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Leptodactylus flavopictus: temporal calling activity and tadpole redescription

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Abstract. Anuran calling activity is linked to adequate environmental conditions, especially relative humidity, temperature, and rainfall. Consequently, the breeding season is frequently concomitant to the rainy season for most anuran species. This applies in particular to species that reproduce in temporary water bodies, such as our focal species *Leptodactylus flavopictus*. We assessed the daily and annual calling activity of *L. flavopictus* through passive acoustic monitoring during a one-year period at Reserva Betary, southeastern Brazil. We redescribe its advertisement call and tadpole, adding details previously unavailable. Adults of *L. flavopictus* presented seasonal activity, with calls being detected mostly between August and December. The most intense calling activity (in terms of greater calling rates) was detected during spring between October and November. Calling activity was predominantly nocturnal, with an increase of call rates soon after sunset, peaking about 6 hours after sunset. The advertisement call of *L. flavopictus* is a short, non-pulsed single, note, low pitched (280–680 Hz), and contains harmonics. The tadpole presented morphological traits similar to those of other leptodactylids from temporary ponds, such as *L. vastus,* for example. The presence of keratinized mouthparts, the shape of labial tooth, and a simple internal oral morphology suggest that this species is at least facultatively carnivorous. During this study, we collected more than 200 hours of soundscape recordings, which included vocalizations of other animals (as birds, other frogs, and insects) that may be used for future community ecology research. We, therefore, reinforce the use of passive acoustic monitoring for studies on anuran behaviour, and the inclusion of advertisement calls and tadpole descriptions in taxonomic and systematic studies, as these features have shown to be important in elucidating questions on anuran evolution.

Key words. Amphibia, Anura, Leptodactylidae, Atlantic rainforest, bioacoustics, internal oral morphology, amphibian larvae, passive acoustic monitoring.

Introduction

Acoustic signalling is the main means of communication used by anurans for their intraspecific interactions (Wells 1997, 2007), with few exceptions of mute species (Emerson & Inger 1992, Augusto-Alves et al. 2018). Hence, call detection, as a proxy to measure activity, is widespread in anuran biology studies (e.g., Bertoluci & Rodrigues 2002, Crouch III & Paton 2002, Boquimpani-Freitas et al. 2007, Canavero et al. 2008, Dorcas et al. 2009). Anuran calling activity is generally related to adequate environmental conditions, such as relative humidity, temperature and rainfall (Wells 2007, Steelman & Dorcas 2010, Llusia et al. 2013). For example, most anuran species depend on water bodies to reproduce and, therefore,

their breeding seasons are concurrent with the rainy season (e.g., Aichinger 1987, Bertoluci & Rodrigues 2002, Prado et al. 2005, Ximenez & Tozetti 2015, Ferreira-Silva et al. 2016). This is particularly true for species that use temporary water bodies for their reproduciton, such as our focal species *Leptodactylus flavopictus* (Supplementary Fig. 1), which reproduces in temporary or small permanent ponds (Bokermann 1957, Haddad et al. 2013, Martins et al. 2020).

Besides the need for adequate environmental characteristics, the activity pattern of each species is influenced by the assamblage structure. Especially for anurans, acoustic activity may be influenced by the calling activity of sympatric males of sympatric species (Protázio et al. 2015). For this reason, it is common to identify spatial/temporal

partitioning in the activity patterns of different species that share the same breeding habitat (Sinsch et al. 2012, Protázio et al. 2015, Bignotte-Giró et al. 2018). The activity pattern of each species may be indicative of relationships between species and the environments, and thus it is relevant to understand the structure and habitat use in natural communities (Gibbons & Bennett 1974).

In order to assess the activity of anuran species we must have proper descriptions, besides of adult morphotypes, of their calls and tadpoles. Information on vocal repertoire can be used to distinguish species, since the advertisement call is used to attract mates (Toledo et al. 2015, Köhler et al. 2017) and acts as a prezygotic barrier (Lodé & Pagano 2000, Pröhl et al. 2006). It is, therefore, under sexual and natural selection (Forester & Czarnowsky 1985, Tobias et al. 2011). Features of tadpoles are also helpful for identifying species, and traits related to the internal oral morphology have been useful for the differentiation of species within the family Leptodactylidae (Ruggeri & Weber 2012).

Although brief descriptions of the advertisement call of *L. flavopictus* (de Sá et al. 2014) and tadpole (Bokermann 1957, Martins et al. 2020) are available, detailed descriptions are still wanting, as is specific ecological and natural history information. Here, we describe the calling activity pattern, annual and daily, of *L. flavopictus* through passive acoustic monitoring (PAM). Based on the data obtained, we also compare the advertisement calls of two isolated populations. Additionally, we provide for the first time a description of the internal oral morphology of tadpoles, which should facilitate future taxonomic studies.

Methods
Acoustic monitoring and advertisement call description

To assess the calling activity of *Leptodactylus flavopictus* we installed an autonomous acoustic recorder (Song Meter SM4) in an area of occurrence of this species in the Reserva Betary, Instituto de Pesquisa da Biodiversidade (IPBio: 24°35'26.39" S, 48°38'1.33" W; 99 m a.s.l.), Iporanga, São Paulo, southeastern Brazil. This area is covered with dense vegetation and has a deep layer of leaf litter, where water accumulating during the rainy season forms temporary ponds (Supplementary Fig. 2). The autonomous acoustic recorder was configured to record 10 minutes of audio with 24 kHz and 16-bit resolution per hour, during 24 consecutive hours. Recordings were made on one day per week from 21 March 2017 (sampling week 1) to 13 March 2018 (sampling week 52), i.e., for a full year (Supplementary Table 1). We analysed the recordings with Raven 1.5 PRO (Bioacoustics Research Program 2014), using the following configuration: 60% contrast, 50% brightness, and 1024 FFT. Each call was visually identified based on spectral and temporal parameters (see below). We considered recordings with no *L. flavopictus* calls as inactivity of this species, and recordings with calls (call

rate = calls/minute) to evaluate its annual and daily calling activity.

