

Temporal variation, duty cycle, and absolute calling effort during sustained calling of *Leptodactylus mystacinus* (Anura: Leptodactylidae)

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Abstract. We studied the within-night temporal call variation of *Leptodactylus mystacinus* using automated bioacoustic survey and pattern recognition techniques. Based on ca 50,000 detected calls we report on large-scale variation of dominant frequency, call duration, peak power, call interval, call period, and call rate. The “absolute” calling effort (as absolute seconds per hour spent on calling calculated as the sum of all detected calls during one hour) was between 165 and 716 s/hour (535 ± 191) with a peak at around 23:00 h, followed by a decrease until the end of the calling period. This might be due to vocal fatigue. In comparison with literature, the overall call production was higher than expected, but calling effort is comparable to estimates from the literature for other leptodactylid frogs. We hypothesize that species-specific mating tactics might be employed to achieve high calling effort during sustained calling. Further intra- and interspecific studies should aim at studying contest behaviour in mating frogs to test this hypothesis.

Key words. Amphibia, advertisement call, amplitude detection, automated bioacoustic survey, Bolivia, contest behaviour, sustained calling, overall call production.

Introduction

By using automated survey methods, scientists have to an increasing extent demonstrated that bioacoustics can be used as a key tool in assessing and measuring biodiversity (PIJANOWSKI et al. 2011, DEPRAETERE et al. 2012, GASC et al. 2013). In taxonomy, many scientists use species-specific animal communication signals for species delimitation, and especially in frogs, where no learning effect of the acoustic signal is known, descriptions of species-specific frog calls are commonly included in species descriptions as diagnostic characters (e.g., SCHNEIDER et al. 1993, HEYER et al. 1996, ANGULO & REICHEL 2008, LEMMON et al. 2008, MORAVEC et al. 2008, PADIAL et al. 2008, DE CARVALHO & GIARETTA 2013; see SCHNEIDER & SINSCH 2007 for a review). Hereby, frog calls have recently been used in combination with other lines of evidence, such as morphology and DNA taxonomy, to discover morphologically look-alike cryptic species (e.g., GLAW et al. 2010, JANSEN et al. 2011, FUNK et al. 2012, PANSONATO et al. 2013, FOUQUET et al. 2014, WANG et al. 2014).

Regarding large-scale variation of frog calls, however, only limited information is available. Although there is a huge body of literature on individual, intra- and interspecific call variation (e.g., SCHNEIDER 1967, 1968, 1978, SCHNEI-

DER & NEVO 1972, GERHARDT 1991, BEE & GERHARDT 2001, BEE et al. 2001, 2010, 2013, MARQUEZ & BOSCH 2001, BEE 2004, GASSER et al. 2009, RODRIGUEZ et al. 2010), as well as on temporal calling variation of anurans (e.g., BRIDGES & DORCAS 2000, BROOKE et al. 2000, TODD et al. 2003, DE SOLLA et al. 2006, WOGEL et al. 2006, BENEVIDES et al. 2009, JANSEN 2009, LLUSIA et al. 2013a,b, OSPINA et al. 2013, STEEN et al. 2013, AKMENTINS et al. 2014, WILLACY et al. 2015), however, only few studies exist on the individual temporal variation during sustained calling. For example, BREPSON et al. (2013) analysed in a laboratory study on average 11,500 calls per individual emitted in one night (in total more than 4.5 million calls from 36 males of *Hyla arborea*) to study the management of calling under energetic constraints.

Knowledge of the temporal variation in call traits might be relevant for taxonomists (e.g., for the delimitation of species based on call traits) on the one hand. On the other, calling is the most expensive behaviour in the lifespan of a male frog (e.g., TAIGEN & WELLS 1985, WELLS & TAIGEN 1986, 1989), and information on the individual calling effort is important for our understanding of sexual selection, contest behaviour, or energetic trade-offs (BREPSON et al. 2013, DYSON et al. 2013).

