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Original Article

Artificial light at night alters activity, body mass, and corticosterone level in a tropical anuran

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Photoperiod is a major factor regulating biological rhythms in animals and plants. At low latitudes, annual variation in daylength is low and species are expected to strongly rely on photic cues to reset their circadian clocks. A corollary is that individuals should be strongly affected by sudden changes in the photic regime as those generated by artificial light at night (ALAN). We tested this hypothesis in an anuran in Costa Rica (10°N). Using an outdoor experimental design, we exposed adult cane toads *Rhinella marina*, a broadly distributed tropical anuran species to two ALAN intensities (0.04 and 5 lx). Locomotor activity was reduced at the lowest intensity, and the activity pattern shifted from crepuscular to nocturnal. Contrary to humans and mice in which ALAN favor obesity, toads from the two exposed groups did not gain mass whereas controls did. Corticosterone was reduced at the highest intensity, a possible consequence of the reduced activity of toads or the altered regulation of their circadian pattern. Thus, the behavioral and physiological disruption that we observed supports the hypothesis of the strong reliance on photic cues to regulate circadian rhythms and control homeostasis in this intertropical anuran. Furthermore, our results suggest that the negative effects of ALAN on physiology, in particular body mass regulation, may differ between vertebrate groups, thus preventing anticipated generalization before more comparative studies have been carried out. We stress the importance of considering the impact of the changing nocturnal environment in the intertropical zone which host the largest fraction of biodiversity.

Key words: **activity pattern, amphibian, light pollution, metabolism, *Rhinella marina*, stress.**

INTRODUCTION

Photoperiod is the main environmental cue that regulates circadian and seasonal rhythms. Photic cues contribute to the daily resetting of physiological and behavioral processes, and to the timing of major life-history traits such as reproduction or migration (Coppack and Pulido 2004; Hut and Beersma 2011; Hut et al. 2013; Gaston et al. 2017; Helm et al. 2017). It has been argued that daylength was not a key cue for synchronizing biological rhythms at low latitudes because of its stability throughout the year (Dorado-correa et al. 2016; Gaston et al. 2017; Helm et al. 2017). This prediction is intuitive for annual rhythms, but it is not a general rule since some tropical birds initiate breeding based on daylength variation shorter than 1 h (Hau et al. 1998). Expectations may differ for circadian rhythms as the annual stability of photoperiod at lower latitudes may favor a stricter dependence on photic cues, and thus limit the possibility for individuals to compensate for the effects generated by altered photoperiod caused by artificial light at night (ALAN). Only very few studies indirectly addressed this hypothesis (Erkhert 1976; Thakurdas et al. 2009) though.

ALAN, that is mostly generated by urban areas and transport infrastructures, raises night brightness and alters the photoperiod regime beyond its natural range (Cinzano et al. 2001; Longcore and Rich 2004). Such an environmental change induces many behavioral and physiological effects in vertebrates (Longcore and Rich 2004; Gaston et al. 2013; Bennie et al. 2015; Hölker et al. 2015; Bennie et al. 2017). Regarding behavior, circadian patterns are altered in various ways. Dawn singing in diurnal passerines is advanced in several European passerine. The time shift is species-dependent (Kempenaers et al. 2010) and varies with the population latitude (Da Silva and Kempenaers 2017). In contrast, the onset of foraging is delayed in nocturnal species like bats (Stone et al. 2009)

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and lemurs (Le Tallec et al. 2013). Such changes in time budget are likely to affect energy metabolism (Pulgar et al. 2019; Touzot et al. 2019), reproductive life-history traits (Ouyang et al. 2015), and ultimately fitness (Touzot et al. 2020). As for physiology, light at night suppresses melatonin, a key hormone regulating circadian rhythms (Hardeland et al. 2012). This phenomenon is wavelength, intensity, and duration-dependent. Blue light is more effective but wavelengths across a large part of the visible range suppress melatonin if exposure duration is long enough (Thapan et al. 2001). Melatonin suppression generates many metabolic alterations (Fonken and Nelson 2014). In particular, links between ALAN exposure and body mass gain have been suggested in humans, questioning the impact of exposure to light at night and obesity (Wyse et al. 2011; Fonken and Nelson 2014; McFadden et al. 2014). Mass gain can result from changes in caloric intake or energy expenditure, but also from time shift of the foraging period (Fonken et al. 2010; Dominoni et al. 2016). For instance, a link between physical activity and metabolism has been found in mice. Laboratory mice exposed for 8 weeks to 5 lux shifted their nocturnal foraging time to the previously inactive part of the diurnal period. The elevation of food intake during the photophase increased body mass and induced metabolic alterations, such as impaired glucose tolerance (Fonken et al. 2010). However, this pattern is not observed in all vertebrates as ALAN did not modify food intake or body mass in common toads, *Bufo bufo* (Touzot et al. 2019) and mouse lemurs, *Microcebus murinus* (Le Tallec et al. 2013).

