



## Vocal activity of the Ferruginous pygmy-owl (*Glaucidium brasilianum*) is strongly correlated with moon phase and nocturnal temperature

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Bird vocal activity is affected by endogenous and exogenous factors. Owl surveys are mainly based on the detection of nocturnal calls, and therefore, the impact of exogenous factors on owls' vocal activity may have consequences in conservation planning and behavioural studies. However, our current knowledge about the impact of climatic factors and the moon phase on owl calling behaviour is very limited, especially in the Neotropics. We used autonomous recording units to evaluate the effect of air temperature, rainfall, relative air humidity, and percent of the moon illuminated on the vocal occurrence (active/inactive) of the Ferruginous pygmy-owl (*Glaucidium brasilianum*) over three consecutive moon cycles in the Brazilian Pantanal. Vocal activity was positively associated with the percent of the moon illuminated, with 75% of the nights on which the species was vocally active having a moon illumination percentage higher than 77%. The vocal activity of the species was negatively associated with the nocturnal air temperature, with more vocal activity observed on cooler nights. Relative air humidity and daily rainfall were not associated with the vocal activity of the Ferruginous pygmy-owl. Our study improves the knowledge about the impact of exogenous factors on the calling behaviour of Neotropical owls. We conclude that future surveys aiming to detect the Ferruginous pygmy-owl should be carried out on nights with a high percent of moon illumination (>75%) and nights with low average temperature (< 18 °C).

KEY WORDS: autonomous recording unit, bird, Brazil, call, Kaleidoscope Pro, Pantanal, passive acoustic monitoring.

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## INTRODUCTION

Bird vocalisations are primarily used for territory establishment and mate attraction but also play a role in other daily activities, such as maintaining group contact and signalling about food or threat (Marler 2004; Catchpole & Slater 2008). Bird vocal activity is affected by a large number of endogenous (e.g., mating status and hormones; Bottjer & Johnson 1997; Amrhein et al. 2002) and exogenous factors, such as climatic conditions (Robbins 1981), moon phase (York et al. 2014; Pérez-Granados & López-Iborra 2020) or photoperiod (Rowan 1929; Ball & Hulse 1998). Several studies have assessed the relationship between bird vocal activity and endogenous and exogenous factors in passerines of northern temperate areas (Catchpole & Slater 2008). However, there is very little information about how climate and moon phase alters the vocal activity and detectability of tropical non-passerines, such as Neotropical owls (Braga & Motta-Junior 2009).

Monitoring programs aiming to determine the presence, abundance, and population trends of owls are based on the detection of their calls uttered spontaneously or as a response to broadcasted recorded calls (e.g., Zuberogoitia & Campos 1998; Shyry et al. 2001; Flesch & Steidl 2006). Therefore, assessing the relationship between exogenous factors and owl vocal activity is desirable for conservation monitoring and behavioural studies. Moreover, the calling rate of owls is usually low (Shonfield et al. 2018), which makes it important to perform surveys during periods of maximum vocal activity to improve the probability of detecting the target species (Conway & Simon 2003). Habitat loss and scarce knowledge about owls in the Neotropics make the development of rigorous survey methods even more urgent (García-Moreno et al. 2007; Braga & Motta-Junior 2009). In recent years, passive acoustic monitoring has allowed researchers to monitor the vocal activity of a large number of owls (Shonfield & Bayne 2017; Shonfield et al. 2018; Domahidi et al. 2019; Wood et al. 2019; Marín-Gómez et al. 2020). The use of autonomous recording units has been applied, for example, to assess the response of owls to background noise (Shonfield et al. 2018) and artificial light at night (Marín-Gómez et al. 2020) and to assess the relationship between weather and vocal activity of northern temperate owls (Ševčík et al. 2019). However, to our knowledge, this technique has never been applied to study the impact of climatic conditions and the moon phase on the vocal activity of Neotropical owls.

In this study, we aimed to increase our knowledge about the relationship between climate and moon phase and the spontaneous vocal activity of a Neotropical owl, the Ferruginous pygmy-owl (*Glaucidium brasilianum*) (FPO hereafter). Through passive acoustic monitoring, we monitored the vocal activity of the species over three consecutive moon phases in the Brazilian Pantanal. We evaluated whether the effects of climatic predictors (air temperature, relative air humidity, and rainfall) and moon illumination are correlated with the vocal activity of the species. Cooler temperatures at night may improve sound transmission (Henwood & Fabrick 1979; Larom et al. 1997), and therefore, birds may prefer to vocalize under cooler temperatures to reach more receivers more efficiently (La 2012; Mennill 2014). Indeed, prior studies found high owl vocal activity at low temperature (Vazquez-Perez & Enriquez 2016). According to these findings, we predicted a negative association between the vocal activity of the FPO and the nocturnal air temperature (but see Norambuena & Muñoz-Pedrerros 2018 for a positive relationship between the vocal activity of owls and daily temperature). Based on previous studies on owls, we predicted a positive relationship between the vocal activity of the FPO and the relative air