Here we use automated bioacoustic survey and pattern detection methods to study sustained calling of the South American frog *Leptodactylus mystacinus*. Besides testing techniques for the recording and analysis of large numbers of frog calls, the present study aims at providing information on individual and temporal call trait variation during hours of sustained calling, and providing for the first time data of the actual, i.e., calculated or “absolute”, calling effort per hour of a leptodactylid frog in the wild.

Material and methods

Leptodactylus mystacinus (Fig. 1A), a frog of the *L. fuscus* species group of the species-rich family Leptodactylidae, is distributed from southeastern Bolivia and eastern Brazil to Uruguay and through Paraguay to central Argentina (FROST 2014, DE SÁ et al. 2014). The advertisement call of *Leptodactylus mystacinus* was previously described from populations in Argentina (BARRIO 1965, DE SÁ et al. 2014), Paraguay (HEYER et al. 2003), and central Brazil (OLIVEIRA FILHO & GIARETTA 2008). JANSEN et al. (2011) stated that genetic and morphological data suggest that Bolivian populations might be referable to an unnamed lineage, however, this could not be confirmed until now.

Our study site was near the Biological Station “Centro de Investigaciones Ecológicas Chiquitos” on the cattle ranch San Sebastián (−16.3622°, −62.00225°, 500 m a.s.l.), 24 km south of the town of Concepción, Province of Ñuflo de Chávez, Santa Cruz Department, Bolivia. Currently, more than 40 species of frogs are known from this area (JANSEN 2009, JANSEN et al. 2009, 2011, SCHULZE et al. 2009, 2011, own publ. data).

Our recordings were made on 24 January 2012 with a weatherproof Song Meter SM2 digital audio field recorder (Wildlife Acoustics 2014) connected to a 12V car battery as power source (Fig. 1B). After detecting a calling male *Leptodactylus mystacinus* in the field, the Song Meter was

set up on the ground and connected to two omnidirectional microphones, with one being about 1.5 m from the calling frog. The recordings were automatically digitised at a sampling frequency of 22,050 Hz and 16 bit resolution. Recordings were stored on 32 GB SD cards in .wav format. We recorded from 22:00 to 6:00 h, resulting in a total of 480 minutes of recording. Temperature was recorded with the built-in temperature sensor and data logger of the Song Meter (measurements every 5 min).

Leptodactylus mystacinus is a territorial frog that usually calls from entrances of small channels or caves in termite mounds that are used for sheltering during the heat of the day (JANSEN, own data). The advertisement call of *Leptodactylus mystacinus* consists of a single note per call (see Fig. 2 for three typical calls) that are emitted continuously in long series, i.e., without being arranged in obvious call bouts, at a call rate of 250–400 calls per minute (HEYER et al. 2003). HEYER et al. (2003) reported on a call duration of 0.04–0.06 s and a dominant frequency (= fundamental frequency) that ranged from 2050–2500 Hz. The focal species is an ideal model system for studying the above mentioned questions, because the following reasons facilitate the automated detection of single signals by means of software: (1) the frog has a rather simple call (unpulsed, simple structure), (2) it is a solitary, territorial species (and not a chorusing frog), and (3) there is low ambient noise in its preferred habitat (e.g., few or no other syntopic frog species). Because the voucher was not collected, we identified the species according to the call description given by (HEYER et al. 2003).

Recordings were analysed on a personal computer using the software Raven Pro v 1.4 (Bioacoustics Research Program 2011). Frequency information was obtained through Fast Fourier Transformation (FFT, width 256 points). Spectrograms were obtained using the Hanning window function with 256 bands resolution. We filtered below 1,100 Hz and above 3,000 Hz to eliminate ambient noise. Using Ravens’ amplitude detectors (settings: amplitude threshold



Figure 1. A) Male *Leptodactylus mystacinus* (not the specimen recorded herein) from the study area at San Sebastián, Department of Santa Cruz, Bolivia; B) call perch (termite mound) and recorder setup in the field. Photos: Martin Jansen.

