

# Calling behavior of males and females of a Bornean frog with male parental care and possible sex-role reversal

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## Abstract

In many species that use acoustic signals for mate attraction, males are usually the most vocal sex. In frogs, females typically remain silent, while males produce advertisement calls to attract mates. In some species, females vocalize, but usually as a response to an initial male advertisement call. The smooth guardian frog (*Limnonectes palavanensis*), found on Borneo, has exclusive paternal care while the females mate and desert after laying the clutch. Males provide care to the eggs until hatching and then they transport the tadpoles to small bodies of water. The vocal repertoire of this species has never been described. Males have a distinctive advertisement call to attract females, but produce the call very infrequently. We found that females of *L. palavanensis* not only respond to male advertisement calls but also vocalize spontaneously, forming lek-like aggregations around a single male. Males may or may not respond to a particular female with a short courtship call, which is elicited only by the female call and not the male advertisement call. The calling rate of females is consistently higher throughout the night compared with the calling rate of males. These observations suggest that this species exhibits a

reversal in calling behavior and possibly a sex-role-reversed mating system.

## Significance statement

Exceptional cases of species with a sex-role reversed mating system have been observed in fishes and birds, but not in frogs. For sex-role reversal to occur, there must be intense parental care by the males and a surplus of females. Additionally, females should exhibit characteristics that are usually observed in males in species with conventional sex roles. We found that in *L. palavanensis*, females are highly vocal, exhibiting higher calling rates compared with the calling rates of the males. This behavior, where females out-signal males has not been observed in anurans. This female calling behavior coupled with observations of several females approaching a male provides evidence of a female-biased operational sex ratio, a characteristic of a sex-role-reversed mating system. Thus, this study provides quantitative evidence that *L. palavanensis* exhibits various aspects consistent with a sex-role reversed mating system.

**Keywords** Female calling · Frogs · Sex-role reversal · Vocal behavior · Calling rate · *Limnonectes palavanensis*

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## Introduction

In most birds, acoustic insects, and frogs, acoustic signals are used to attract or court prospective mates (Andersson 1995; Wagner and Reiser 2000; Gerhardt and Huber 2002; Searcy and Nowicki 2005; Rebar et al. 2009). In most cases, males devote more time and effort to acoustic displays than do females. Nevertheless, there is considerable variation in signaling sex roles within and among clades. For example, in passerine birds, species with male-only song are common in the

temperate zone. In contrast, in tropical passerines, females sometimes sing as much as males and both sexes often participate in synchronized duets (Grafe and Bitz 2004; Slater and Mann 2004). In some clades, singing by both males and females appears to be the ancestral condition, with female song having been lost in species that moved into the temperate zone (Price et al. 2009). In many acoustic insects, females respond to the male advertisement signal with a courtship song, which may be similar to calls of males or different (Gerhardt and Huber 2002). In some other cases, females respond shortly after the male's call, leading to a duet that is essential for successful courtship and mating (Wells and Henry 1992; Cooley and Marshall 2001; Rodríguez et al. 2004). Examples of acoustic insects in which females call more often than males are unknown.

In birds, cases of females signaling more frequently than males are rare and often associated with partial or complete reversal of the usual sex roles in courtship and mating (Goymann et al. 2004; Ekstrom et al. 2007; Price et al. 2008). Such sex-role reversal is related to males assuming expensive parental care duties, which removes them from the pool of mates available to females, or to population sex ratios heavily skewed toward females. Thus, males become a limiting resource for females, and females are expected to compete for access to males (Trivers 1972; Emlen et al. 1998; Kokko and Jennions 2008). A well-studied species in which the mating system is sex-role reversed is the African black coucal (*Centropus grillii*). In this species, females are larger than males and aggressively defend territories, whereas males perform all of the parental care. In a given season, males can raise up to four clutches, whereas females on average produce nine clutches. Hence, the potential reproductive rate of females is higher than that of males. In addition, females are more conspicuous and their vocal activity substantially exceeds that of the males, a behavior typically associated with a sex-role reversed mating system (Andersson 1995; Geberzahn et al. 2009, 2010; Illes and Yunes-Jimenez 2009).

In most species of frogs, males produce advertisement calls to attract conspecific females or advertise ownership of territories to other males (Wells 1977, 2007). In some species, males produce a distinct courtship call when females approach their calling sites, possibly providing cues that assist females in locating males (Gerhardt and Huber 2002; Wells 2007). Female frogs of a few species also vocalize, despite having a relatively under-developed larynx compared to that of males (see Suthers et al. 2006 for a case of reverse sexual dimorphism in larynx size). In species with female calling, the most common context is for females to respond to the male advertisement call with a courtship call (Wells 2007). Generally, these calls are distinct from male calls, being shorter and of much lower amplitude. Female frogs seldom call spontaneously or in the absence of males. Often female courtship calls stimulate the males, which sometimes respond

with a call distinct from the advertisement call (Given 1993; Judge and Swanson 2000). In some cases, the female call may serve as a signal of reproductive receptivity or reveal the female's location (Emerson 1992; Bush et al. 1996). Male vocal responses to female calls probably assist females in locating males in hidden locations or acoustically complex environments (Marquez and Verrell 1991; Tobias et al. 1998; Bosch 2001; Shen et al. 2008).

Clear cases of sex-role reversal in frogs have not been reported, despite the prevalence of paternal care and the wide variety of mating systems observed in anurans. In some species of dendrobatids with male parental care, females actively court the males and sometimes engage in female–female agonistic behaviors. However, males still do most of the calling to attract mates, compete with each other for mating opportunities, and can care for more than one clutch at a time, meaning that parental care is not limiting mating opportunities for males (Wells 1978, 1980; Summers 1989; Ursprung et al. 2011). In the Majorcan midwife toad, *Alytes muletensis*, males call from crevices and hidden locations, often widely separated from one another. Females respond to the male advertisement call with a soft courtship call. This reciprocal call aids both males and females in finding each other. Females of *A. muletensis* sometimes initiate courtship by calling spontaneously, but they only do so when they are heavily gravid and in danger of losing their eggs (Bush et al. 1996). During the breeding season, the operational sex ratio (OSR) can vary, and when the OSR is female biased, females wrestle in contests for access to males, although males also can fight for access to females (Bush 1997; Bush and Bell 1997). After amplexus and oviposition, males intertwine the eggs around their legs and brood them until the eggs are ready to hatch. Males in this population rarely care for more than one clutch at a time, even though a female-biased sex ratio should increase mating opportunities for males (Bush 1996). Thus, males may be a limiting resource for females, leading to sex-role reversal in some aspects of courtship and mating.

We studied the smooth guardian frog (*Limnonectes palavanensis*) (Anura: Dicroglossidae) on the island of Borneo. Parental care is performed exclusively by males, which not only care for eggs but also transport tadpoles to water (Inger et al. 1986; Inger and Voris 1988; Goyes Vallejos 2016). Males give advertisement calls to attract mates, but do so very infrequently from widely spaced locations on the forest floor. Females respond to male advertisement calls with a soft short-range call, but they also call spontaneously in the absence of males. With male parental care and female calling behavior, *L. palavanensis* offers a unique opportunity to investigate the possibility of a reversal of the typical anuran sex roles in calling behavior. The aims of this study were to (1) characterize the vocal repertoire of males and females of *L. palavanensis*, (2) determine the behavioral contexts in which males and females vocalize and interact with

one another, and (3) quantify calling rates of males and females in the field. From the sex-role reversal hypothesis, we predict that (1) females will vocalize more frequently than males, and (2) calling females will be more abundant than calling males.

