

The effects of environmental cues on chorusing onset in a tropical frog assemblage

Sergio C. Gonzalez, Venetia S. Briggs-Gonzalez*

Department of Wildlife Ecology and Conservation, Fort Lauderdale Research and Education Center,
University of Florida, Fort Lauderdale, FL 33314, USA

*Corresponding author; e-mail: vsbriggs@ufl.edu

ORCID iD: Briggs-Gonzalez: 0000-0001-7748-7986

Received 21 September 2021; final revision received 28 January 2022; accepted 30 January 2022;
published online 14 February 2022

Associate Editor: Julian Gos

Abstract. There are extensive studies on frog calling behaviours, including the effects of environmental variables, however, there are no known studies to explore the specific proximate cues that stimulate the onset of calling in an individual on a given night. The aim of this study is to identify and quantify the species-specific set of environmental variables that stimulate males to produce mating calls under natural conditions. Call surveys were conducted at an active breeding pond on the edge of Parque Nacional Soberanía, Panama, during the breeding seasons of 2009 and 2010. Observations were made on 20 anuran species at the study site and we examined the onset of calling in nine species that were active and most consistently present during breeding seasons. We used logistic and linear regression models to investigate environmental conditions that affect calling for each species. The initiation of chorusing differed by species and key factors included ambient light, rainfall, and lunar cycle. Our data define the margins of a behavioural-environmental envelope that is species-specific and is not related to calling behaviour itself but is rather defined by physiological constraints related to environmental exposure.

Keywords: abiotic cues, anuran, frog calls, mating behaviour, sexual selection.

Introduction

General relationships between environmental factors and activity patterns of most amphibian and reptilian species are well documented. For example, the effects of rainfall and temperature on the intensity of breeding activity of frogs have been documented in several studies (Brooke, Alford and Schwarzkopf, 2000; Oseen and Wassersug, 2002; Gottsberger and Gruber, 2004; Steelman and Dorcas, 2010; Schalk and Saenz, 2016). Daily rainfall patterns have also been correlated with varying levels of breeding in the salamander *Plethodon hubrichti* (Reichenbach and Sattler, 2007). Similarly, canopy cover has been shown to affect the spatial distribution of breeding amphibians (Werner and Glennemeier, 1999). Steelman and Dorcas (2010) observed the effects of environmental factors on chorus intensity

with the goal of determining when best to conduct call surveys for frog monitoring programs. Despite these observations, relatively little is known about which cues might trigger specific behaviours at the organismal level and thus be relevant to individual reproductive success.

Activity patterns of other nocturnal ectothermic taxa have been linked to temperature, light, and moonphase (Lillywhite, 1971; Jaeger and Hailman, 1981; Brischoux and Lillywhite, 2011; Weaver, 2011; Sperry, Ward and Weatherhead, 2013). In mammals, a similar pattern is manifested as circadian rhythms (Heldmaier et al., 1982; Gachon et al., 2004). Calling in spotted owls increased in intensity during the last quarter through new moon phases [which is at reduced ambient light levels, and lessen chances of being visually perceived by prey (Ganey, 1990)] and calling also increased on calm nights with no precipitation. Courtship behavior in

guppies differs under variable ambient light conditions (Endler, 1991). These responses can often be linked to environmental influence on an organism's metabolic rate, exposure to predation risk, or risk of detection by prey. For example, wire-tailed manakins displayed mainly in the shade because this affected their visibility and conspicuousness (Heindl and Winkler, 2003). Because anurans are generally nocturnal, small in size, and possess semipermeable skin, their behaviour may be even more strongly dictated by immediate environmental conditions (Oseen and Wassersug, 2002).

Anuran vocalization has been the focus of extensive studies in the contexts of sexual selection and male-male competition (Ryan, 1988; Briggs, 2008, 2010), predation risk tradeoffs (da Silva Nunes, 1988; Bernal, Rand and Ryan, 2007), and even speciation (Rand, 1985; Ryan, Cocroft and Wilczynski, 1990; Richardson et al., 2008; Bonachea and Ryan, 2011). Environmental conditions that maximize breeding activity (and therefore detection or capture by field herpetologists) on a nightly basis are relatively understood for some species (Oseen and Wassersug, 2002; Steelman and Dorcas, 2010; Kusano et al., 2015). Other works have examined the effects of seasonal variation in environmental factors that influence breeding activity (Gottsberger and Gruber, 2004; Schalk and Saenz, 2016). Much of the work relating environmental conditions with anuran breeding calls, however, have measured activity as whether or not a species is calling during a given time period, often by automated means (as in Oseen and Wassersug, 2002; Steelman and Dorcas, 2010). Environmental variables such as air and water temperature, rainfall, relative humidity, photoperiod, and moon phase (Hatano, Rocha, and Van Sluys, 2002; Oseen and Wassersug, 2002; Gottsberger and Gruber, 2004; Steelman and Dorcas, 2010; Kusano et al., 2015) have been identified as important to promoting chorus intensity (number of calling

individuals). The identification of these important variables and relating remote weather station data to a categorical assignment of calling intensity characterizes the weather which is favorable for breeding. Much attention has also been given to the physiology of anuran vocalization (Ryan, 1988; Wells, 2001), weather affecting chorus intensity (Henzi et al., 1995; Brooke, Alford and Schwarzkopf, 2000), and the role of call traits on sexual selection and niche differentiation (Briggs, 2010; Bignotte-Giró and López-Iborra, 2019), however no studies have attempted to identify the environmental cues that stimulate calling in an individual on a given night.