Table 1. Definitions and calculation formulae of temporal characters measured for this study.

Character	Definition respectively calculation formula	Unit of measure
call period	interval between the beginning of call n to the beginning of call n+1	ms
instantaneous call rate	(inverse of the interval between the beginning of call n to the beginning of call n+1 [ms]) \times 3600	1/hour
instantaneous calling effort or call duty cycle	call rate \times call duration or call duration/call period	s/hour or dimensionless (percentage as ratio of sound to silence)
absolute calling effort per hour	sum of all durations of calls that were emitted during one hour	s/hour

= 2500 units; smoothing = 0.046 s; delay compensation = 0.005 s), we automatically detected a total of 50,092 calls. From those, we deleted ca 600 false positives by choosing selections with (1) durations far below the minimum duration of the call, (2) relative peak amplitudes below the minimum peak amplitude of the call in our recording. Every case of a putative deletion was carefully checked manually. In addition, we checked the whole recording visually for false negatives and additionally selected ca 100 calls manu-

ally, all of them were less intense (usually at the beginning of a new series) and thus were not detected by the software, leading to a total of 49,573 calls for analysis. We assigned all the calls to one individual, because we found the possibility of callers switching unlikely. *Leptodactylus mystacinus* is bound to particular calling sites near a shelter and a switch in calling males would most probably result in some kind of male–male combat. However, all calls in our recording were continuously emitted (larger breaks in the series were