Glucorticoid (e.g., cortisol or corticosterone) regulation may be also altered by light at night. These hormones modulate diverse functions to maintain organismal energy balance (Hau and Goymann 2015). They exhibit circadian variation and a concentration peak occurs around the onset of the wake period in humans and nocturnal rodents (Oster et al. 2017) or fishes (Brüning et al. 2015). Glucocorticoids are entraining signals for peripheral circadian oscillators (Pezük et al. 2012) and a positive relationship between corticosterone and the photic entrainment of locomotor activity has been observed in rats (Sage et al. 2004) which outlines the importance of monitoring both in exposed subjects. Glucocorticoids are also involved in the stress response which study is of interest in the context of global change (Angelier and Wingfield 2013). In rats, exposure to stress can cause a reduction of body mass well after the stressor disappeared (Harris et al. 1998). Prolonged elevation of glucocorticoids circulating concentrations due to chronic stress may inhibit resource allocation to reproductive or immune activities and eventually lower fitness (McEwen and Wingfield 2003). The influence of ALAN on glucocorticoids has been studied in various species. Even if Nile grass rats, *Arvicanthis niloticus,* exposed to ALAN increased serum corticosterone after chronic exposure (Fonken et al. 2012), corticoids concentrations remained unchanged in Siberian hamsters, *Phodopus sungorus* (Bedrosian et al. 2011), laboratory mice (Fonken et al. 2010), and Indian crows, *Corvus splendens* (Taufique et al. 2019). The contrasting effect of artificial light on glucocorticoids levels could be related to experimental differences (spectrum and intensity of experimental light treatments) as well as biological differences among species (for instance diurnal *vs.* nocturnal species) (Bedrosian et al. 2016; Ouyang et al. 2015; Alaasam et al. 2018).

We investigated the consequences of the alteration of the natural photoperiod by ALAN on the behavior and physiology of a tropical anuran the cane toad *Rhinella marina.* This species is a widely distributed anuran across Central and South America that experiences small annual daylength variation. Individuals tend to avoid light (Davis et al. 2015) and their physical activity tend to be negatively related to moonlight intensity, although the effect is not strong and was observed only in the post-wet season in Australia (Muller et al. 2018). Nevertheless, the species is known to colonize areas near human settlements subjected to ALAN (González-Bernal et al. 2016). An experimental study showed that ALAN generated by point light sources increased food intake while ambient light generated by skyglow tended to reduce it, possibly by reducing insect availability (Komine et al. 2020). Observed or potential effects of ALAN on the physiology and behavior of amphibians have been reported (Buchanan 2006; Wise and Buchanan 2006; Perry et al. 2008) but they have rarely been linked. Three recent studies addressed this issue and focused on the larval stage (Dananay and Benard 2018; May et al. 2019; Forsburg et al. 2021). Investigating the adult stage in amphibians is important too as its vital rates contributes more to population growth in some species (Biek et al. 2002; Schmidt et al. 2005). They also used very high intensities that are not ecologically realistic (190 and 300 lx for two of them) or on the higher range of values that may be encountered in few localized sites. However, characterizing response to low light intensities to which individuals are the most likely to be exposed is essential. We tested here light intensities that have been observed in breeding sites of amphibians (Secondi et al. 2017). In one experimental treatment intensity was two order of magnitudes lower than the lowest used in the other studies and below the intensity of a full moon. We monitored locomotor activity, body mass, and salivary corticosterone concentration in this amphibian as these proxies are functionally linked, contribute to fitness, and are affected by light pollution. We predicted that realistic levels of ALAN will affect circadian activity pattern, glucocorticoid concentration, and ultimately generate metabolic alteration which are globally captured by body mass change. Moreover, these effects are expected to increase with light intensity at night.