## Methods

### Study species

*L. palavanensis* is a small leaf-litter frog found in primary and old secondary rainforests of Borneo and the Palawan Island of the Philippines (Fig. 1). Males call at night from the leaf litter on the forest floor, although the structure of the call has not been published. *L. palavanensis* belongs to a genus of Southeast Asian frogs that has some unusual sexual characters. Males in some species of the genus lack vocal sacs and have been called “voiceless frogs” (Emerson 1992), although males in some species without vocal sacs produce advertisement calls (Matsui 1995; Orlov 1997). The clade also has been grouped under the name “fanged frogs,” because many species of *Limnonectes* have bony projections on the lower jaw, in addition to enlarged heads and hypertrophied jaw muscles used in male–male combat over access to females (Emerson et al. 2000). Females of *Limnonectes* typically lack fangs and are smaller than males, a pattern opposite to that generally observed in anurans; usually, females are larger than males (Wells 2007). Both males and females of *L. palavanensis* lack both vocal sacs and fangs, males do not have enlarged heads, and females are larger than males, the reverse of the pattern normally seen in this clade.

### Study area

Fieldwork was conducted at the Kuala Belalong Field Studies Centre (KBFSC), a research facility at the heart of the Ulu Temburong National Park, Temburong District of the Sultanate of Brunei Darussalam. This largely pristine forest is located on the Northwest coast of the island of Borneo and comprises 50,000 ha of lowland mixed dipterocarp rainforest. This forest is one of the tallest tropical forests in the world, with trees being 30–40 m tall, although individual trees can reach heights over 50 m. The Institute of Biodiversity and Environmental Research (IBER) and the Universiti Brunei Darussalam manage the KBFSC, located at 115° 109' E, 4° 33' N and about 50–200 m above sea level. The topography around the station is steep, with several slopes and with a loose layer of clay soil. There are no ponds or bodies of standing water except for ephemeral rain pools and bearded-pig wallows. The mean temperature during the sampling time (1700–0000 h) was  $25.2 \pm 1.9$  °C (mean  $\pm$  SD) with a relative humidity of 90% (JGV, pers. obs.). The yearly rainfall at the site



**Fig. 1** An adult female of *Limnonectes palavanensis* from Brunei Darussalam

varied between 4900 and 6800 mm without a well-defined dry season, although the driest period tends to be between June and August (KBFSC Weather Data 2005–2014). A population of *L. palavanensis* is found in the forest surrounding the research station. This study took place from June to July 2012 and from July to November 2013.

### Behavioral observations

Our study area was adjacent to the field station and encompassed approximately 1.3 ha of forest. The low density of understory vegetation allowed us to survey the area systematically making use of three main transects as our starting points and making a series of hairpin turns perpendicular to the transects. We surveyed this area almost every night (145 nights in total) searching for calling individuals of *L. palavanensis*. The acoustic encounter surveys began at 1700 h and continued until 0000 h and involved walking slowly along the main transects listening carefully for calls. It is nearly impossible to locate either males or females that are not calling, although a few were found through chance encounters. It was not possible to record data blind because our study involved focal animals in the field. When a calling individual was found, we performed focal observations *ad libitum*. We recorded time of day, temperature, and number of individuals within a 2-m radius around the focal frog, along with other natural history notes. In addition to behavioral observations, whenever possible, we made sound recordings of calling individuals for the acoustical analysis portion of this study. The snout–urostyle length (SUL) of the focal individual was measured with a caliper (to the nearest 0.1 mm) and its mass obtained with a portable digital scale (to the nearest 0.01 g). We calculated a Body Condition Index using the residuals of the linear regression of SUL and mass. The tip of a toe of each front foot and hind foot were clipped for individual identification. To aid in the collection of natural history observations, a few individuals were taken to the laboratory at the research station and kept in captivity in a glass terrarium for one night.

## Sound recordings

We recorded 562 calls from 33 individual males and 26 females at night starting at 1700 h until 2300 h (mean temperature  $25.2 \pm 1.9$  °C, range 22–28 °C) using a Marantz PMD 661 recorder (44.1 kHz sample rate, 16-bit resolution; Marantz America, LLC, Mahwah, NJ, USA) and a Sennheiser ME 66 directional microphone (Sennheiser USA, Old Lyme, CT, USA). In all cases, the microphone was positioned 20–30 cm away from the calling individual. Sound pressure level measurements (db SPL re 20  $\mu$ Pa, C frequency weighting, fast response) were taken at a distance of 50 cm from each calling male using a digital sound level meter Extech 407730 (Extech Instruments, Waltham, MD). It was not possible to obtain the measurements of the sound pressure level of female calls due to their low amplitude and short duration. For every individual, we recorded whether calls were given in the presence or absence of other individuals and the sex of any other individuals that were present.

## Acoustical analysis

We used Raven Pro v1.4 software (Cornell Laboratory of Ornithology, Ithaca, NY, USA) to measure spectral properties such as dominant frequency, fundamental frequency, frequency modulation, number of harmonics, and the harmonic of the dominant frequency. We defined harmonics as the spectral components appearing as integer multiples of the fundamental frequency, with the fundamental frequency being the first harmonic. The dominant frequency was measured using Raven's Maximum Frequency function over the entire duration of each call, while the fundamental frequency was obtained using the same function, but selecting only the first harmonic in Raven's spectrogram view. We also measured temporal properties such as call duration, call rise time, and call fall time (defined as the time from the onset of the first pulse to the offset of the last pulse, the time from the onset of the first pulse to the peak of maximum amplitude, and the time from the peak of maximum amplitude to the offset of the last pulse, respectively), using Raven's waveform view. The advertisement call of males of *L. palavanensis* is a trill (call with multiple sequential pulses), so we measured additional temporal properties that included pulses per call, pulse rate (calculated as the number of pulses minus 1 divided by call duration following Bee et al. 2013), pulse duration, pulse rise time, pulse fall time, and interpulse interval. The spectrograms were generated using a 512-point fast Fourier transform (FFT) for male calls and 256-point FFT for the female calls. A smaller FFT is recommended for shorter lower amplitude calls like the female call of *L. palavanensis* for a better time domain resolution.

All statistical analyses were conducted using R version 3.2.2 (Development Core Team 2015). We report the mean and standard deviation for each measured acoustic property using a mixed effects linear model with frog ID as the random effect to correct for

the fact that some individuals call more often than others, resulting in more calls to analyze for some individuals. For count data (number of harmonics, number of pulses), we report the median and interquartile range. We described the frequency modulation of the male advertisement call by plotting every pulse in a call versus dominant frequency at each pulse for each advertisement call of all the sampled males. Then, we fitted a third degree polynomial using a linear mixed effects model with individual frog as the random effect. We report coefficients of variation within ( $CV_w$ ) and among individuals ( $CV_a$ ) as percentages ( $CV = 100\% \times SD/mean$ ) using the standard deviation within individuals and among individuals from the model. We assessed the relationship between dominant frequency and body mass, snout–urostyle length and body condition for both males and females. In addition, we assessed the relationship between male trill duration and mass, snout–urostyle length, and body condition. We tested for significance of the linear regression fitted with a linear mixed effects model using a Kenward-Roger approximate *F* test. Because the temperature at the time each of the recordings that was made was fairly constant (22–26 °C), we disregarded the effect of temperature on the properties of the calls.