humidity (Clark & Anderson 1997; Braga & Motta-Junior 2009), while we predicted that the amount of nocturnal rainfall would have a negative effect on the vocal activity of the species (Lengagne & Slater 2002; Zuberogoitia et al. 2019). Prior research has found a positive association between moon phase and the number of calls emitted by a large number of owls (Penteriani et al. 2010; Mori et al. 2014; Vazquez-Perez & Enriquez 2016; but see Rosado Hidalgo 2018). In agreement with these results, we expected to find a positive relationship between the vocal activity of the FPO and the percent of the moon that was illuminated.

## MATERIALS AND METHODS

### *Study species*

We selected the FPO as a study model to evaluate the effects of climate and moon phase on owl vocal activity. We chose this species because it is a common resident owl in the study area (Brazilian Pantanal, see below). The vocal activity of the species is concentrated during the crepuscular periods, with a high vocal activity after midnight (61.1% of the 394 calls recorded over a year in the monitored station using an autonomous recording unit were registered between 0 a.m. and 5 a.m., authors' unpublished data). The calling activity of the species is seasonal, with a peak in activity from June to September (90.9% of the 394 calls recorded over a year in the monitored station using an autonomous recording unit were registered during this 4-month period, authors' unpublished data). The FPO is a widespread, year-round resident of lowlands spanning from the southern United States to central Argentina (König & Weick 2008). The typical territorial call of the FPO is a long series of 8–30 monotonal hoots emitted at low frequencies (Fig. S1 in Supplemental Material). Only males utter territorial calls, usually while perched near the nest (Proudfoot et al. 2020).

### *Study area*

The study area was located in the northeastern part of the Brazilian Pantanal (Pantanal Matogrossense), close to the SESC Pantanal (Poconé municipality, Mato Grosso, Brazil; 16°30'S, 56°25'W, Fig. S2 in Supplemental Material). This area is composed of a mosaic of forested and savanna areas (Junk et al. 2006), which represents potential habitat for the FPO (König & Weick 2008). The area was located on the floodplain of the Cuiabá River (a tributary of the Paraguay River), which is seasonally inundated (October–April) due to the flooding of the Paraguay River (Junk et al. 2006). The regional climate is tropical and humid; the average annual rainfall is 1,000–1,500 mm, and the mean annual temperature is ~ 24 C. Detailed information about the local avian community and floristic elements of the study area can be found in de Deus et al. (2020).

### *Acoustic recording*

We placed one Song Meter SM2 recorder in the study area (Wildlife Acoustics, USA, [www.wildlifeacoustics.com](http://www.wildlifeacoustics.com)) at 1 m height attached to a tree. The recorder was active, always in the same location, from 16 June 2015 to 13 September 2015. This period corresponds to three complete moon cycles (see Table S1 in Supplemental Material). The recorder was programmed to record (in stereo and .wav format) the first 15 min of each hour from 00:00 to 05:00 and was configured with a sampling rate of 48 kHz and a resolution of 16 bits per sample. We selected this recording schedule (15 min per hour) for logistical reasons since we were able to visit the study

area only once per week and recording on continuous mode would have required extra visits. Recordings were stored on SD memory cards capable of storing ~ 250 hr of recordings. The recorder was powered by four 1.5 V alkaline batteries (Duracell MN13000) (~ 160 hr autonomy) and checked weekly to download data and change batteries. No device failures were detected. Based on previous studies with birds using the Song Meter SM2 recorder, we expected that the effective detection radius of the recorder for detecting FPO vocalizations might be around 160–200 m (Rempel et al. 2013; Pérez-Granados et al. 2019a).

