Journal of Natural History

ISSN: 0022-2933 (Print) 1464-5262 (Online) Journal homepage: www.tandfonline.com/journals/tnah20

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JOURNAL OF NATURAL HISTORY
2019, VOL. 53, NOS. 43–44, 2699–2710
https://doi.org/10.1080/00222933.2020.1728409

Elucidating the diel and seasonal calling behaviour of *Elachistocleis matogrosso (Anura: Microhylidae)*

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ABSTRACT

Acoustic monitoring provides the opportunity to study ecological processes that are difficult to assess with traditional surveys. *Elachistocleis matogrosso* is an anuran species, described in 2010, for which limited biological information is available. This study investigated the calling activity of the species in the north-eastern portion of the Pantanal, Brazil, a wetland area with marked seasonality between the dry and wet seasons. The calling activity of *E. matogrosso* was monitored using automated digital recorders in combination with automated signal recognition software over two different annual cycles. The species was vocally active only during the wet season (October – April), with a peak in November-December during the 2013–2014 annual cycle and in February-March during the 2015–2016 annual cycle. The peak calling activity occurred at dusk. This species has nocturnal habits and an explosive breeding activity. The detection of the species was intermittent, which suggests that environmental predictors or site-specific conditions might play an important role in species detection. Moreover, this intermittent occupancy indicated that surveys that employ traditional field techniques would likely fail to detect this species. We describe an effective protocol for detecting *E. matogrosso* with acoustic monitoring, which requires recording during 20 days in February from 17:01 to 05:00. Our procedure would be easy to adapt to other anuran species, and it could be used for investigating new localities and assessing population changes over time.

ARTICLE HISTORY
Received 8 June 2019
Accepted 6 February 2020

KEYWORDS
Anurans; acoustic monitoring; automated recorder; calling activity; Microhylidae; Pantanal; monitoring protocol

Introduction

The use of automated recorders has revolutionised the way we understand soundscapes (Pijanowski et al. 2011) and the way we monitor vocally active wildlife species (Sugai et al. 2019). This transformation has produced remarkable results for monitoring

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Supplementary material data for this article can be accessed here.

Published online 17 Feb 2020

species that are active under challenging circumstances (Ospina et al. 2013), such as at night and at sites that are inhospitable or difficult to access (Forest 2007; Willacy et al. 2015). Furthermore, this technique has allowed researchers to increase the spatial and temporal coverage of monitoring programmes and unravel ecological patterns that are difficult to ascertain with traditional methods (Willacy et al. 2015). However, the ability to manage large amounts of data remains a challenge (De Oliveira et al. 2015; Browning et al. 2017), and thus few studies have covered wide spatial and temporal scales at the same time (Sugai et al. 2019).

Amphibians are considered the most threatened vertebrate group globally, and among them, anurans exhibit the most rapid declines (Stuart et al. 2004; Scheele et al. 2019). Therefore, it should be a conservation priority to increase our knowledge regarding anurans and develop effective protocols for monitoring them. Anurans are typically vocally active only at night during the breeding season (Lemckert and Mahony 2008; Willacy et al. 2015), when they usually engage in countersinging night choruses (Hödl 1977; Arzabe 1999). Calls from anuran males are species-specific, and they are typically heard at long distances (Hödl 1977). Consequently, male anurans are excellent for monitoring with acoustic techniques and automated signal recognition software (Waddle et al. 2009). Indeed, acoustic monitoring has proven to be an efficient method for monitoring and conserving different anuran species. For example, this technique has been used to improve the probability of detecting rare or threatened anurans (Forest 2007), to study the impact of invasive species on native frogs (Tennessen et al. 2013), and to assess climate change impacts (Llusia et al. 2013). The collection of recordings on long temporal scales provides the opportunity to assess variations in anuran calling activity over time (Akmentins et al. 2015; Willacy et al. 2015). In fact, seasonal calling variation has been employed to assess frog reproductive phenology (e.g. Bridges et al. 2000; Lemckert and Mahony 2008; Willacy et al. 2015) and measure habitat suitability for reproduction (Hilje and Aide 2012).

