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The effect of cave illumination on bats

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ABSTRACT

Artificial light at night has large impacts on nocturnal wildlife such as bats, yet its effect varies with wavelength of light, context, and across species involved. Here, we studied in two experiments how wild bats of cave-roosting species (*Rhinolophus mehelyi*, *R. euryale*, *Myotis capaccinii* and *Miniopterus schreibersii*) respond to LED lights of different colours. In dual choice experiments, we measured the acoustic activity of bats in response to neutral-white, red or amber LED at a cave entrance and in a flight room – mimicking a cave interior. In the flight room, *M. capaccinii* and *M. schreibersii* preferred red to white light, but showed no preference for red over amber, or amber over white light. In the cave entrance experiment, all light colours reduced the activity of all emerging species, yet red LED had the least negative effect. *Rhinolophus* species reacted most strongly, matching their refusal to fly at all under any light treatment in the flight room. We conclude that the placement and light colour of LED light should be considered carefully in lighting concepts for caves both in the interior and at the entrance. In a cave interior, red LED light could be chosen – if needed at all – for careful temporary illumination of areas, yet areas important for bats should be avoided based on the precautionary principle. At cave entrances, the high sensitivity of most bat species, particularly of *Rhinolophus* spp., towards light sources almost irrespective of colour, calls for utmost caution when illuminating cave entrances.

1. Introduction

Human activities influence natural processes and ecosystems all over the globe to such a degree that the current geologic epoch is considered as the Anthropocene (Crutzen and Stoermer, 2000). A well-recognised anthropogenic driver in reducing or altering biodiversity is artificial light at night (ALAN), defined by the spread of artificial lighting at night which eliminates natural darkness (Longcore and Rich, 2004; Hölker et al., 2010; Gaston et al., 2013; Davies and Smyth, 2018). The proportion of artificially lit surface on earth increases by more than 2 per cent each year (Kyba et al., 2017) with street lamps as the main source of light pollution (Gaston et al., 2012; Kyba et al., 2014). ALAN has mostly negative consequences on wildlife (Longcore and Rich, 2004; Hölker et al., 2010), ranging from influencing wildlife behaviour (Dwyer et al., 2013), reproduction (Robert et al., 2015) and physiology (Dominoni et al. 2013, 2016; Dominoni, 2015) to shifting animal community compositions

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(Arlettaz et al., 2000; Manfrin et al., 2018). Light-emitting diodes (LED) have already been installed inside a great number of caves, mostly for aesthetic purposes to render them more attractive for visitors, but LED lights are also increasingly applied for outdoor lighting given that the use of these energy efficient light sources does not involve large costs (Gaston et al., 2012; De Almeida et al., 2014). However, the impact of LED lights on wildlife using these caves has still received little attention, which is a crucial gap in our knowledge as cave-dwelling wildlife comprise often rare or threatened species (Arita, 1996).

Bats are highly adapted to a life in darkness (Fenton et al., 1995; Voigt and Lewanzik, 2011) and a vast number of bat species are cave-dwelling, whether only seasonally during e.g. hibernation, or throughout the full year (Kunz, 1982). Hence, they are a particularly relevant taxon for studying the effect of ALAN on cave-dwelling wildlife. While some bat species can benefit from concentrated prey insects attracted to streetlights (Rydell, 1992; Blake et al., 1994; Gaisler et al., 1998, van Langevelde et al. 2011), the growing body of literature suggests that bats respond to light in a species-specific, light-type, colour- and context-dependent manner (Stone et al., 2015a, b; Rowse et al., 2016; Spoelstra et al., 2017; Lewanzik and Voigt, 2017). For instance, even though the acoustic activity of some bat species like *Pipistrellus* spp. increased in the presence of light sources (Stone et al., 2015a; Spoelstra et al., 2017), particularly when lanterns illuminated trees (Matthews et al., 2015; Straka et al., 2019), they still seem to prefer dark areas during their commute in urban areas (Hale et al., 2015). Other species such as bats from the genera *Rhinolophus* and *Myotis* are particularly light-averse when commuting under white LED lights (Stone et al., 2012; Azam et al., 2018). In the Netherlands, Spoelstra and colleagues showed in field experiments with three light colour treatments (white, green and red, compared to dark control) that all local bats were insensitive to red light while green or white light conditions resulted in reduced activity in so-called light-averse species of the genera *Myotis* and *Plecotus* (Spoelstra et al., 2017). Further, Stone and colleagues (2015 b) found in the U.K. that bat activity differed among light types with *Pipistrellus pipistrellus, P. pygmaeus* and *Nyctalus/Eptesicus* spp. showing higher activity at metal halide than at low-pressure sodium lights, suggesting that different light technologies can trigger specific cascading effects on lower trophic levels.