This approach assumes a highly mechanistic view of behaviour as it implies the existence of hardwired acute responses to specific abiotic stimuli, which we know exists of feeding behavior (Wassersug, Naitoh and Yamashita, 1999) and predator avoidance (Yamashita, Naitoh and Wassersug, 2000). Excluding the social aspect of male-male interference calls or breeding calls produced in response to hearing other males, there likely exists specific external stimuli that prompt the first male to initiate the chorus. For example, Henderson and Nickerson (1976), demonstrated that manipulation of light levels to specific thresholds could stimulate specific activity level responses in three species of blunt-headed tree snakes (*Imantodes* sp.). Phototactic behavior has also been examined in the laboratory in several frog species, including some tropical species that we present calling onset for (see Jaeger and Hailman, 1971, 1973, 1976), and results predicted that species' activity would be limited to a narrow range of ambient light, presumably within which their eyes are best adapted (Jaeger and Hailman, 1981). Jaeger and Hailman (1981) also determined species specific thresholds of ambient light which prompted feeding activity. Beyond these studies, proximate environmental triggers for specific behaviors have not been documented

in other vertebrates. Demonstrating such a relationship may alter how we perceive reproductive behaviour and specifically the decision to initiate breeding activity in anurans.

The aim of this study is to identify the species-specific set of environmental variables that stimulates the initiation of mating calls under natural conditions. This study operates on a fine scale where relatively rapid environmental changes occur during the crepuscular hour when focal species begin breeding activity. The first call of the night is the target of our study because calling after that may be a social response to the first male's call (Richardson et al., 2008; Höbel, 2017). We investigate whether frog species respond to different thresholds of ambient light and explore the effects of other environmental variables on the initiation of calling. We contextualize our findings with species' life histories and discuss how differences may reflect breeding niche differentiation that allow dozens of anuran species to partition spatio-temporal space in a tropical forest.

Materials and methods

Study site

Call surveys ($N = 71$) were conducted at Experimental Pond at the Smithsonian Tropical Research Institute facilities in Gamboa, Panama between May and August 2009 ($N = 38$), and between June and July 2010 ($N = 33$). The pond is at an elevation of 50 m and is on the edge of Parque Nacional Soberanía (N 9°07.286', W 79°42.182'). Experimental Pond is a man-made, concrete pond surrounded by potted and naturally growing vegetation constructed specifically to facilitate the study of anuran ecology and behaviour. The pond measures approximately 5 m × 10 m with a maximum depth of 1.5 m. Egg clutches of *Dendropsophus ebraccatus* (Cope, 1874) and *Agalychnis callidryas* (Cope, 1862) are frequently collected from vegetation around the pond for experiments during the rainy season. Numerous species have colonized and use the pond and the immediate terrestrial area for breeding. Forest canopy cover around the pond is between 62% on the south edge and 85% on the north edge, as measured by a spherical convex densitometer (Lemmon, 1956). Leaf litter and debris fall into the pond naturally and emergent vegetation grows from the shallow edges.

Survey methods

Pilot surveys showed that many of the nocturnal frog species in the area began calling as the sun was setting. Surveys were conducted before dusk, from about 1800 h until 1900 h, just after sunset. When the opportunity arose, surveys started earlier. Surveys were not conducted during heavy rains which impaired the surveyor's hearing, as based on North American Amphibian Monitoring Program protocol (Weir and Mossman, 2005). Surveys were conducted from the same spot on the eastern edge of the pond using a red headlamp to lessen effects of surveyor's presence on anuran behaviour. Start time, Julian calendar day, day of moon phase (0-28 or 0-30), ambient temperature, percent (%) relative humidity, ambient light levels (foot candles, ft c. measured using a Fisher Scientific Enviro-meter), and sky conditions were registered at the beginning of each survey.

Species already calling upon arrival were noted (but not included in the analysis). During surveys, the time, ambient temperature, % relative humidity, and ambient light levels at first call were registered for each species. When applicable, the time at which a species stopped calling and the corresponding environmental data were also registered. Data points were documented if a time gap greater than 10 minutes during which no new species joined the chorus. This helped maintain a record of how ambient conditions changed as the sun set during each survey and further served to provide null data points for logistic models. Daily rainfall data were obtained from weather station data kept in Gamboa, Panama.