Elachistocleis, Parker 1927, (Microhylidae) is an anuran genus that comprises 18 species distributed throughout Central and South America (Frost 2019). *Elachistocleis matogrosso* Caramaschi, 2010 is a species of Least Concern species on the IUCN Red List (IUCN Amphibian Specialist Group 2013), and there is a need to gain more knowledge about its life history, ecology, monitoring protocols and distributional range.

The present study aimed to describe, based on acoustic monitoring, the diel and seasonal calling activity patterns of *E. matogrosso*. Our objective was to gain insights into the breeding biology and calling behaviour of this species. We also propose an effective monitoring protocol for species detection, using automated recorders and automated signal recognition software. This protocol could be useful for future studies conducted in new localities inhabited by *E. matogrosso* and for long-term monitoring programmes conducted to detect population changes over space and time. Moreover, the procedure applied in this study might also be adapted to monitoring other anuran species.

Methods

Study species

The known distributional range of *E. matogrosso* is restricted to a few localities in the states of Mato Grosso and Mato Grosso do Sul, midwestern Brazil (Caramaschi 2010; Pansonato et al.

2011; Dorado-Rodrigues et al. 2018) and to a single locality in northern Paraguay (Brouard et al. 2015). Currently, there is some basic knowledge about its morphology (Caramaschi 2010), its advertisement call (Marinho et al. 2018; Pansonato et al. 2018) and the impact of environmental predictors on its calling activity (Pérez-Granados et al. 2019). The species can be identified by the presence of an immaculate venter and a small body size (it is the smallest among its congeners with immaculate bellies, with mean length from snout to vent of 23 mm and 30 mm for males and females, respectively; Caramaschi 2010; but see Nunes-de-Almeida and Toledo 2012) (Online Resource 1). The advertisement call of this species is unique and different from that of all other *Elachistocleis* species (Marinho et al. 2018; Pansonato et al. 2018) (Online Resource 1), lasting from 1.3 to 3.6 s and uttered at a very narrow bandwidth (the dominant frequency is between 4,000 and 4,800 Hz; Marinho et al. 2018; Pansonato et al. 2018).

Study area

This study was carried out in the north-eastern part of the Brazilian Pantanal (Pantanal Matogrossense) which is seasonally inundated due to the flooding of the Paraguay River (Junk et al. 2006). The investigated area comprised seven acoustic monitoring sites placed around the SESC Pantanal (Mato Grosso, Brazil; 16°39′S, 56º47′W, Online Resource 1). The acoustic monitoring stations at each site were separated by 300–3,300 m (Online Resource 2). The area was located in the floodplain of the Cuiabá River, one of the main tributaries of the Paraguay River within the Pantanal. The study site comprised a mosaic of different forested and savanna areas (Junk et al. 2006). The regional climate is tropical and humid; the average annual rainfall is 1000–1500 mm, and the mean annual temperature is approximately 24ºC. The study area exhibits marked seasonality, with a pronounced terrestrial phase from May to September and an aquatic phase from October to April (Junk et al. 2006).

Acoustic recording

The acoustic monitoring stations operated daily for an entire year; thus, one complete annual cycle was recorded at each site. One of the stations (Site A) was operational from 15 November 2013 to 14 November 2014; the other six stations (Sites B-G) operated from 8 June 2015 to 2 June 2016. At each acoustic monitoring station, we placed one Song Meter SM2 recorder (Wildlife Acoustics). At Site A, the recorder was programmed for continuous recording (.wav format), in 24/7 mode; at Sites B-G, the recorders were also programmed to operate in 24/7 mode, but recorded only the first 15 min of each hour. The recorders were always set to winter local time (GMT-4). The sampling rate was 48,000 Hz, with a resolution of 16 bits per sample.

