poor job of placing the three call types into distinct clusters (Figure S3).

Advertisement calls

Actively calling males that were undisturbed by females or intruding males produced advertisement calls (Figure 1(A)) at a mean rate of 4.8 \( \pm 0.9 \) calls/min (\( N = 14 \) males).
Table 2. Descriptive statistics and results of repeated measures ANOVAs comparing the acoustic properties of advertisement, territorial, and encounter calls based on using within-individual medians (N = 14 individuals).

<table>
<thead>
<tr>
<th>Category of acoustic property</th>
<th>Properties</th>
<th>Advertisement call</th>
<th>Territorial call</th>
<th>Encounter call</th>
<th>F_{2,26}</th>
<th>P-value</th>
<th>Effect size ($\eta^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temporal call properties</strong></td>
<td>Call duration (ms)</td>
<td>941.1 ± 164.4^a (653.0–1178.0)</td>
<td>164.3 ± 28.0^b (109.0–209.0)</td>
<td>210.0 ± 45.2^b (130.5–272.0)</td>
<td>245.65</td>
<td>&lt;0.001</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Elements per call</td>
<td>4.0, 4.0–5.0^a (3.0–5.0)</td>
<td>2.0, 2.0–2.0 (2.0–2.0)</td>
<td>8.0, 6.3–9.0 (4.0–10.0)</td>
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</tr>
<tr>
<td></td>
<td>Element rate (notes or pulses/s)</td>
<td>4.5 ± 0.3^a (4.1–4.8)</td>
<td>12.6 ± 2.3^b (9.6–18.3)</td>
<td>35.2 ± 4.1^c (30.7–43.1)</td>
<td>540.99</td>
<td>&lt;0.001</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Temporal element properties</strong></td>
<td>E1 duration (ms)</td>
<td>137.7 ± 20.4^a (116.0–182.0)</td>
<td>137.7 ± 27.5^a (87.0–187.0)</td>
<td>21.1 ± 3.0^b (17.0–28.0)</td>
<td>158.97</td>
<td>&lt;0.001</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>E1 rise time (ms)</td>
<td>16.4 ± 6.3^a (8.5–32.0)</td>
<td>100.9 ± 30.7^b (37.0–153.0)</td>
<td>3.1 ± 1.9^a (1.0–7.5)</td>
<td>125.33</td>
<td>&lt;0.001</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>E1 fall time (ms)</td>
<td>49.9 ± 11.0^a (35.0–74.5)</td>
<td>36.3 ± 31.6^b (8.5–123.0)</td>
<td>17.9 ± 2.8^a (14.0–24.0)</td>
<td>9.08</td>
<td>0.007</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>E2 duration (ms)</td>
<td>128.3 ± 9.3^a (115.0–152.5)</td>
<td>19.1 ± 3.6^b (14.0–27.0)</td>
<td>21.0 ± 2.6^a (17.0–26.0)</td>
<td>1755.64</td>
<td>&lt;0.001</td>
<td>0.99</td>
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<tr>
<td></td>
<td>E2 rise time (ms)</td>
<td>122.0 ± 3.8^a (8.5–22.0)</td>
<td>5.2 ± 1.0^b (3.5–7.0)</td>
<td>2.8 ± 1.9^a (1.0–8.0)</td>
<td>46.03</td>
<td>&lt;0.001</td>
<td>0.78</td>
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<tr>
<td></td>
<td>E2 fall time (ms)</td>
<td>51.5 ± 11.8^a (36.5–71.5)</td>
<td>14.0 ± 3.4^b (9.0–22.0)</td>
<td>18.4 ± 3.0^a (14.0–24.0)</td>
<td>108.27</td>
<td>&lt;0.001</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Spectral call properties</strong></td>
<td>Low peak frequency (Hz)</td>
<td>593.7 ± 31.9 (549.0–657.6)</td>
<td>572.3 ± 57.7 (493.0–724.9)</td>
<td>577.3 ± 31.2 (537.8–638.6)</td>
<td>1.74</td>