#### *Acoustic data analyses*

The left channel of the recordings was scanned using Kaleidoscope Pro 5.1.9 (Wildlife Acoustics, USA, [www.wildlifeacoustics.com](http://www.wildlifeacoustics.com)), which is an automated signal recognition software that can detect a target signal according to the introduced parameters. According to calls recorded in the study area (see Table S2 in Supplemental Material), we selected the following signal parameters for detecting the FPO: minimum and maximum frequency range of 1150 and 1450 Hz, respectively; minimum and maximum length of detection of 2 and 24 sec, respectively; and a maximum intersyllable gap of 0.7 sec. The maximum intersyllable gap is considered the maximum allowable gap between syllables (hoots for the FPO); thus, hoots separated by less than 0.7 sec were considered to be part of the same call. Therefore, in this study, we considered the whole sequence of hoots as a unique call (see Fig. S1 in Supplemental Material). Kaleidoscope Pro also requires an extra parameter for scanning the recordings, which is the “maximum distance from the cluster center to include outputs” (Wildlife Acoustics 2020). This parameter ranges from 0 to 2, a higher parameter value yields more candidate sounds. Larger parameter values also result in a greater number of false positives (nontarget sounds, see Pérez-Granados et al. 2020). This parameter was set to 2, since we aimed to detect as many calls as possible.

All candidate sounds detected by Kaleidoscope Pro were visually and/or acoustically checked, always by the same observer (G. Pérez-Granados), to remove false positives from the data.

#### *Environmental variables*

We collected environmental data from a weather station located in the study area at a distance of 1,800 m from the recorder location (Fig. S2 in Supplemental Material). The following hourly information was gathered: air temperature (°C), rainfall (mm) and relative air humidity (%). In posterior analyses, we employed averaged values of air temperature and air humidity per night while accumulated rainfall during the night was used as variable in posterior analyses. We included the daily percent of the moon that was illuminated from the US Naval Observatory (<http://aa.usno.navy.mil/data/docs/MoonFraction.html>).

#### *Statistical analyses*

To identify which environmental variables had a major correlation on whether the FPO was vocally active at night, we employed a hierarchical partitioning approach (hereafter HP). The HP method can identify the independent contributions of the considered variables to the vocal activity of the species each night (Mac Nally 2002, see applications in bioacoustics studies in Pérez-Granados et al. 2019b). We used a logistic regression method with a log-likelihood function as the goodness-of-fit measure and tested the statistical significance of the independent contribution of each environmental variable based on a randomisation procedure with 999 bootstraps (Mac Nally 2002).

All statistical analyses were performed in R 3.6.2 (R Development Core Team 2019). The level of significance was  $P < 0.05$ , and the results were expressed as the mean  $\pm$  SE. We used the package “hier.part” for HP (Walsh & Mac Nally 2008).

## RESULTS

Kaleidoscope detected a total of 6,557 candidate sounds. After visually and/or acoustically checking these events, a total of 210 events were identified as calls of the FPO. The species was detected on 19 of the 90 monitoring nights (21.1%).

We found that the vocal activity of the FPO was positively correlated to the percent of the moon that was illuminated (Table 1). The percentage of the illuminated moon explained more than 71% of the vocal activity of the species. The FPO was more vocally active on nights with a high proportion of illuminated moon (Fig. 1). A total of 73.7% of the active nights (14 out of 19) had a moon illumination percent greater than 77%. Additionally, the nocturnal air temperature was negatively correlated to the vocal activity of the species (individual contribution of 14.5%, Table 1). The FPO was significantly more vocally active on nights with low nocturnal air temperatures, with only five vocally active nights (26.3% of the nights detected), and an average temperature higher than 18 °C (Fig. 1). The other two examined environmental predictors (air humidity and rainfall) were not significantly correlated with the vocal activity of the species (Table 1).

## DISCUSSION

Our study is the first to use passive acoustic monitoring to assess the relationship between the vocal activity of a Neotropical owl and climate predictors and the percent of the moon illuminated. The nocturnal air temperature was negatively

Table 1.

Environmental variables related to the vocal activity of the Ferruginous pygmy-owl in the Brazilian Pantanal. The vocal activity (active/inactive) was monitored with 15-min recordings obtained every hour from 00:00 a.m. to 05:00 a.m. from 16 June 2015 to 13 September 2015. The species was vocally active in 19 of the 90 monitoring days. The individual contribution of each environmental predictor is shown as a percentage (I%) of the total deviance explained by the predictors. The signs of the effects were obtained from univariate regression models. The z-score column shows the values of the randomised z-tests for the independent contributions, while the  $P$  column shows the significance level of the z-tests (\*\* $P < 0.01$ , and \*\*\* $P < 0.001$ ). Deviance refers to the percentage of deviance accounted for by a logistic regression including all variables.