Temperature and precipitation data were provided by the Instituto Nacional de Meteorologia (INMET 2019) from its closest meteorological station (about 75 km straight-line distance) in the municipally of Registro.

We analysed calls from two different populations of *L. flavopictus*: Reserva Betary and Ribeirão Branco (24°14' S, 48°45' W), São Paulo, Brazil. For the population from Reserva Betary, we selected five different recordings containing advertisement calls (acquired with PAM) for analyses. For the Ribeirão Branco population, we obtained two recordings from the sound archive Fonoteca Neotropical Jacques Vielliard (FNJV 34032 and 34033). We normalized the calls using Adobe Audition, removing DC offset (mean amplitude displacement from zero), centring on vertical and to the maximum amplitude of -1.0 dB. We then analysed these calls in Raven 1.5 PRO (using 60% contrast, 50% brightness, and 1024 FFT). Calls were characterized using the following parameters: call duration; inter-call interval; call rate; peak of dominant frequency (peak frequency function in Raven); minimum frequency (frequency 5% function in Raven); maximum frequency (frequency 95% function in Raven); and frequency bandwidth (BW 90% function in Raven). All our recordings from Reserva Betary are now deposited at the Fonoteca Neotropical Jacques Vielliard (FNJV 41388-43828).

Tadpole description

Tadpoles were collected in the Reserva Betary at a temporary pond (the same site at which calls were recorded) on 30 November 2017. Some of the tadpoles were raised to metamorphosis and then compared to a juvenile preserved in the amphibian collection of the Museu de Zoologia "Prof. Adão José Cardoso", Universidade Estadual de Campinas (ZUEC-AMP 24300) to confirm its specific identity. On 23 December 2017, we collected eggs from a foam nest found in a temporary pond of about 20 cm in depth at the same locality. Later, it was verified that the tadpoles from this nest were identical to those previously collected, and they were subsequently preserved in 8% formalin. The description presented herein is based on a series of 22 tadpoles between the stages 28–39 (Gosner 1960) and measurements are provided for 11 specimens at stages 35–37. Abbreviations of measurements (in mm) are: total length (TL), body length (BL), body height (BH), body width (BW), tail length (TAL), maximum tail height (MTH), tail muscle height (TMH), tail muscle width (TMW), maximum lower fin height (LFH), maximum upper fin height (UFH), eye diameter (ED), nostril diameter (ND), interorbital distance (IOD), internarial distance (IND), snout–narial distance (SN), eye–snout distance (ES), snout–spiracle distance (SS), and oral disc width (ODW). Measurements follow Haas & Das (2011) and Lavilla & Scrocchi (1986) and were obtained using a Zeiss stereomicroscope (Stemi DV4) with millimetric ocular, except for TL, BL, and TAL

which were taken with a digital calliper. For examining the internal oral morphology, we dissected two specimens at Stages 35 (ZUEC-AMP 24301, 24465) following the procedures described in Wassersug (1976). Photographs of oral discs were taken with a digital camera AxioCam attached to the stereomicroscope steREO Discovery.V20, except for those of the labial tooth that were taken under a Zeiss Axio Imager M2 light microscope equipped with DIC and a digital AxioCam MRC5.

Statistical analyses

We fitted a logistic regression using a General Linear Model (GLM) to correlate *L. flavopictus* calling activity with accumulated rainfall and maximum and minimum temperatures on the day of sampling. To test for a correlation between call rate and rainfall, we ran a Spearman's rank correlation test. Statistics were performed in R v. 3.5.1 (R Core Team 2018).

Results
Advertisement call

In both the Iporanga and Ribeirão Branco populations the advertisement call of *L. flavopictus* was a non-pulsed single note (= simple calls) with harmonics (Fig. 1). The spectral and temporal parameters analysed present similar values between populations and are presented in Table 1.
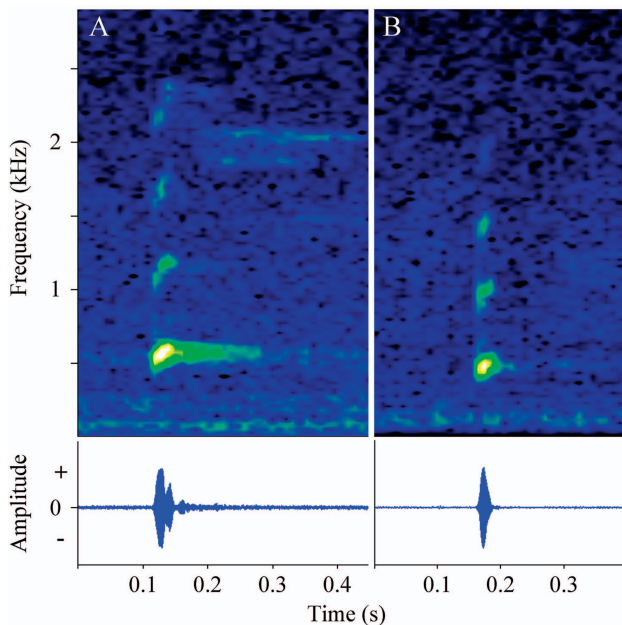

Figure 1. Spectrogram (top) and waveform (bottom) of the advertisement call of *Leptodactylus flavopictus* recorded in the municipally of Iporanga (A) and Ribeirão Branco (B), both in the state of São Paulo, Brazil.