Acoustic data analyses

The recordings were scanned with Kaleidoscope Pro 5.1.8, an automated signal-recognition software programme provided by Wildlife Acoustics. Kaleidoscope can examine recordings for signals of interest, based on the following signal parameters: minimum and maximum frequency ranges (Hz), minimum and maximum times of detection (s), and a maximum inter-syllable gap (ms). We used recent descriptions of *E. matogrosso* advertisement calls to select the optimal signal parameters. In previous studies, the minimum and maximum call durations for this species were 1.26 and 3.63 s, respectively; the minimum and maximum frequencies were 3,610 and 4,823 Hz, respectively (Marinho et al. 2018; Pansonato et al. 2018); and the minimum gap detected between two successive calls was 4.3 s (Pansonato et al. 2018). Pansonato et al. (2018) and Marinho et al. (2018) described *E. matogrosso* advertisement calls based on single individuals calling at close distances, recorded with directional microphones. Therefore, we relaxed the range of signal parameters slightly to maximise the possibility of detecting weaker, overlapping, and successive calls uttered by multiple individuals. For that purpose, we adjusted the following signal parameters in Kaleidoscope as follows: the minimum and maximum frequencies were 3,600 and 6,100 Hz, respectively; the minimum and maximum detection durations were 0.76 and 15 s, respectively; and the maximum inter-syllable gap: 0.03 ms. Kaleidoscope required one additional parameter for the recording analyses: the distance from the cluster centre. This value ranges from 0 to 2, and it impacts the number of detected signals that could be introduced in the output. Large values result in a large number of detected target signals, which increase the number of false positives (misclassified signals). A value between 1.0 and 1.4 is recommended by Wildlife Acoustics. We used a value of 1.8. Although the selection of this value could increase the number of false positives, our goal was to detect as many *E. matogrosso* calls as possible. Events detected by Kaleidoscope were visually and/or acoustically checked, always by the same observer (CPG), to separate false positives from true positives (correct classifications).

Monitoring protocol analyses

We divided the one-hour recordings collected at Site A into four 15-min recordings. Then, we selected the first 15-min recording from each hour to standardise the recordings collected under the different schedules. Therefore, all our monitoring analyses and recommendations were based on a recording period of 15-min per hour. Next, we estimated the minimum number of monitoring days needed to detect the presence of the species during January and February with 95% probability, as the species was detected only during these months at all monitored sites (Online Resource 3). The probability of recording the presence of the species was estimated by fitting a logistic regression model to the data; the dependent variable was the detection of the species at each site on each date (yes/no), and the predictor variable was the date. Data analyses were conducted in R 3.4.1 (R Core Team 2016).

We also assessed the need to record in 24/7 mode to detect the presence of the species. To do so, the following three categories of recording periods were created: from 17:01 to 23:00 (sunset recordings), from 23:01 to 05:00 (night recordings), and from 05:01 to 17:00 (day-time recordings). Next, we assessed the probability of detecting the species based on only sunset recordings, only sunset and night recordings, or the whole dataset. The results are expressed as the mean ± standard error (SE).

Results

Kaleidoscope reported a total of 297,477 events that matched the signal parameters. A total of 16,755 events were classified as *E. matogrosso* advertisement calls.

Diel and seasonal patterns

Elachistocleis matogrosso was detected at all seven acoustic monitoring sites (Table 1). The mean number of days on which the species was found per site was 24.7 ± 6.0 (range: 10–47, Table 1). Detections were intermittent at all sites; the mean number of consecutive days that *E. matogrosso* was detected was 6.4 ± 1.5 (2–12 range, Table 1).