## Calling rates of males and females

In 2013, the study area surrounding the KBFSC was visited almost every night to locate calling individuals through acoustic encounter surveys. We obtained these data concurrently while doing the sound recordings of calling individuals. When an individual was located, we would remain quiet to avoid disturbing the individual and registered the number of calls *ad libitum* until the individual stopped calling. We broke off the observation if the individual did not resume calling within 30 min. For males, only advertisement calls were included in the samples, as males do not give courtship calls spontaneously. The number of calls of each individual per recording time period was standardized to number of calls per hour to calculate the average calling rate of males and females throughout the sampling period (1700–2300 h). We fitted a generalized linear model predicting the number of calls as a function of sex (male, female). Because of the variable sample periods for each individual (up to 1 h for individual females, and up to 2 h for individual males), we used an offset of the log number of minutes and fit the entire model as a quasipoisson distribution using a log link function. Inference was based on the mean calling rate per sex as estimated by the model at the average start time of observations.

## Results

### Behavioral observations

From June 5 to July 24, 2012 and July 7 to November 20, 2013, we observed 44 females and 35 males of *L. palavanensis* ( $N = 79$ ). The mean ( $\pm$ SD) snout–urostyle



length (SUL) of adults was  $30.7 \pm 1.2$  mm for females (range 27.9–33.1 mm) and  $26.4 \pm 1.6$  mm for males (range 21.1–30.6 mm); females were significantly larger than males (Mann–Whitney test,  $U = 25$ ;  $P < 0.001$ ). Besides size, there are no other sexually dimorphic morphological traits to distinguish males from females. However, it was evident that all calling females were gravid, because the white eggs filling the abdominal cavity were easily observed through the skin of their bellies.

Males of *L. palavanensis* were highly dispersed in the forest, with individual males separated by many meters ( $>10$  m). The frogs called from the ground and did not use elevated perches nor did they form aggregations or choruses. They did not call frequently, but their calls could be heard from 20 m away by an observer. No more than four male frogs were found calling on a given night in the study area (ca. 1.3 ha), and there were many nights on which males failed to call at all. The number of calling females per courtship event was significantly greater than the number of calling males (Wilcoxon signed rank test;  $P < 0.001$ ). We observed 29 cases of more than one female calling within 2 m of the same male (range = 2–4), 32 cases of a single female and a single male interacting with each other, and only four cases of two males and one female calling to each other.

### Calling behavior of males

The advertisement call of *L. palavanensis* is a short trill (call with multiple sequential pulses) of moderate intensity (70 dB SPL re 20  $\mu$ Pa at 50 cm). Over the course of a few seconds before calling, the male inflates his whole body by inhaling air several times. At the end of this process, the flanks are visibly expanded. During calling, the whole body of the frog shakes fiercely for the duration of the trill (see Online Resource 1). After each advertisement call, it can take from 1 min to a full hour for a male to give another call. Their low calling rates and their cryptic coloration render the males very difficult to find.

### Calling behavior of females

Females called in response to the male advertisement call with a single-note squeak or chirp. We could hear the calls of an approaching female only when she was less than 3 m from the calling male. Once a female was within ca. 1 m, the male sometimes responded to the female with a short courtship call (single note call) (Fig. 2; Online Resource 1). This short male call was often given as an immediate response to the female call (response time mean =  $0.7 \pm 0.09$  s, median = 0.5 s, range = 0.2–2.2 s,  $N = 79$  calls from 13 individuals) (Fig. 3; Online Resource 1). Males did not approach the females during our observations, and they did not give the courtship call in the absence of a female nearby. We observed that females called antiphonally when more than one calling female was

present, but we were unable to obtain recordings of these interactions due to the very low intensity of the female calls. Females also were found alone calling spontaneously ( $N = 21$ ) at various times throughout the night in the absence of males or male advertisement calls. To confirm that females call spontaneously, 12 females were kept in the laboratory at the KBFSC field station in isolated conditions for one night. All 12 captive females called spontaneously at various times throughout the evening (1600–0000 h). Descriptive statistics and coefficients of variation for the spectral and temporal properties measured for all *L. palavanensis* calls are presented in Table 1.

### Male advertisement call

We recorded 26 individuals giving the advertisement call, but only recorded the SUL of 16 of them. The other males escaped immediately after the sound recording. The male advertisement call had an average duration of  $1899.3 \pm 267.2$  ms, with a dominant frequency of  $1888 \pm 81$  Hz and a fundamental frequency of  $611 \pm 22$  Hz. The median number of pulses per call was 28 pulses (interquartile range 27–31 pulses), with the first pulse being significantly longer ( $77.8 \pm 42.4$  ms) and with a slower rise time compared to the rest of the pulses ( $19.2 \pm 3.0$  ms) (paired  $t$  test  $t_{59} = 5.14$ ;  $P < 0.0001$ ) (Table 2). The average pulse rate was 14 pulses/s (range 10–17 pulses/s). The advertisement call had a median of six harmonics (interquartile range 5–12), with the third harmonic being the dominant frequency. The call had an average upward frequency modulation between the 1st and 28th pulse of  $388 \pm 97$  Hz,  $N = 21$  (Fig. 4).

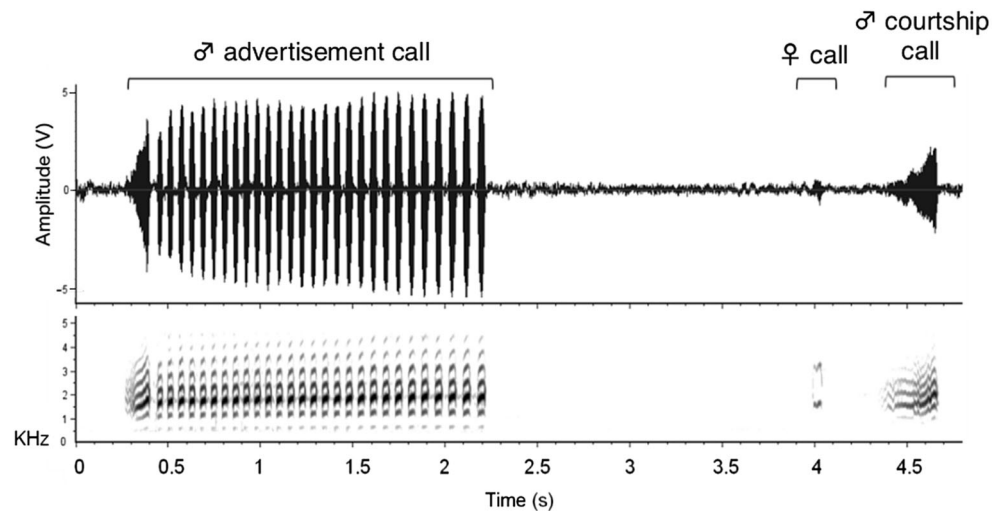
### Female call

The female call was a short chirp, lasting on average  $56.8 \pm 9.0$  ms. The first harmonic was the dominant frequency, which makes the fundamental frequency and the dominant frequency the same ( $1608 \pm 67$  Hz). The median number of harmonics was eight (interquartile range 4–15) (Fig. 5a).