Daily and annual calling activity

Adults of *L. flavopictus* from the Reserva Betary presented a seasonal activity, with calls being detected mainly between August (sampling week 23) and December (sampling week 38). The most intense activity, i.e., with the greatest call rates, was detected in spring, between October (sampling week 30) and November (sampling week 35). During the other periods of the year, calls were either absent or sporadic (Fig. 2A). The best model to explain the presence of a calling male included rainfall and minimum temperature (AIC = 74.27, P = 0.03). However, the logistic regression showed that neither rainfall nor minimum temperature were significantly influencing the presence of a call (β = -0.1 ± 0.05, Z = -1.8, P = 0.06, 95% CI -0.02, 0). Also, the Spearman's correlation indicated a negative relationship between call rate and rainfall (ρ = -0.31, P = 0.03).

Calling activity was mostly nocturnal, with an increased call rate soon after sunset, peaking about 6 hours after sunset and diminishing considerably or ending during or just before sunrise (Fig. 2B).

Tadpole

External morphology. Average measurements of 11 tadpoles at stages 35–37. Mean total length 53.96 mm ± 3.7 (50–60). Tadpoles elongated, wider than high (BH/BW = 0.88 ± 0.05). Body ovoid in dorsal view and elliptic in lateral view (Fig. 3A); length about 40% of total length (BL/TL = 0.4 ± 0.02). BH about 33% of body length (BH/BL = 0.33 ± 0.03), and BW about 37% of body length (BW/BL = 0.37 ± 0.04). Snout short and rounded (Fig. 3B). Nostrils positioned dorsally, rounded, directed dorsolaterally, closer to the snout than to the eyes; diameter 3% of body with (ND/BW = 0.03 ± 0.01); IND 25% of body with (IND/BW = 0.25 ± 0.03). Eyes large, positioned dorsally and directed dorsolaterally (Fig. 3B); diameter approximately 10% of body width (ED/BW = 0.11 ± 0.05), IOD almost half of body width (IOD/BW = 0.46 ± 0.03). Spiracle single, lateral, sinistral, short and tubular, directed posterodorsally; its inner wall fused to body without free extremity; located nearly in the middle of the body (SSD/BL = 0.40 ± 0.02). Vent tube long, wide and located medially, attached to the ventral fin, with medially-directed opening. Intestine mass horizontally positioned (Fig. 3C). Tail long, almost as high as the body (TAH/BH = 0.92 ± 0.10). Dorsal fin slightly convex, originating behind the body–tail junction, higher than ventral fin (VF/DF = 0.84 ± 0.07) and gradually diminishing toward the tip; 30% of TAH (DF/TAH = 0.32 ± 0.05) in its larger portion. Ventral fin straight, almost parallel to the tail musculature. Tail musculature well developed and robust (TMH/TAH = 0.61 ± 0.08, TMW/BW = 0.52 ± 0.04), finely reticulated with myomeres, v-shaped, orientated towards the tail tip, reaching the rounded tip of the tail. Lateral line system visible. Oral disc anteroventral (Fig. 3C); its width approximately 30% of BW (ODW/BW = 0.32 ± 0.04). Labial tooth row formula (LTRF) 2(2)/3(1),

Table 1. Spectral and temporal parameters of the advertisement call of *Leptodactylus* spp. of the *L. pentadactylus* species group. Values are presented as mean ± standard deviation (range, sample size).