MATERIAL AND METHODS

Subjects and experimental setup

We captured 60 nonbreeding adult cane toads at the Biological Station of La Selva (Puerto Viejo de Sarapiquí, Costa Rica, 10.433412, -84.003345) on 24–26 October 2017. The area is covered by a lawn, trees, and bushes, and surrounded by mature tropical forest. Cane toads breed in many habitats from rainforest to savanna woodland, and are more common in open habitats and around human settlements (Zug and Zug 1979). Individuals were housed singly in boxes for 14 days (L64 cm × l46 cm × H40 cm) and fed every other day with about 3% of their mass with cockroaches *Nauphoeta cinerea* and crickets *Acheta domesticus*. In all groups, individuals were commonly observed capturing both prey types during daytime within a few seconds after these were introduced in their box confirming that light does not impair feeding. In each box, we laid 4 cm of local soil, that we kept wet, and a 15-cm section of PVC tubing (diameter 10 cm) for shelter. Boxes were covered with a shade net to dim sunlight and keep toads and preys, and located in the shade under the canopy of a wooded area. After 2 days of acclimation in their box, toads were exposed to experimental conditions for 12 days and then released at their original site. Individuals were collected from 15 m to 150 m away from the place where the boxes were installed, that is, in the habitat actually exploited by toads in the research station. Our experimental setup ensured that individuals experienced the natural fluctuations

in temperature, humidity, or acoustic conditions (regarding local wildlife) they experienced prior to capture.

An important issue is to select experimental light intensities that are consistent with the range of ALAN individuals can be potentially exposed to. We selected three levels of light intensity at night and tested 20 toads per group. Individuals were weighed before the experiment and assigned alternatively to each experimental group by increasing weight. The spatial arrangement of treatments is presented in the Supplementary Material 1. The first two groups were respectively exposed to 5 lx, hereafter referred as the "High" group, corresponding to side street lighting, and 0.04 lx, hereafter referred as the "Low" group, corresponding to skyglow (Nowinzsky et al. 1979; Gaston et al. 2013)). Skyglow, that is, the light refracted by atmosphere aerosols, generates light levels of the same magnitude order than the maximal natural levels which can be experienced dozens of kilometers away from cities (Longcore and Rich 2004). This value also lies at the lower range of illuminance for full moon nights (Kyba et al. 2017). The third group, hereafter referred as the "Control"group, was exposed to *in situ* natural illumination which corresponded to a dark sky (range 19.25–24.08 magarc.s^{-2}, see below for explanation about the unit).

We did not have a lux meter to set the light level inside the boxes. Instead, we used a SQM-L (Unihedron) light meter which is largely used for measuring light pollution levels across the World (de Miguel et al. 2017). Measurements are given in magarc.s^{-2} not in lux. Thus, we determined the SQM-L values for the High and Low group in the lab prior to the experiment using a lux meter (Illuminance meter T-10A, Konica Minolta, sensitivity threshold 0.01 lx). The light intensity as measured with the SQM-L was 12.34 magarc.s^{-2} for the High group, 17.61 magarc.s^{-2} for the Low group and 21.43 magarc.s^{-2} for the Control group. We additionally measured on several days the variation of light level at night in habitats used by the toad population in La Selva biological station. Light intensity above the control boxes 2 m above the ground was within the same range of values as those measured under deep forest canopy (Supplementary Material 2). On 6 November 2017, one of the darkest night of the study period, light level under the forest canopy was 23.8 magarc.sec^{-2}, that is, close to the detection limit of the lightmeter, and it was even below that detection threshold inside the control boxes.

Boxes were illuminated by battery-operated LED-ribbons (2800–3200k, 60 LED/m) from 18:00 to 07:00. The LED peak wavelength (590 nm) was close to the yellow-orange peak of sodium lamps that still generates the dominant color of skyglow across the World (Supplementary Material 3). Selecting a yellow light is a conservative approach as long wavelengths induce a milder physiological disturbance in humans, and this characteristics is expected to be conserved in vertebrates (Brainard et al. 2001). As far as we are aware white LEDS were not widely used in the urbanized areas close to the biological station (J.S., personal observation).

Corticosterone measurement

Corticosterone was sampled on the mornings (09:00–12:00) of the first and the last day of the experiment. We quantified the corticosterone levels in saliva using a noninvasive method developed for steroid detection in wild-caught vertebrates. Corticosterone was analyzed via enzyme-linked immunoassay (EIA) using protocols optimized for amphibians (Janin et al. 2012; Troianowski et al. 2017). The EIA method was validated for *R. marina* by demonstrating parallelism between serial dilutions of two saliva samples and the standard curve obtained with a calibrated solution of corticosterone (Supplementary Material 4).

