



Ecological light pollution affects anuran calling season, daily calling period, and sensitivity to light in natural Brazilian wetlands

Karina Soares Dias¹ · Elisa Stuani Dosso¹ · Alexander S. Hall² · André Passaglia Schuch³ · Alexandro Marques Tozetti⁴

Received: 22 March 2019 / Revised: 24 June 2019 / Accepted: 25 June 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Ecological light pollution alters an environment's light cycle, potentially affecting photoperiod-controlled behavior. Anurans, for example, generally breed nocturnally, and the influence of light pollution on their natural history may therefore be especially strong. In this study, we tested this hypothesis by measuring male calling behavior of anuran communities in natural wetlands in southern Brazil exposed or not exposed to street lights. We recorded seasonal and diel calling activity and calling response to a light pulse. The peak calling season differed between continuously lit and unlit locations with most species in illuminated wetlands shortening their calling season and calling earlier in the year. In unlit breeding sites, *Boana pulchella*, *Pseudis minuta*, and *Pseudopaludicola falcipes* confined their calling activity to well-defined hours of the night, but in continuously lit areas, these species called more continuously through the night. A 2-minute light pulse inhibited calling, but only in unlit wetlands. After a light pulse, frogs quickly resumed calling—suggesting acclimatization to brief artificial light exposure. Our field experiment presents a convincing example of ecological light pollution showing that artificial light alters the seasonal and diel calling time of some South American wetland anurans. It also documents their acclimatization to brief lighting when being continuously exposed to light.

Keywords Amphibians · Night lighting · ALAN · Behavior · Vocalization · RAMSAR

Introduction

The effects of artificial light on ecosystems are called ecological light pollution (Longcore and Rich 2004). Ecological light

Communicated by: Matthias Waltert

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00114-019-1640-y>) contains supplementary material, which is available to authorized users.

✉ Karina Soares Dias
karinasdias_@hotmail.com

¹ Universidade Federal do Rio Grande – FURG, Campus Rio Grande, Rio Grande, Rio Grande do Sul 96203-900, Brazil

² Thermo Fisher Scientific, 16700 Park Row Dr., Houston, TX 77084, USA

³ Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS 97110-970, Brazil

⁴ Laboratório de Ecologia de Vertebrados Terrestres, Universidade do Vale do Rio dos Sinos – UNISINOS, Campus São Leopoldo, Rio Grande, Rio Grande do Sul 93020-190, Brazil

pollution is mainly caused by public lighting of streets, parks, buildings, and by vehicle lights (Longcore and Rich 2004). One of the main ways that this pollution affects ecosystems is through changes to the natural light-dark cycles of wildlife (Wise 2007). Changes in the natural light-dark cycles in ecosystems caused by artificial light may mask the natural changes of the photoperiod over the seasons and generate an imbalance in the biological cycles of some species (Gaston et al. 2013). Amphibians, for example, use the variations in photoperiod as seasonal cues which drives the rhythm of their reproductive cycle (Canavero and Arim 2009). Photoperiod is potentially a trigger for the reproductive season of many species (Hsu et al. 2006; Both et al. 2008), which is evidenced by the existence of seasonality in their calling activity (Wells 2007).

The impacts of light on reproductive cycles have been observed in some taxa, such as mammals, in which photoperiodic changes interfere with the mating period (LeTallec et al. 2015) and the birth of offspring (Robert et al. 2015). Furthermore, light pollution can alter the circadian rhythm of individuals, as reported in birds, which initiate their morning singing activities earlier in places with artificial lighting

(Kempnaers et al. 2010). An artificial light at night can be considered a direct disturbance, affecting antipredator behavior (Rand et al. 1997; Buchanan 2006) and decreasing metamorphic time length and growth rate of frog species (Dananay and Benard 2018). Adult anurans are mostly nocturnal animals (Buchanan 2006), increasing the likelihood of many species to be negatively affected by ecological light pollution.

Despite the increased concern about the threats to which amphibian populations are being exposed (e.g., habitat loss, habitat fragmentation, invasive species, diseases), little attention has been given to the role of artificial lighting in natural areas (Wise 2007). Considering that the levels of light pollution are increasing at a rate of about 6% every year (Hölker et al. 2010), studies of the effects of artificial lighting on reproductive behavior of anurans are important, providing relevant information for management actions (Buchanan 2006; Wise 2007). In an attempt to address these questions, we conducted field surveys and experiments to test the following hypotheses: (a) considering that artificial lighting (public lighting) is an interference to the natural photoperiod, we expected to find differences in the seasonal and diel calling activity pattern between species from wetlands with and without artificial lighting; and (b) considering that artificial light is a disturbance, the introduction of a light pulse during the night should reduce the number of vocalizing individuals.

Material and methods

Study area

The study was performed on the coastal plains of southern Brazil's Rio Grande municipality. This region belongs to the Pampa biome (IBGE 2004). The surveyed region's landscape is flat and dominated by flooded grasslands located approximately at sea level (Waechter 1985). The vegetation is mainly composed of herbaceous (grass) and aquatic plants (emergent and floating macrophytes; Burguer and Ramos 2006). The climate is humid subtemperate, with two well-defined seasons: warm (September to March) and cold (April to August). The mean annual temperature is 18.1 °C and the mean annual rainfall is 1162 mm distributed homogeneously over the year (Maluf 2000).

When present, the artificial lighting in a surveyed area refers to public lighting, and is generated by the street lights equipped with high-pressure sodium lamps (PMDRG 2011; Online Resource 1). The study areas are subjected to the same temperature and rainfall regimes. Thus, the main environmental variables considered to trigger anuran vocalization activity were considered operationally identical with the exception of photoperiod. The area selected for sampling includes well-preserved marshes that are near or inside a Conservation Site (Online Resource 1). The selected study sites, as well as their

surroundings, have a low level of urbanization and the vehicle traffic is low; so, light poles—whenever present—are the only continuous source of artificial light.