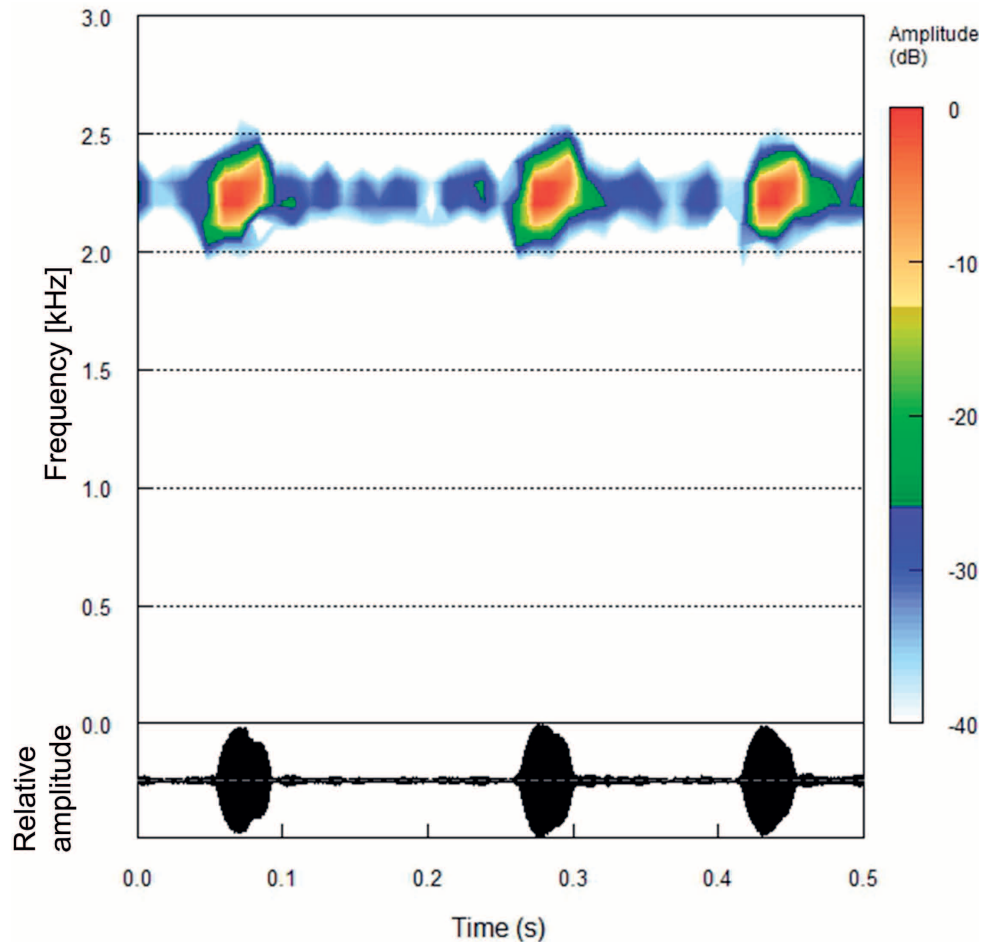


Figure 2. Spectrogram (above) and corresponding waveform (below) of a typical section of three calls of *Leptodactylus mystacinus*. Taken from the recording analysed herein (San Sebastián, Department of Santa Cruz, Bolivia, 24 January 2012, 25–26°C).

Table 2. Call parameters of a 194-min calling period ($n = 49,573$ calls) of one individual of *Leptodactylus mystacinus* from Bolivia.

	Mean (\pm SD)
dominant frequency	2024–2282 Hz (2136 \pm 36)
call duration	0.014–0.053 s (0.043 \pm 0.004)
peak power	70.8–107.3 dB (103 re 1 \pm 2)
call interval	0.085–56.564 s (0.192 \pm 0.482)
call period	0.127–56.603 s (0.235 \pm 0.482)
instantaneous call rate	64–28,347/hour (18,438 \pm 4073)
call duty cycle	2–1282 s/hour (796 \pm 191)
absolute calling effort	165–716 s/h (535 \pm 250.7)

lacking), no emitted territorial calls could be found during the recording session, and the relative amplitude of the calls was constant throughout the recording.

For each call, we measured the following call parameters with Raven: dominant frequency (Hz, the frequency at which the maximum power is seen, taken for the entire call), call duration (s, time from the beginning to the end of the vocalization), and peak power (dB re 1 dimensionless sample unit; in a greyscale spectrogram, the peak power is the power at the darkest point). We calculated the call period (interval between the beginning of call n to the beginning of call $n+1$). Based on this measurement, we estimated the instantaneous call rate (calls per hour) ([inverse of the interval between the beginning of call n to the beginning of call $n+1$] \times 3600), and the instantaneous call effort or call duty cycle (sensu KLUMP & GERHARDT 1992) as the product of call rate and call duration (e.g., TAIGEN & WELLS 1985, TARANO & FUENMAYOR 2014), respectively the ratio of call duration to call period (e.g., GERHARDT et al. 2000). In addition, we calculated the “absolute call effort” per hour, which we define here as an absolute measurement of time spent calling or seconds per one hour called (sum of all durations of calls that were emitted during one hour). See Table 1 for the definition and calculation formulae of measured temporal characters within this study.

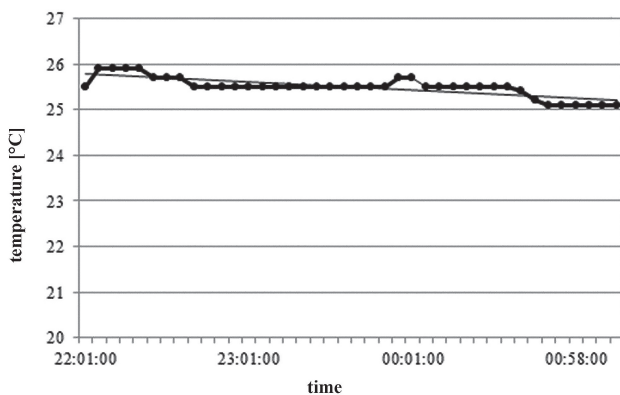


Figure 3. Temperature during calling activity measured with the built-in temperature sensor and data logger of the recording device (see text for details).

Statistical analysis was performed in R (R Development Core Team 2010, version 3.0.2). For smoothening the data to means of 5 minutes, we used the R package “Openair” (CARSLAW & ROPKINS 2012). To visualize audiospectrograms and waveforms, we used the R package “Seewave” (SUEUR et al. 2008).