Briefly, salivary samples were taken using cotton balls that were placed in microtubes and stored in a freezer (−20 °C) until the quantification of corticosterone concentration (Supplementary Material 3). Cotton balls were weighed before and after taking saliva samples to determine the mass using a precision scale (Mettler AE 100, precision 0.0001 g.) Saliva was extracted from the cotton ball with the addition of 150 µL of phosphate buffer (1 M phosphate solution containing 1% BSA, 4 M sodium chloride, 10 mM ethylene-diaminetetraacetic acid [EDTA], and 0.1% sodium azide) to the microtube and centrifugation at 8000 rpm for 5 min at 4 °C. Corticosterone analysis was carried out using a colorimetric 96-well EIA Assay Kit (n°501320, Cayman Chemical Company, Ann Arbor, MI). This method is based on the competition between corticosterone and a corticosterone–acethylcholinesterase conjugate for a limited number of corticosterone polyclonal antiserum binding sites. The color reaction was developed using Ellman's reagent containing acetylthiocholine and 5,5′-dithio-bis-(2-nitrobenzoic acid). Saliva samples were run in duplicates on each plate, and corticosterone concentrations were calculated using a standard curve. The interassay variation and intra-assay variation was inferior to 5%. The assay detection threshold was 8 pg/mL. In our experiment, no value under the threshold was observed ensuring that concentration values were accurate (Supplementary Material 4).

Quantification of ALAN effects and statistical analyses

From day 2 to day 14, we recorded toad activity for each treatment. Four boxes were placed below a digital camera, located 2 m above the ground, which took pictures in the visible range during the day and in the infrared range at night (Digital trail camera, B01MXSF377, Coolife). We took one picture every 5 min from 16:00 to 11:00. We analyzed frame-to-frame changes in the position of toads and scored as a movement any change as long as one body length between two frames. Image analysis was carried out blindly by a naive observer (V.G.) to the experiment. All individuals were recorded twice on two separate days, except four individuals that were recorded three times, between 17:00 and 06:00. For each individual, we summed the number of movements recorded per hour, and these results were averaged over the number of days an individual has been recorded. Hence, we measured as activity variables the total number of movements, the hour of the activity peak, and the intensity of the activity peak which corresponds to the number of movements during the peak hour during the 17:00–06:00 period. This period corresponds to nighttime which is the normal activity period for cane toads. To retrieve the nocturnal activity pattern, we kept for analysis the hours for which the individuals was out of its shelter in at least 10 pictures out of the 12 taken in a given hour. For each individual, we also recorded body mass with a digital scale to the nearest 0.01 g, body size (i.e., snout-urostyle length) to the nearest millimeter using a ruler, and corticosterone level in saliva on the mornings of the first and the last day of the experiment. We computed the difference in corticosterone concentration (final value-initial value) and the relative variation (variation/initial value × 100) in body mass for these two variables.

For activity variables, we used either linear models or generalized linear model with a Gamma distribution and an inverse link function. The full models included treatment, body size, and their interaction. For the analysis of relative body mass variation, we used a

linear model with relative body mass variation as the response variable and treatment, nighttime activity, initial corticosterone, relative corticosterone variation, and the interaction between treatment and body size as predictors. For the analysis of corticosterone, we used a linear model using treatment, nighttime activity, initial corticosterone concentration, relative body mass variation, and the interactions between treatment and body size, treatment and relative body mass variation, treatment and initial corticosterone concentration as predictors. However, we could not use the relative corticosterone variation as a response variable because its distribution was very skewed and its range included negative values that prevented the use of Gamma GLM. We therefore used the variation in concentration as response variable.

For all analyses, we selected the best models using a backward selection procedure based on *F*-tests. We checked the dispersion of residuals using graphic diagnostic plots and Shapiro test for linear models. Multiple comparisons between exposure groups were performed using post hoc Tukey tests for all response variables. All analyses and graphics were performed using R v.3.5.0 (R Core Team 2016) and R Studio v.1.0.143 (RStudio 2018), and R packages *stats* (base), *multcomp* (posthoc test), *faraway*(model diagnostic), *effects*, and *margins* (extraction of partial effects).