<td>0.203</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>High peak frequency (Hz)</td>
<td>2494.8 ± 136.5^a (2252.1–2722.6)</td>
<td>2271.1 ± 240.2^b (1815.1–2621.8)</td>
<td>2404.5 ± 273.0^ab (1865.5–2812.3)</td>
<td>7.41</td>
<td>0.003</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Low peak harmonic number*</td>
<td>2.0, 2.0–2.0 (2.0–2.0)</td>
<td>2.0, 2.0–2.0 (2.0–2.5)</td>
<td>2.0, 2.0–2.0 (2.0–2.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High peak harmonic number*</td>
<td>8.0, 8.0–9.0 (8.0–9.0)</td>
<td>8.0, 8.0–8.0 (6.0–10.0)</td>
<td>8.5, 7.6–9.0 (7.0–10.0)</td>
<td>0.82</td>
<td>0.449</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Relative amplitude (dB)</td>
<td>4.1 ± 4.2^a (-4.5–9.4)</td>
<td>-1.8 ± 6.6^b (-18.1–5.4)</td>
<td>2.9 ± 2.8^b (-2.5–7.6)</td>
<td>10.57</td>
<td>0.001</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Amplitude property</strong></td>
<td>Peak power (dB)</td>
<td>90.1 ± 1.5^a (87.7–93.0)</td>
<td>81.8 ± 2.8^b (76.9–86.1)</td>
<td>85.9 ± 4.4^c (73.9–91.4)</td>
<td>33.11</td>
<td>&lt;0.001</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Notes: Shown here are the means ± SDs (minimum–maximum) or, where indicated by asterisks (*), the median and inter-quartile range (minimum–maximum). Different letters (a, b, c) indicate significant differences in Tukey HSD posthoc tests.
The advertisement call is a multi-note call typically consisting of four or five consecutive notes delivered in just under 1 s (mean note rate = 4.5 notes/s; Table 2). This call is relatively longer than both territorial and encounter calls. Each note within a call contains a pulsed and noisy onset followed by a harmonic stack (Figure 1(A)). The pulsed nature of the onset is typically more pronounced in notes following the first note. A typical note is about 130 ms in duration (Table 2). In the call’s bimodal frequency spectrum, the mean low peak was 593.7 Hz and the mean high peak was 2494.8 Hz. The low peak frequency corresponded to the second harmonic of an average fundamental frequency near 297 Hz, and the high peak harmonic number corresponded to either the eighth or ninth harmonic (Table 2). Frequency increased slightly over the duration of a note. The extent of modulation was most pronounced in the first note of the call and was, on average, 505.7 ± 141.6 Hz measured from the lowest to the highest points along the contour of the high peak frequency in spectrograms. On average, the high peak frequency had a relative amplitude that was about 4.1 dB greater than that of the low peak frequency. Based on individual median values, the high peak frequency was the ‘dominant frequency’ (i.e. the spectral component having the greatest relative amplitude) for 12 of 14 individuals. On average, the peak power of advertisement calls was 8.3 and 4.2 dB greater than that of territorial calls and encounter.
calls, respectively, produced by the same male during the same recording session. None of the properties we measured for our sample of advertisement calls were significantly related to SVL, mass or condition (Table S5).