Results

Within the 480 min of recording, the frog was active for 194 min and 10.9 s. Temperature varied from 25.1 to 25.9°C (25.5 ± 0.2) during the calling period (Fig. 3). During this period, we detected 49,573 emitted calls. Three typical successive calls are shown in Figure 2, and a typical five-minute section is shown in Figure 4. Call parameters of this 194-min calling period are shown in Table 2: We found an overall dominant frequency of 2,024 to 2,282 Hz, a call duration of 0.014 to 0.053 s, a peak power of 70.8 to 107.3 dB $^{-1}$, and a mean call interval of 0.192 s (Table 2). In addition, we measured a mean call period of 0.235 s, a mean instantaneous call rate of 18,438 calls per hour, a mean call duty cycle of 796 s/h, and an absolute calling effort of 535 s/hour (Table 2).

There was a high variation in all of these values during one night (Figs 5A–D), and all values seem to be affected by the time of the night. In dominant frequency, call duration and call duty cycle, there was an increase from 22:00 h to around 23:00 h, followed by a decrease to around midnight, and then a smaller second peak around 01:00 h and a decrease towards the end of the night. The absolute call effort (as the sum of all call durations during one hour) varied from 164.9 to 715.9 s/h between hours, had a peak of activity around 23:00 h, and was followed by an overall decrease (Fig. 5D).

Discussion

The specific characters of the advertisement call of *Leptodactylus mystacinus* of this study (Table 2) agree well with those revealed by previous studies. According to literature, the advertisement call consists of a single, unpulsed note emitted at rates of 213 (OLIVEIRA FILHO & GIARETTA 2008) to 250 to 400 calls per minute (BARRIO 1965, HEYER et al. 2003, DE SÁ et al. 2014) (these equal 12,780, respectively 15,000 to 24,000, calls per hour). The values of call duration in the literature vary: HEYER et al. (2003, as well as DE SÁ et al. 2014) reported on a call duration of 0.04–0.06 s, OLIVEIRA FILHO & GIARETTA (2008) measured a call duration of 0.04 s ($n = 18$ calls, three males, SD = 0), and BARRIO (1965) gave a value of 0.10 s (without providing sample size or SD). HEYER et al. (2003) suggested that the high values given by BARRIO (1965) could be due to over-recording or microphone ringing. HEYER et al. (2003) measured a dominant frequency (= fundamental frequency) that ranges from 2,050–2,500 Hz [BARRIO (1965): 2,200–2,500 Hz], without harmonics. OLIVEIRA FILHO & GIARETTA (2008)

stated 2,239 Hz as dominant frequency, and a mean call interval of 0.18 s ($n = 18$ calls, three males, $SD = 0.12$). However, it has to be mentioned that BARRIO (1965) and HEYER et al. (2003) did not give the sample size of analysed calls, and no sample size for each individual is given by OLIVEIRA FILHO & GIARETTA (2008) either.

Even though species-specific, advertisement calls may exhibit considerable variability due to ambient temperature and individual body size (e.g., ZWEIFEL 1959, 1968, GERHARDT & MUDRY 1980, RODRIGUEZ et al. 2015; see reviews in GERHARDT & HUBER 2001, SCHNEIDER & SINSCH 2007). However, the influence of temperature and individual body size on call traits can be ignored in our data set, because temperature hardly varied during that night (Fig. 3) and body size of the studied individual was constant as well. Instead, our results showed a significant influence of time on the variation in call traits. For example, the dominant frequency deviated by about 12% from the mean during the night, and call duration by 90% from the mean. Similar to our results, CASTELLANO & GAMBA (2011) found that call duration and pulse rate, both of which are commonly used as diagnostic characters in species descriptions, were variable during sustained calling of *Hyla intermedia*. They hypothesized that this might be due to different strategies