RESULTS

ALAN altered the activity pattern of toads. The timing and the intensity of activity peak were significantly affected by increasing ALAN intensity (Table 1; Figure 1). Toads from the High group exhibited a significant delay of the activity peak compared with the control and the Low group but the Low lux group did not differ from the Control. Activity at peak hour was higher in the control than in the High group and the Low group was intermediate and did not differ from the other groups (Table 1). Body size and ALAN treatment significantly affected nighttime activity (Table 1; Figure 2a). The number of movements was negatively related to body size (estimate = −3.873 ± 1.039 SE) and decreased with ALAN level (Table 1). The High group was less active than the control and the Low group, and the Low lux group was less active than the control group (Figure 2a; Supplementary Material 5). In contrast to all other activity variables, the time spent in the shelter did not differ significantly between groups and no minimal model could be selected (Supplementary Material 6). Finally, the number of days since the start of the experiment did not affect the activity response for any of the three groups (Supplementary Material 7).

Table 1

Best linear models explaining the variation in activity, body mass and corticosterone of adult cane toads exposed to artificial light at night (High = 5 lx, Low = 0.04 lx, Control = no artificial light). Tukey post hoc tests have been used for the pairwise comparisons of groups

Peak hour of activity	F(df)/z	P
Treatment	11.664 (2,57)	<0.001
Control-High	4.432	<0.001
Control-Low	1.454	0.309
High-Low	3.236	0.003
Activity at peak hour		
Treatment	3.886 (2,57)	0.026
Control-High	2.723	0.023
Control-Low	1.879	0.154
High-Low	0.844	0.676
Nighttime activity		
Treatment	12.85 (2,56)	<0.001
Control-High	5.070	<0.001
Control-Low	2.490	0.041
High-Low	2.650	0.028
Body size	13.905 (1,56)	0.004
Time spent in shelter	no minimal model	
Body mass		
Treatment	10.82 (2,57)	<0.001
Control-High	3.637	0.02
Control-Low	4.33	<0.001
High-Low	0.692	0.769
Corticosterone concentration		
Treatment	14.809 (2,56)	<0.001
Control-High	5.049	<0.001
Control-Low	1.461	0.404
High-Low	4.443	<0.001
Nighttime activity	6.45 (1,55)	0.014
Initial corticosterone	189.6 (1,55)	<0.001

Treatment groups did not differ in initial body mass (High group: 167.3 g ± SD 70.7 g, Low group: 175.0 g ± SD 71.3 g, control: 173.3 g ± SD 58.5 g) ($F_{2,57}$ = 0.407, P = 0.667) but were significantly different for final body mass ($F_{2,57}$ = 13.83, P < 0.001). The gain in body mass was small in individuals from the Low group (2.16% ± 7.09% SE) and the High group (1.59% ± 1.04% SE) whereas the change was more pronounced in the Control group (11.1% ± 2.28% SE) (Figure 2b). The relative change in body mass between the start and the end of the experiment was explained by treatment only (Figure 2b). The gain in body mass was significantly higher in the Control than in the Low group and the High group. The two groups exposed to ALAN did not exhibit difference in relative variation in body mass (Table 1).

The initial corticosterone level did not differ between the three groups (High group: 11.31 ± SD 5.2 pg/mg of saliva, Low group: 11.67 ± SD 9.4 pg/mg, Control: 14.37 ± SD 9.7 pg/mg) (Kruskal–Wallis test: χ^2 = 1.726, df = 2, P = 0.422). The best model accounting for the variation in corticosterone concentration between the start and the end of the experiment retained treatment, nighttime activity, and initial corticosterone concentration ($F_{4,54}$ = 51.46, P < 0.0001, r^2 = 0.792). There was significant variation across the three treatment groups. The High group experienced a stronger reduction in concentration than the Control and the Low group (Figure 2c; Table 1). However, these two groups did not differ significantly. Corticosterone variation also increased with nighttime activity (estimate = 0.131 ± SE 0.052) and initial corticosterone concentration (estimate = 0.981 ± SE 0.071). Individual 42 from the control group was excluded from the analysis based on the model's diagnostic plots and Shapiro normality tests on model residuals (W = 0.874, P < 0.001). Removing this value allowed to meet model's assumptions about the normality of residuals (W = 0.984, P = 0.632) and did not change the outcome of the model selection. Only, nighttime activity had then a marginal nonsignificant effect on the response variable ($F_{56,1}$ = 2.479, P = 0.121).