Survey design

Based on a pilot study during which we checked the main electrical transmitting lines surrounding local wetlands, we selected survey sites according to their distance from light poles. Sites were classified in the following manner: (a) “sites with lighting”: sites whose anuran breeding sites were between 50 and 100 m from the light poles; (b) “sites without lighting”: sites whose breeding sites were at least 2 km away from any light pole. These definitions were adopted after an evaluation of the incidence of light from poles to the ground. We measured illuminance using a lux meter (Instrutemp model ITLD270) positioned at different distances from the poles. The dissipation of light makes its detectability vary nonlinearly as one moves away from its source, with a sudden decrease in incidence after 100 m. In order to standardize the surveys, we surveyed portions of sampling sites within a distance of 50 to 100 m from the poles, where the light incidence was as homogeneous as possible.

We conducted surveys between September 2016 and August 2017 at six sites, three with lighting and three without lighting (Fig. 1). Each survey site was used as a breeding site by anurans, had an area of about 1 ha, and was located at least 1 km apart from the others. The sites without lighting were inside a conservation unit, the Taim Ecological Station (ESEC Taim), that is a RAMSAR site (RAMSAR 2018). All sites have the same configuration regarding vegetation cover, climate, hydroperiod (Cordazzo 2002; Pereira et al. 2012), and composition of anuran species. A total of 12 anuran species distributed in three families (Leptodactylidae, Hylidae, and Odontophrynidae) occur at the survey sites, which are *Boana pulchella*, *Dendropsophus minutus*, *Dendropsophus sanborni*, *Leptodactylus gracilis*, *Leptodactylus latrans*, *Leptodactylus latinasus*, *Physalaemus spp.*, *Pseudis minuta*, *Pseudopaludicola falcipes*, *Scinax granulatus*, *Scinax squalirostris*, and *Odontophrynus sp.* Two of those species (*S. granulatus* and *D. minutus*) occur only in sites with illumination, and *Leptodactylus latinasus* is the only species that occurs solely in sites without public lighting. This similarity favors the comparison of vocalization activity among the communities, since the composition of species could affect the pattern of vocalization activity.

Field procedures

In order to standardize the level of natural light, we only conducted surveys during a new moon (Grant et al. 2012). During all surveys, we measured environmental illuminance (with a lux meter; instrument model ITLD270), temperature, and

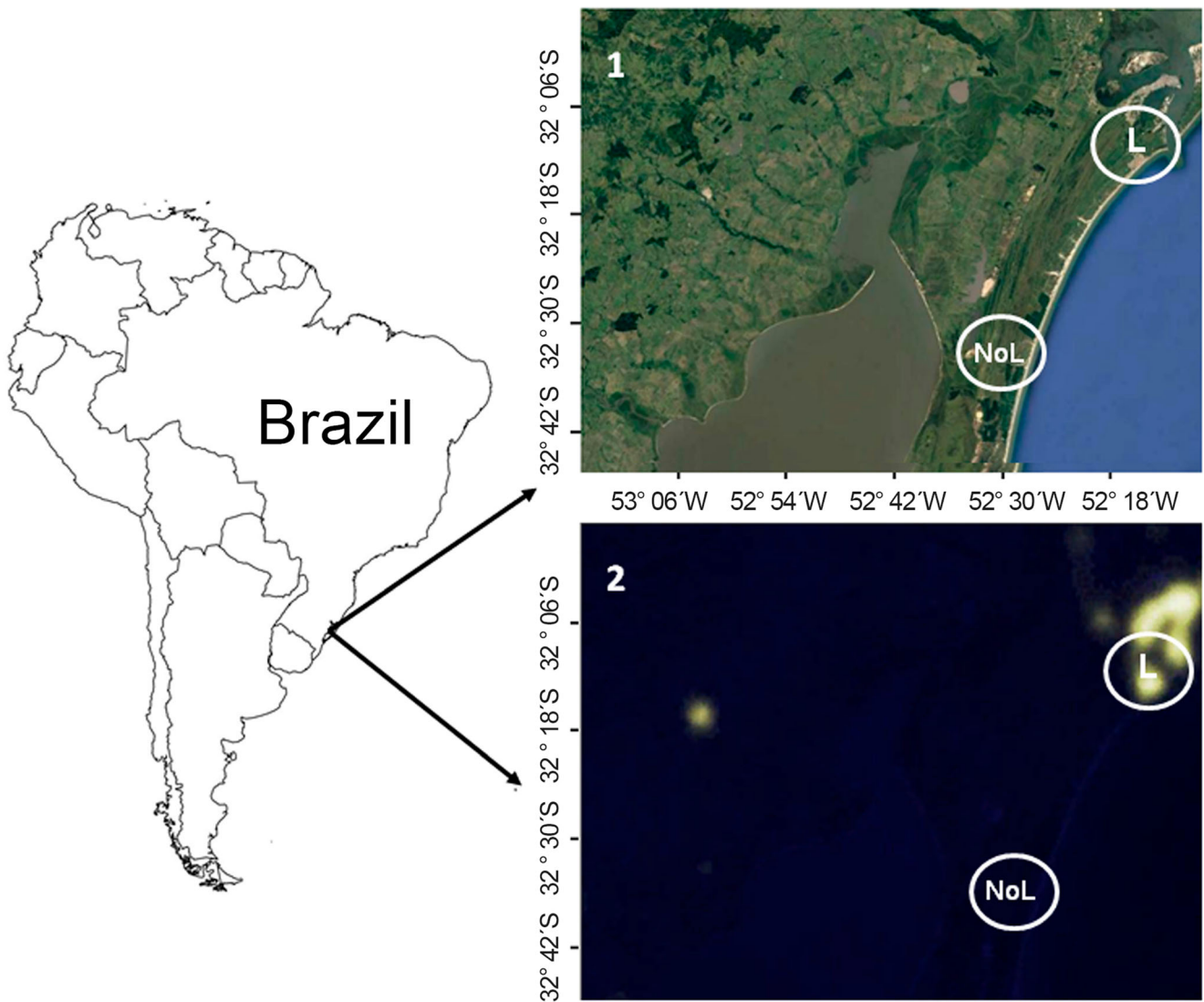


Fig. 1 Geographic location of the study area. Image 1 shows the sites with lighting “L” located around the urban area of the city of Rio Grande, and the survey sites without lighting “NoL” located in the Taim Ecological Station. Both areas are in the state of Rio Grande do Sul,

Brazil. Image 2 is of the same area as Image 1, but at night and showing the light pollution in the sites of point “L” and the absence of light pollution in point “NoL”

relative humidity in each of the sites. Data collection was performed in two consecutive nights monthly at each site. We used two sampling protocols, one for each sampling night as described below.