Data analysis

We explored the variability of light level at first call for each species and tested whether data adhered to assumptions of normality. We tested the hypothesis that different species respond to different light thresholds using an analysis of variance (ANOVA) and Tukey's HSD *post-hoc* tests to locate the origin of differences. For species with small variance in light levels at first call, logistic regressions that included light as an independent variable were used to model the environmental cues that stimulate calling (i.e., we modeled binomial calling with our environmental data). We conducted linear regressions to model light level (a proxy for time) at onset of calling for species that displayed a high degree of variability in light levels at first call and that engaged in sporadic calling during daytime showers outside of our survey period. Independent variables included in the global models were % relative humidity, ambient light levels (ft.c.), days until full moon (Full = 0), air temperature, daily total precipitation (mm), total precipitation over the preceding 48 h period (mm), number of consecutive days of rainfall prior to calling activity, as well as number of consecutive days without rainfall prior to calling activity. We used a Pearson's correlation coefficient to test for correlations between rain and humidity variables ($r < \pm 0.2$). We used backwards stepwise regression to build optimized models for each species. For *Craugastor fitzingeri* we were able to model both the onset and cessation of calling.

Results

Species-specific observations

Frog species observed or heard near our survey site included *Agalychnis callidryas* (Cope, 1862), *Rhinella horribilis* (Wiegmann, 1833), *Rhinella alata* (Thomiot, 1884), *Chiasmocleis panamensis* (Dunn et al., 1948), *Dendropsophus ebraccatus*, *Dendropsophus microcephalus* (Cope, 1886), *Diasporus diastema* (Cope, 1875), *Craugastor fitzingeri* (Schmidt, 1857), *Pristimantis taeniatus*, *Boana rosenbergi* (Boulanger, 1898), *Boana rufitelus* (Fouquette, 1961), *Leptodactylus insularum* (Barbour, 1906), *Leptodactylus fragilis* (Brocchi, 1877), *Engystomops pustulosus* (Cope, 1864), *Scinax boulengeri* (Cope, 1887), *Scinax ruber* (Lorenti, 1768), and *Smilisca sila* (Duellman and Trueb, 1966). Other species observed in Gamboa, Panama but not included in this study were *Dendropsophus phlebodes* (Stejneger, 1906), *Leptodactylus pentadactylus* (Lorenti, 1768), and *Trachycephalus venulosus* (Laurenti, 1768). *Scinax ruber* and *B. rufitelus* were not heard at the survey site during the 2009 field season but were both heard and observed in 2010 with a single male present for approximately 5 days, after which, it was no longer heard. This species is known from other parts of the Parque Nacional Soberanía but had not been documented at this site (Ibáñez, Rand and Jaramillo, 1999).

Males of *C. fitzingeri*, *Diasporus diastema*, and *Dendropsophus ebraccatus* were usually the first to begin calling and would sometimes begin calling prior to the start of the survey (constraining what can be deduced from our models for these species). The effects of rainfall, and humidity were most noticeable in these three species when the onset of calling was delayed on days without rainfall. *Pristimantis taeniatus* would only begin calling when light levels were below 1.0 ft.c., and usually at 0.0 ft.c. *Boana rosenbergi* would also consistently begin calling when light levels were below 4.0 ft.c., with few exceptions. *Rhinella*

horribilis was never heard calling during the survey period, nor was it heard before 2000 h in the Gamboa area. The onset of breeding seasons of *R. alata* and *S. boulengeri* were documented during both years, as well as a pause in *S. boulengeri*'s breeding activity in 2010.

Agalychnis callidryas responded negatively (reduced activity) to rain during the survey period and to night-time rainfall, but positively to diurnal rain. This contrasts with what has been observed in some other parts of its range (Briggs, 2008). In general, chorus intensity was noticeably diminished on days without any precipitation for all species. We observed 20 species during the study period of which approximately half were consistently present at the study site, and we collected data on nine species to generate optimized models to evaluate the cues associated with onset of calling. We list these nine focal species with environmental data for ambient light, relative humidity, and temperature along with pertinent natural history details in table 1.

Species responses to light levels

Results of the ANOVA indicated that focal species responded differently to light levels ($F = 39.39_{9,569}$, $P < 0.001$). Based on results of Tukey's HSD, we sorted species into groups that responded similarly to light levels (i.e., not significantly different from each other within a group; fig. 1). Group A is less sensitive to light as demonstrated by the high ambient light levels at which they would begin calling and with high variance in the data (fig. 1). Species in Group B began calling at lower ambient light levels with less variance (fig. 1). The species in Group C were most sensitive to light levels (as shown by much lower variance) requiring darkness to begin calling (fig. 1).

Environmental cues that induce calling

Light levels had significant negative effects on the initiation of calling in all species, except *Dendropsophus ebraccatus*. Days until

Table 1. Means of environmental variables measured at first call for focal anuran species, alongside average size (as snout-vent length – SVL according to Ibáñez et al., 1999), natural history details, and light-sensitivity grouping. Group A = low light sensitivity, group B = medium light sensitivity, Group C = very light sensitive. Light = foot candless; RH % = percent relative humidity, Temp = temperature (F).