| Species	Call duration (s)	Intercall interval (s)	Call rate (call/m)	Peak of dominant frequency (Hz)	Minimum frequency (Hz)	Maximum frequency (Hz)	Frequency bandwidth (Hz)	Pulsed call	Harmonic structure	Reference
L. fallax	0.18–0.20	–	up to 42	500–1500	–	–	–	yes	no	Kaiser (1994)
L. flavopictus (Iporanga)	0.35±0.06 (0.17–0.56)	1.46±0.83 (0.31–4.83)	30. 65±15.94 (17.78–56.93)	592.67±53.34 (281.20–679.70)	535.34±52.25 (257.80–562.50)	664.14±44.37 (375.00–1007.80)	128.87±51.64 (93.80–492.20)	no	yes	This study
L. flavopictus (Ribeirão Branco)	0.17±0.13 (0.07–0.44)	1.47±0.76 (0.65–3.65)	44.76±24.58 (27.38–62.14)	565.56±63.58 (492.20–632.80)	524.80±62.17 (445.30–585.90)	605.80±57.38 (515.60–679.70)	81.02±13.74 (70.30–117.20)	no	yes	This study
L. flavopictus	0.06–0.09	–	–	650	–	–	–	no	not evidentiated	de Sá et al. (2014)
L. knudseni	0.16–0.43	–	16–66	340–700	–	–	–	yes	yes	Heyer & Heyer (2006)
L. labyrinthicus	0.17±0.03	1.00±0.13 (0.72–1.35)	45.1±6.9	391.3±42.4	208.1±38.8	1298.8±56.8	–	no	yes	Zina & Haddad (2005)
L. lithonaetes	0.62–0.83	1.9–126	–	596–3187	462–897	–	–	yes	yes	Heyer & Barrio-Amorós (2009)
L. myersi	0.33–0.36	–	36	600–690	–	–	–	yes	not evidentiated	Lescure & Marty (2000)
L. pentadactylus	0.18–0.40	–	4–37	680–1030	340–860	–	–	yes	not evidentiated	Heyer (2005)
L. peritoaktites	0.20–0.30	–	34–37	~860	550–690	–	–	yes	not evidentiated	Heyer (2005)
L. rhodomystax	0.12–0.23	–	13.8	3700–5770	–	–	–	no	yes	Zimmerman & Bogart (1988)
L. rhodonotus	0.05 (0.04–0.07)	–	173.2±31.7 (106–214)	1680–2530	–	–	–	yes	yes	Köhler & Lötters (1999)
L. rugosus	0.78–0.91	–	–	609–2842	484–913	–	–	yes	yes	Heyer & Barrio-Amorós (2009)
L. savagei	0.24–0.42	–	40–49	350–520	300–345	–	–	yes	yes	Heyer et al. (2010)
L. stenodema	~0.36	–	–	760–900	–	–	–	yes	no	Heyer (1979)
L. turimiquensis	0.33	–	–	~400	–	–	–	yes	yes	Heyer (2005)
L. vastus	0.20 (0.19–0.21)	–	54	390	300	–	–	yes	yes	Jansen & Schulze (2012)
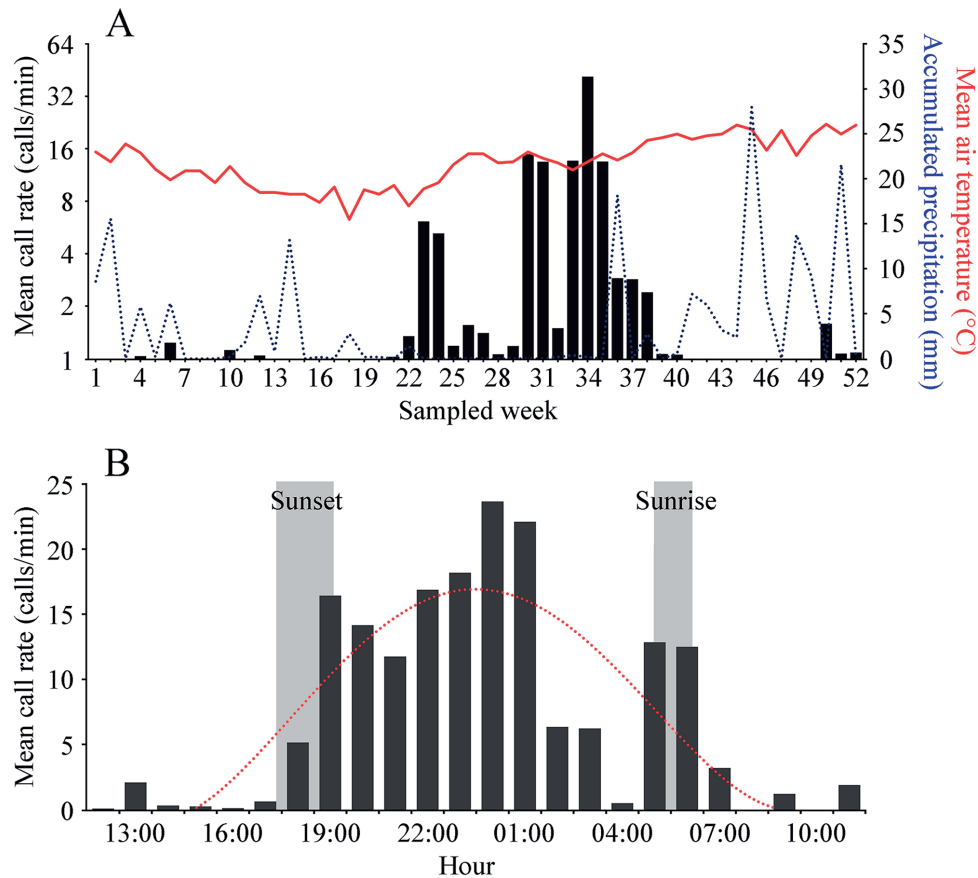
Figure 2. Call rate (calls/minute) of *Leptodactylus flavopictus* from 21 March 2017 to 13 March 2018: A) The red continuous line represents the mean air temperature, the blue dotted line accumulated precipitation; B) daily call rate based on the period with call activity, 22 August to 05 December 2017.

where P1 = P2 and P3 is slightly smaller (Fig. 4A). Teeth density on P1 68–70 teeth/mm (n = 3), curved towards oral opening. Each labial tooth is elongated and thin, with distal cusps at the tip (Fig. 4B). One row of marginal papillae projecting posteriorly and laterally in alternation, emulating two rows; papillae small and round, with a wide mental gap on anterior labium; submarginal papillae absent. Upper jaw sheath arc-shaped and lower jaw sheath V-shaped, both finely serrated and moderately keratinized (Fig. 4B). Measurements (in mm) of each tadpole (stages 28–39) are presented in Table 2.