to avoid vocal fatigue, a phenomenon that might be widespread among species with high vocal activity during mating (HUMFELD 2013, PITCHER et al. 2014). Our study found a peak of calling effort during one nightly activity period and most probably, because it is related to this (see below), a peak in the metabolic rate at the same time. The decrease towards the end of the night might as well be due to vocal fatigue or body condition, or to a change in motivation for some or other reason, however, this hypothesis needs to be tested. As to how far such temporal variation or plasticity of call traits (CASTELLANO & GAMBA 2011, present study) might actually affect the practical use of frog calls by taxonomists for species delimitation has to be tested in future studies and should be analysed based on more data from different individuals and species.

Several studies have demonstrated that calling effort is a determinant of oxygen consumption in frogs (e.g., *Hyla arborea*: BREPSON et al. 2013; *Hyla versicolor*: TAIGEN & WELLS 1985, WELLS & TAIGEN 1986; *Dendropsophus microcephalus*: WELLS & TAIGEN 1989, SCHWARTZ et al. 1995; *Engystomops pustulosus*: e.g., BUCHER et al. 1982, POUGH et al. 1992; *Pseudacris crucifer*: WELLS et al. 1996). However, although these studies suggest that calling is probably the energetically most expensive activity in the lifetime of

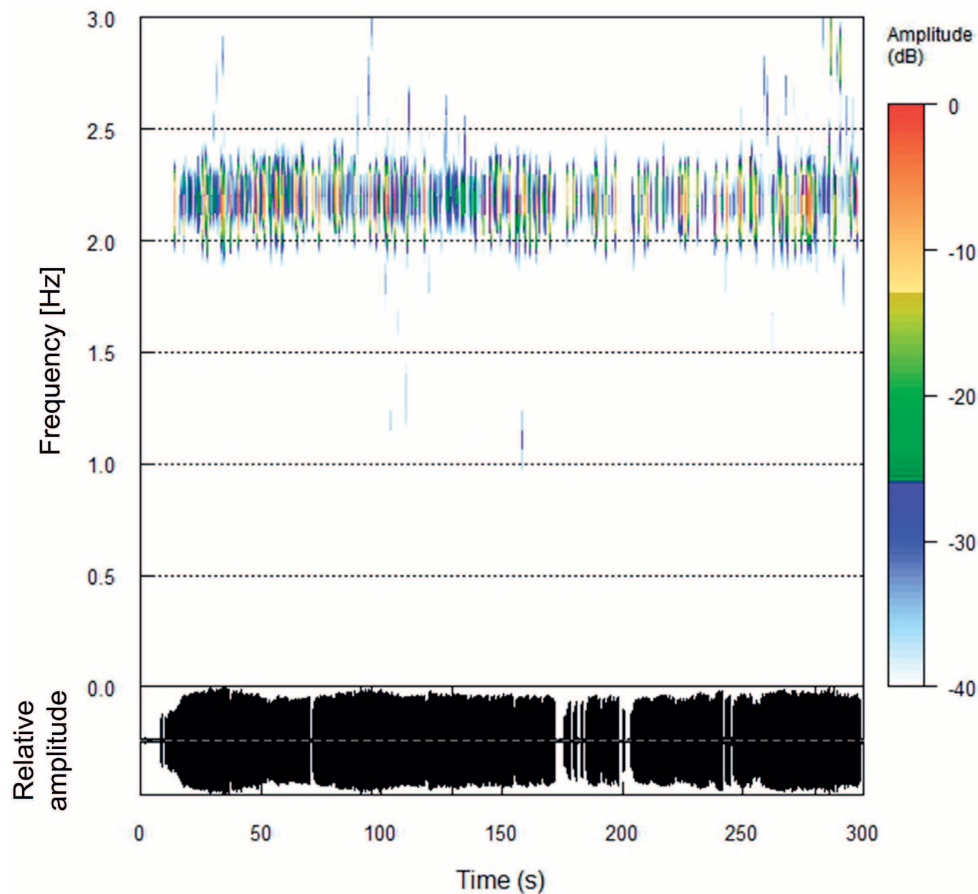


Figure 4. Spectrogram (above) and corresponding waveform (below) of a 5-minute section of calls of *Leptodactylus mystacinus*. Taken from the recording analysed herein (San Sebastián, Department of Santa Cruz, Bolivia, 24 January 2012, 25–26°C).