Light sensitivity group	Species	Light		RH %		Temp		Mean SVL (cm) male/female	Forest level	Clutch deposition site
		Mean	SD	Mean	SD	Mean	SD			
A	<i>Dendropsophus ebraccatus</i>	29.54	23.98	0.82	0.05	82.03	1.91	2.8/3.7	Midstory, call from emergent vegetation	Emergent vegetation, in or out of water
A	<i>Diasporus diastema</i>	29.19	22.19	0.83	0.03	81.83	1.84	2.1/2.5	Midstory to canopy	Protected site in tree, undergo direct development
A	<i>Craugastor fitzingeri</i> (start)	40.07	30.93	0.82	0.03	82.26	1.98	3.5/5.3	Understory, low bushes	On leaf litter, undergo direct development
C	<i>C. fitzingeri</i> (stop)	0.17	0.43	0.85	0.03	80.57	1.77			
B	<i>Rhinella atata</i>	7.67	12.93	0.84	0.02	81.18	1.54	4.3/5.5	Terrestrial	In water
B	<i>Dendropsophus microcephalus</i>	7.52	10.11	0.82	0.04	81.35	1.84	2.5/3.1	Midstory, call from emergent vegetation	In water
B	<i>Scinax boulengeri</i>	14.4	16.47	0.83	0.03	79.97	10.57	4.9/5.3	Midstory, low bushes	In water
C	<i>Agalychnis callidryas</i>	3.26	5.46	0.84	0.03	80.91	1.75	5.6/7.1	Arboreal, Midstory-canopy	Vegetation overhanging pond
C	<i>Boana rosenbergi</i>	1.87	0.84	0.84	0.03	80.82	1.88	9.2/9.5	Arboreal, males call on ground	Mud basin near water
C	<i>Pristimantis taeniatus</i>	0.37	0.55	0.83	0.02	80.82	1.76	2.5/3.2	Terrestrial, low bushes	Unknown

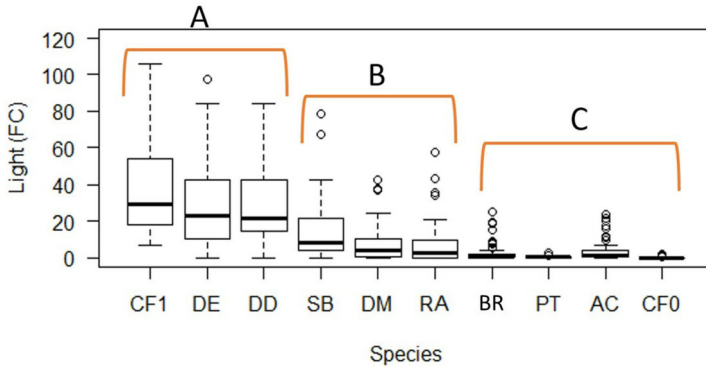


Figure 1. Ambient light level at first call* by tropical frog species, Gamboa, Panama. Groupings A, B, C are based on results from Tukey's HSD test with similarly behaving species grouped together. Box plots represent quartile ranges for each species. *CF1 refers to onset of *Craugaster fitzingeri*, CF0 refers to cessation of calling by *C. fitzingeri*. DE – *Dendropsophus ebraccatus*, DD – *Diasporus diastema*, SB – *S. boulengeri*, DM – *Dendropsophus microcephalus*, RA – *R. alata*, BR – *B. rosenbergi*, PT – *P. taeniatus*, AC – *Agalychnis callidryas*.

full moon was a significant predictor of call initiation in four of the ten logistic models included (table 2). Logistic models for *D. ebraccatus* and *Diadophis diastema* do not include sporadic daytime calling. Similarly, the logistic model for *C. fitzingeri* may not be accurate because the onset of calling could not always be captured (table 3). These three species fall into Group A, for which light level may not be a significant stimulus to calling behavior and other cues may be more important in stimulating the first call.

Onset of calling for *A. callidryas* was negatively affected by ambient light, and days from full moon tended to have a negative effect. The model for *B. rosenbergi* included a negative effect of ambient light and a strong positive effect of relative humidity. For *Pristimantis taeniatus*, the onset of calling was only predicted by light levels. The first call of *Rhinella alata* was predicted by number of consecutive days with rain and ambient light tended to have a negative effect on start of calling. The model for *D. microcephalus* included only the effects of ambient light on first call. The model for *S. boulengeri* included ambient light levels, number of days to full moon, total precipitation (mm) in the preceding 48 h and tended to be affected by number of consecutive days with rain (table 2).

Because the breeding activity of *C. fitzingeri* is strictly crepuscular, we modeled both the onset and cessation of calling with logistic models (table 3). Cessation of calling was only dependent on ambient light levels, whereas onset was more complex. The model predicting onset of calling was based on data collected on days in which the species began calling later (after the start of this survey). Variables included in this model were ambient light, consecutive days without rain, and number of days to full moon (not significant). The model shows that a period without rain delays the initiation of calling in this species.