Environmental predictor	Sign	I%	z-score	$P$
Mean air temperature	–	14.5	1.91	**
Air humidity	–	5.0	0.18	
Rainfall	–	9.3	0.55	
% of the moon illuminated	+	71.2	12.56	***
Deviance (%)		70.7		

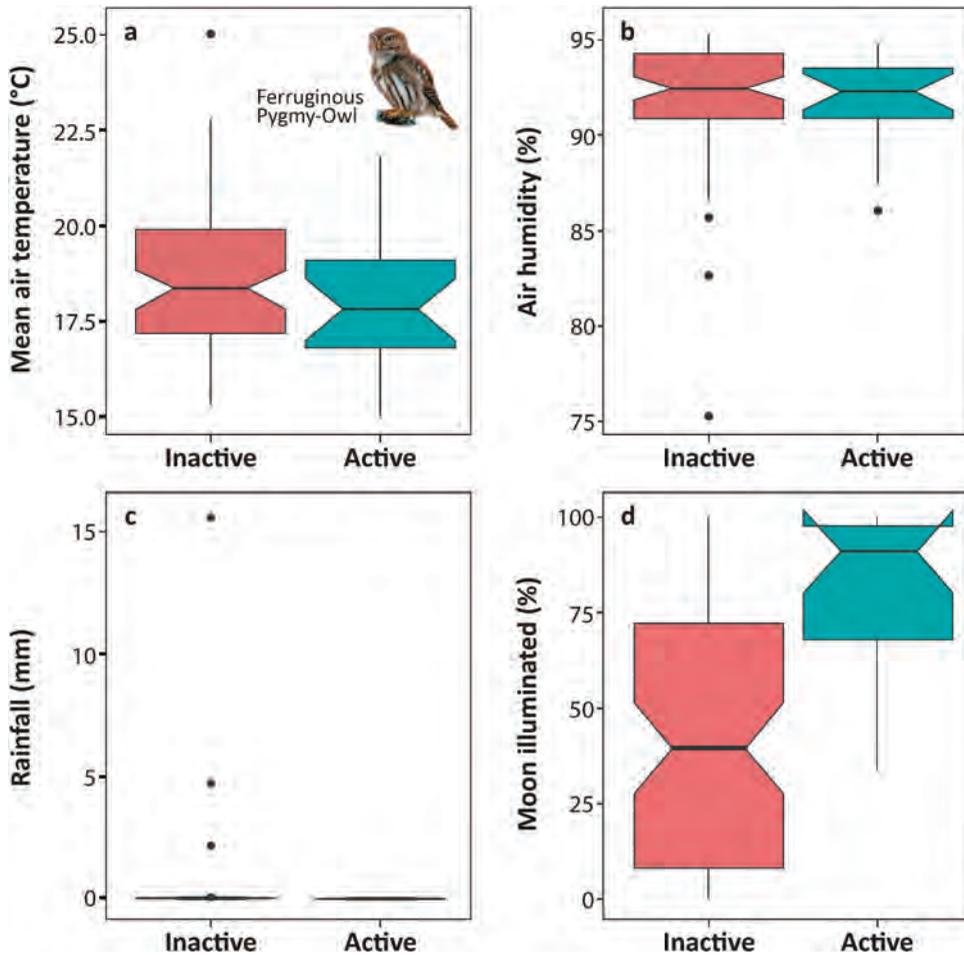


Fig. 1. — Boxplot showing the vocal activity (vocally active or inactive) of the Ferruginous pygmy-owl in the Brazilian Pantanal as a function of the environmental variables (mean air temperature, relative air humidity, accumulated rainfall) and percent of the moon illuminated. The vocal activity (active/inactive) was monitored with 15-min recordings obtained every hour from 00:00 a.m. to 05:00 a.m. from 16 June 2015 to 13 September 2015. The species was vocally active in 19 of the 90 monitoring days. The central band of boxplots are showing the median, boxes indicate the first (25%) and the third (75%) quartiles of the distribution, while vertical lines correspond to a 95% confidence interval. Points indicate outliers.

and significantly associated with the vocal activity of the FPO. This finding is in agreement with our prediction and previous studies that also found high vocal activity of owls (Vazquez-Perez & Enriquez 2016) and other non-passerine birds at low temperatures (Digby et al. 2014; Mennill 2014). Since improved sound transmission is expected in cooler temperatures (Henwood & Fabrick 1979; Larom et al. 1997; Mennill 2014), the FPO may prefer to vocalise on cooler nights to reach receivers more efficiently (i.e., the enhanced sound transmission hypothesis, La 2012). Daily minimum air temperature is usually reached at dawn, when the vocal activity of the species

is at a maximum level, and may contribute to explaining the significant impact of the nocturnal air temperature on the vocal activity of the FPO.