Protocol 1

This protocol was applied during the first sampling night each month and aimed to evaluate the influence of artificial lighting on (a) the seasonality in the vocalization activity, (b) the diel calling phenology, and (c) the extension of the vocalization season. We evaluated the calling activities by means of recording the environmental sound using an automated recording system (Bridges and Dorcas 2000;

Maldalozzo et al. 2017) equipped with a recorder (Sony ICD-PX312/PX312F). We installed the recorders at 1 m from the ground in each of the six sites. We programmed the recorders to record the environmental sound between 7:00 p.m. and 8:00 a.m. local time of the following day (13 h of continuous recording). This period includes the calling activity of all anuran species of this region (Ximenez and Tozetti 2015). For each survey site, we generated one audio file for each surveyed night. We later extracted audio excerpts with duration of 3 min counting from the start of each surveyed hour. This yielded 13 audio surveys for each of the 12 recorded nights for each survey site. Species recorded were identified with the use of a database of records of the study area as a sound-based

species guide (Haddad et al. 2005; Kwet and Márquez 2010). We estimated the abundance of vocalizing individuals in each survey in four categories: A) 1–4, 5–9, 10–20, or > 20 vocalizing individuals (Bertoluci and Rodrigues 2002; Ximenez and Tozetti 2015).

Protocol 2

This protocol aimed to evaluate the effect of an introduced light pulse on the vocalization behavior of the species. As it was an intervention on the local light conditions, protocol 2 was always performed one night after protocol 1. To generate the light pulse, we used a portable device composed of four 70-W halogen lamps (OSRM; Online Resource 2). This device was suspended by a rod that allowed it to be lifted such that the light reached the animals with approximately 60 lx—illuminance similar to that at the base of the light poles. This lamp model was selected after analyzing its spectra in tests conducted at Laboratório de Física da Universidade do Rio dos Sinos. We certified that selected lamps had the same properties to those used in roads poles (high-pressure sodium lamps), which include a broad spectrum, a wide wavelength (344 to 2398 nm), and an emission peak close to 1000 nm (Online Resource 3).

Before running the experiment, we placed the light in survey sites in a way so as to point towards the highest concentration of vocalizing individuals. We then waited until the males' vocalization activity returned to levels close to those before installing the light. The light was set to emit a single, continuous pulse directed towards the anurans for 2 min. We evaluated calling activity in three phases: 3 min before the pulse, during the pulse, and 3 min after the pulse. We recorded the number of calling male anurans of each species during each phase following the same abundance classes of protocol 1. Only anurans vocalizing within the first 100 m² of the illuminated area were counted. Human observers recorded observations on site. This allowed accurately constraining records to individuals located in the illuminated area.

Data analysis

We performed analyses using only species present in both site types (with and without light) and that reached the minimum n for respective tests. All circular analyses were performed using software Oriana (v. 4.02). The other statistical testing was performed in IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Released 2013, Armonk, NY).

Analysis of seasonality in vocalization activity

We applied circular statistics to test for existence and variation in anuran calling seasonality. To do this, each species' mean

abundance values per month were converted to relative values. The maximum abundance value found (10) was considered 100%, from which the respective percentages were defined based on the mean abundance of the species in each month in each type of sample unit. Each month was transformed into angles with intervals of 30° (January = 0° and December = 330°). In order to verify whether the abundance of vocalizing males throughout the months was equally distributed, we used Rao's spacing tests (Kovach 2011). A significant Rao test result ($\alpha = 0.01$) indicates seasonality for this species. We observed that no species' data followed a Von Mises distribution (Watson's U^2 tests). Thus, we conducted Watson-Wheeler tests to compare each species' annual vocalization activity distribution between sites with and without lighting ($\alpha = 0.05$). Only species shared among sites and that were recorded more than ten times were used in these analyses. Our methods followed Prado et al. (2005) and Caldart et al. (2016). The comparative parameters generated by the circular analyses were as follows: (1) mean vector (μ), which corresponds to the mean angle or time of the year when most males vocalized, (2) circular standard deviation (SD), and (3) the vector r , an index that measures the concentration of data around the circle (year), which varies from 0 (disperse data) to 1 (data concentrated in the same direction).

By listening to the 13 3-min audio surveys per sampling night, we filled a table with each species' abundances per day and per survey hour. Using this table, we calculated the number of months that each species vocalized. This should test for a change in the length of the vocalization season. We performed this evaluation with all nine species shared between the two site types.

Influence of artificial lighting in the diel calling phenology

We evaluated the variation in abundance of calling males throughout the night using circular analysis. Surveyed hours from 7:00 p.m. to 8:00 a.m. were transformed into angles, from 0 to 360°, with an interval of 27.69° between survey times. Additionally, we calculated the call frequency per hour and binned these data into their corresponding angles (h). The analyses were the same as described for the seasonal circular analysis. We used Rao's tests to evaluate calling activity uniformity over the night per species and per lighting condition ($\alpha = 0.01$). We also used Watson-Wheeler test to compare the night period of the peak number of calling males of each species between the two types of sites ($\alpha = 0.05$). The analyses described above related to the nocturnal variation of vocalization between the sites with and without lighting were performed with data of species shared between all sampling sites and that reached a sufficient number to conduct the analyses ($n \geq 10$).

Table 1 Results of the circular statistics to verify the occurrence of seasonality in the abundance of vocalizing males of different anuran species throughout the year in marshes with and without interference of artificial lighting (public lighting) in southern Rio Grande do Sul, Brazil