Internal oral morphology. Based on two specimens at stage 35 (GOSNER 1960). Ventral features (Fig. 4C) – Buccal floor triangular, wider than long. Four infralabial papillae, two fused at midline; the two lateral infralabial papillae are large with a single, medially-directed, apical projection each; the medial papillae are large, flap-like, located in the inferior plane relative to the lateral ones, directed upwards. Elliptical tongue anlage with three lingual papillae; medial papilla distinctly bifurcate; the other two papillae attenuate, directed upwards, posterolateral to medial papillae. Buccal pockets wide, perforated, transversely orientated.
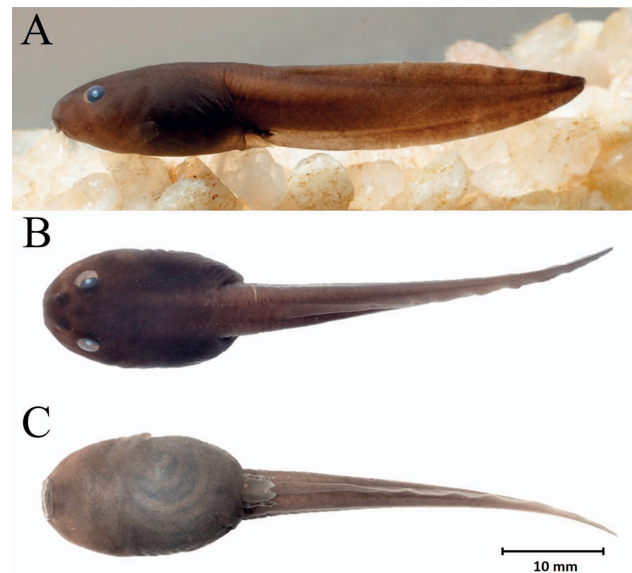

Figure 3. *Leptodactylus flavopictus* tadpole (ZUEC 24301; Gosner's stage 36) in A) lateral, B) dorsal, and C) ventral views.

Three small prepocket papillae on each side. Buccal floor arena (BFA) somewhat triangular, with about 15 BFA papillae on each side, varying in shape and size; randomly scattered pustulations anterior to buccal pockets and within the BFA. Ventral velum round in the superior part, with three long projections, directed posteromedially, and with marginal projections. Median notch wide, with two thin papillae on each side of the median notch. Secretory pits of velar surface conspicuous.

Dorsal features (Fig. 4D) – Buccal roof longer than wide. Prenarial arena trapezoid, without papillae or pustulations. Nares elliptical, orientated parallel relative to the buccal opening, positioned in the anterior third of buccal roof; narial valves thin with no distinct projections. Postnarial arena with two stout irregular papillae with rounded apices. A linear series of six short flattened papillae in the centre of the arena. Median ridge trapezoid with pustulated irregular margins. One large lateral-ridge papilla on each side of the median ridge, located far lateral and anterior to median ridge. Oval buccal roof arena (BRA) delimited by four attenuate papillae on each side, directed toward the centre of the BRA; several randomly distributed pustulations throughout the BRA. Glandular zone conspicuous. Dorsal velum widely interrupted on midline.

Tadpole coloration in preservative. Dorsum dark brown and venter translucent, permitting partial view of intestine tube; spiracle greyish; iris black, tail musculature yellowish. Dorsal and ventral fins greyish and translucent (Fig. 3).

Discussion

The reproductive site used by the population from Reserva Betary encompasses a set of temporary ponds, thus, the accumulation of water precursory rainfalls is a factor necessary to the start the reproductive season. Toledo &
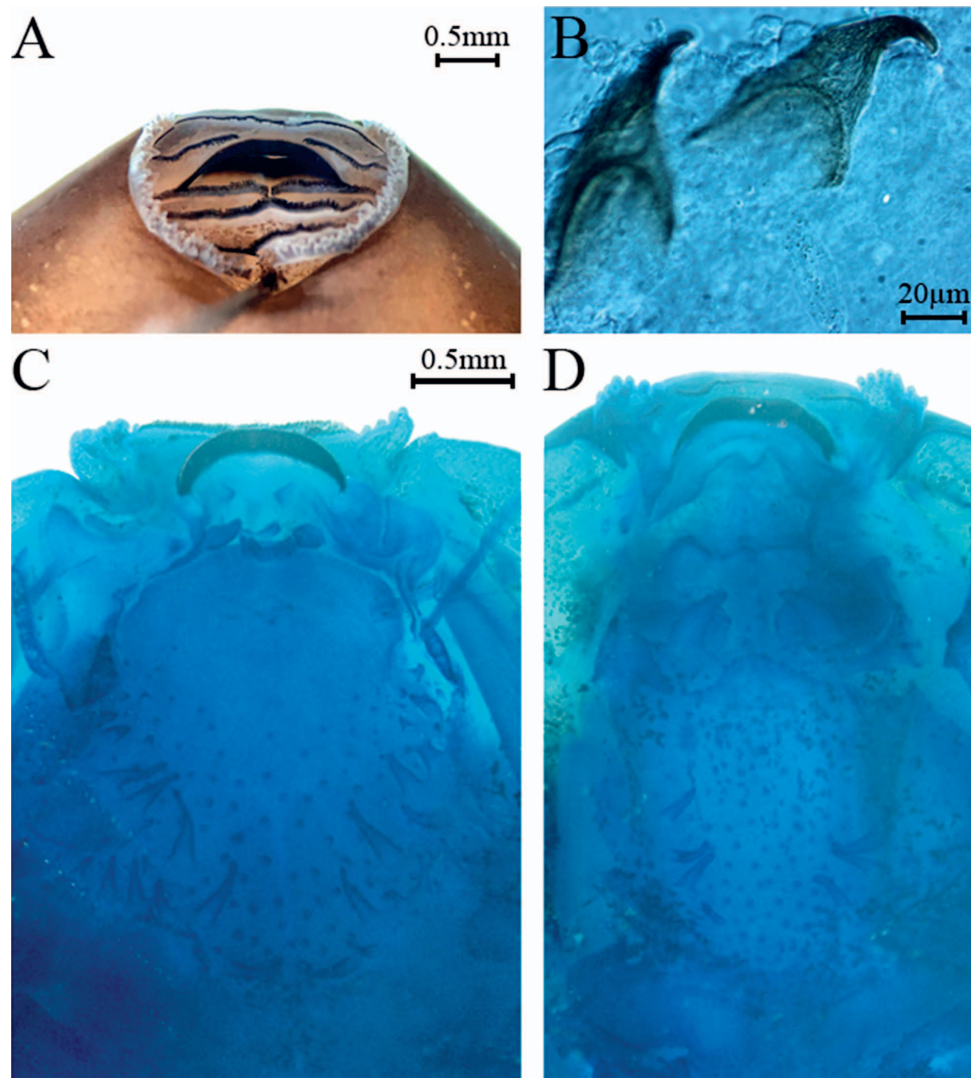