### Male courtship call

We recorded 17 individuals giving the courtship call. This call was always given in response to a female call and was a soft single note squeak with an average dominant frequency of  $1752 \pm 86$  Hz and an average fundamental frequency of  $514 \pm 48$  Hz. The call had an average duration of  $309 \pm 126$  ms and consisted of a single note, but there were instances in which the males gave two consecutive calls ( $N = 5$ ). In one exceptional case, a male gave the courtship call four consecutive times (Fig. 5b). The median number of harmonics was eight (interquartile range 5–13), with the third harmonic being the dominant frequency.

**Fig. 2** Oscillogram (*top*) and spectrogram (*bottom*) of the male advertisement call, female call, and male courtship call of *L. palavanensis*. Males respond with a courtship call immediately after a female call. All three calls are represented in real time. Note the difference in amplitude between the male advertisement call, the female call, and the male courtship call. Both oscillogram and spectrogram are on the same time scale

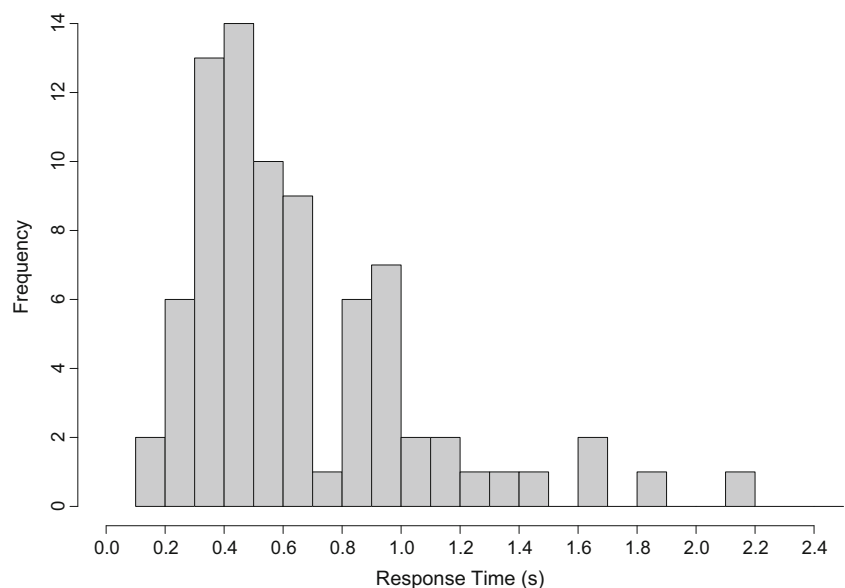


### Call variation

We also explored the variation within and among individuals in the different spectral and temporal parameters of the calls. Differences in spectral and temporal properties of the call among individuals can influence mate choice by females (Gerhardt 1991). Following Gerhardt and Huber (2002) and Pettitt et al. (2013), we classified the acoustic parameters as “static” if the values of  $CV_w$  were less than 7% and “dynamic” if the values of  $CV_w$  are greater than 12%. In general, static acoustic properties are related to species recognition and are constrained by physical and physiological processes. The dynamic acoustic properties are more dependent on the social context that elicits the vocalization. Based on the  $CV_w$  values, dominant frequency and the fundamental frequency were static properties for the three types of

vocalizations in *L. palavanensis*, while call duration, call rise time, and call fall time were the dynamic properties based on the  $CV_w$  values. These results are similar to those observed in other frogs (Gerhardt and Huber 2002) where there is low variation in general in the spectral properties. Within the pulse properties of the male advertisement call, pulse rate, interpulse duration, and pulse duration comprise the static properties, whereas the number of pulses per call and the duration of the first pulse were the dynamic properties. Overall, there was little variation among individuals in the values of dominant frequency and fundamental frequencies for all three of the call types and for the pulse rate in the male trill ( $CV_a < 10\%$ ). The greatest variation among individuals was found in the temporal parameters for both male advertisement call and female call. In the male courtship call, there was more variation within individuals than among individuals.

**Fig. 3** Histogram of the response times of males *L. palavanensis* giving courtship calls after a female call ( $N = 79$  courtship calls from 13 individuals, median = 0.5, range = 0.2–2.2 s)



**Table 1** Description of temporal and spectral properties measured for the three different calls of *Limnonectes palavanensis*

Acoustic property	Male advertisement call				Female call				Male courtship call			
	$\bar{x} \pm \text{SD}$	Min–max	CV <sub>w</sub>	CV <sub>a</sub>	$\bar{x} \pm \text{SD}$	Min–max	CV <sub>w</sub>	CV <sub>a</sub>	$\bar{x} \pm \text{SD}$	Min–max	CV <sub>w</sub>	CV <sub>a</sub>
Spectral parameters												
Dominant frequency (Hz)	1888 $\pm$ 81	1604–2062	3.2	4.3	1608 $\pm$ 67	1378–1787	2.6	4.2	1753 $\pm$ 86	1464–2143	7.1	4.9
Fundamental frequency (Hz)	611 $\pm$ 22	569–660	1.9	3.7	1608 $\pm$ 67	1378–1787	2.6	4.2	514 $\pm$ 47	379–624	8.0	9.2
Temporal parameters												
Call duration (ms)	1899 $\pm$ 267	773–2691	13.8	14.2	57 $\pm$ 9	34–99	11.6	15.8	310 $\pm$ 126	120–1146	46.5	40.7
Call rise time (ms)	812 $\pm$ 284	225–1733	37.1	35.0	32 $\pm$ 7	5–55	17.8	20.7	239 $\pm$ 68	43–510	28.0	28.3
Call fall time (ms)	1074 $\pm$ 242	222–2107	34.0	22.5	25 $\pm$ 6	13–66	28.2	25.4	41 $\pm$ 18	10–316	97.2	43.8

### Relationship between acoustic properties and morphology

Dominant frequency was not significantly associated with mass, body size, or condition for either males or females (all  $P > 0.05$ ). Male advertisement call pulse rate was not associated with any of the parameters tested. However, call duration was positively associated with body size ( $F_{1, 12.8} = 5.09$ ;  $P < 0.05$ ) (Fig. 6).

### Relative calling rates of males and females

In 2013, we estimated the calling rates of 94 individuals (54 males, 40 females) throughout the evening over the course of 5 months. Females started calling at about 1700 h and continued to call throughout the evening (Fig. 7). Both females calling spontaneously and interacting with males were included in the dataset, as they were not apparent differences between the two groups. Males started producing advertisement calls sporadically after sunset (1700 h), but usually stopped calling by 2200 h. Some males had calling rates as low as one call per hour ( $N = 3$ ). Females sometimes continued to call for up to an hour after males had ceased calling. Females also

called sporadically without a particular bout pattern, calling on average three times per minute, but they sometimes called up to five times per minute ( $N = 5$ ). Males had significantly lower calling rates than females throughout the evening ( $\beta_{\text{male number of calls}} = -3.3 \pm 0.2$ ;  $P < 0.001$ ). Neither females nor males call constantly for an hour; hence, we present the average calling rate as calls per 10 min. The predicted mean female calling rate was  $30.3 \pm 3.7$  (mean  $\pm$  SE) calls/10 min, whereas the predicted mean male calling rate was  $1.1 \pm 0.2$  (mean  $\pm$  SE) calls/10 min.