DISCUSSION

A 2-week exposure to moderate and weak levels of ALAN-induced behavioral and physiological effects in adult cane toads. Regarding the behavioral effects, the activity peak was delayed by 4.5 h and its intensity, that is, the number of movements recorded during this hour, was reduced by 21% between the Control and High groups. Another study reported a decrease of 73% of activity in *Bufo bufo*, another bufonid, when exposed to the same light level at night (Touzot et al. 2019). Overall, individuals shifted from a

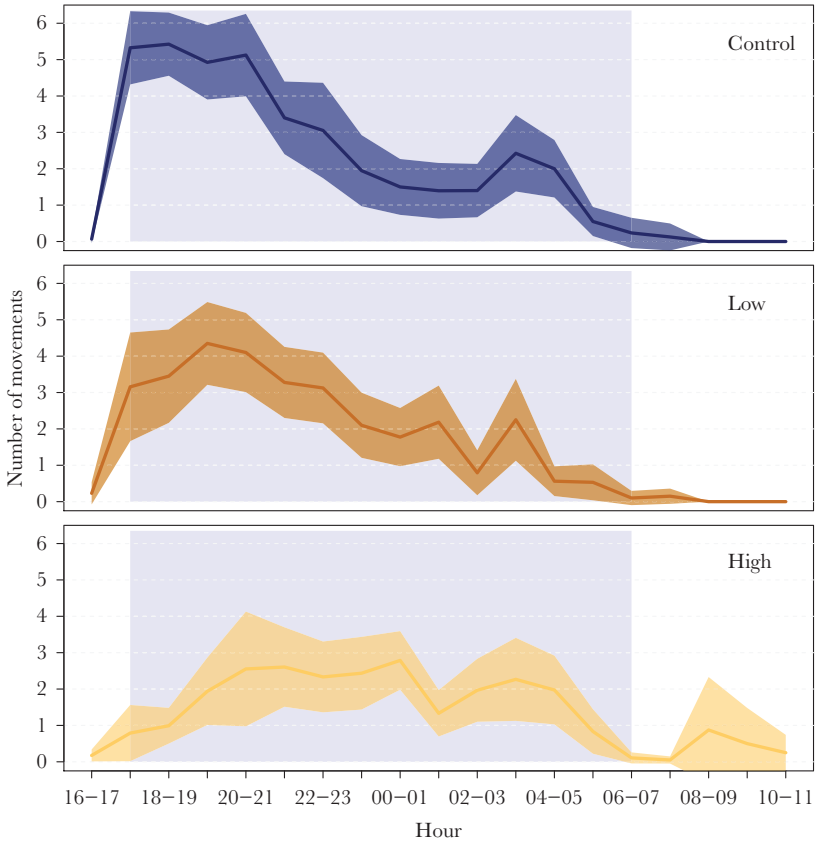

Figure 1
Effect of artificial light at night on the activity pattern of adult cane toads in Costa Rica. The graph shows the progressive shift from a pattern of crepuscular activity to a lower and more uniform activity throughout the night. Lines represent the mean number of movements per hour. Colored envelopes around lines correspond to the 95% confidence intervals. The shaded areas show the night time period, which is the period that we considered for analyses.

crepuscular pattern to a more uniform nocturnal activity, the dawn and dusk peaks gradually disappearing with increasing light intensity. Thus, our study suggests that light is used as a time indicator to determine the onset of the foraging periods, and that ALAN strongly disrupts the activity period (Rich and Longcore 2006). ALAN maintained a low and uniform activity pattern in toads that is normally experienced only during a fraction of the lunar cycle. It is noteworthy that this change in activity pattern was not caused by toads seeking to avoid light as the treatment had no effect on the time spent under the shelter. Individuals stayed active but at a lower level. This lack of strong aversion to light is consistent with the behavior of this species that is commonly found near human settlements (González-Bernal et al. 2016) and can efficiently forage under strong levels of ALAN (Komine et al. 2020). Cane toads forage in open habitats and can naturally be exposed to illuminance level higher than 0.04 lx (Low group). Thus, according to our results, a negative relationship between moonlight intensity and activity could be predicted under natural conditions as already observed in amphibians (Deeming 2008; Vignoli and Luiselli 2013), including in the invasive range of the Cane toad in Australia where this relationship was found to be weak and restricted to a part of the year (Muller et al. 2018). Interestingly, in amphibians, the opposite pattern has been found with some species being more active during bright nights, probably to avoid nonvisual predators like snakes (Grant et al. 2013).