Species	Light in survey site	<i>n</i>	Mean vector (μ)	Mean vector date (rounded)	Mean vector length (<i>r</i>)	Circular standard deviation (SD)	Rao (<i>P</i>)	Watson-Wheeler
<i>Physalaemus</i> spp.	With	140	220.5°	10 August	0.941	20°	<i>U</i> = 349.7 <i>P</i> < 0.01	<i>W</i> = 20.2 <i>P</i> < 0.05
	Without	60	254.6°	15 September	0.762	42.2°	<i>U</i> = 32 <i>P</i> < 0.01	
<i>P. minuta</i>	With	58	262.7°	23 September	0.693	49°	<i>U</i> = 322.7 <i>P</i> < 0.01	<i>W</i> = 10 <i>P</i> < 0.05
	Without	203	257.6°	18 September	0.805	37.8°	<i>U</i> = 345.8 <i>P</i> < 0.01	
<i>S. squalirostris</i>	With	178	180.3°	30 June	0.558	61.9°	<i>U</i> = 341.8 <i>P</i> < 0.501	<i>W</i> = 3 <i>P</i> > 0.05
	Without	156	180.5°	30 June	0.548	62.9°	<i>U</i> = 336.9 <i>P</i> < 0.01	
<i>D. sanborni</i>	With	60	263.4°	23 September	0.616	56.4°	<i>U</i> = 318 <i>P</i> < 0.01	<i>W</i> = 49.9 <i>P</i> < 0.05
	Without	113	285.3°	15 October	0.889	27.7°	<i>U</i> = 340.9 <i>P</i> < 0.01	
<i>B. pulchella</i>	With	200	142.7°	23 May	0.614	56.6°	<i>U</i> = 343.8 <i>P</i> < 0.01	<i>W</i> = 10.5 <i>P</i> < 0.05
	Without	351	158.6°	9 June	0.744	44°	<i>U</i> = 350.8 <i>P</i> < 0.01	
<i>L. gracilis</i>	With	37	348.9°	19 December	0.849	32.8°	<i>U</i> = 321 <i>P</i> < 0.01	<i>W</i> = 1.2 <i>P</i> > 0.05
	Without	43	350°	20 December	0.763	42.1°	<i>U</i> = 318. <i>P</i> < 0.01	
<i>P. falcipes</i>	With	13	309.7°	10 November	0.91	24.9°	<i>U</i> = 276.9 <i>P</i> < 0.01	<i>W</i> = 0.3 <i>P</i> > 0.05
	Without	100	311.1°	11 November	0.883	28.6°	<i>U</i> = 358.2 <i>P</i> < 0.01	

Significant values (*P* < 0.01 and *P* < 0.05) are shown in italic font

Influence of a light pulse on calling behavior

To verify whether the light pulse modified the number of vocalizing individuals, we compared the number of calling males between the three phases of our experiment: before, during, and after the light pulse. As these data did not support the normality assumptions based on the Shapiro-Wilk test, we used Wilcoxon tests (the non-parametric equivalent of the paired *t* test) for these comparisons.

Results

Influence of artificial light on the seasonality of calling activity

Calling activity was seasonal at all sites, regardless of ambient illumination (values of *r* > 0.5, *P* < 0.01; Table 1). Annually, all species limited their calling season to a specific period (Figs. 2 and 3). Importantly, the seasonal timing of the peak number of

calling males differed between sites with and without ambient lighting in four species (Table 1): *B. pulchella* (*W* = 10.541, *P* = 0.005), *D. sanborni* (*W* = 49.961, *P* < 0.01), *Physalaemus* spp. (*W* = 20.154, *P* < 0.01), and *P. minuta* (*W* = 10.11, *P* = 0.006). The peak calling activity of *B. pulchella*, *D. sanborni*, and *Physalaemus* spp. moved earlier when lit (approximately 23 May, 23 September, and 10 August, respectively) compared with unlit (approximately 9 June, 15 October, and 15 September, respectively; Table 1). The opposite response occurred for *P. minuta*: peak calling activity shifted later in lit sites (from 18 September to 23 September; Table 1). In Online Resource 4, we report the mean abundance of each species in all surveyed months.

Five of nine species that called in both site types exhibited longer vocalization seasons in the sites without lighting (*L. gracilis*, *Odontophrynus* sp., *Physalaemus* spp., *P. minuta*, and *S. squalirostris*). Two species, *D. sanborni* and *P. falcipes*, showed longer vocalization seasons in the sites with lighting. Annually, the other two species had similar behavior between site types (Fig. 2).

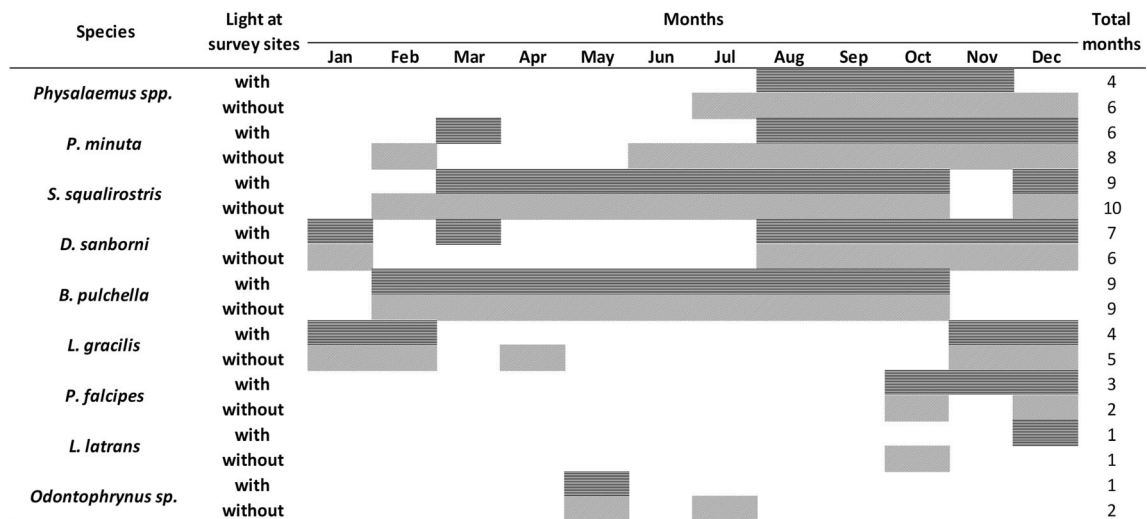


Fig. 2 Extension of the vocalization season of anuran species in marshes with influence of artificial lighting (public lighting) and marshes without influence of artificial lighting. Sites were located around the urban zone of

the city of Rio Grande, RS, Brazil, and in the Taim Ecological Station, RS, Brazil, respectively, and monitored between September 2016 and August 2017