### Discussion

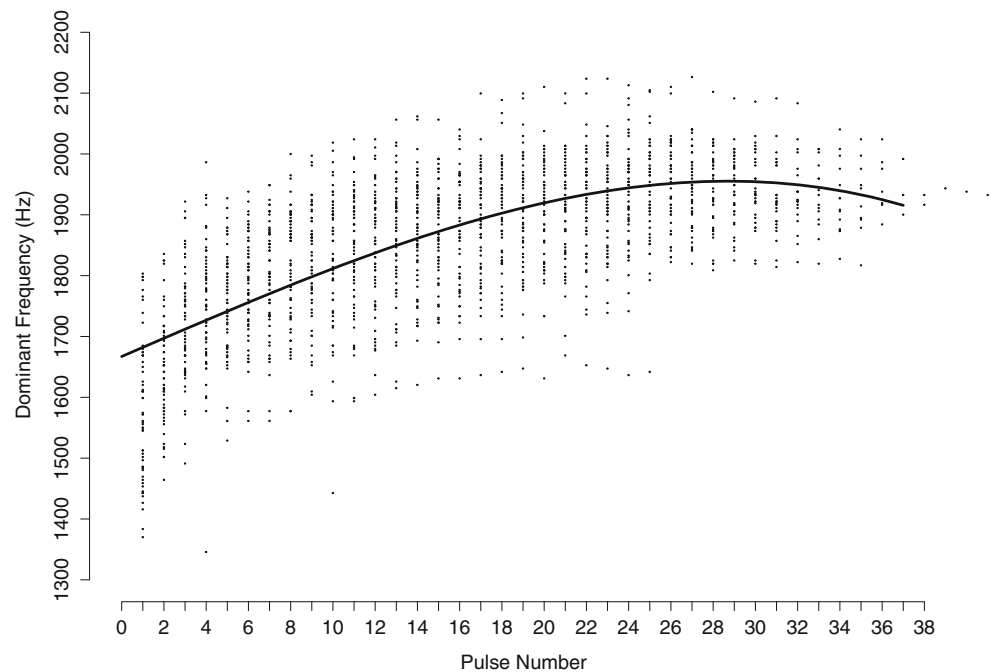
In addition to high paternal investment, in species with a sex-role reversal mating system, there is a surplus of females in the population and these are generally more conspicuous and vocal than the males. Male-only parental care in anurans is a widespread phenomenon. Although some species exhibit aggressive competition among females, receptive males usually outnumber females and therefore are not a limiting resource for females (Summers 1989). In this study, we tested two of

**Table 2** Pulse temporal properties in *Limnonectes palavanensis* male advertisement calls

Temporal parameter	$\bar{x} \pm \text{SD}$	Min–max	CV <sub>w</sub>	CV <sub>a</sub>
Pulses per call (k)	28 (27–31) <sup>a</sup>	9–38	15.7	15.6
Pulse rate (pulses/s)	14 $\pm$ 1	10–17	5.7	8.6
First pulse duration (ms)	78 $\pm$ 42	29–349	48.7	54.5
First pulse rise time (ms)	60 $\pm$ 44	18–320	58.1	73.9
First pulse fall time (ms)	20 $\pm$ 4	9–33	22.8	22.8
Interpulse duration (ms)	25.6 $\pm$ 4	19–40	5.3	15.6
Pulse duration (ms)	42 $\pm$ 3	34–49	2.5	8.4
Pulse rise time (ms)	19 $\pm$ 3	14–28	6.9	15.7
Pulse fall time (ms)	22 $\pm$ 3	15–31	7.5	14.0

<sup>a</sup> For pulses per call, we report the median and the interquartile range

**Fig. 4** Upward frequency modulation of the male advertisement call of *Limnonectes palavanensis*. Fitted line was obtained by fitting a third degree polynomial using a linear mixed effects model with individual frog as the random effect (mean  $\pm$  SD frequency modulation of  $388 \pm 97$  Hz,  $N = 21$ )



the predictions of the sex-role reversal hypothesis in *L. palavanensis*: calling females outnumber calling males, and females vocalize more intensively than males do. Our characterization of the vocal repertoire of *L. palavanensis*, especially the female calling behavior, provides support for these predictions. We found that females not only call in response to a male advertisement call, but also initiate calling without a male acoustic stimulus. Female calling in *L. palavanensis* has not been previously reported. Spontaneous female calling has been observed also in *Limnonectes leporinus* (formerly *blythii*, Emerson 1992), but it has not been reported in other members of the genus. In general, calls of female frogs usually are very soft and hard to hear; therefore, their absence is more difficult to document than their presence (Wells 2007). Thus, it is possible that female calling is found in other species of *Limnonectes*, but has yet to be reported. Some hypotheses about the function of female calling include the idea that females initiate calling to aid in localization when males are widely dispersed in the breeding area (Given 1993). In *L. palavanensis*, males not only are scattered throughout the forest, but they also seem to be the less abundant sex, considering that when a male gives an advertisement call, it can attract more than one female at a time, despite calling infrequently.

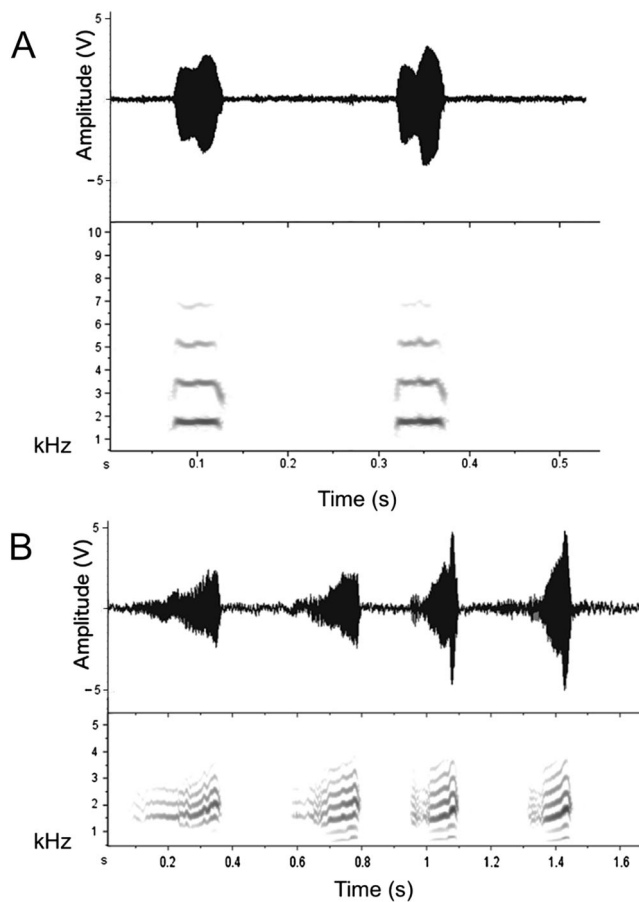
Females gather around a specific male and start calling spontaneously, in a manner reminiscent of leks in other species. This female lek-like behavior has never been observed before in anurans. Situations where more than two males are courting a female are the exception in *L. palavanensis*. In fact, more than two females calling around a male constituted 44.6% of our observations. This variation in the number of

mates available of each sex can influence mate choice. For example, in some populations of pipefish and seahorses where females are more abundant, several females approach a male for mating opportunities and compete with each other over access for a mate (Vincent 1992; Smith 1979). Thus, when females are more abundant, males become the choosy sex, usually choosing larger females (Berglund and Rosenqvist 1993; Berglund 1995; Monteiro et al. 2017). Our observations of up to four females around a calling male constitute evidence of an excess of females available for mating per night in this species, supporting our first prediction.