a male frog (see also POUGH et al. 1992, PRESTWICH 1994, WELLS 2001), practically nothing is known about the influencing external (e.g., climate, social context) and internal (e.g., metabolic rate, energy reserves, body condition) factors of the absolute, i.e., not estimated, individual calling effort in the wild (e.g., SCHWARTZ et al. 1995, DYSON et al. 2013). For example, no absolute measurements of call production or calling effort of other *Leptodactylus* species in the wild have been recorded until now. However, WELLS (2007, Table 5.2) compiled some estimates of the duty cycle of selected frog species based on calling rates and call duration from the literature, including one congener, *Leptodactylus fragilis* (original data from BEVIER 1995). According to WELLS (2007), the duty cycle (estimated calling effort per hour) of *L. fragilis* is 690 s/h, and the estimated number of “notes” per night is 16,450 (as the call of *L. fragilis* is like the call of *L. mystacinus* composed of

unpulsed and single notes, with “note” being synonymous with “call”). The duty cycle of *L. mystacinus* found herein is slightly higher (mean 796 s/hour), and the absolute calling effort per hour is slightly lower (mean 535 s/h). Regarding the absolute number of emitted calls (or notes) emitted during one night, our results are limited. Recording only started when the frog was detected (at 22:00 h), but this species usually already starts calling ca three hours earlier, namely approximately one hour after sunset (M. JANSEN, own data; in the present case around 19:00 h). Extrapolation from the results of the present study (i.e., the detected 50,000 calls in ca three hours plus ca three hours of calling with another 50,000) gives an estimate of around 100,000 calls per night for this individual. Preliminary studies on another leptodactylid frog from Bolivia, *L. syphax*, revealed a mean absolute calling effort of 800–1100 s/h with 10,300 to 11,550 calls per night of one single male (M. JANSEN, own