Influence of artificial lighting in the diel calling phenology

We noted different diel calling phenology (daily behavior) between sites with and without lighting in four species: *B. pulchella*, *P. minuta*, *P. falcipes*, and *Physalaemus spp.* In the first three species, and exclusively in sites without lighting, the calling activity was concentrated to a specific period of the night ($P < 0.01$; Table 2). In sites with lighting, these species showed calling activity evenly distributed between 7:00 p.m. and 8:00 a.m. (Table 2). *Physalaemus spp.* showed the inverse tendency: even calling in sites without lighting and concentrated to a certain period in sites with lighting ($P < 0.01$; Table 2). The peak calling activity of these four species did not vary between sites with and without lighting ($P > 0.05$), only the spread. The other three species (*D. sanborni*, *L. gracilis*, and *S. squalirostris*) showed equally even distributions of vocalization in both site types (Table 2). Online Resource 5 shows the frequencies used to conduct the circular analyses. Significant values ($P < 0,01$ and $P < 0,05$) are shown in italic font

Influence of a light pulse on the calling behavior

Only anuran communities in sites without artificial lighting exhibited a behavioral change when exposed to a light pulse. In these sites, the introduction of a light pulse led to an immediate reduction in the number of calling males. Before the pulse, there were on average 2.19 ± 2.4 (SD) individuals vocalizing. This was reduced to 1.26 ± 1.6 (SD) individuals when illuminated ($Z = -2.871$, $P = 0.004$, $n = 21$). Overall, the number of calling males immediately re-established after

the pulse without variation between the number of calling males before and after the pulse ($Z = -1.604$, $P = 0.109$, $n = 21$). In sites exposed to ambient lighting, the pulse did not change the number of calling males ($Z = -1.342$, $P = 0.18$, $n = 23$). In these sites, the calling abundance before and after the light pulse did not change ($Z = 0$, $P = 1$, $n = 23$). The mean number of vocalizing individuals was 1.87 ± 1.7 (SD) before and after the pulse.

Discussion

Our field experiment shows that calling activity in some South American anuran species changes due to artificial light at night. This is a clear example of ecological light pollution (Longcore and Rich 2004). The light pollution affected seasonal and diel anuran breeding activity. One unexpected change was an anticipation of peak calling season in ambiently lit locations. Changes in calling season timing, if not accompanied by adjustments in the physiology of the female reproductive cycle, could lead to lower reproductive efficiency in these populations. Additionally, we speculate that altered breeding seasons may affect the juvenile recruitment period. All else equal, adjusted breeding seasons may expose juveniles to less favorable climates or resource availability. Although speculative, similar events are known from a widely cited report in birds (Kempenaers et al. 2010).

Lighting also affected daily calling habits (i.e., diel calling activity phenology). Calling activity in *B. pulchella*, *P. minuta*, and *P. falcipes* was concentrated to a specific period of the night only in the sites without lighting, but was

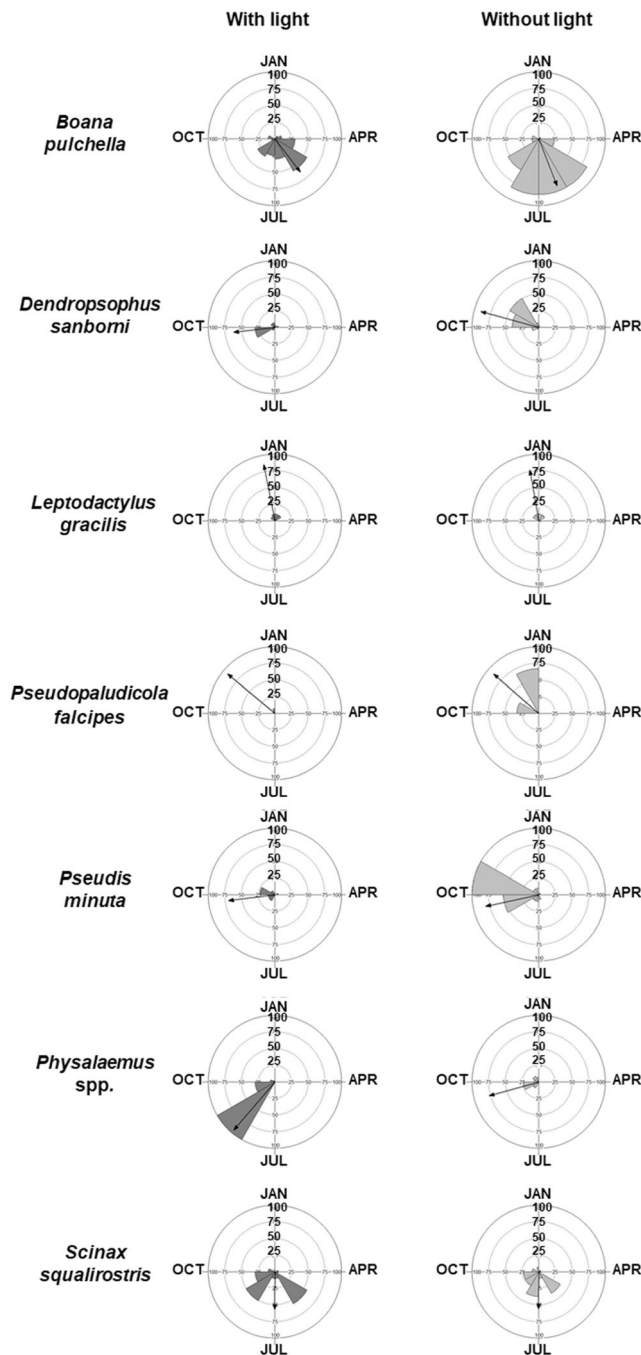


Fig. 3 Circular diagrams representing the mean monthly abundance of vocalizing males of *Boana pulchella*, *Dendropsophus sanborni*, *Leptodactylus gracilis*, *Pseudopaludicola falcipes*, *Pseudis minuta*, *Physalaemus* spp., and *Scinax squalirostris*. Calls were recorded between January (0°) and December (330°; intervals of 30° between the months) in sites with and without influence of artificial lighting (public lighting) in southern Rio Grande do Sul, Brazil. The black arrow corresponds to the vector r and points to the angle (month) with the average concentration of abundance of individuals. The length of the vector r indicates the concentration of individuals around the mean angle (smaller, scattered; larger, concentrated). Scales range from 0 to 100

continuous in sites with light. Despite this general pattern, the species-specific responses were highly variable; thus, this

suggests differences regarding each species' sensitivity to changes in natural light. Artificial light can, supposedly, reduce a frog's capacity to perceive subtle changes in the natural light. This fact may explain changes in diel or seasonal pattern of calling activity when the artificial light is present.