**Territorial calls**

The 14 males from which we recorded advertisement calls also produced between 1 and 8 territorial calls ($\bar{X} = 3.5$ calls/male). These calls were produced in response to intrusions by other males and were usually embedded within a longer sequence of advertisement calls. Territorial calls (Figure 1(B); Table 2) were short (e.g. 109.0–209.0 ms) and always consisted of two notes, including a harmonic stack lasting about 137.7 ms and a shorter single pulse lasting about 19.1 ms. Computed over the entire call duration, the frequency spectrum of territorial calls, similar to that of advertisement calls, had a characteristic bimodal distribution of energy, with a mean low peak frequency of 572.3 Hz and a mean high peak frequency of 2271.1 Hz. The high peak harmonic number corresponded to the sixth to tenth harmonic (Table 2). On average, the low peak frequency had a relative amplitude that was about 1.8 dB greater than that of the high peak frequency. On average, the peak power of territorial calls was 8.3 and 4.1 dB less than those of advertisement calls and encounter calls, respectively (Table 2). None of the properties we measured for territorial calls were significantly related to SVL, mass or condition (Table S5).

**Encounter calls**

During aggressive interactions, males also produced an encounter call that differed from advertisement and territorial calls in having a distinctly pulsatile temporal structure (Figure 1(C)). The 14 males from which we recorded advertisement and territorial calls also produced between 1 and 6 encounter calls ($\bar{X} = 2.3$ calls/male). Somewhat longer than territorial calls, but shorter than advertisement calls, encounter calls were about 210.0 ms in duration and were composed of a series of 4.0–10.0 pulses produced at a rate of about 35.2 pulses/s (Figure 1(C); Table 2). A typical pulse (e.g. E1) is about 21.0 ms in duration. In the bimodal spectrum of encounter calls, the mean frequencies of the low peak and high peak were 577.3 and 2404.5 Hz, respectively. The high peak harmonic number corresponded to seventh to tenth harmonic (Table 2). On average, the high peak had a relative amplitude that was about 3 dB greater than that of the low peak, but which peak was the dominant frequency was variable across calls and individuals. On average, the peak power of encounter calls was 4.2 dB less than advertisement calls and 4.1 dB greater than territorial calls.

The number of pulses per call in encounter calls was significantly positively related to mass and condition (Table S5; Figures 4(A and B)). The correlated property of call duration (Table S3) was also significantly positively related to condition (Table S5). Some temporal properties of the individual pulses (E1 and E2) in encounter calls, such as duration, onset time or offset time, were also significantly positively related to mass or condition (Table S5). No properties of encounter calls were related to SVL, and none of the other measured properties were related to mass or condition.

**Amplectant call**

We opportunistically recorded two males that produced vocalizations while in amplexus with a female. Each vocalization was associated with a characteristic elevation of the male’s head due to inflation of the vocal sac, an observation also reported in at least one other frog
Following Toledo et al. (2014), we provisionally term these vocalizations as amplectant calls (Figure 1(D)). Based on an analysis of at least 20 calls per male, the 2 males produced amplectant calls at rates of about 7.7 calls/min and 24.7 calls/min, respectively. Acoustically, amplectant calls were very similar to territorial calls (cf. Figures 1(B and D)). Amplectant calls were 143.8 ± 14.5 ms in duration (cf. 164.3 ms for territorial calls), and similar to territorial calls, they consisted of two distinct notes, an initial harmonic stack and a subsequent pulse. The harmonic stack (E1) had a mean duration of 110.8 ± 11.7 ms (cf. 137.7 ms in territorial calls), and the subsequent pulse (E2) was relatively shorter, having a mean duration of 18.5 ± 0.7 ms (cf. 19.1 ms in territorial calls). Amplectant and territorial calls were grouped together in a cluster analysis based on the temporal properties of calls and elements E1 and E2 that included advertisement, territorial, encounter and amplectant calls (Figure S4). Amplectant calls also had a bimodal frequency spectrum, with a mean low peak frequency of 556.6 ± 8.4 Hz and a mean high peak

![Graph showing the relationship between condition and pulses per encounter call.](image1)

Figure 4. Body size and condition predict some features of encounter calls. Depicted here are scatterplots showing the relationship between the median numbers of pulses per call in the encounter calls of 14 individuals and (A) mass and (B) condition. The trend line represents the best-fit regression line based on least squares. Additional details for these and other regression analyses are reported in Table S5 in the supplemental online material.
frequency of $1883.6 \pm 462.2$ Hz. The latter had a mean relative amplitude that was $2.4 \pm 6.8$ dB higher compared with the low peak frequency.