Figure 4. Oral morphology of the *Leptodactylus flavopictus* tadpole (ZUEC 24301) at Gonsner's stage 35: A) Oral disc; B) labial teeth; C) buccal floor; D) buccal roof.

Table 2. Measurements (in mm) of *Leptodactylus flavopictus* tadpoles (ZUEC 24301, 24465). See Methods for abbreviations.

	Stage	TL	BL	BH	BW	TAL	TH	TMH	TMW	UFH	LFH	ED	ND	IOD	IND	SN	ES	SS	ODW
ZUEC 24301	31	46.6	18.8	5.84	6.4	27.8	5.2	3.36	3.2	1.6	1.2	1.04	0.24	3.2	1.6	0.8	2.4	6.8	2.24
	33	49.7	17.5	6	7.36	32.2	5.2	3.36	3.52	1.6	1.2	1.2	0.32	3.36	1.76	0.8	2.4	7.2	2.4
	33	45.7	16.7	5.76	6.56	29	4.88	3.2	3.36	1.6	1.2	1.2	0.24	3.2	1.6	1.04	2.4	6.8	2.4
	35	52.3	20.3	6.8	7.2	32	6.08	4	4	2	1.76	1.2	0.24	3.36	1.6	0.8	2.4	7.6	2.24
	35	50.6	18.6	6.4	7.84	32	5.6	3.2	3.6	1.6	1.2	1.2	0.24	3.36	1.84	1.2	2.8	8	2.4
	36	57.8	20.9	6.8	7.6	36.9	6.4	4	4	2	1.6	1.2	0.24	3.6	1.76	0.8	2.4	7.6	2.4
	36	50.2	17.8	6.4	7.6	32.4	5.6	4	3.84	1.6	1.2	1.2	0.24	3.6	1.84	1.2	2.4	8	2.4
	37	52.7	19.8	6.8	7.6	32.9	5.6	4	3.84	1.6	1.2	1.2	0.24	3.6	1.76	1.2	2.4	8	2.4
ZUEC 24465	28	33	13	3.6	4.4	21	3.6	1.6	1.5	0.9	0.7	0.3	0.2	2.7	1.3	0.7	5.2	5.2	1.4
	28	34	13	3.3	4.4	22	3.3	1.6	1.4	0.9	0.8	0.4	0.2	2.8	1.3	0.5	5.2	5.2	1.4
	30	42	15	4.4	5.3	28	3.2	2	2.2	1.3	1.3	0.4	0.2	2.4	1.6	1.1	6	6	1.8
	32	45	18	5.2	6	30	3.6	2.4	2.8	1.8	1.6	0.4	0.2	2.2	1.5	1.1	6.4	6.4	2
	34	51	19	5.2	6.4	35	5.2	2.8	2.7	1.8	1.6	0.4	0.2	3	1.8	1.1	7.2	7.2	2.4
	34	50	18	5.1	6	35	5.1	2.8	3	1.6	1.3	0.5	0.2	3	1.8	1	7.6	7.6	2.4
	35	51	20	6.8	6.8	39	6.8	3.2	3.4	2.2	1.9	0.5	0.2	3.4	2	1.1	8.2	8.2	2.4
	35	52	19	5.8	6.1	38	5.8	3.2	3	1.7	1.5	0.5	0.2	3	1.8	1.1	7.8	7.8	2.2
	36	59	21	6	7	40	6	3.5	3.8	1.8	1.6	0.4	0.2	3.2	1.9	1.1	8.2	8.2	2.6
	36	60	21	6.5	7.4	40	6.5	3.6	4.2	1.9	1.8	0.5	0.2	3.2	2	1.2	8.4	8.4	2.6
	37	57	21	6	7.4	38	6	3.6	4.2	1.8	1.6	0.5	0.2	3.2	2	1.2	8.4	8.4	2.6
	37	51	20	7.6	8.8	41	5.2	3.6	4	2.4	2	0.5	0.2	3.6	2	1.2	8	8	2
	38	65	21	7.6	8.8	46	4.6	3.6	4.4	2.2	2	0.6	0.2	3.8	2	1.1	8	8	2.8
	39	64	29	7	7.6	42	7	4	4.5	2	1.8	0.5	0.3	4.5	2	1.2	8.8	8.8	2.6