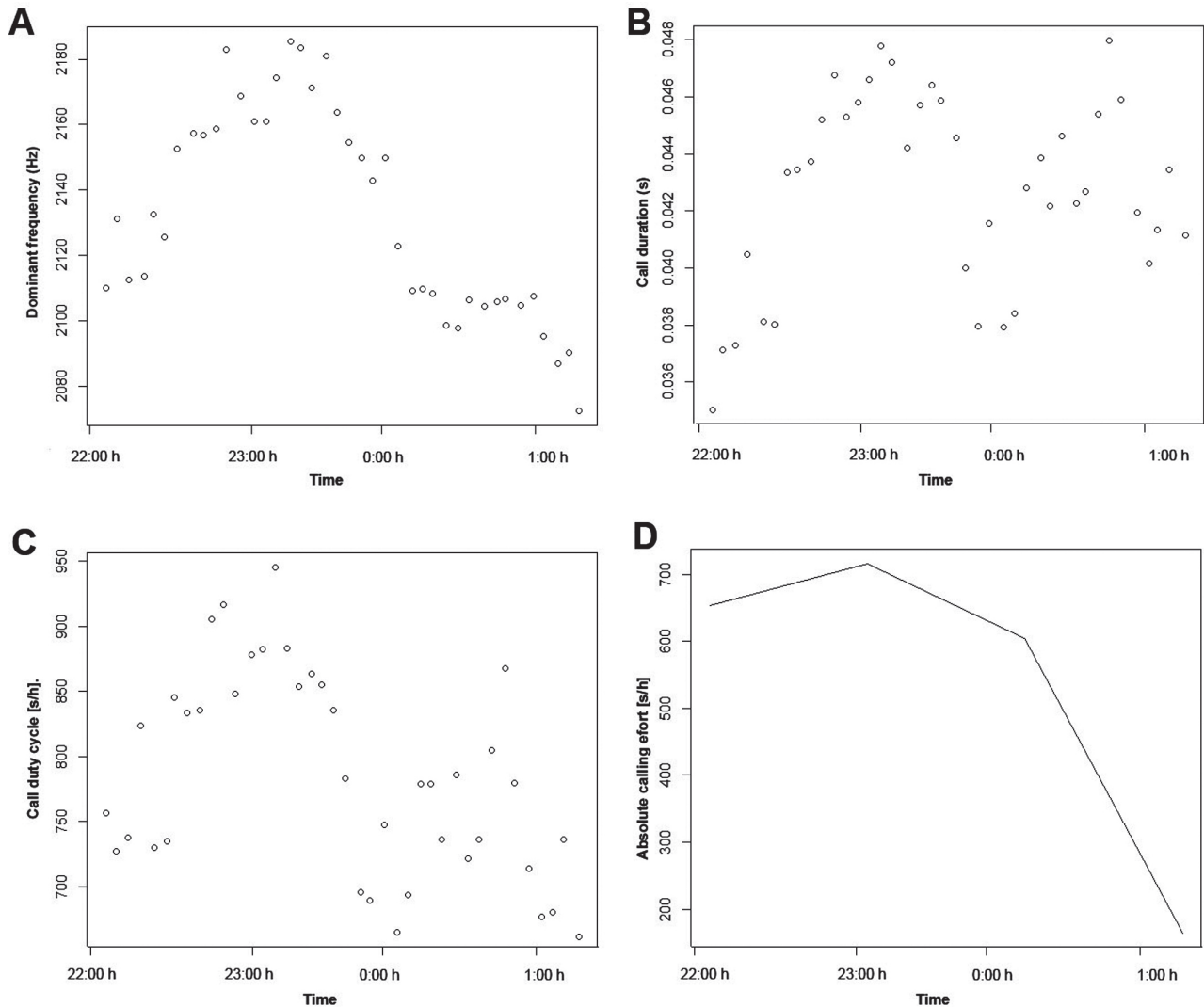


Figure 5. Graphs showing variation in calls and calling activity of the recorded individual of *Leptodactylus mystacinus* during one night (A–C smoothened data, see text): A) dominant frequency [Hz]; B) call duration [ms]; C) call duty cycle or instantaneous calling effort [s/h]; D) absolute call effort per hour [s/h], shown for every hour.

data). Even higher absolute numbers of calls per night are expected from a close relative of *L. mystacinus*: CASSINI et al. (2014) reported the call rate of *L. cupreus* as amounting to 14 calls/s, which would result in 50,400 calls per hour, and – given that the frog calls continuously throughout 4 to 6 hours – which amounts to an impressive number of 200,000 to 300,000 calls per night. However, all-night recordings of this and other species are needed to prove such extremely high calling performances. Further, it can be hypothesized that there might be species-specific mating tactics to achieve a high calling effort during sustained calling (e.g., many short calls like in *L. mystacinus* versus fewer but longer calls in *L. syphax*, own data). Nevertheless, far more data are needed to comprehensively rank and compare absolute calling efforts and mating tactics in selected frog species (M. JANSEN, in prep.).

Data on the variation in the hourly, daily, or seasonal calling effort of individual frogs are important, because, e.g., “until such information is available, it will be impossible to construct reliable annual energy budgets” for frogs (WELLS 2007: 229). Field measurements of the absolute calling effort are an indirect measurement of the metabolic rate, and therefore could shed some light on the management and strategy of calling, their underlying principles of physiological constraints and consequences for fitness, as well as different evolutionary scenarios. Future studies should include long-term studies of individual frogs (e.g., from one year to another as suggested by DYSON et al. 2015) to study contest behaviour during mating season in the context of evolution and sexual and natural selection.

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