**Distress calls**

We opportunistically recorded distress calls (Figure 1(E)) from a single male that had been attacked and was being consumed by a predatory water snake, *Sinonatrix percarinata suriki*. Each of the distress calls made by this animal consisted of a single note ($N = 9$ calls), and the calls were produced at a mean rate of about 5 calls/min. On average, distress calls were $227.3 \pm 48.0$ ms (range: 148.0–279.0 ms), with a mean onset time of $155.6 \pm 45.6$ ms (range: 78.0–197.0 ms) and a mean offset time of $71.6 \pm 10.8$ ms (range: 61.0–87.0 ms) (Figure 1(E)). The frequency spectrum was broadband and consisted of a harmonic stack with a single dominant frequency peak centred on $2375.7 \pm 305.8$ Hz (range: 2005.5–2851.5 Hz) when averaged over the duration of the call. Across the nine calls, this dominant peak corresponded to the seventh to tenth harmonic. Compared with other calls in the repertoire, there was marked frequency modulation in distress calls. Measured from the minimum to the maximum frequency values along the contour of the dominant peak in the spectrogram, the mean magnitude of frequency modulation was $1085.4 \pm 306.8$ Hz (range: 630.0–1569.0 Hz).

**Alarm call**

While conducting this study, we observed that individuals we startled would commonly emit a single, short ‘chirp’ sound while simultaneously jumping away from us. Similar sounds have been described as warning calls (Capranica 1965) or alarm calls (Bogert 1960) in other species, and we adopt the term alarm call here. We opportunistically recorded four alarm calls (Figure 1(F)) from four individuals (1 call/frog). On average, alarm calls were $80.8 \pm 50.4$ ms in duration (range: 36.0–32.0 ms) and had mean onset and offset times of $35.3 \pm 26.5$ ms (range: 14.0–73.0 ms) and $25.8 \pm 11.5$ ms (range: 20.0–43.0 ms), respectively. The spectrum was broadband and lacked the harmonic structure present in other vocalizations. Nevertheless, the spectrum was bimodal and had a mean low peak frequency of $597.9 \pm 84.6$ Hz (range: 483.3–672.3 Hz) and a mean high peak frequency of $1774.4 \pm 223.1$ Hz (range: 1647.0–2107.4 Hz). The high peak frequency had a mean relative amplitude that was $1.4 \pm 9.1$ dB greater than that of the low peak frequency (range: −4.8–14.8 dB).

**Discussion**

In this study, we described six call types in the vocal repertoire of *B. adenopleura* based on their acoustic properties and the behavioural contexts in which they were delivered. Many species of frogs have repertoires of similar size (e.g. Brunetti et al. 2015; Hutter et al. 2013), although much larger repertoires have been described for some species (e.g. Narins et al. 2000; Christensen-Dalsgaard et al. 2002; Rowley et al. 2011). The repertoire we describe here for *B. adenopleura* is broadly similar to those described previously for other territorial frogs in the family Ranidae. For example, North American bullfrogs, *Rana (Lithobates) catesbeiana*, and green frogs, *Rana (Lithobates) clamitans*, both produce advertisement calls consisting of three or four repeated notes, each having a broad, bimodal spectrum comprising many harmonics (Capranica 1965; Bee and Gerhardt 2001a; Bee et al. 2001). Both species also produce one or more aggressive vocalizations directed towards other males (Wells 1978; Ramer et al. 1983; Bee and Perrill 1996; Bee et al. 1999;
Owen and Gordon 2005). Both species also produce short, chirp-like alarm calls when startled, and bullfrogs also produce a frequency-modulated distress call when being predated by snakes (M.A. Bee, personal observations).

Although we have assigned putative functions to several call types by giving them names (e.g. advertisement call, territorial call, encounter call and alarm call), we emphasize that these names actually represent hypotheses about the behavioural functions of each of call type. To be sure, we can have some level of confidence in the hypothesized functions of some call types based on the behavioural contexts in which they were produced and on previous studies of related frog species. Nevertheless, testing hypothesized functions will generally require playback experiments to demonstrate the intended receivers of each signal, to describe the information conveyed to receivers by each call type and to characterize the responses of receivers. With these caveats in mind, we offer the following discussion of the putative functions of the call types we recorded.