Haddad (2005) demonstrated the necessity of accumulated water to start the reproductive season of *Scinax fuscomarginatus*, presenting differences in the breeding period for two populations, and they linked the beginning of call activity with the formation of temporary ponds. Continued precipitation in the area appeared to be important for the maintenance of adequate temporary habitats. As expected, and reported here, reproductive activity was linked to the beginning of the rainy season, corroborating what has been reported for several *Leptodactylus* species, e.g., *L. labyrinthicus* (Zina & Haddad 2005, Silva & Giaretta 2008), *L. knudseni* (Gascon 1991), *L. furnarius* (Giaretta & Kokubum 2004), *L. mystacinus* (Oliveira Filho & Giaretta 2008), and *L. fuscus* (Lucas et al. 2010). Besides this, our analyses showed a negative correlation between call rate and daily precipitation, meaning that individuals are more acoustically active on non-rainy days during the rainy season. Calling activity cessation during rain was also observed in *L. labyrinthicus* (Zina & Haddad 2005), which might be the result of acoustic interference. The dominant frequency of the advertisement call of *L. flavopictus* is low (ranging 280–680 Hz) as is the noise produced by rain, with frequencies of 0–5 kHz (Krause & Farina 2016). Therefore, the noise produced by rain may represent an acoustic barrier, masking the signal transmission of *Leptodactylus* spp. This was also demonstrated for the tawny owl (*Strix aluco*) whose calls are also low pitched (80% of the call energy is within frequencies of 500–1100 Hz) and its audible broadcast area decreases around 69-fold when its rains (Lengagne & Slater 2002).

We observed that call activity stopped before the end of the rainy season. This may be related to the conditions necessary for the complete tadpole development in temporary habitats soon turning unfavourable, reducing the probability of tadpole mortality due to the desiccation of temporary ponds being certain (Oliveira Filho & Giaretta 2008). This change in conditions may be relevant for *L. flavopictus*, since this species has a prolonged larval developmental, reported to be about 5 months from egg to imago in captivity (Bokermann 1957).

The advertisement call of *L. flavopictus* was briefly described by de Sá et al. (2014), however there is no information about the locality of the recording and the description is simplistic. It is possible to recognize the resemblance of the dominant frequency, around 600 Hz, but call durations are different. Also, we report here the presence of harmonics in the calls of individuals from the two populations analysed, whereas harmonics were not evidentiated by de Sá et al. (2014). Such difference may be a result of different software or recording settings. Geographic variation on advertisement call parameters were previously reported for some *Leptodactylus* species (e.g., Heyer & Reid 2003, Zina & Haddad 2005, Heyer 2005, Kok et al. 2007, Heyer & Barrio-Amorós 2009) and it was also reported for other anuran species (e.g., Pimenta et al. 2008, Tessarolo et al. 2016, Zornosa-Torres & Toledo 2019). These

Table 3. Tadpole characteristics of the species of the *Leptodactylus pentadactylus* species group (de Sá et al. 2014).

Species	LTRF	Oral Disc	Marginal Papillae	Feeding habits	Reference(s)
L. fallax	1/0	Terminal	Not described	Obligatorily oophagous	Lescure & Letellier (1983), Davis et al. (2000)
L. flavopictus	2(2)/3(1)	Anteroventral	Single row	?/ Facultatively carnivorous	Bokermann (1957), Martins et al. (2020), present study
L. knudseni	2(2)/2–3(1)	Anteroventral / terminal	Single row	Facultatively oophagous / carnivorous	Hero & Galatti (1990), Duellman (2005), Heyer & Heyer (2006)
L. labyrinthicus	1/2(1)	Almost terminal	Single row	Facultatively oophagous	Vizotto (1967), Rossa-Feres & Nomura (2005)
L. lithonates	2(2)/3[1]	Ventral	Single row	?	Heyer & Heyer (2001), Heyer (1995)
L. pentadactylus	1/2(1) or 2(2)/3(1)	Terminal	Single row	Facultatively oophagous	Hero & Galatti (1990), Menin et al. (2010)
L. rhodomystax	2(2)/3	Anteroventral	Double row	Oophagous: both intra- and interspecific eggs	Rodrigues et al. 2007
L. rhodonotus	2(2)/3[1]	Anteroventral	Single row, double row laterally	Facultatively oophagous	Duellman (2005), Heyer (1969)
L. rugosus	2(2)/3[1]	Ventral	Not described	?	Heyer (1995), Duellman (1997), Heyer & Thompson (2000)
L. savagei	2(2)/3(1)	Terminal	Single row, double row posteriorly	Facultatively oophagous / carnivorous	Heyer et al. (2010)
L. vastus	1(2)/1 or 1/2–3(1)	Terminal	Double row, single row laterally	Facultatively oophagous	Vieira et al. (2007), Schulze et al. (2015)

variations might occur for many different reasons, such as due to the influence of climate, community structure, or even geographic and genetic isolation (Pröhl et al. 2007, Ohmer et al. 2009, Forti et al. 2016, Köhler et al. 2017). Except for *L. paraensis* and *L. rhodomerus,* all other species allocated to the *L. pentadactylus* species group have had their advertisement calls described (Tab. 1). Therefore, it is currently possible to use bioacoustic information not only for species differentiation, but also in studies on the evolution of acoustic characteristics, especially as these species present clear variations in note structure, spectral and temporal traits (Table 1).