The effect on body mass was less expected. Unlike controls, exposed toads did not gain mass, even in the Low group, despite the fact that their nighttime activity was lower than controls. In this study, toads were fed ad libitum and our results show that individuals were not subjected to food deprivation. Instead, we interpret them as evidence of the alteration of energetic and metabolism processes. As a support to this hypothesis, a previous study on a temperate bufonid, *B. bufo*, showed that the basal metabolism of toads increased and motor activity decreased with nocturnal light intensity (Touzot et al. 2019), thus revealing some level of uncoupling between these two variables. Yet, body mass variation did not differ across treatments in this study. In birds, one study on *Parus major* showed that exposed nestlings did not gain mass, whereas the control group did (Raap et al. 2016), like in the present study. Furthermore, our results strongly differ from what has been previously observed in mammals like mice (Fonken et al. 2010) and humans (McFadden et al. 2014), where ALAN exposure has been associated to mass gain. Note that these studies were lab studies conducted in very controlled environment. The physiological response of the cane toad to ALAN highlights the diversity of responses in vertebrates exposed to this environmental disturbance. ALAN has been suggested as an aggravating factor leading to obesity in humans but, clearly, the same phenomenon does not induce the same disturbance across species. Whether there is a phylogenetic component to the physiological effects of ALAN is an important issue that remains to be addressed. Currently, the species coverage of ALAN studies is too small and other factors, such as the experimental setup (lab vs. field approach for instance), need to be accounted for before getting a clear picture. Even in the Bufonid family, we observed no change in body mass in *Bufo bufo* (Touzot et al. 2019). This could be at least partly due

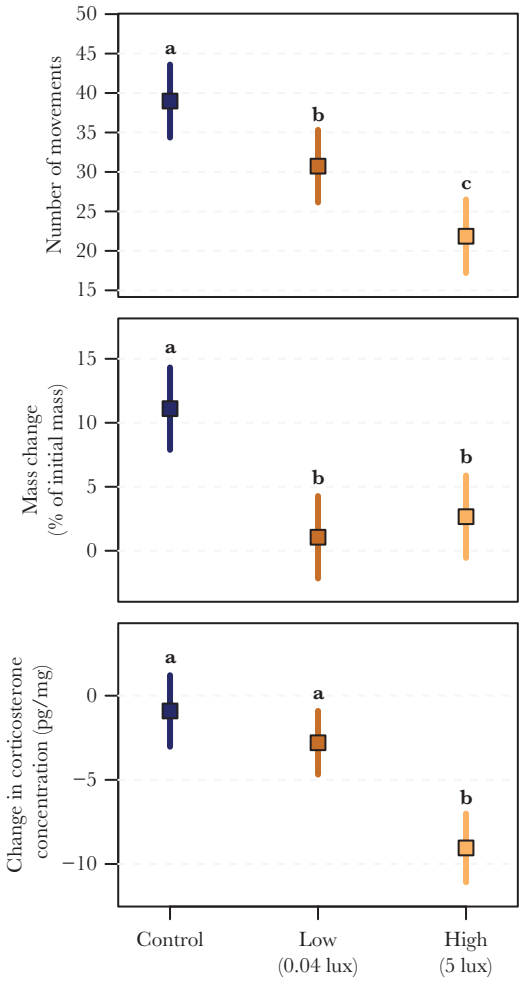

Figure 2
Effect of artificial light at night on motor activity (up), percentage variation in body weight (middle), and change in salivary corticosterone concentration (lower left panel) in adult cane toads in Costa Rica Rica (models predictions are represented with 95% confidence intervals).

to the influence of the photic signal for circadian rhythms at different latitudes. Again, studies across species and latitudes are currently too scarce to infer the mechanisms explaining the diversity of responses. Nevertheless, these few studies suggest that there is no generic response across vertebrates and that identifying surrogate species especially for health studies is not straightforward. At least, caution should be taken not to anticipate the physiological consequences of ALAN until comparative analyses have been carried out in a more systematic way.

Corticosterone is involved in the stress response and in the normal transition between rest and wake in vertebrates. At least in rodents, the concentration of this hormone peaks at the onset of the wake or active period early in the morning for diurnal species and at early nighttime for nocturnal species (Oster et al. 2017). Both salivary corticosterone concentration and the crepuscular activity peaks of toads were reduced in the High group. This result suggests that constant light suppressed or dampened the diel pattern of corticosterone expression. If the process is the same as in rodents, the reduction of peak concentration at the onset of the activity period may drive the reduction of the activity peak and nighttime activity. Another explanation is that the expression peak of corticosterone is shifted relative to controls and that the shift depends on light intensity. Then, because all groups are sampled during the same time period a more intense exposure to ALAN would shift the peak further away from the normal peak time, but will appear as a milder disruption. However, this peak shift may not account for the reduced activity. As for body mass, ALAN has been reported to elevate, reduce or not to affect the glucocorticoid level (Fonken and Nelson 2014). This endocrine parameter seems species-dependent and strongly linked to the spectral composition of light and exposure duration, and it is currently difficult to understand how the physiological processes involved in the stress response are affected as long as the temporal component of the disruption are not understood.