Five of nine species shortened their vocalization season when lit by artificial lighting. Overall, these five species (*L. gracilis*, *Physalaemus* sp., *P. minuta*, *S. squalirostris*, and *Odontophrynus* sp.) exhibited a response to light that likely led to fewer opportunities for attracting female mates. Potentially, this could reduce their reproductive success, though this was not directly measured in this study. In contrast, the two species *D. sanborni* and *P. falcipes* showed opposite responses: longer vocalization seasons in sites with lighting. It is worth noting that *D. sanborni* can exhibit certain level of tolerance from light since many *Dendropsophus* are well adapted to urban environments (Santos et al. 2008; López et al. 2015). We also found another species of the genus (*D. minutus*) only in the units with lighting. Besides that, *P. falcipes* often vocalizes during the day (Witt 2013), and may hence be less affected by light than nocturnal species (Buchanan 2006; Wise 2007).

We note that our results could be evaluated in terms of photoperiod change (Buchanan 2006; Both et al. 2008) thus affecting the circadian rhythm of amphibians. Photoperiod is an important biological rhythm marker that regulates the reproductive activity of amphibians by marking seasons and adequate reproductive periods (Canavero and Arim 2009). Reproductive and circadian rhythms in some taxa change in response to artificial lighting via an inhibition of melatonin production (D'Istria 1994; Brüning et al. 2015; Ouyang et al. 2018), such as in mammals (LeTallec et al. 2015; Robert et al. 2015) and fish (Brüning et al. 2015). In anurans, inhibiting melatonin affects gonad development (Delgado et al. 1983; Alonso-Bedate et al. 1988; Udaykumar and Joshi 1997).

We must be cautious before attributing all observed changes to light pollution. On the other hand, it is worth highlighting that the surveyed breeding sites with and without lighting were remarkably similar in regard to vegetation cover (microhabitat), were subjected to the same climatic conditions (Cordazzo 2002; Pereira et al. 2012), and shared basically the same anuran assemblages. Additionally, our data collection on both sites happened on consecutive days to avoid great differences in temperature and humidity between surveys. Thus, we were strongly convinced that artificial light was the most relevant distinguishing factor between sites.

We also note that repeated statistical tests across many different species will inevitably increase the chance of a type I error (false positive), and that some tests will be significant only due to chance. We feel that aggressive multiple test corrections (cf. Bonferroni) would be overly punishing for the amount of variance typically seen in field experiments. At $\alpha = 0.05$, we expected two significant tests due to chance. A Bonferroni correction for 51 tests would have led to a

Table 2 Results of the circular statistics for the analysis of the abundance distribution of vocalizing males throughout the night (1900 to 0700) in marshes with and with interference of artificial lighting (public lighting) in southern Rio Grande do Sul, Brazil

Species	Light in survey site	<i>n</i>	Mean vector (μ)	Mean vector hour-p.m. (rounded)	Mean vector length (<i>r</i>)	Circular standard deviation (SD)	Rao (<i>P</i>)	Watson-Wheeler
<i>Physalaemus spp.</i>	With	44	77.8°	10:00	0.083	127.8°	<i>U</i> = 253.6 <i>P</i> < 0.01	<i>W</i> = 0.09 <i>P</i> > 0.05
	Without	12	107.9°	11:00	0.154	110.9°	<i>U</i> = 53 <i>P</i> > 0.01	
<i>P. minuta</i>	With	21	126.8°	11:30	0.142	113.2°	<i>U</i> = 137.1 <i>P</i> > 0.01	<i>W</i> = 0.869 <i>P</i> > 0.05
	Without	34	85.9°	10:00	0.213	100.7°	<i>U</i> = 222.3 <i>P</i> < 0.01	
<i>S. squalirostris</i>	With	18	83.1°	10:00	0.046	142.1°	<i>U</i> = 100 <i>P</i> > 0.01	<i>W</i> = 0.56 <i>P</i> > 0.05
	Without	18	106.8°	11:00	0.266	93.2°	<i>U</i> = 120 <i>P</i> > 0.01	
<i>D. sanborni</i>	With	15	70.6°	9:30	0.161	109.3°	<i>U</i> = 72 <i>P</i> > 0.01	<i>W</i> = 0.936 <i>P</i> > 0.05
	Without	19	85.4°	10:00	0.393	78.2°	<i>U</i> = 151.6 <i>P</i> > 0.01	
<i>B. pulchella</i>	With	15	75.9°	10:00	0.189	104.6°	<i>U</i> = 72 <i>P</i> > 0.01	<i>W</i> = 0.885 <i>P</i> > 0.05
	Without	35	78.8°	10:00	0.446	72.7°	<i>U</i> = 236.6 <i>P</i> < 0.01	
<i>L. gracilis</i>	With	10	124.6°	11:30	0.277	91.7°	<i>U</i> = 74.8 <i>P</i> > 0.01	–
	Without	8	121.8°	11:30	0.433	74.1°	<i>U</i> = 103.8 <i>P</i> > 0.01	
<i>P. falcipes</i>	With	10	124.6°	11:30	0.277	91.7°	<i>U</i> = 74.8 <i>P</i> > 0.01	<i>W</i> = 1.95 <i>P</i> > 0.05
	Without	42	53.6°	5:00	0.467	70.6°	<i>U</i> = 257.1 <i>P</i> < 0.01	

Significant values ($P < 0.01$ and $P < 0.05$) are shown in italic font

vanishingly small corrected alpha unrealistic for behavioral testing. In spite of increased type I errors, statistically significant tests converged on a fairly cohesive response to seasonality, diel phenology, and light pulse response. Thus, we infer that detected patterns of changes in anuran calling activity were real and not entirely attributable to spurious significance.