Advertisement calls were, by far, the most commonly produced call type. Our description of this call is similar to that by a previous study of a population of B. adenopleura in northern Taiwan, some 160 km from our study site (Matsui and Utsunomiya 1983). Although males in our population tended to produce more notes per call, reports of note rate (i.e. element rate), note duration, fundamental frequency, high peak frequency or harmonic and frequency modulation are broadly similar between the two populations. The advertisement calls of B. adenopleura also bear some acoustic similarities to those described previously for several congeners, including Babina daunchina (Chen et al. 2011), Babina okinavana (formerly, Rana psaltes) (Kuramoto 1985) and Babina (formerly, Rana) lini (Chou 1999). One interesting difference between B. adenopleura and most other Babina species is that males of the former produce advertisement calls floating on the water’s surface (Chuang et al. 2013), whereas most congeners produce advertisement calls from inside excavated burrows and rarely call from outside their burrows (Kuramoto 1985, Chou 1999, Chuaynkern et al. 2010; Chen et al. 2011; Cui et al. 2012). How these behavioural differences might influence the acoustic design of vocalizations across the genus is an interesting question for future comparative studies (e.g. Cui et al. 2012).

As in other territorial frogs (Wells 2007), the advertisement call of B. adenopleura likely functions both to attract females and to announce possession of a territory to rival males. The role of this signal in long-distance communication is suggested by its greater relative amplitude compared with, for example, territorial and encounter calls. What role the acoustic features of advertisement calls might play in both female mate choice and male–male interactions remains to be explored in B. adenopleura. In previous work with this species, we have successfully observed a large number of mate choice events, quantified mating and fertilization success under natural conditions and measured important features of male territories, such as the quantity of vegetation (Chuang et al. 2013). By quantifying mating and fertilization success as functions of both territory quality and advertisement call features in this species, it might become possible to disambiguate, for the first time in frogs, the relative roles of male vocal behaviour and territory quality on female mating decisions.

Interestingly, we found no relationship between the acoustic properties of advertisement calls and the signaller’s size or condition. This was unexpected, given that spectral properties exhibit strong negative correlations with body size in other ranid frogs (Bee and Perrill 1996; Bee and Gerhardt 2001a). Whether female ranid frogs prefer to mate with larger males based on the assessment of his vocalizations remains unknown, although such preferences have been reported in other frogs (Gerhardt and Schwartz 2001). In some ranids, male frogs use the body size information conveyed in
advertisement calls to assess their rival’s fighting ability (Bee et al. 1999, 2000), although other ranids appear to ignore this information (Bee 2002).

Males produced two types of calls – territorial and encounter calls – to which we have assigned an aggressive function. Some previous studies have suggested that the use of different aggressive calls corresponds to the degree of aggressive escalation. For example, in their study of the red-spotted glass frog, *Nymphargus grandisonae*, Hutter et al. (2013) suggested that “the context of the encounter call might be peak aggression (i.e. before combat), whereas the territorial call might represent a wider range of aggression levels leading up to peak aggression.” This might also be true for *B. adenopleura*. Similar to other ranid frogs (Wells 1977a; Howard 1978a, 1978b; Wells 1978), the *B. adenopleura* mating system is resource-defence polygyny (Chuang et al. 2013). Males aggressively defend their territories using a combination of vocalizations and physical fighting. Playbacks of putative advertisement calls to territorial males reliably elicit territorial calls and encounter calls. Moreover, playbacks of advertisement calls elicit territorial calls at lower broadcast sound pressure levels than are required to elicit encounter calls (Chuang et al. unpublished data). These observations are consistent with the notion that both territorial and encounter calls function in aggressive contexts but that encounter calls may be used in more escalated interactions compared with territorial calls. There was no indication that territorial calls function as a more ‘long-distance’ aggressive signal than encounter calls. Compared with encounter calls, territorial calls were actually produced at lower amplitudes, as indicated by their lower peak power. Interestingly, only encounter calls had acoustic properties (e.g. number of pulses/call) that were significantly related to the body size (mass) and condition of signalers. To the extent that greater mass and better condition correlate positively with fighting ability, as might be expected (e.g. Howard 1978a), we suggest the acoustic properties of encounter calls might be used in assessing a competitive rival’s resource holding potential (Parker 1974).