Although females of *L. palavanensis* call more frequently than males, females calling to a male and males giving the courtship call are ultimately more difficult to detect due to the close-range nature of both calls. Males can often be detected up to 20 m away (JGV, pers. obs.) when they produce the advertisement call, while detection distances for females were typically about 3 m. Therefore, we believe that the number of events where more than one female is calling around a male is likely to be higher due to these sex-specific differences in the detectability of calling individuals.

When one or more calling females approach a calling male, the male produces a different call as the females get closer. This vocalization was previously unknown, and we describe it as a male courtship call, since it is given only as a response to the female call. In some species, males produce courtship calls, possibly to make themselves more conspicuous to a given female (Gerhardt and Huber 2002; Wells 2007). Females of the torrent frog *Odorrana tormota* elicit a distinct vocalization from the males, similar to what we observed in *L. palavanensis*. Males of *O. tormota* also exhibit precise





**Fig. 5** **a** An oscillogram (top) and spectrogram (bottom) showing two calls of the same female *Limnonectes palavanensis*. Both oscillogram and spectrogram are on the same time scale. **b** An oscillogram (top) and spectrogram (bottom) of four consecutive courtship calls from a single male *L. palavanensis* given as a response to a single female calling (not shown). Both oscillogram and spectrogram are on the same time scale

phonotaxis toward female calls. Thus, this behavior seems to be more related to males being able to locate females in a noisy environment than to mate choice (Shen et al. 2008). In *L. palavanensis*, the exact function of the male courtship call is not clear, although it is only produced after a female has called. In species where both males and females are highly dispersed, female calling may have evolved to aid in location and signaling of reproductive status (Bush 1997). Female calling behavior may increase predation risk, because calling makes females more conspicuous; therefore, the trade-off has to have some compensatory benefits, e.g., increasing a female's ability to locate a high quality mate. In *L. palavanensis*, males do not always respond to a female call, but when they do, they respond within a narrow window of time. So, spontaneous female calling may aid in male localization if it successfully elicits male courtship calling.

When more than one female approaches a male *L. palavanensis*, an initial female call causes other females to start calling antiphonally. In males of the poison dart frog *Allobates femoralis*, antiphonal calling behavior is a sign of

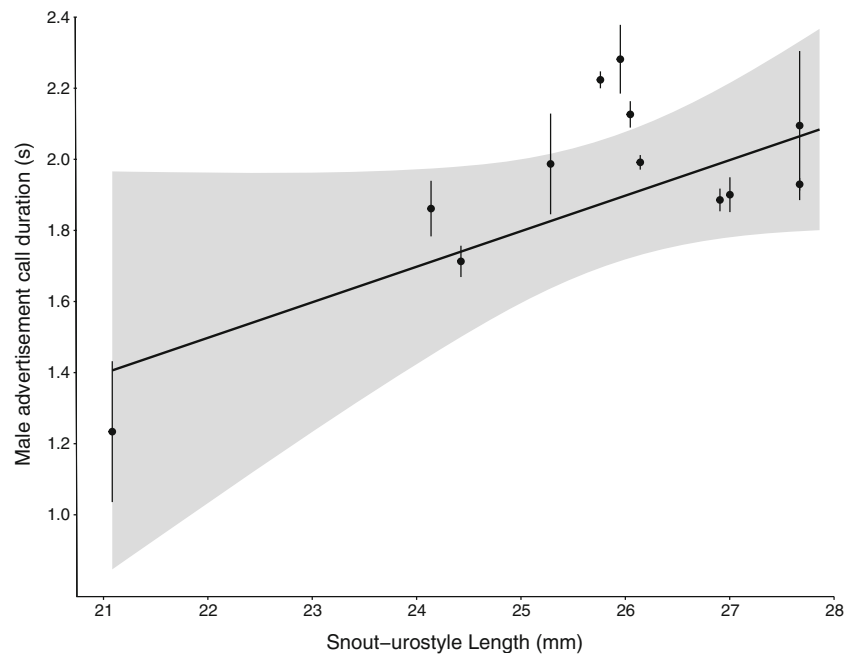
competition among males, and usually precedes physical aggression (Roithmair 1994). While we did not observe physical contact among females of *L. palavanensis*, females eavesdrop on other calling females approaching a nearby male and start calling themselves. This form of acoustic competition has also been observed in *Clinotarsus curtipes* (formerly *Rana curtipes*), where females compete with each other for mating opportunities when males are scarce (Krishna and Krishna 2005).

Advertisement calls signal to competitors to keep away while simultaneously attracting mates (Wells 1977). The term “female advertisement call” has been proposed before in other anurans (Given 1993; Tobias et al. 1998; Toledo et al. 2014). In *L. palavanensis*, the female call may be serving the dual function of eliciting a response from the male while simultaneously alerting other females of their presence. However, we refrain from using the term advertisement call for females of *L. palavanensis* since we do not have experimental evidence of the function of this call in mate attraction, female–female competition, or male mate choice.

Reports of female calling in frogs have increased recently (Boistel and Sueur 2002; Toledo et al. 2014). However, reports of spontaneous female calling are remarkably scarce (Boistel and Sueur 1997; Krishna and Krishna 2005). This unusual feature of the calling behavior of *L. palavanensis* females allowed us to estimate female calling rates and compare them with those of the males. To the best of our knowledge, this is the first instance of an anuran species in which female calling rates dramatically exceed male advertisement calling rates, results consistent with our second prediction. In birds, only in a few species do females commonly sing more often than males. In the Streak-backed Oriole (*Icterus pustulatus*), females call more often than males, probably as an aggressive signal while defending territories (Price et al. 2008). In the sex-role reversed African black coucal (*C. grillii*), females use vocalizations to defend large territories that encompass smaller male territories (Goymann et al. 2004), although, this is not the case for females of *L. palavanensis*, since they do not attract males to territories, but rather move through the forest floor homing in on the male advertisement calls.

Most aspects of the vocal behavior of species within the genus *Limnonectes* remain unknown. There are very few descriptions of the male advertisement calls of most species, and some of them are still considered voiceless (Emerson 1992), despite growing evidence of calling in species lacking vocal sacs (Matsui 1995; Rowley et al. 2014). The advertisement call of *L. palavanensis* is a moderately loud trill used in mate attraction. In contrast to other leaf-litter species with terrestrial egg deposition, which form clusters of calling males throughout the forest or near temporary ponds (Hauselberger and Alford 2005; Kaefer et al. 2012), males of *L. palavanensis* were not observed forming aggregations or choruses. Males of *L. palavanensis* called infrequently and on very few nights during the study period. Forest and leaf-litter species often