However, ALAN might not only affect bats while foraging and commuting. At two daytime roosts in the U.K., artificial light reduced the number of bats emerging from a building irrespective of colour, yet white light had the most intensive effect (Downs et al., 2003). The sensitive response of bats towards illumination of roost entrances is underlined by a study in the U.K. showing the negative long-term effect of artificial light on house-dwelling bats (Boldogh et al., 2007) and by a study on *Plecotus auritus* in Sweden. Rydell et al. (2017) showed unambiguously that *P. auritus* abandoned its roosts in historical churches after buildings became illuminated by outdoor aesthetic lights. Synthesising these studies, the absence of ALAN, i.e. the preservation of natural darkness, is most preferable for the protection of bats (Zeale et al., 2018; Voigt et al., 2018). However, it is still unknown how artificial light might affect bats when roosting in caves or when emerging from a cave entrance, even though previous studies indicated that bats might respond sensitive to human activities at or in caves (Mann et al., 2002).

In two choice experiments, we tested if cave-dwelling bats prefer one out of three light colours (neutral-white, amber, and red) emitted by LED lamps. The flight room experiments mimicked the conditions of a cave interior, whereas the cave entrance represented a natural situation for bats when emerging from a cave. We decided against performing our lighting experiments in actual cave interiors to protect cave-roosting bats from undue stress caused by experimental illumination of their roosting environment. Neutral-white is a colour often encountered in street LED lightings (Kyba et al., 2017). Research has shown that amber is less disruptive to some insects such as fireflies compared to artificial light with shorter wavelength (Longcore et al., 2018), whereas so far just anecdotal evidence suggested that amber is supposed to be less disturbing to bats. Red light is often used by bat researchers to minimise bat disturbance and is effective for reducing the negative impact on bats when used as streetlight (Spoelstra et al., 2017). We selected bat species that presumably varied in their sensitivity to ALAN, with the following presumed increasing sensitivity: *Miniopterus schreibersii*, *Myotis capaccinii* and two species of rhinolophids (*Rhinolophus euryale* and *R. mehelyi*). We expected that rhinolophid species were least tolerant towards ALAN (Stone et al., 2009; Froidevaux et al., 2018) and that light spectra with longer wavelengths (amber and red) would be less disturbing to bats overall. Understanding if cave-dwelling bats react differentially to light of certain colours is a crucial contribution to the ongoing development of bat-friendly lighting concepts, particularly with the increase of ALAN due to the increasing density of human activities (Kyba et al., 2014).

2. Methods

2.1. Study area and bat species

The study took place near the Siemers Bat Research Station in Tabachka, northern Bulgaria (*43°36.6′N, 25°58.4′E*). The region is characterised by a karst landscape with a large number of natural and artificial caves. For the flight room experiment, bats were captured between 14th to 20th June 2017 in the nearby Gabarnika cave in which Schreiber's bent-winged bats (*Miniopterus schreibersii*) and long-fingered bats (*Myotis capaccinii*) roost. Bats were captured in the afternoon from the cave and trials were performed the same evening between 10 p.m. and 3 a.m. Bats were released at the capture site on the following night. For the cave entrance experiment, we selected the Zorovitsa cave as Mediterranean horseshoe bats (*Rhinolophus euryale*), Mehely's horseshoe bats (*R. mehelyi*), *M. schreibersii* and *M. capaccinii* occur in this cave (Ivanova, 2005). The cave entrance experiments took place on five evenings between the 25th June and 12th July 2017 with a break of one to eight days in between. Experimental work was conducted under the permit #711/June 12, 2017 (MOEV-Sofia and RIOSV-Ruse).