We found clear diel and seasonal calling activity patterns for *E. matogrosso*. The calling activity peaked at dusk; 44.4% of all calls were detected between 18:00 and 21:00 (Figure 1). More specifically, the highest calling activity occurred at 19:00 (21.3% of the total, Table 1), with lower, but relevant calling activity between 21:00 and 04:00 (Figure 1). After that, a predominantly quiet period occurred (fewer than 6% of all calls) between 04:00 and 15:00 (Figure 1). The seasonal plot showed a unimodal pattern (Figure 1), with calling activity restricted to the wet season (October-April). We found large differences between the two monitored annual cycles. During the annual cycle of 2013–2014, the highest number of calls was detected during November and December (95.0% of all calls detected), while during the annual cycle of 2015–2016, most of the calls were detected during February and March (86.5% of calls detected) (Figure 2). The species was vocally inactive from early May to late October during both annual cycles (Table 1). The species was identified at all studied sites only during January and February, but the number of months during which the species was found differed among sites (Online Resource 3).

Monitoring protocol

We encountered 88 detections of *E. matogrosso* during January and February, across all sites. The logistic regression indicated that it is necessary to record for the first 29.78 (30) and 19.54 (20) days of January or February, respectively, to detect the presence of the species with 95% confidence (Figure 3). The best time for detecting the presence of *E. matogrosso* was during the sunset period, when the species was detected on 48 out of 88 times (55.5%). When the night and sunset recordings were combined, the species was recorded 82 times (93.2%). The remaining 6 detections were identified in the day-time recordings (6.8%).

Discussion

This study was the first to analyse the calling activity pattern of *E. matogrosso*. We found that the calling activity pattern of this species varied with the time of day and the season. Most calling activity was nocturnal, with a peak at dusk, consistent with the typical diel vocalisation cycles described for most anuran species (e.g. Bridges et al. 2000; Hilje and Aide 2012; Akmentins et al. 2015). This vocalisation cycle has been suggested to be an adaptation related to avoiding visual predators and skin desiccation (Pechmann and Semlitsch 1986; Santos and Grant 2011). In prior studies, *E. matogrosso* individuals were always found during night surveys, consistent with our results (Carrillo 2017; Marinho et al. 2018; Pansonato et al. 2018).

Elachistocleis matogrosso calling activity primarily occurred from November to April, which corresponds to the main period of the wet season in the study area (Junk et al. 2006). The finding of high vocal activity during the wet season is consistent with the pattern described for populations of *Elachistocleis bicolor* in Argentina (Martori et al. 2005)

Table 1. Summary of *Elachistocleis matogrosso* calling activity at seven sites monitored with acoustic monitoring in Pantanal Matogrossense, Brazil.

Recording site[a]	First call	Last call	Most active day	Most active hour[b]	Most active month	Number of days detected	Consecutive days detected
A	28-Oct	15-Feb	25-Dec	19	December	38	5
B	06-Jan	28-Feb	25-Feb	19	February	10	2
C	19-Jan	05-May	16-Feb	23	February	28	9
D	23-Jan	02-May	26-Feb	1	February	19	10
E	11-Dec	08-Apr	09-Mar	19	March	19	4
F	06-Jan	11-Apr	19-Feb	22	February	12	3
G	06-Dec	23-Apr	29-Mar	19	March	47	12

[a]Site A was monitored from 15 November 2013 to 14 November 2014; Sites B-G were monitored from 8 June 2015 to 2 June 2016.
[b]Hours represent the time of day, based on the 24-h clock notation, with minutes omitted (all are 00 min).