A linear model using light levels at first call as the response variable included days to full moon and a negative effect of consecutive days of rain. The seemingly conflicting effects of days of rain and no rain between the linear and logistic models on calling activity can be reconciled as such: periods of no rain were relatively few during the sample period and usually lasted for one or two days, with only one three-day and a four-day dry spell across both sampling seasons. The duration of consecutive rainfall was much more variable, spanning up to 25 days. The logistic model captures the high sensitivity of *C. fitzingeri* to rare dry spells which reduce the instantaneous probability of the onset of chorusing at any given time. The linear model

explaining the timing (as light levels) of the call shows that a delay in onset of calling can occur during prolonged wet periods, while indicating that calling behavior is hindered by the full moon.

Both *Dendropsophus ebraccatus* and *Diadophis diastema* were heard calling during day-light hours during light showers. Therefore, because ambient light did not appear to be a significant driver of their behavior, as also evidenced by the greater variance in light level at first call for these species (fig. 1), the better question to ask was which suite of variables predicts when they would begin calling. The logistic model for *D. ebraccatus* included days to full moon, total daily precipitation, and consecutive days with rain (table 2). The linear model for *D. ebraccatus* included temperature, days of no precipitation, and relative humidity (table 3). The positive coefficients of the variables in the logistic model indicate that rainfall correlates with the likelihood of calling. The linear model is in agreement, predicting a delayed response in the absence of precipitation and earlier calling brought on by higher humidity.

The logistic model of *D. diastema* included ambient light, days to full moon, and relative humidity (table 2). Ambient light was a significant predictor of calling onset in this species and relative humidity was not a significant predictor. This lends support to our rationale for using linear models to understand the behavior of these two species. The linear model for *D. diastema* appears more consistent with our observations and included days without precipitation and relative humidity (table 3).

Discussion

Our results indicate that the initiation of calling behaviour in male frogs can be triggered by environmental stimuli. Specific light-level thresholds that stimulate calling behaviour could be identified in several species, similar to how specific light thresholds have previously been demonstrated to induce foraging activity

in tropical tree snakes (Henderson and Nickerson, 1976) and anurans (Jaeger and Hailman, 1981). Jaeger and Hailman (1981) hypothesized that photic cues could allow for sympatric anuran species to partition the foraging environment both temporally and by microhabitat; and may be the main factor explaining the seasonal variation of anuran activity (Both et al., 2008; Canavero and Arim, 2009). While many of the species we examined appear to respond to photic cues, sensitivity to those cues appears to be species specific and modified by other environmental conditions, behavioral context, and life history.

Focal species could be grouped by sensitivity to light. Group A had the least sensitivity to light levels and (fig. 1) includes the smallest species, Group B had more sensitivity to light levels and included intermediate sized species, and Group C was the most sensitive to light levels and included the largest species. The size-sensitivity correlation is independent of calling or egg deposition strategy (table 1). Simultaneously, the fact that species that fell into Group A are sometimes heard calling in broad daylight during rain showers should also be considered. We suggest that, while predation risk is a trade-off associated with many types of behaviours (especially those requiring relocation or repositioning), male advertisement calls make individuals especially conspicuous (Ryan, Tuttle and Rand, 1982; Bernal, Rand and Ryan, 2007). Large species are more vulnerable to detection by visual predators and may only initiate breeding activity at very low light levels, when the chance of successfully breeding outweighs the risk of detection by a predator.

Conversely, as light levels become less important in the decision to initiate calling among smaller species, other environmental factors appear more important. Here, we begin to see how breeding strategy dictates which environmental cues are more important stimuli. For instance, *R. alata* and *S. boulengeri* (fig. 1, Group B) are explosive breeders and deposit eggs directly in the water, likely requiring cer-

Table 2. Logistic regression results for predicting the onset of calling in tropical frog species, Gamboa, Panama.

Species	Variable	Estimate	SE	P
<i>Agalychnis callidryas</i>	Intercept	0.271	0.035	<0.001*
	Ambient light	-0.004	0.001	<0.001*
	Days from full moon	-0.005	0.004	0.156
<i>Boana rosenbergi</i>	Intercept	-0.656	0.324	0.043*
	Ambient light	-0.002	0.001	<0.001*
	Relative humidity	0.967	0.386	0.013*
<i>Pristimantis taeniatus</i>	Intercept	0.144	0.015	<0.001*
	Ambient light	-0.003	0.001	<0.001*
<i>Rhinella margaritifera</i>	Intercept	0.135	0.032	<0.001*
	Ambient light	-0.002	0.001	0.077
	Consec. days with rain	0.031	0.009	<0.001*
<i>Dendropsophus microcephalus</i>	Intercept	0.229	0.023	<0.001*
	Ambient light	-0.003	0.001	<0.001*
<i>Smilax boulengeri</i>	Intercept	0.204	0.043	<0.001*
	Ambient light	-0.001	0.001	0.014*
	Days from full moon	-0.009	0.004	0.021*
	48 h precipitation (mm)	0.002	0.001	0.003*
	Consec. days with rain	-0.097	0.006	0.0862
<i>Craugastor fitzingeri (start)</i>	Intercept	0.956	0.101	<0.001*
	Ambient light	-0.004	0.001	<0.001*
	Days from full moon	0.015	0.009	0.098
	Consec. days without rain	-0.149	0.041	<0.001*
<i>Caugastor fitzingeri (stop)</i>	Intercept	0.123	0.015	<0.001*
	Ambient light	-0.003	0.007	<0.001*
<i>Dendropsophus ebraccatus</i>	Intercept	0.153	0.072	0.034*
	Days from full moon	0.020	0.009	0.022*
	24 h precipitation (mm)	0.006	0.003	0.064
	Consecutive days with rain	0.048	0.015	0.002*
<i>Diasporus diastema</i>	Intercept	-0.786	0.811	0.333
	Ambient light	-0.003	0.001	0.002*
	Days from full moon	0.014	0.033	<0.001*
	Relative humidity	1.682	0.968	0.084