2.2. LED lights

We used three different colours of LED light provided by Innolumis Public Lighting BV (Amersfoort, The Netherlands) in both experiments: neutral-white (broad spectrum of ~420–700 nm with peaks around 450 and 540–620 nm), red (spectrum between 620 and 640 nm with a peak around 630 nm) and amber (so-called ‘Batlamp’, spectrum between 580 and 610 nm with a peak around 597 nm, see Supplementary Material Figs. S1–3). The illuminance of each single lamp was calibrated to approximately 23 lux in the flight room experiment and to 10–15 lux in the cave entrance experiment (lower illuminance was chosen to increase the chances of bats leaving the cave) with a luxmeter (MS-200LED, VOLTCRAFT, Conrad Electronics SE, Wernberg-Köblitz) held perpendicularly 2.0 m below the lamp.

2.3. Video and acoustic recording

We recorded the bats' behaviour in the flight room with two video cameras (HDR-CX550VE Handycam, Sony) under infrared illumination (ABUS TV6815, 880 nm). We recorded the bats’ echolocation calls with two omnidirectional (flight room; FG-O, Avisoft Bioacoustics) and directional (cave entrance; CM16/CMPA, Avisoft) microphones via an UltraSoundGate 416H soundcard (Avisoft; settings: 30 dB gain to maximize sensitivity, 250 kHz sampling rate, 16 bit resolution) to a laptop computer (see experimental descriptions below for recording details).

2.4. Flight room experiment

A sound and echo attenuated flight room (8.0 m length, 4.0 m width, and 2.4 m height, Fig. 1) was separated into two same-sized compartments by a felt-like divider (‘Velter’, thickness 5 mm, Arbanasy EOOD) reaching from the ceiling to the floor (the same material covered the flight room walls). Two vertical gaps of about 40 cm width between the fabric and the long walls on each side allowed the bats to switch between compartments. In each compartment, three LED lamps (white, red and amber) were mounted together on a tripod at 2.0 m height. Lamps were oriented downwards, and light intensity was set for each light colour to the same level prior to each trial (23 lux). In each compartment, one omnidirectional microphone was placed on the ground in the middle of the opposing ends of the flight room, pointing 45° upwards into the centre of the room (Fig. 1). To monitor the switching activity of bats between compartments, one infrared-sensitive digital camera and infrared lamp were set up in each compartment and directed at the gaps on either side (Fig. 1). The experimenter was sitting on the ground in one of the vertical gaps between both compartments (also see Supplementary Material Fig. S4).

We conducted this experiment with 16 *Miniopterus schreibersii* (males, adults), and 21 *Myotis capaccinii* (males, adults). We also tested *R. ferrumequinum* and *R. euryale*, yet individuals of those two species repeatedly kept resting after a short period. Hence, we excluded rhinolophids from this experiment. Each bat was tested individually once for 14 min in the flight room with the following procedure: first, the experimenter randomly released a bat from the observation point into one compartment while all lights were turned off, allowing the bat to habituate and explore the room for 5 min in darkness. Subsequently, we presented nine consecutive treatments of light colour combinations for 1 min each. The ten treatments (i.e.