Each species' life history and micro-habitat selection preferences may affect its susceptibility to the impacts of artificial light. Among the species in which we recorded the greatest changes in the vocalization behavior due to artificial light, some vocalize on the water surface (*P. minuta* and *Physalaemus spp.*) while others vocalize when perched on vegetation (*B. pulchella* and *D. sanborni*; Witt 2013). *L. gracilis*, which apparently was one of the species being least affected by artificial light, vocalizes inside burrows constructed on the ground (Witt 2013). This may protect *L. gracilis* from artificial light at night, at least with regard to reproductive activity. In this way, we may have observed that artificial light differently affected the vocalization behavior of species occupying different microhabitats in the same environment. However, *L. latinasus* also calls from burrows and seems to be affected by the presence of

artificial light. We believe that other ecological traits of *L. gracilis* made this species prone to live in altered habitats as showed by Santos et al. (2008).

The light pulse experimentally introduced inhibited vocalization activity only in the sites without lighting. This behavior was already recorded by Hall (2016) in some North American pond-dwelling frogs. This provides further evidence for acclimatization to a temporary light due to the continuous presence of artificial lighting. Additionally, we observed that males in ambiently lit sites rapidly resumed vocalizing after the pulse. This intriguing finding suggests that brief and interleaved lighting, such as that generated by car headlights, could affect reproductive activity by decreasing the vocalization time of males in naturally dark places where pulses of light occur frequently (Baker and Richardson 2006). An obvious example is breeding sites near rural roads.

Our results demonstrate a clear case of ecological light pollution in South American frog communities. Artificial lighting near anuran reproductive sites may compromise the reproductive success of some species, but this is still an untested assumption. To verify this important connection between light pollution and anuran fitness, it will be important

to conduct experimental studies in the field and in controlled conditions to extract how this type of pollution interacts with the natural history of a species. At present, our study is one of the most comprehensive field experiments to demonstrate an interaction between light pollution and response to a light pulse in natural anuran communities.

Acknowledgments The Taim Ecological Station permitted us to carry out the research in their locality and provided accommodations. We are also grateful to colleagues Nathan Arocha, Saulo Pino, and Sandra Halfen for assisting us with field work. Finally, we thank Cassio Hervé for help with the analysis of the lamps.

Funding information We thank the Coordination of Improvement of Higher Education Personnel for the financial support.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

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

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Permit number: 54857 issued by Sisbio, Brazil.

References

- Alonso-Bedate M, Delgado MJ, Carballada R (1988) In vivo effect of melatonin and gonadotropin-releasing hormone on testicular function in *Rana temporaria*. *J Pineal Res* 5:323–332. <https://doi.org/10.1111/j.1600-079X.1988.tb00881.x>
- Baker BJ, Richardson JML (2006) The effect of artificial light on male breeding season behaviour in green frogs, *Rana clamitans melanota*. *Can J Zool* 84:1528–1532. <https://doi.org/10.1139/z06-142>
- Bertoluci J, Rodrigues MT (2002) Seasonal patterns of breeding activity of atlantic rainforest anurans at Boracéia, southeastern Brazil. *Amphibia-Reptilia* 23:161–167. <https://doi.org/10.1163/156853802760061804>
- Both C, Kaefler IL, Santos TG, Cechin STZ (2008) An austral anuran assemblage in the Neotropics: seasonal occurrence correlated with photoperiod. *J Nat Hist* 42:205–222
- Bridges AS, Dorcas ME (2000) Temporal variation in anuran calling behavior: implications for surveys and monitoring programs. *Copeia* 2000:587–592. [https://doi.org/10.1643/0045-8511\(2000\)000\[0587:TVIACB\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2000)000[0587:TVIACB]2.0.CO;2)
- Brüning A, Hölker F, Franke S, Preuer T, Kloas W (2015) Spotlight on fish: light pollution affects circadian rhythms of European perch but does not cause stress. *Sci Total Environ* 511:516–522. <https://doi.org/10.1016/j.scitotenv.2014.12.094>
- Buchanan BW (2006) Observed and potential effects of artificial night lighting on anuran amphibians. In: Catherine R, Travis L (eds) *Ecological consequences of artificial night lighting*. Island Press, Washington, D.C., pp 192–220
- Burguer M, Ramos R (2006) Áreas importantes para conservação na Planície Costeira do Rio Grande do Sul. In: Becker FG (ed) *Biodiversidade: Regiões da Lagoa do Casamento and dos Butiazais de Tapes, planície costeira do Rio Grande do Sul. Série Biodiversidade*, 25. Ministério do Meio Ambiente, Brasília, Brazil.
- Caldart VM, Iop S, Lingnau R, Cechin SZ (2016) Calling activity of a stream-breeding frog from the austral Neotropics: temporal patterns of activity and the role of environmental factors. *Herpetologica* 72: 90–97. <https://doi.org/10.1655/HERPETOLOGICA-D-15-00029>
- Canavero A, Arim M (2009) Clues supporting photoperiod as the main determinant of seasonal variation in amphibian activity. *J Nat Hist* 43:2975–2984. <https://doi.org/10.1080/00222930903377539>
- Cordazzo CV (2002) Effect of seed mass on germination and growth in three dominant species in southern Brazilian coastal dunes. *Braz J Biol* 62:427–435. <https://doi.org/10.1590/S1519-69842002000300005>
- Danayay KL, Benard MF (2018) Artificial light at night decreases metamorphic duration and juvenile growth in a widespread amphibian. *Proc R Soc B* 285:20180367. <https://doi.org/10.1098/rspb.2018.0367>
- Delgado MJ, Gutierrez P, Alonso-Bedate M (1983) Effects of daily melatonin injections on the photoperiodic gonadal response of the female frog *Rana ridibunda*. *Comp Biochem Physiol* 76A:389–392. [https://doi.org/10.1016/0300-9629\(83\)90343-2](https://doi.org/10.1016/0300-9629(83)90343-2)
- D'Istria M, Monteleone P, Serino I, Chieffi G (1994) Seasonal variations in the daily rhythm of melatonin and NAT activity in the Harderian gland, retina, pineal gland, and serum of the green frog, *Rana esculenta*. *Gen Comp Endocrinol* 96:6–11. <https://doi.org/10.1006/gcen.1994.1153>
- Gaston KJ, Bennie J, Davies TW, Hopkins J (2013) The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biobehav Rev* 88:912–927. <https://doi.org/10.1111/brv.12036>
- Grant R, Halliday T, Chadwick E (2012) Amphibians' response to the lunar synodic cycle - a review of current knowledge, recommendations, and implications for conservation. *Behav Ecol* 24:53–62. <https://doi.org/10.1093/beheco/ars135>
- Haddad C, Giovanelli J, Giasson L, Toledo L (2005) Guia sonora dos anfíbios anuros da Mata Atlântica. Biota FAPESP, São Paulo, Brazil
- Hall AS (2016) Acute artificial light diminishes central Texas anuran calling behavior. *Am Midl Nat* 175:183–193. <https://doi.org/10.1674/0003-0031-175.2.183>
- Hölker F, Moss T, Griefahn B, Kloas W, Voigt CC, Henckel D, Hänel A, Kappeler PM, Völker S, Schwöpe A, Franke S, Uhrlandt D, Fischer J, Klenke R, Wolter C, Tockner K (2010) The dark side of light: a transdisciplinary research agenda for light pollution policy. *Ecol Soc* 15(13) <http://resolver.sub.uni-goettingen.de/purl?gs-1/7268>
- Hsu MY, Kam YC, Fellers GM (2006) Temporal organization of an anuran acoustic community in a Taiwanese subtropical forest. *J Zool* 269:331–339. <https://doi.org/10.1111/j.1469-7998.2006.00044.x>
- IBGE [Instituto Brasileiro de Geografia and Estatística] (2004) Mapa de biomas do Brasil. <https://ww2.ibge.gov.br/home/presidencia/noticias/21052004biomashtml.shtm>. Accessed 5 June 2017
- Kempnaers B, Borgström P, Loës P, Schlicht E, Valcu M (2010) Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds. *Curr Biol* 20:1735–1739. <https://doi.org/10.1016/j.cub.2010.08.028>
- Kovach WL (2011) Oriana – circular statistics for Windows, ver. 4. Kovach Computing Services, Pentraeth, Wales, U.K.
- Kwet A, Márquez R (2010) Sound guide of the calls of frogs and toads from southern Brazil and Uruguay. Alosa: sons de la natura, Fonoteca, Madrid, Spain audio CD
- LeTallec T, Théry M, Perret M (2015) Effects of light pollution on seasonal estrus and daily rhythms in a nocturnal primate. *J Mammal* 96: 438–445. <https://doi.org/10.1093/jmammal/gyv047>
- Longcore T, Rich C (2004) Ecological light pollution. *Front Ecol Environ* (4):191–198. [https://doi.org/10.1890/1540-9295\(2004\)002\[0191:ELP\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2004)002[0191:ELP]2.0.CO;2)