*Indicates significance at $\alpha = 0.05$ level.

Table 3. Linear regression results predicting light level (ft.c.) at onset of calling in focal anuran species, Gamboa, Panama.

Species	Variable	Estimate	SE	P
<i>Dendropsophus ebraccatus</i>	Intercept	-508.688	238.901	0.037*
	Temperature	5.108	2.134	0.019*
	Consec. days without rain	-6.585	3.587	0.071
	Relative humidity	149.473	102.52	0.149
<i>Diasporus diastema</i>	Intercept	182.414	55.444	0.001*
	Consec. days without rain	-6.465	2.224	0.004*
	Relative humidity	-172.101	67.329	0.011*
<i>Craugastor fntzingeri (start)</i>	Intercept	63.119	10.043	<0.001*
	Days from full moon	-2.143	0.990	0.034*
	Consec. days with rain	-3.040	1.460	0.043*

tain thresholds of precipitation to fall before investing energy into reproduction. Our results indicated that, in addition to light, rainfall was important for the onset of calling in these

species (table 2). Conversely, during nighttime rain showers, *A. callidryas*, had reduced breeding activity unlike its explosive breeding behaviour in the first rains in Belize (Briggs,

2008) and may be related to availability of vegetation in Gamboa being too wet to deposit egg masses during rain or perhaps more likely, as social interactions with heterospecifics.

The onset of calling in the smaller species of Group A were related to several other parameters including rainfall, relative humidity, and moon phase. The higher sensitivity to moisture in any form than to ambient light, may have more to do with increased desiccation risks for small bodied species with high surface area to mass ratios and relatively exposed eggs laid outside of the water (table 1).

Days from full moon, was included in the logistic models in 5 of the 9 species, and it was a significant variable (p -value < 0.05) in 3 of them (table 2) in predicting the onset of calling. It was also included in the linear model of light level at onset of calling for *C. fitzingeri*. Unfortunately, as with much of the previous literature pertaining to moon phase and animal behavior, we cannot offer much more quantitative and conclusive evidence as to how the moon affects the initiation of breeding calls in anurans. Anecdotally, as the lunar cycle approached full moon, both *A. callidryas* and *B. rosenbergi* noticeably delayed calling. *B. rosenbergi* did not call on 3 of the 4 full moons captured in our data (across both sampling periods). This phenomenon is not reflected in our statistical results, however. We can only suggest that moonlight negatively affects the decision to begin calling.

We also found that ambient light levels induce the cessation of breeding behavior in *C. fitzingeri*. While other species breed late into the night, until females are no longer available or individuals are exhausted from calling, this species' calling is strictly crepuscular and ended with the exhaustion of twilight. *Craugaster fitzingeri* would usually cease calling when or just after ambient light levels reached 0.0 ft.c. Only on three occasions during 2010, one or two individuals could still be heard calling at a rate of approximately 3 calls per hour at the pond at roughly 2200 h. Interestingly, however, on at least five occasions over the course of

both years, individuals were heard calling well after dark from vegetation directly under or near streetlamps in Gamboa. Similarly, the nocturnal *E. pustulosus* would often be heard calling from the darkness within gutters and sewers in the daytime.

Given the anthropogenic causes of amphibian declines, which include habitat loss, the spread of disease, impacts of invasive species, and warming temperatures (Carey, Cohen and Rollins-Smith, 1999; Kiesecker, Blaustein and Belden, 2001), our findings could be beneficial in assessing the impacts of environmental changes on amphibian reproduction. Within a few hundred meters of the site of this study lies the Panama Canal which has been recently widened resulting in the elimination of large swaths of tropical forest. Considering that the edge-to-area ratio of remaining habitat is being increased, a greater proportion of remaining forest is being impacted by edge effects. The associated changes in temperature, humidity, and increased light pollution can negatively impact amphibian reproduction (Demaynadier and Hunter, 1998).