Only a few previous studies have reported the presence of amplectant calls in frogs (Odendaal et al. 1983; Toledo and Haddad 2005; Costa and Toledo 2013). The function of these calls is largely unknown. One hypothesis is that they function to stimulate females to commence oviposition (Odendaal et al. 1983), thereby potentially shortening the overall duration of amplexus. This putative function might be especially important in *B. adenopleura*, in which unpaired males commonly attack males in amplexus, causing a reduction in the paired male’s mating success and fertilization success (Chuang et al. 2013). The duration of amplexus in *B. adenopleura* is among the shortest ever reported for any frog (Chuang et al. 2013). Hence, we hypothesize that amplectant calls function in facilitating the rapid release of eggs by females. An alternative hypothesis is that rival males, and not the amplexed female, are the intended receivers of this vocalization. Amplectant calls are acoustically similar to territorial calls. Given the high prevalence of attacks by unpaired males on males in amplexus (Chuang et al. 2013), the amplectant call may function as a territorial call that deters attack by rival males. Playback tests and manipulations to silence males in amplexus, similar to those used in the classic study of fighting assessment by Davies and Halliday (1978), could reveal whether amplectant calls have a reproductive function, an aggressive function or both.

The distress call is a vocalization common to a diversity of frogs (Bogert 1960; Schmidt 1966; Capranica 1968; Hödl and Gollmann 1986; Hutter et al. 2013; Toledo et al. 2014). Distress calls have been noted for having high amplitudes and frequencies and for being one of the only call types that some frogs can produce with the mouth wide open (Capranica 1965). Bogert (1960) hypothesized that the distress call is a response to a predator causing pain and distress, although the adaptive function of distress calls is still
obscure. Distress calls might function to warn conspecifics, to startle a predator or to attract predators of the animal attacking the frog (Bogert 1960; Schmidt 1966). Playbacks of distress calls to other conspecifics and to potential predators would be useful for determining the intended receivers of these calls, which in turn, could eliminate some competing hypotheses for their function.

Alarm calls similar to those described here might be more common than current evidence would suggest. Similar calls have been reported to occur in some other ranids, such as *R. catesbeiana* (Capranica 1965), and they are also produced by several other species in Taiwan, including *Rana guentheri*, *Rana plancyi* and *Fejervarya cancrivora* (M-F Chuang, personal observation). These calls are produced during the execution of escape behaviours, and the most characteristic features of this sound are its sudden onset, quick offset and brief duration (Capranica 1965, 1968). Whether alarm calls function to warn conspecifics of eminent danger remains to be demonstrated in playback experiments. Unlike the receivers of alarm calls in more social, non-anuran species (e.g. Sherman 1985), there is no evidence to suggest that signalling frogs would be closely related to nearby conspecifics. Hence, a function of alarm calls in warning kin seems unlikely. Indeed, this call may have no function at all and, instead, may be a by-product of rapid escape, lung deflation and tensed muscles (Capranica 1965).

Compared with the number of previous bioacoustic studies of frogs from Europe, Australia and North, Central and South America, we know far less about the vocal behaviours of frogs native to Asia. We suggest the 10 species in the genus *Babina* (Chuaynkern et al. 2010; Frost 2015) found throughout south-eastern and eastern Asia represent a suitable group for future comparative studies of bioacoustics and vocal behaviour. As a first step, we recommend quantitative descriptions of each species’ vocal repertoire, as reported here for *B. adenopleura*.

**Supplementary material**

Supplementary material for this article is available via the supplementary tab on the article’s online page at doi:10.1080/09524622.2015.1076347.

**Acknowledgements**

This study was approved by the Institutional Animal Care and Use Committee of Tunghai University (No: 100–19). We thank the staff of Lien-Hua-Chih Research Center of Taiwan Forestry Research Institute for providing accommodations and permitting us to collect specimens and conduct experiments (Official permitting number: TFRILHC0950001568), and we thank Yi-Huei Chen, Ting-Yun Stella Huang, Jou-Chiieh Lai and Yi-Rou Li for field assistance.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**


The Cornell Lab of Ornithology.