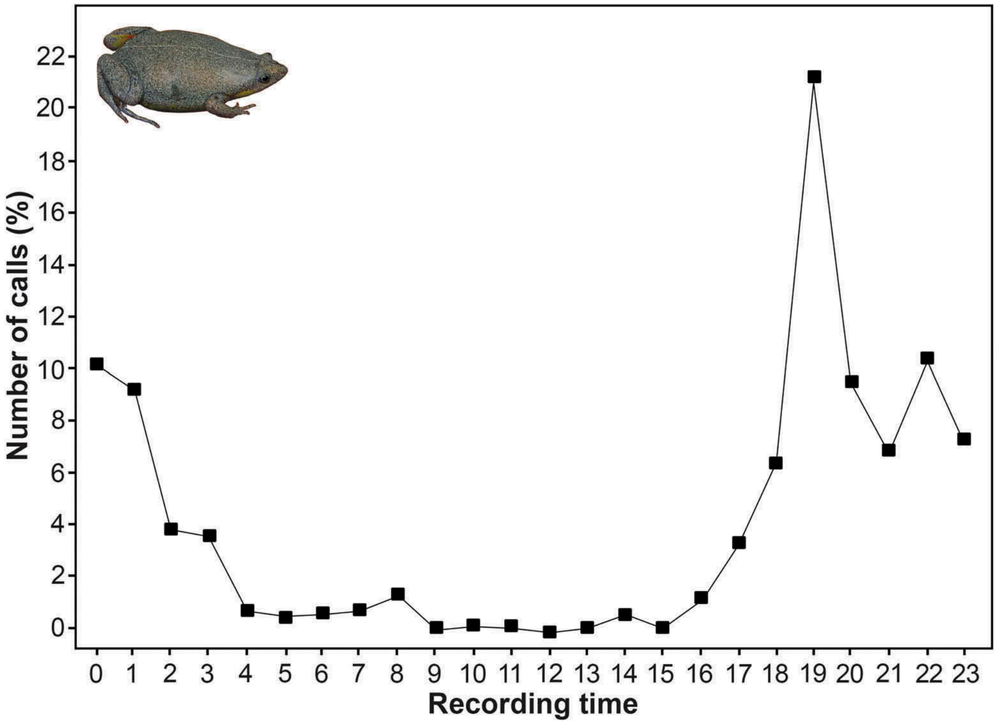

Figure 1. Diel pattern of *Elachistocleis matogrosso* calling activity during an entire year in the Pantanal Matogrossense, Brazil. Calling activity was detected with acoustic monitoring from 15 November of 2013 to 14 November 2014 at one site (a, Table 1) and from 8 June 2015 to 2 June 2016 at six other sites (b–g, Table 1). The diel pattern is expressed as the mean percentage of calls detected at all sites at each recording time. The hours are expressed in 24-h notation of the winter local time (GMT −4).

and the southern Pantanal (Rodrigues et al. 2003; Prado et al. 2005) and with patterns reported for several other populations of Brazilian species of *Elachistocleis* (see Thomé and Brasileiro 2007 and references therein) and most other Neotropical anurans (Moreira and Barreto 1997; Arzabe 1999; Prado 2003; Akmentins et al. 2015). However, several anurans are vocally active throughout the entire year, even during the dry season (Hödl 1977). The seasonal pattern varied between years in this study, with an advanced calling phenology during the annual cycle of 2013–2014 when compared to that of 2015–2016. The mean rainfall in the study area during November and December of 2013 was 142 and 190 mm, respectively, while it declined to 44 and 127 mm during November and December 2015 (authors' own data). This suggests that the calling phenology of the species might be linked to changes in rainfall patterns.

Seasonal calling variation analyses provided the first insights into the calling behaviour and breeding biology of *E. matogrosso*. Wells (1977) classified anuran reproductive activity into prolonged or explosive breeding, according to the duration of the breeding season. Our results suggest that *E. matogrosso* is nocturnal with explosive breeding behaviour due to the nocturnal and intermittent calling pattern observed. This is in agreement with the explosive reproductive activity described for *Elachistocleis bicolor* (Prado 2003; Rodrigues et al. 2003). The intermittent detection of *E. matogrosso* at all monitored sites and the low