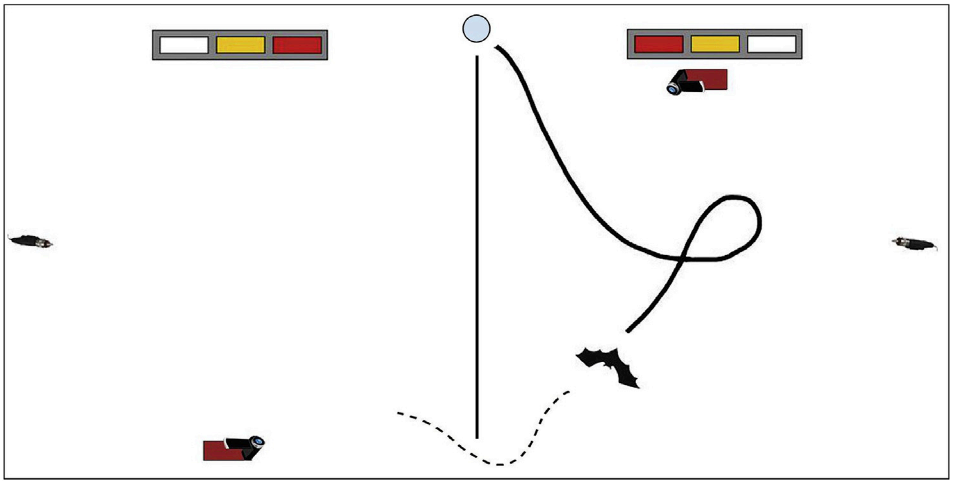

Fig. 1. Top view schematic of the flight room setup. The room was separated into two compartments by a felt divider (black line), leaving 40 cm gaps on each side. White, amber and red LED lights were mounted on tripods in each compartment (grey rectangles with white, amber and red). The behaviour of the bats was recorded with video cameras under infrared illumination and with ultrasonic microphones positioned at both ends of the room. Experimenter position is marked by a circle at the top of the flight room (see also Supplementary Material Fig. S4). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

one dark period of 5 min and nine light treatments à 1 min) consisted of the following colour combinations of the lamps in the two compartments (code indicates light in compartment 1 and 2, respectively): dark-dark (dd), amber-amber (aa), red-red (rr), white-white (ww), amber-red (ar), red-amber (ra), red-white (rw), white-red (wr), amber-white (aw) and white-amber (wa). The nine light treatments were presented in a pseudo-randomised order, ensuring that no colour was presented twice in a row in the same compartment. If bats landed during a treatment, they were stimulated to fly again after the full minute has passed and before a new recording minute began with the next light combination. For each experimental night, a different sequence of treatments was used to prevent possible sequential effects. We recorded a single audio file of 1-min duration for each light treatment and 5 min for the dark-dark treatment by manually starting and stopping the audio recording at the start and end of each treatment (hence, for the dark-dark treatment, we analysed all 5 min). Video was recorded consecutively throughout the full duration of an experiment for each bat.

2.5. *Cave entrance experiment*

The field experiment at the cave entrance was a two-alternative forced choice experiment with wild bats under natural conditions. We separated the cave entrance of the Zorovitsa cave with a 6.0 m long and light-tight felt-like wall into two compartments of 6.0 m length and about 4.0 m width and equipped it symmetrically with LED lights (Fig. 2). Exiting bats were thus forced to decide between one of the two sides during emergence. The bat community in the cave was dominated by two species of horseshoe bats, *Rhinolophus euryale* and *R. mehelyi,* with lower numbers of *Myotis capaccinii* and *Miniopterus schreibersii.*

The three light colours (white, amber and red) were positioned in both compartments on tripods at 2.0 m height. Acoustic recordings were conducted with one microphone on the ground in the middle of each compartment, pointing 45° upwards and towards the emerging bats. During the experiments, emerging bats were filmed by two cameras under infrared illumination (similar as in flight room), placed at the sides of the cave entrance. The physical quasi-symmetry of the setup was only disturbed by a fixed wooden table and a bench in the right compartment, which could not be removed from the test arena (Fig. 2). We regarded the impact of table and bench as negligible, since they did not considerably limit the bats' flight space and the animals were habituated to them. Importantly, we controlled for any potential side effects by alternating all treatments between sides. We conducted the experiments on five evenings between 25th June and 12th July under stable weather conditions of no to low cloud cover and no to low wind. We left one to eight nights without experiments between experimental evenings to assure that the bat colony was not suffering from the light exposure at the cave entrance. Experiments started at dusk when the bats started to emerge (between 9.30 p.m. and 10 p.m.) and ended 64–80 min later (latest 11 p.m. to reduce any potential pressure and allow bats to leave if they had not done so already).