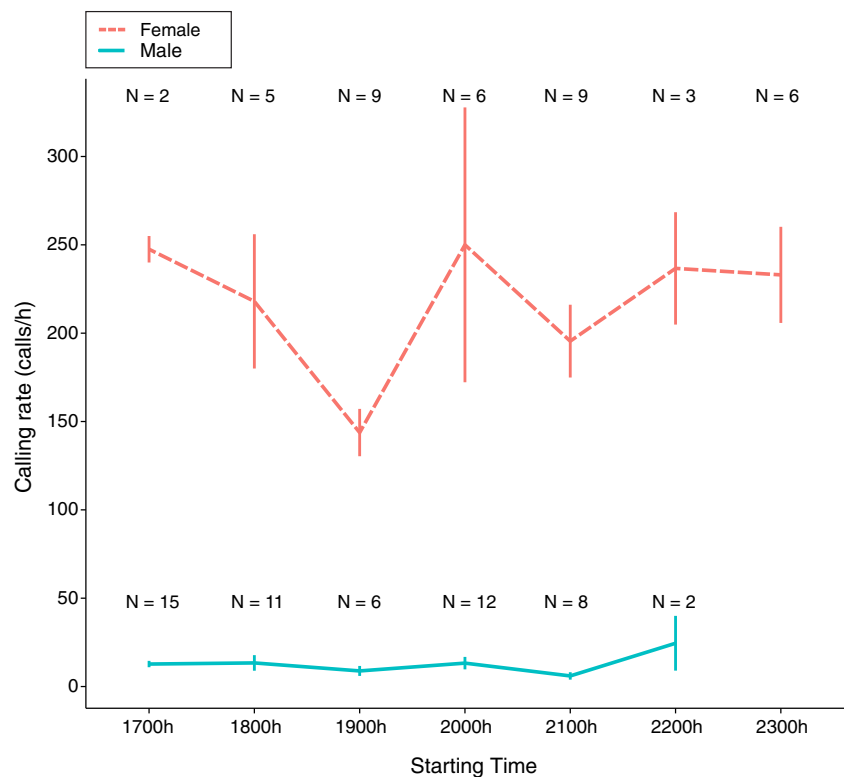
**Fig. 6** Correlation between advertisement call duration of male *L. palavanensis* and their snout–urostyle length. There is a positive correlation between call duration and body size in males ( $N = 16$ ,  $F_{1, 12.8} = 5.09$ ;  $P < 0.05$ ). Confidence intervals (95%) are shown in gray. Bars reflect  $\pm$ standard error



have low calling rates (Zimmerman and Bogart 1984), but the calling rate of *L. palavanensis* males is the lowest ever recorded. Because of this, the energetic costs of producing the advertisement call are likely to be low. Moreover, competition with other individuals is what drives energetic investment in calling behavior (Wells and Taigen 1986), but since the density of calling males per night is low, males are unlikely to invest much energy in calling.

Neither males nor females have true vocal sacs and therefore the ability to produce loud calls efficiently is limited. Males seem to have strong abdominal muscles for call production and bigger males produce longer calls (with more pulses), but females lack large abdominal muscles, evidenced by the fact that the eggs can be observed through the body wall. As a result, the calls of *L. palavanensis* females are shorter and lower in intensity than the male advertisement

**Fig. 7** Average hourly advertisement calling rates of male and female *Limnonectes palavanensis* individuals based on focal observations of 54 males and 40 females ( $N$  represents the number of individuals sampled per time period). Bars reflect  $\pm$ standard error



calls and are composed of a single note because they lack the machinery to produce longer and louder calls. While *L. palavanensis* females have a higher calling rate than males do, ultimately, the energetic costs of calling are likely to be low for both sexes.

Since the mating system of a species is determined in part by which sex is the limiting resource, individual tactics for acquiring mates will be influenced by the number of individuals available for mating at a given time (Emlen and Oring 1977). Despite the fact that male calls are longer, louder, and spectrally more complex than the females' calls, the more repetitive nature of the female calls presumably makes them easier to locate (Gerhardt and Huber 2002). The fact that females call spontaneously in the absence of male calls and call more frequently than males, coupled with the prolonged parental care by males (Goyes Vallejos 2016) and apparent scarcity of males in the habitat, suggests that receptive males are a limited resource for females. These aspects of the reproductive behavior and the calling behavior of *L. palavanensis* are consistent with a sex-role reversed mating system, making this species a good candidate for the first case of sex-role reversal in anurans.

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**Author's Contributions** JGV designed the study, collected and analyzed the data, and drafted the manuscript. TUG helped with the experimental design and data collection and provided comments on the manuscript. HHAS facilitated fieldwork. KDW helped with the experimental design of the study, drafting of the manuscript, and provided funding for fieldwork.

#### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical Approval** All the behavioral observations and experimental methods done in this study were done in accordance with the Institutional Animal Care and Use Committee at the University of Connecticut (Approved Protocol No. A12-028) and followed the guidelines of the Animal Behavior Society (ABS) for the treatment of animals in behavioral research.

## References

- Andersson M (1995) Evolution of reversed sex roles, sexual size dimorphism, and mating system in coucals (Centropodidae, Aves). *Biol J Linn Soc* 54:173–181
- Bee MA, Suyesh R, Biju SD (2013) Vocal behavior of the Ponnudi bush frog (*Raorchestes graminirupes*): repertoire and individual variation. *Herpetologica* 69:22–35
- Berglund A (1995) Many mates make male pipefish choosy. *Behaviour* 132:213–218
- Berglund A, Rosenqvist G (1993) Selective males and ardent females in pipefishes. *Behav Ecol Sociobiol* 32:331–336
- Boistel R, Sueur J (1997) Comportement sonore de la femelle de *Platymantis vitiensis* (Amphibia, Anura) en l'absence du mâle. *CR Acad Sci III-Vie* 320:933–941
- Boistel R, Sueur J (2002) XVIIth symposium of the international bioacoustic council—abstracts. *Bioacoustics* 13:77–102
- Bosch J (2001) Female reciprocal calling in the Iberian midwife toad (*Alytes cisternasi*) varies with male call rate and dominant frequency: implications for sexual selection. *Naturwissenschaften* 88:434–438
- Bush SL (1996) Why is double clutching rare in the Majorcan midwife toad? *Anim Behav* 52:913–922
- Bush SL (1997) Vocal behavior of males and females in the Majorcan midwife toad. *J Herpetol* 31:251–257
- Bush SL, Bell DJ (1997) Courtship and female competition in the Majorcan midwife toad, *Alytes muletensis*. *Ethology* 103:292–303
- Bush SL, Dyson ML, Halliday TR (1996) Selective phonotaxis by males in the Majorcan midwife toad. *Proc R Soc Lond B* 263:913–917
- Cooley JR, Marshall DC (2001) Sexual signaling in periodical cicadas, *Magicicada* spp. (Hemiptera: Cicadidae). *Behaviour* 138:827–855
- R Development Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>
- Ekstrom JMM, Burke T, Randrianaina L, Birkhead TR (2007) Unusual sex roles in a highly promiscuous parrot: the greater vasa parrot *Caracopsis vasa*. *Ibis* 149:313–320
- Emerson SB (1992) Courtship and nest-building behavior of a Bornean frog, *Rana blythii*. *Copeia* 1992:1123–1127
- Emerson SB, Inger RF, Iskandar DT (2000) Molecular systematics and biogeography of the fanged frogs of Southeast Asia. *Mol Phylogenet Evol* 16:131–142
- Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. *Science* 197:215–223
- Emlen ST, Wrege PH, Webster MS (1998) Cuckoldry as a cost of polyandry in the sex-role reversed jacana, *Jacana jacana*. *Proc R Soc Lond B* 265:2359–2364
- Geberzahn N, Goymann W, Muck C, ten Cate C (2009) Females alter their song when challenged in a sex-role reversed bird species. *Behav Ecol Sociobiol* 64:193–204
- Geberzahn N, Goymann W, ten Cate C (2010) Threat signaling in female song—evidence from playbacks in a sex-role reversed bird species. *Behav Ecol* 21:1147–1155
- Gerhardt HC (1991) Female mate choice in treefrogs: static and dynamic acoustic criteria. *Anim Behav* 42:615–635
- Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans. The University of Chicago Press, Chicago
- Given MF (1993) Male response to female vocalization in the carpenter frog, *Rana virgatipes*. *Herpetologica* 44:304–317
- Goyes Vallejos J (2016) Parental care and acoustic communication of the smooth guardian frog *Limnonectes palavanensis*, a Bornean frog with possible sex-role reversal. PhD Dissertation, University of Connecticut