While previous papers target calling frequency, chorus intensity, or time periods of preferred calling, none describe a direct behavioural response prompted by specific environmental cues. Our data quantify the environmental conditions that stimulate the first call in male frogs in a diverse tropical assemblage; thus, defining the margins of a behavioural-environmental envelope. Interestingly, the environmental envelope for calling is not related to the behaviour itself (in a sender/receiver context), but is defined by species' specific physiological constraints and tradeoffs related to environmental exposure (i.e., desiccation risk, oviposition strategy, and predation risk). While our operating assumption of mechanistic responses to abiotic stimuli appears to be confirmed, the environmental stimuli driving the behavioral response in question are mediated by an organism's physiology and social interactions.

Acknowledgements. The research was made possible by Dr. M.A. Nickerson, the Reptile and Amphibian Conservation Corps, the Florida Museum of Natural History, the University of Florida Center for Latin American Studies Panama Canal Research Grant, the University of Florida University Scholars Undergraduate Research Program, Drs. K. Warkentin and J.R. Vonesh, and the Smithsonian Tropical Research Institute.

References

- Bernal, X.E., Rand, S.A., Ryan, M.J. (2007): Sexual differences in the behavioral response of túngara frogs, *Physalaemus pustulosus*, to cues associated with increased predation risk. *Ethology* **113**: 755-763.
- Bignotte-Giró, I., López-Iborra, G.M. (2019): Acoustic niche partitioning in five Cuban frogs of the genus *Eleutherodactylus*. *Amphibia-Reptilia* **40**: 1-11.
- Bonachea, L.A., Ryan, M.J. (2011): Localization error and search costs during mate choice in tungara frogs, *Physalaemus pustulosus*. *Ethology* **117**: 56-62.
- Both, C., Kaefer, I.L., Santos, T.G., Cechin, S.T.Z. (2008): An austral anuran assemblage in the neotropics: seasonal occurrence correlated with photoperiod. *J. Nat. Hist.* **42**: 205-222.
- Briggs, V.S. (2008): Mating patterns of red-eyed treefrogs, *Agalychnis callidryas* and *A. moreletii*. *Ethology* **114**: 489-498.
- Briggs, V.S. (2010): Call trait variation in Morelett's tree frog, *Agalychnis moreletii*, of Belize. *Herpetologica* **66**: 241-249.
- Brischoux, F., Lillywhite, H.B. (2011): Light- and flotsam-dependent float-and-wait foraging by pelagic sea snakes (*Pelamis platurus*). *Mar. Biol.* **158**: 2343-2347.
- Brooke, P.N., Alford, R.A., Schwarzkopf, L. (2000): Environmental and social factors influence chorusing behavior in a tropical frog: examining various temporal and spatial scales. *Behav. Ecol. Sociobiol.* **49**: 79-87.
- Canavero, A., Arim, M. (2009): Clues supporting photoperiod as the main determinant of seasonal variation in amphibian activity. *J. Nat. Hist.* **43**: 2975-2984.
- Carey, C., Cohen, N., Rollins-Smith, L. (1999): Amphibian declines: an immunological perspective. *Dev. Comp. Immun.* **23**: 459-472.
- da Silva Nunes, V. (1988): Vocalization of treefrogs (*Smilisca sila*) in response to bat predation. *Herpetologica* **44**: 8-10.
- Demaynadier, P.G., Hunter, M.L. (1998): Effects of sylvicultural edges on the distribution and abundance of amphibians in Maine. *Conserv. Biol.* **12**: 340-352.
- Endler, J.A. (1991): Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Research* **31**: 587-608.
- Gachon, F., Nagoshi, E., Brown, S.A., Ripperger, J., Schibler, U. (2004): The mammalian circadian timing system: from gene expression to physiology. *Chromosoma* **113**: 103-112.
- Ganey, J.L. (1990): Calling behavior of spotted owls in northern Arizona. *The Condor* **92**: 485-490.
- Gottsberger, B., Gruber, E. (2004): Temporal partitioning of reproductive activity in a Neotropical anuran community. *J. Trop. Ecol.* **20**: 271-280.
- Hatano, F.H., Rocha, C.F.D., Van Sluys, M. (2002): Environmental factors affecting calling activity of a tropical diurnal frog (*Hylodes phyllodes*: Leptodactylidae). *J. Herpetol.* **36**: 314-318.
- Heindl, M., Winkler, H. (2003): Interacting effects of ambient light and plumage color patterns in displaying wire-tailed manakins (Aves, Pipridae). *Behav. Ecol. Sociobiol.* **53**: 153-162.
- Heldmaier, G., Steinlechner, S., Rafael, J., Latteier, B. (1982): Photoperiod and ambient temperature as environmental cues for seasonal thermogenic adaptation in the Djungarian hamster, *Phodopus sungorus*. *Int'l. J. Biometeor.* **26**: 339-345.
- Henderson, R.W., Nickerson, M.A. (1976): Observations on the behavioral ecology of three species of imantodes (Reptilia, Serpentes, Colubridae). *J. Herpetol.* **10**: 205-210.
- Henzi, S.P., Dyson, M.L., Piper, S.E., Passmore, N.E., Bishop, P. (1995): Chorus attendance by male and female painted Reed frogs (*Hyperolius marmoratus*): environmental factors and selection pressures. *Funct. Ecol.*: 485-491.
- Höbel, G. (2017): Social facilitation is a better predictor of frog reproductive activity than environmental factors. *Biotropica* **49**: 372-381.
- Ibáñez, R.A., Rand, S., Jaramillo, C.A. (1999): Los anfibios del Monumento Natural De Barro Colorado, Parque Nacional Soberanía y áreas adyacentes. The amphibians of Barro Colorado Nature Monument, Soberania National Park and adjacent areas. Fundación Natura, Círculo Herpetológico de Panamá, Smithsonian Tropical Research Institute.
- Jaeger, R.G., Hailman, J.P. (1971): Two types of phototactic behaviour in anuran amphibians. *Nature, Lond.* **230**: 189-190.
- Jaeger, R.G., Hailman, J.P. (1973): Effects of intensity on the phototactic responses of adult anuran amphibians: a comparative survey. *Z. Tierpsychol.* **33**: 352-407.
- Jaeger, R.G., Hailman, J.P. (1976): Phototaxis in anurans: relation between intensity and spectral preferences. *Copeia* **1976**: 92-98.
- Jaeger, R.G., Hailman, J.P. (1981): Activity of Neotropical frogs in relation to ambient light. *Biotropica* **13**: 59-65.
- Kiesecker, J.M., Blaustein, A.R., Belden, L.K. (2001): Complex causes of amphibian population declines. *Nature* **410**: 681-684.
- Kusano, T., Miura, T., Terui, S., Maruyama, K. (2015): Factors affecting the breeding activity of the Japanese common toad, *Bufo japonicus formosus* (Amphibia: Bufonidae) with special reference to the lunar cycle. *Current Herpetol.* **34**: 101-111.
- Lemmon, P.E. (1956): A spherical densiometer for estimating forest overstory density. *Forest Sci.* **2**: 314-320.
- Lillywhite, H.B. (1971): Temperature selection by the bullfrog, *Rana catesbeiana*. *Comp. Biochem. Physiol. - Part A: Physiol.* **40**: 213-227.