We assessed daily and annual calling activity using PAM. This method (reviewed by Sugai et al. 2019) facilitates data gathering, as it allows the monitoring of one or more species over a long period of time and collecting great amounts of data; besides, it decreases the need for time spent by researchers in the field, and also forestalls possible observer's influence on the behaviour of the organisms that are monitored (Peterson & Dorcas 1992, Bridges & Dorcas 2000, Dorcas et al. 2009, Brauer et al. 2016, Sugai et al. 2019). For these reasons, PAM is increasingly seen in studies with different aims, such as for estimating population density (Marques et al. 2013, Stevenson et al. 2015), monitoring endangered species (Blumstein et al. 2011, Moskwik et al. 2013), measuring daily and annual activity patterns (Bridges & Dorcas 2000, Saenz et al. 2006, Ospina et al. 2013), as well as for assessing the consequences of climate change on activity patterns (Krause & Farina 2016). Using PAM in this study, we were able to produce around 208 hours of soundscape recordings. Also, these records contain sounds produced by several animals, such as insects, birds, and other anuran species, like *Adenomera marmorata*, *Leptodactylus notoakitites*, *Physalaemus spiniger*, that could serve to further a better understanding of the community ecology in the area where our study was conducted.

The tadpole of *L. flavopictus* has an elongated body, ovoid in dorsal view, a long tail, sinistral spiracle, and a long vent tube, which are features typically found in pond-dwelling larvae of the Leptodactylinae subfamily (de Sá et al. 2014, Schulze et al. 2015). However, the only species of the *L. pentadactylus* group that might co-occur with *L. flavopictus* is *L. labyrinthicus* (de Sá et al. 2014, Frost 2020). Although tadpoles of the two species are morphologically similar, they can be distinguished by their oral discs, which is more ventrally located in *L. flavopictus,* and almost terminal in *L. labyrinthicus* (de Sá et al. 2014), and also by their LRTF, 2(2)/3(1) in *L. flavopictus* and 1/2(1) in *L. labyrinthicus* (Rossa-Feres & Nomura 2005). The presence of keratinized mouthparts, shape of labial tooth, and a simple internal oral morphology in *L. flavopictus* tadpoles are characteristic of carnivorous species (Wassersug & Heyer 1988, Vera Candioti & Altig 2010), suggesting that, like other tadpoles in the group, this species is at least facultatively carnivorous (Wassersug & Heyer 1988,

Table 4. Internal oral morphology of tadpoles of the *Leptodactylus pentadactylus* species group (DE SÁ et al. 2014). IP: Number of infralabial papillae; LP: number of lingual papillae; PpP: number of prepocket papillae; BFA papillary: number of papillae in the buccal floor arena; MR: shape of median ridge; BRA: shape of buccal roof arena; BRA papillary: number of papillae in the buccal roof arena.

Species	IP	LP	PpP	BFA papillary	MR	BRA shape	Reference
L. flavopictus	4	3	3 small papillae; pustulations	15-20 papillae	Trapezoid	Circular	Present study
L. knudseni	5	3	2–3 small papillae	10 papillae	Trapezoid	Triangular	WASSERSUG & HEYER (1988)
L. labyrinthicus	4	3	4–5 small; pustulations	6 papillae	Semi-circle	Triangular	MIRANDA & FERREIRA (2008)
L. pentadactylus	4	4	0; several pustulations	6 papillae	Small, round	Poorly defined	WASSERSUG & HEYER (1988)
L. vastus	4	3	4–5; several pustulations	8 long + 12–14 Shorter papillae	Trapezoid	Circular	VIEIRA et al. (2007)

VIEIRA et al. 2007, MIRANDA & FERREIRA 2008). Although most species of this group have had their tadpoles described (Table 3), information on internal oral morphology is scarce and is only available for *L. knudseni* and *L. pentadactylus* (WASSERSUG & HEYER 1988), *L. vastus* (VIEIRA et al. 2007), *L. labyrinthicus* (MIRANDA & FERREIRA 2008), and *L. flavopictus* (present study). However, internal oral morphology does not seem to vary much between the species of this group (Tab. 4), as all species have in common 4–5 infralabial papillae, 3–4 labial papillae, no papillae or pustulations in the prenarial arena, and two papillae in the postnarial arena.

Recently, MARTINS et al. (2020) redescribed the tadpole of *L. flavopictus* based mainly on specimens at stage 40 (GOSNER 1960). The external morphologies of the tadpoles of that population and the one characterized herein are similar, except that we observed the spiracle to be fused to the body without a free extremity, contrary what was reported by MARTINS et al. (2020). Such variation could either represent an inter-populational difference or a specific variation. Therefore, this is another example highlighting the relevance of describing larval features from different populations, which could enhance the quality of the traits subsequently applied to anuran systematics. Finally, understanding temporal patterns of anuran call activity are relevant for a vast field of studies. It may allow researchers to assess the impact of threats to the studied assemblage by detecting changes in activity calling due to climate changes (KRAUSE & FARINA 2016, SUEUR et al. 2019) or even detecting invasive species such as the bullfrog (*Lithobates catesbeianus*), which is known to cause damage to natural anuran communities (BOTH & GRANT 2012, RUGGERI et al. 2019, RIBEIRO et al. 2019).

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Supplementary data

The following data are available online:

Supplementary document Table 1. Sampling days of the study.

Supplementary document Figure 1. Adult *Leptodactylus flavopictus*.

Supplementary document Figure 2. One of the temporary ponds used by *Leptodactylus flavopictus* as breeding site.