- Goymann W, Wittenzellner A, Wingfield J (2004) Competing females and caring males. Sex-role reversal in African black coucals, *Centropus grillii*. Anim Behav 68:733–740
- Grafe TU, Bitz JH (2004) Functions of duetting in the tropical boubou, *Laniarius aethiopicus*: territorial defence and mutual mate guarding. Anim Behav 68:193–201
- Hauselberger KF, Alford RA (2005) Effects of season and weather on calling in the Australian microhylid frog *Austrochaperina robusta*. Herpetologica 61:349–363
- Illes AE, Yunes-Jimenez L (2009) A female songbird out-sings male conspecifics during simulated territorial intrusions. Proc R Soc Lond B 276:981–986
- Inger RF, Voris HK (1988) Taxonomic status and reproductive biology of Bornean tadpole-carrying frogs. Copeia 1988:1060–1061
- Inger RF, Voris HK, Walker P (1986) Larval transport in a Bornean ranid frog. Copeia 1986:523–525
- Judge KA, Swanson SJ, Brooks RJ (2000) *Rana catesbeiana* (Bullfrog): female vocalization. Herpetol Rev 31:236–237
- Kaefer IL, Montanarin A, da Costa RS, Lima AP (2012) Temporal patterns of reproductive activity and site attachment of the brilliant-thighed frog *Allobates femoralis* from Central Amazonia. J Herpetol 46:549–554
- Kokko H, Jennions MD (2008) Parental investment, sexual selection, and sex ratios. J Evol Biol 21:919–948
- Krishna S, Krishna S (2005) Female courtship calls of the litter frog (*Rana curtipes*) in the tropical forests of Western Ghats, South India. Amphibia-Reptilia 26:431–435
- Marquez R, Verrell PA (1991) The courtship and mating of the Iberian midwife toad *Alytes cisternasii* (Amphibia: Anura: Discoglossidae). J Zool 225:125–139
- Matsui M (1995) Calls produced by a “voiceless” frog, *Rana blythi* Boulenger 1920, from Peninsular Malaysia (Amphibia, Anura). Trop Zool 8:325–331
- Monteiro N, Carneiro D, Antunes A, Queiroz N, Vieira M, Jones A (2017) The lek mating system of the worm pipefish (*Nerophis lumbriciformis*): a molecular maternity analysis and test of the phenotype-linked fertility hypothesis. Mol Ecol 26:1371–1385
- Orlov NL (1997) Breeding behavior and nest construction in a Vietnam frog related to *Rana blythi*. Copeia 1997:464–465
- Pettitt BA, Bourne GR, Bee MA (2013) Advertisement call variation in the golden rocket frog (*Anomaloglossus beebei*): evidence for individual distinctiveness. Ethology 119:244–256
- Price JJ, Yunes-Jimenez L, Osorio-Beristain M (2008) Sex-role reversal in song? Females sing more frequently than males in the streak-backed oriole. Condor 110:387–392
- Price JJ, Lanyon SM, Omland KE (2009) Losses of female song with changes from tropical to temperate breeding in the New World blackbirds. Proc R Soc Lond B 276:1971–1980
- Rebar D, Bailey NW, Zuk M (2009) Courtship song’s role during female mate choice in the field cricket *Teleogryllus oceanicus*. Behav Ecol 20:1307–1314
- Rodríguez RL, Sullivan LE, Crocroft RB (2004) Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). Evolution 58: 571–578
- Roithmair ME (1994) Male territoriality and female mate selection in the dart-poison frog *Epipedobates trivittatus* (Dendrobatidae, Anura). Copeia 1994:107–115
- Rowley JLL, Le DTT, Hoang HD, Altig R (2014) The breeding behaviour, advertisement call and tadpole of *Limnonectes dabanus* (Anura: Dicroglossidae). Zootaxa 3881:195–200
- Searcy WA, Nowicki S (2005) The evolution of animal communication: reliability and deception in signaling systems. Princeton University Press, Princeton, NJ
- Shen JX, Feng AS, Xu ZM, Yu ZL, Arch VS, Yu XJ, Narins PM (2008) Ultrasonic frogs show hyperacute phonotaxis to female courtship calls. Nature 453:914–916
- Slater PJB, Mann NI (2004) Why do the females of many bird species sing in the tropics? J Avian Biol 35:289–294
- Smith RL (1979) Paternity assurance and altered roles in the mating behaviour of a giant water bug, *Abedus herberti* (Heteroptera: Belostomatidae). Anim Behav 27:716–725
- Summers K (1989) Sexual selection and intra-female competition in the green poison-dart frog, *Dendrobates auratus*. Anim Behav 37:797–805
- Suthers RA, Narins PM, Lin WY, Schnitzler HU, Denzinger A, Xu CH, Feng AS (2006) Voices of the dead: complex nonlinear vocal signals from the larynx of an ultrasonic frog. J Exp Biol 209:4984–4993
- Tobias ML, Viswanathan SS, Kelley DB (1998) Rapping in the African clawed frog *Xenopus laevis*. P Natl Acad Sci USA 95:1870–1875
- Toledo LF, Martins IA, Bruschi DP, Passos MA, Alexandre C, Haddad CFB (2014) The anuran calling repertoire in the light of social context. Acta Ethol 18:87–99
- Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) Sexual selection and the descent of man. Aldine-Atherton, Chicago, pp 136–179
- Ursprung E, Ringler M, Jehle R, Hödl W (2011) Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. Mol Ecol 20:1759–1771
- Vincent ACJ (1992) Prospects for sex role reversal in teleost fishes. Neth J Zool 42:392–399
- Wagner WE Jr, Reiser MG (2000) The importance of calling song and courtship song in female mate choice in the variable cricket. Anim Behav 59:1219–1226
- Wells KD (1977) The social behaviour of anuran amphibians. Anim Behav 25:666–693
- Wells KD (1978) Courtship and parental behavior in a Panamanian poison-arrow frog, *Dendrobates auratus*. Herpetologica 34:148–155
- Wells KD (1980) Social behavior and communication of a dendrobatid frog (*Colostethus trinitatis*). Herpetologica 36:189–199
- Wells KD (2007) The ecology and behavior of amphibians. The University of Chicago Press, Chicago
- Wells MM, Henry CS (1992) The role of courtship songs in reproductive isolation among populations of green lacewings of the genus *Chrysoperla* (Neuroptera: Chrysopidae). Evolution 46:31–42
- Wells KD, Taigen TL (1986) The effect of social interactions on calling energetics in the gray treefrog (*Hyla versicolor*). Behav Ecol Sociobiol 19:9–18
- Zimmerman BL, Bogart JP (1984) Vocalizations of primary forest frog species in the Central Amazon. Acta Amaz 14:473–519