In the first night, the lighting scheme at the cave entrance was similar to the flight room experiment, presenting light simultaneously in both compartments, with interspersed dark control periods. However, only few bats emerged from the cave when both compartments were lit, and mainly emerged during the dark control periods. Hence, we adapted our approach to a one-sided treatment by lighting only one compartment while keeping the other compartment dark. Each light treatment lasted for 2 min, and the lit compartment was alternated between treatments. Each sequence of treatments started with a period of darkness (2 min) on both sides to assess baseline bat activity. Then, we followed with a combination of darkness on one side and light on the other, alternating the lit compartment between left and right. After another period of darkness we repeated the light sequence from before but this time the light was presented in the opposite compartment as before. Hence, in total one experiment lasted for 16 min and contained the following combinations (code indicates light in left

Fig. 2. View from the outside into the cave with red light in the left and white light in the right compartment (treatment 'rw'). Note that this treatment with two lit compartments was not used in the final experiment, but only during the first night, see text. We chose this photograph with both lit compartments for illustrative purposes to show the whole cave entrance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and right side, respectively): twice dark-dark (dd), dark-white (dw), white-dark (wd), dark-red (dr), red-dark (rd), dark-amber (da) and amber-dark (ad). This sequence of treatment was then repeated up to five times per night until most bats had left the cave. Thus, each bat was only tested once per night, which made a randomisation of light treatments unnecessary since bats flew out and away from the cave during emergence, so each emerging bat only experienced one particular light treatment per night (in contrast to ten different sequential treatments for one bat in the flight room). Typically, bats tend to gather and circle in the interior part of the cave behind the entrance, until eventually leaving the cave. This behaviour could in theory lead to us recording the calls of an individual circling bat multiple times. We thus placed our microphones at the beginning of our divider (approximately 1 m into the cave), to record mostly those individuals that had chosen to fly through the tunnel. Even if we recorded the same individual multiple times while circling, any reduction in acoustic activity would indicate that the bats gathering in the cave interior kept a larger distance away from the lit compartment.

2.6. Data analysis

We analysed the echolocation calls in the audio recordings of each compartment as a proxy for the bat activity in this compartment. Specifically, we calculated for each treatment (of 1 or 2 min duration) and for each compartment the average sound pressure level received at the microphone in that compartment over the full duration of that treatment. The average sound pressure level increases when a bat spends more time in the respective compartment (flight room experiment) and as more bats emerge via a respective compartment (cave entrance experiment). The average sound pressure level was calculated as the root mean square (RMS), also called the quadratic mean, of all digital amplitude values of the WAV-file, resulting in a single RMS value per treatment and compartment, termed "acoustic activity". The RMS is a standard measure to describe the average value of a signal that fluctuates around zero, such as fluctuating voltage generated by a microphone in response to impinging sound waves. If no or faint sound arrives at a microphone (e.g., when the bat is in the other acoustically isolated compartment), this results in a low RMS. The closer the bat gets to the microphone, the higher is the received sound pressure, the generated voltage signal and thus the RMS. Furthermore, RMS is the acoustic parameter that correlates best with the amount of bats exiting a roost (Kloepper et al., 2016). We confirmed visually by inspecting the call recordings that the divider between the compartments also worked as an acoustic divider, so that calls recorded in one compartment were not recorded in the other one.

All acoustic analyses were performed with MATLAB (version R2015a, The MathWorks Inc., Natick, MA, USA). We first filtered all recordings to exclude background-noise and, for the cave entrance experiment, the calls of other species (zero-phase 8th-order butterworth filters: *M. schreibersii*: 45 kHz high-pass (flight room) and 50−55 kHz band-pass (cave entrance), *M. capaccinii*: 35 kHz high-pass (flight room) and 40−49 kHz band-pass (cave entrance), *Rhinolophus* spp: 100−115 kHz band-pass (cave entrance)). We then calculated the root mean square (RMS) of each filtered call recording to estimate the acoustic activity per treatment and compartment (linear values theoretically ranging from 0 to 1, but practically <1 as digital amplitude values are <1). We expressed the bats' preference for a particular light colour as the "relative acoustic activity", which we calculated by dividing the acoustic activity in one compartment by the sum of the acoustic activity of both compartments (RMS1/(RMS1+RMS2)). A value of 0.5 indicates equal acoustic activity between both compartments, and thus no preference for one over the other light colour. Values of > 0.5 and < 0.5 indicate a preference for the light colour named first and second in the treatment abbreviation, respectively.