- Oseen, K., Wassersug, R. (2002): Environmental factors influencing calling in sympatric anurans. *Oecologia* **133**: 616-625.
- Rand, S.A. (1985): Tradeoffs in the evolution of frog calls. *Proceedings: Animal Sci.* **94**: 623-637.
- Reichenbach, N., Sattler, P. (2007): Effects of timbering on *Plethodon hubrichti* over twelve years. *J. Herpetol.* **41**: 622-629.
- Richardson, C., Lena, J.P., Joly, P., Lengagne, T. (2008): Are leaders good mates? A study of call timing and male quality in a chorus situation. *Animal Behav.* **76**: 1487-1495.
- Ryan, M.J. (1988): Energy, calling, and selection. *Am. Zool.* **28**: 885-898.
- Ryan, M.J., Tuttle, M.D., Rand, A.S. (1982): Bat predation and sexual advertisement in a Neotropical anuran. *The Am. Nat.* **119**: 136-139.
- Ryan, M.J., Cocroft, R.B., Wilczynski, W. (1990): The role of environmental selection in intraspecific divergence of mate recognition signals in the cricket frog, *Acris crepitans*. *Evol.* **44**: 1869-1872.
- Schalk, C.M., Saenz, D. (2016): Environmental drivers of anuran calling phenology in a seasonal Neotropical ecosystem. *Austral Ecol.* **41**: 16-27.
- Sperry, J.H., Ward, M.P., Weatherhead, P.J. (2013): Effects of temperature, moon phase, and prey on nocturnal activity in ratsnakes: an automated telemetry study. *J. Herpetol.* **47**: 105-111.
- Stelman, C.K., Dorcas, M.E. (2010): Anuran calling survey optimization: developing and testing predictive models of anuran calling activity. *J. Herpetol.* **44**: 61-68.
- Wassersug, R.J., Naitoh, T., Yamashita, M. (1999): Turning bias in tadpoles. *J. Herpetol.* **33**: 543-548.
- Weaver, R.E. (2011): Effects of simulated moonlight on activity in the desert nightsnake (*Hypsiglena chlorophaea*). *NW Sci.* **85**: 497-500.
- Weir, L.A., Mossman, M.J. (2005): North American Amphibian Monitoring Program (NAAMP). In: *Amphibian Declines: Conservation Status of United States Amphibians*, p. 307-313. Lannoo, M., Ed., University of California Press, Berkeley, California, USA.
- Wells, K.D. (2001): The energetics of calling in frogs. In: *Anuran Communication*, p. 45-60. Ryan, M.J., Ed., Smithsonian Institution Press, Washington, DC.
- Werner, E.E., Glennemeier, K.S. (1999): Influence of forest canopy cover on the breeding pond distributions of several amphibian species. *Copeia* **1999**: 1-12.
- Yamashita, M., Naitoh, T., Wassersug, R.J. (2000): Startle response and turning bias in *Microhyla* tadpoles. *Zool. Sci.* **17**: 185-189.