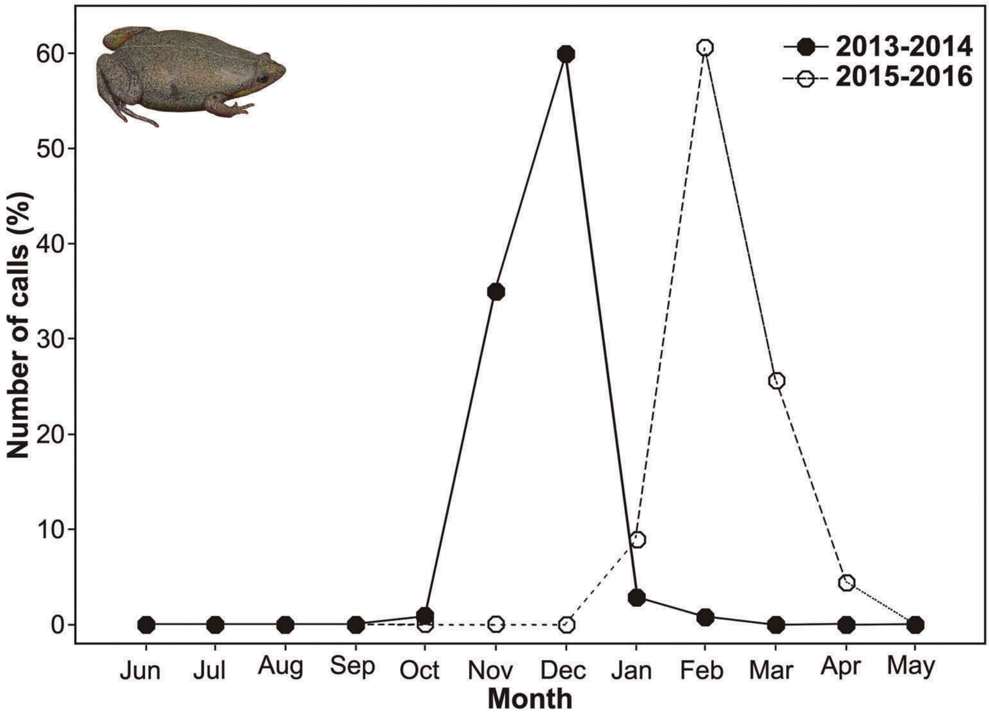

Figure 2. Seasonal pattern of *Elachistocleis matogrosso* calling activity during two entire years in the Pantanal Matogrossense, Brazil. Calling activity was detected with acoustic monitoring from 15 November of 2013 to 14 November 2014 at one site (a, Table 1) and from 8 June 2015 to 2 June 2016 at six other sites (b–g, Table 1). The seasonal pattern is expressed as the percentage of calls detected per month (2013–2014) or as the mean percentage of calls detected at all sites per month (2015–2016).

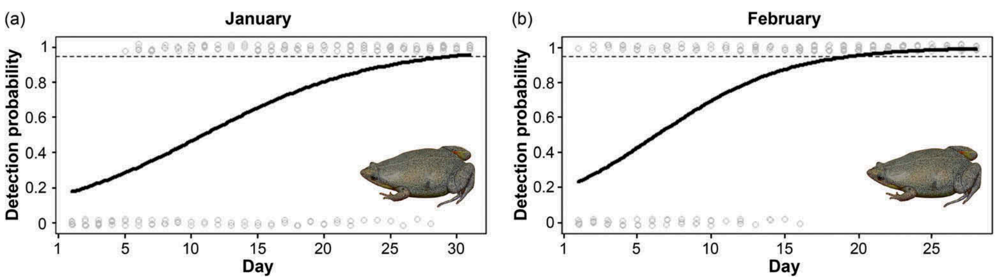

Figure 3. Estimated probability of detecting the presence of *Elachistocleis matogrosso* during (a) January and (b) February. Calculations were based on logistic regression, with the detection/lack of detection of the species at the seven monitored sites as the dependent variable and the date as the predictor variable. Dashed lines show the 95% probability of detecting the species.