- López JA, Scarabotti PA, Ghirardi AR (2015) Amphibian trophic ecology in increasingly human-altered wetlands. *Herpetol Conserv Biol* 10: 819–832
- Maluf JRT (2000) Nova classificação climática do Estado do Rio Grande do Sul. *Revista Brasileira de Agrometeorologia* 8:141–150
- Madalozzo B, Santos TG, Santos MB, Both C, Cechin S (2017) Biodiversity assessment: selecting sampling techniques to access anuran diversity in grassland ecosystems. *Wildl Res* 44:78–91. <https://doi.org/10.1071/wr16086>
- Ouyang JQ, Davies S, Dominoni D (2018) Hormonally mediated effects of artificial light at night on behavior and fitness: linking endocrine mechanisms with function. *J Exp Biol* 221:jeb156893. <https://doi.org/10.1242/jeb.156893>
- Pereira AS, Trindade CR, Albertoni EF, Palma-Silva C (2012) Aquatic macrophytes as indicators of water quality in subtropical shallow lakes, Southern Brazil. *Acta Limnol Bras* 24:52–63. <https://doi.org/10.1590/S2179-975X2012005000026>
- PMDRG (2011) Prefeitura Municipal do Rio Grande. Prefeitura do Rio Grande. <http://www.riogrande.rs.gov.br/pagina/index.php/noticias/detalhes+a751,,em-breve-municipio-podera-dar-inicio-ao-projeto-reluz.html#.WqdEjyjwbIU>. Accessed 10 June 2017
- Prado CPA, Uetanabaro M, Haddad CFB (2005) Breeding activity patterns, reproductive modes, and habitat use by anurans (Amphibia) in a seasonal environment in the Pantanal, Brazil. *Amphibia-Reptilia* 26:211–221. <https://doi.org/10.1163/1568538054253375>
- RAMSAR (2018) Wetland of international importance. <https://www.ramsar.org/>. Accessed 15 March 2018
- Rand AS, Bridarolli ME, Dries L, Ryan MJ (1997) Light levels influence female choice in Túngara Frogs: predation risk assessment. *Copeia* (2):447–450. <https://doi.org/10.2307/1447770>
- Robert KA, Lesku JA, Partecke J, Chambers B (2015) Artificial light at night desynchronizes strictly seasonal reproduction in a wild mammal. *Proc R Soc B* 282:20151745. <https://doi.org/10.1098/rspb.2015.1745>
- Santos TG, Kopp K, Spies MR, Trevisan R, Cechin SZ (2008) Distribuição temporal e espacial de anuros em área de Pampa, Santa Maria, RS. *Iheringia, Sér Zool* 98:244–253. <https://doi.org/10.1590/S0073-47212008000200013>
- Udaykumar K, Joshi BN (1997) Effect of exposure to continuous light and melatonin on ovarian follicular kinetics in the skipper frog, *Rana cyanophlyctis*. *Biol Signals* 6:62–66. <https://doi.org/10.1159/000109110>
- Waechter JL (1985) Aspectos ecológicos da vegetação de restinga no Rio Grande do Sul, Brasil. *Comunicações do Museu de Ciências da PUCRS, série Botânica*, vol 33, pp 49–68
- Wells KD (2007) The ecology and behavior of amphibians. The University of Chicago Press, Chicago
- Wise S (2007) Studying the ecological impacts of light pollution on wildlife: amphibians as models. In: Cipriano M, Jafar J (eds) *Starlight: a common heritage*, pp 209–218
- Witt PBR (2013) Fauna e flora da reserva biológica Lami José Lutzenberger. Porto Alegre: Secretaria Municipal do Meio Ambiente, Rio Grande do Sul, Brazil
- Ximenez SS, Tozetti AM (2015) Seasonality in anuran activity and calling season in a Brazilian subtemperate wetland. *Zool Stud* 54:1–9. <https://doi.org/10.1186/s40555-015-0125-8>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.