Effects on activity and body mass occurred at a lower illuminance than usually reported in ALAN studies (Longcore and Rich 2004). Some authors have suggested that physiological effects occur at low light intensities (Dominoni et al. 2013; de Jong et al. 2016) or below the lowest values they had selected (Brüning et al. 2016). Our study supports the view that prolonged exposure affects organisms, even at light levels they naturally experience at night (Evans et al. 2007). This is the current challenge for ALAN studies to determine the lower threshold at which biological effects are triggered. It has far-reaching consequences for conservation because lower illuminance sensitivity thresholds will determine larger areas across which organisms are potentially exposed to ALAN. The relationship between corticosterone and activity or body mass may be functionally linked but the relationship between body mass and nighttime activity is not clear as individuals did not need to be active to forage in their boxes. To unravel this functional link, a measure of metabolism energetics is required (Touzot et al. 2019), which was not possible in this study. Our results are at odds with the fact that *Rhinella marina* often occurs near human settlements and is exposed to ALAN, which is consistent with the lack of aversion to light observed in our study. Nevertheless, even if not repelled by a light source, exposure has physiological consequences. Individuals move less so that they could either capture less preys if they stay in a low quality food patch, or fail to put on mass when food is available, which can eventually reduce their breeding potential or their capacity to pass through periods of food shortage (Werner and Anholt 1993, Lima 1998). It is interesting to note that depressed corticosterone level may increase the capacity to face pathogens or colonize new environments. As noted above, the cane toads, like other bufonids, often colonizes human settlements and gets exposed to artificial lights (Zug and Zug 1979). A low stress response may facilitate the colonization of these disturbed environments. More largely, this study highlights an apparent paradoxical effect of ALAN on the physiology and behavior of a species prone to colonize modern human settlements. It remains to be seen if this effect is still observed in populations that are non-naive to ALAN.

One limitation to the environmental assessment of ALAN is the diversity of light spectra to which organisms are potentially exposed and whether the effects of ALAN would. We used a light with a peak in the yellow-orange range which light color has been historically dominant and is still largely used across the world. Our light source does not have the blue peak or the broad spectrum of white LEDs, which are more and more used worldwide. These LEDs emit a peak in the blue range that is known to strongly affect the regulation of circadian clocks through the suppression of melatonin (Thapan et al. 2001). Both differ from the spectra of natural light sources. Moonlight for instance has a broad spectrum like LEDs but it has no blue peak. However, effects on physiology have been observed for any wavelength

in the visible range (Prayag et al. 2019). Regarding melatonin suppression, longer exposure or more intense light sources are required for longer wavelengths to obtain the same suppression level as shorter wavelength. We thus believe that our results are conservative and that only stronger effects could be expected. The major difference between moonlight and ALAN lies in the fact that the latter can reach higher intensities than full moon. It is emitted every night during the whole nocturnal period and is amplified when the sky is overcast. For these reason, the effects of ALAN are of particular concern for animal populations, especially those living in areas where cloud cover is frequent at night like in Costa Rica.

Our study and others (Hau et al. 1998) contradict the general opinion that the importance of the transition between night and day as a time giver is reduced at lower latitudes, and question the view that biological rhythms may be less disrupted by ALAN in the intertropical zone. Because most studies have focused on temperate species, we think that we have not yet fully considered the global consequences of ALAN for biodiversity. This phenomenon has already been shown to affect foraging in tropical frugivorous bats which may disrupt crucial ecosystem processes like plant dispersal (Lewanzik and Voigt 2014). Whether the observed effects occur in other intertropical groups of vertebrates or are idiosyncratic to the cane toad or related species remains to be tested. Nevertheless, the high sensitivity of this tropical species to ALAN and the potential effects raise questions about the consequences for exposed populations in areas where diversity is the richest (Secondi et al. 2020). This study highlights our current limited capacity to predict the effects of changing photic conditions on organisms across the globe.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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ETHICS STATEMENT

All applicable institutional and/or national guidelines for the care and use of animals were followed. Experiments were carried out in agreement with the capture and ethics permits issued by Ministerio de Ambiente y Energía de Costa Rica (N° SINAC ACC-052-2017).

CONFLICT OF INTEREST

No competing interests declared.

Data availability: Analyses reported in this article can be reproduced using the data provided by Secondi et al. (2021).

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