number of consecutive days with calling activity at a site (maximum of 12) suggests that environmental predictors and site-specific conditions (e.g. the creation of water bodies after raining) might play an important role in species occupancy and calling behaviour. Indeed, a prior study testing the effects of environmental predictors on the calling activity of *E. matogrosso* showed that the daily occurrence of the species was positively associated with a high minimum air temperature and high rainfall, while calling production was positively associated with the daily rainfall and rainfall accumulated during the last three days (Pérez-Granados et al. 2019). Therefore, the intermittent occupancy of *E. matogrosso* is likely related to the availability of water bodies after rainfall, similar to what was reported for *Elachistocleis bicolor* in the southern Pantanal (Rodrigues et al. 2003).

This study described an effective protocol for detecting *E. matogrosso* by applying acoustic monitoring. To the best of our knowledge, this protocol was the first to describe concrete times and define a definite number of monitoring days necessary for detecting a selected anuran species using acoustic monitoring. Although our estimates cannot be directly extrapolated to other anuran species, the approach and the statistical analyses described might guide future studies in other taxa. The recommendations regarding the best times and dates for detecting the species could be used to improve traditional monitoring survey techniques. Monitoring efforts can be maximised during the month of February, the only month when the species was detected at all sites. During February, recording for 15 min per hour between 17:00 and 05:00 for 20 days was sufficient for detecting the presence of *E. matogrosso* with greater than 93% confidence. This protocol could be used to detect population changes over time (Royle 2004), discover new localities occupied by the species (e.g. the expected presence of *E. matogrosso* in eastern Bolivia; Frost 2019), and check the actual identity of possibly misidentified populations. Moreover, the monitoring protocol might be particularly useful for assessing *E. matogrosso* occurrence between the single known locality in northern Paraguay and sites in midwestern Brazil (Brouard et al. 2015). We are aware that our protocol is based on data from two years and that there is interannual variation in the calling phenology of the species. Therefore, we cannot rule out that best monitoring period or number of days needed to detect the species may differ among years according to weather conditions.

Acoustic monitoring, coupled with automated signal recognition software, was useful for describing the diel and seasonal calling activity patterns and detecting the presence of the poorly known *E. matogrosso*. The low percentage of events classified as *E. matogrosso* by the automated signal recognition software is due to the signal parameters included in the sound analyses and the methods employed (e.g. the large 'distance from the cluster centre' used). We used Kaleidoscope as an activity detector with relaxed parameters, since we aimed to detect as many calls as possible. The error rate might have been significantly reduced by creating one recogniser after labelling the events, but this was outside of the goals of the manuscript. This method is well suited for monitoring this particular species because of its intermittent activity, which makes it difficult to detect with traditional field surveys, unless intense fieldwork is applied. Acoustic monitoring also obviated the necessity of performing night surveys in the study area, and due to difficult accessibility, it minimised the associated human risk.

Acknowledgements

We thank the SESC Pantanal, Mato Grosso, for permission to conduct research on their property and for their logistical help with our fieldwork. This study is part of the biodiversity monitoring project: Sounds of the Pantanal – The Pantanal Automated Acoustic Biodiversity Monitoring of INAU, Cuiabá, Mato Grosso, Brazil, conducted under SISBIO permit no. 39095 (KLS).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

We greatly appreciate the financial support of the following institutions: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil [CAPES – INCT/MCTI/CNPq/CAPES/FAPs no.16/2014]; Instituto Nacional de Ciência e Tecnologia em Áreas Úmidas [INAU/UFMT/CNPq]; Centro de Pesquisa do Pantanal (CPP); Brehm Foundation for International Bird Conservation (BF), Bonn, Germany; Conselho Nacional de Desenvolvimento Científico e Tecnológico for a research fellowship to CS [CNPq no. 3123038/2018-1].

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Data availability

Data supporting the results and analyses presented in the paper can be found at: https://doi.10.6084/m9.figshare.8246123.v1

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