In contrast to our prediction and previous studies on owls, we did not find any relationship between the relative air humidity and the vocal activity of the FPO (Clark & Anderson 1997; Braga & Motta-Junior 2009). The lack of relationship between these variables in our study might be related to the fact that the relative air humidity was very high and constant during the study period ( $91.9\% \pm 3.2$ , mean  $\pm$  SD, Fig. 1). Therefore, the low daily variation in air humidity and the fact that air might have been hypersaturated throughout the study period may have made it difficult to detect an association between FPO vocal activity and relative air humidity. Similarly, the very low number of rainfall events during the study period (only 3 nights had daily rainfall events higher than 1 mm, Fig. 1) may clearly explain the lack of relationship found between nocturnal accumulated rainfall and vocal activity of the FPO, in contrast to our prediction and prior research that found a negative association between owl vocal activity and rainfall (e.g., Lengagne & Slater 2002; Zuberogoitia et al. 2019). Therefore, our results regarding the association between air humidity and rainfall with owl vocal activity are not conclusive, and further research should evaluate the association between the vocal activity of the FPO or other owls with air humidity and rainfall in areas with more variability of air humidity and during periods with more frequent rainfall events.

The percent of the moon illuminated was the variable with the highest correlation with the vocal activity of the FPO. Our results are in agreement with a large number of studies that also found a positive association between the percent of the moon illuminated and owl vocal activity (e.g., Braga & Motta-Junior 2009; Penteriani et al. 2010; Mori et al. 2014) as well as with artificial light at night (Marín-Gómez et al. 2020). Similarly, moonlight has been linked to owls' dispersal movements (Penteriani et al. 2014) and foraging efficiency (Clarke 1983). The light level during full moon nights is 10 times higher than that on nights with new moons (Kronfeld-Schor et al. 2013). The elevated light level on nights with a high percent of moon illumination may allow the FPO to extend their crepuscular routine into the night period, which may partly explain the positive association found between the percent of the moon illuminated and FPO vocal activity. Our results suggest the possibility of the existence of a circalunar clock, by which the FPO may regulate its vocal activity according to the moon phase (Goymann & Helm 2014) as reported in previous bird migration studies (Norevik et al. 2019; Evens et al. 2020). Previous studies have shown a significant impact of the moon position on the nocturnal vocal avian activity with birds calling earlier and more often under full moon nights only when the moon was above the horizon (York et al. 2014). In our analyses, we did not consider the effect of moon position above or below the horizon, since the moon was exposed (above the horizon) between 00:00 and 05:00 in 70% of the monitored nights. The percent of the moon illuminated during the nights that the moon was below the horizon was always lower than 55%, and the FPO was vocally active only at one of the 27 nights (3.7%) when the moon was below the horizon. Therefore, the low vocal activity of the FPO during nights with low percent of the moon illuminated and with the moon below the horizon supports the positive and significant relationship found between vocal activity of the FPO and percent of the moon illuminated and suggests that this relationship was driven by light intensity rather than by other factors associated with moon phase. We are aware that we did not control for cloud cover, which is a factor that may have influenced the moonlight perceived by the FPO and therefore may have distorted the

relationship between the percent of the moon illuminated and the vocal activity of the FPO (Digby et al. 2014). Further research analysing the impact of moonlight on owls' vocal activity should try to include cloud cover as covariate.

Our study increased our understanding of the effect of exogenous factors on the vocal activity of tropical birds, for which limited information is available; additionally, this study is the first to use passive acoustic monitoring to assess the relationship between climatic predictors and the percent of the moon illuminated with the spontaneous vocal activity of a Neotropical owl. Our results might be useful for improving the detection probability of the species without the need for playback experiments. According to our results, we suggest that future surveys aiming to detect the presence of the species should be carried out on nights with a moon illumination percent of at least 75% and, if possible, on nights with an average temperature lower than 18 °C. Future studies should attempt to include information about exogenous factors that were not tested in our study (e.g., cloud cover, background noise) as well as information about the impact of endogenous factors, such as hormone concentration and breeding status of recorded males, to improve our knowledge about the impacts of exogenous and endogenous factors on the vocal activity of owls.

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#### DISCLOSURE STATEMENT

The authors declare that there is no conflict of interest.

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#### AUTHOR CONTRIBUTIONS

(1) Conceived the idea, design, experiment (supervised research, formulated question or hypothesis): C. Pérez-Granados, K.-L. Schuchmann, M.I. Marques. (2) Performed the experiments (collected data, conducted the research): K.-L. Schuchmann, M.I. Marques. (3) Wrote

the paper (or substantially edited the paper): C. Pérez-Granados, K.-L. Schuchmann, M.I. Marques. (4) Developed or designed methods: C. Pérez-Granados. (5) Analyzed the data: C. Pérez-Granados. (6) Contributed substantial materials, resources, or funding: K.-L. Schuchmann, M.I. Marques.

#### SUPPLEMENTARY MATERIAL

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