Statistical analyses were performed in R (Version 3.5.1, R CORE Team (2018), Vienna, Austria) using the packages 'nlme' (Pinheiro et al., 2016) for linear mixed effects models, and 'effects' (Fox and Hong, 2009), 'splines' (R core team 2018), 'plyr' (Wickham, 2011) and 'reshape2' (Wickham, 2007) to create plots.

2.7. Flight room experiment

For the flight room experiment, we pooled both side-reversed combinations of two light colours into one treatment. As a result, we ended up with three mixed-light combination treatments with two repeated measurements each ('ar' and 'ra', 'rw' and 'wr', 'aw' and 'wa'), as well as one control treatment including dark and same-light combinations ('dd', 'rr', 'aa', 'ww'). The mixed-light combination treatments are hereafter labelled as 'ar', 'rw' and 'wa', and we report the relative acoustic activity for the light colour at the first position. We fitted linear mixed effects models for *Myotis capaccinii* and *Miniopterus schreibersii* separately, with 'relative acoustic activity' as response variable, 'treatment' as predictor variable and 'ID'/'minute' (individual number/chronological position at which the respective combination was applied) as random factor. The random factor was selected based on the AICc (Burnham and Anderson, 2002) with either 'minute' (chronological position at which the respective combination was applied within the trial), the individual 'ID' number or both (see Supplementary Material Table S1). The 'relative acoustic activity' was normally distributed in both bat species (Shapiro-Wilk test, $p > 0.05$ for *Myotis capaccinii* and *Miniopterus schreibersii*). The 'treatments' consisted of four levels with the three light treatments 'ar', 'rw' and 'wa' as well as a 'control' consisting of the dark-dark- and same-light combinations ('dd', 'aa', 'rr', 'ww'). We analysed only those treatments where bats were flying continuously (scored from the video recordings), resulting in 14 out of 16 *M. schreibersii* and 19 out of 21 *M. capaccinii*. The 14 individuals of *M. schreibersii* flew continuously and hence, we used all treatments of these 14 individuals. In contrast, we could only use 29.5% (56 out of 190) of the treatments with *M. capaccinii* because these bats frequently perched on the wall or due to recording errors in five cases.

2.8. Cave entrance experiment

For the cave entrance experiment, we used only the first 40 min of each night for better comparability among nights. We fitted linear mixed effects models with the ‘relative acoustic activity’ of each species as response variable and ‘treatment’ as fixed. As random effect we used ‘date’ for *Myotis schreibersii* and ‘minute’/‘date’ for *Rhinolophus* spp. and *Myotis capaccinii* based on the AICc (see supporting material Table S2). Note that ‘date’ could have been included as a predictor variable to investigate differences of bat activity among experimental nights. However, as our main question was whether bats prefer one out of three light colours (neutral-white, amber, and red), we decided to include date (and/or, based on the AICc, ‘minute-|date’) as random variable in our model. ‘Treatments’ included one light on either side of the compartment (white left, white right, amber left, amber right, red left and red right) and darkness on both sides (control treatment).

3. Results

3.1. Flight room experiment

Both species tested in the flight room, *M. schreibersii* and *Myotis capaccinii*, reacted in a similar way to the different light combinations. The strongest preference of one light colour compared to another was found in the red versus white treatment. Here, the relative acoustic activity was higher in the compartment with red light (55–60%) compared to the compartment with white light (40–45%, Fig. 3, treatment ‘rw’; *M. schreibersii*: beta = 0.082, t (119) = 2.94, p = 0.004; *M. capaccinii*: beta = 0.05, t (107) = 2.37, p = 0.02). In contrast, the bats' relative acoustic activity was about the same in both compartments for all other light combinations, i.e., the same-light controls and the amber-red and white-amber treatments (Fig. 3; same-light controls (‘ww’, ‘aa’, ‘rr’ and ‘dd’ combined): *M. schreibersii*: beta = 0.51, t (119) = 31.20, p < 0.001; *M. capaccinii*: beta = 0.50, t (107) = 38.09, p < 0.001; amber-red: *M. schreibersii*: beta = −0.003, t (119) = −0.11, p = 0.91; *M. capaccinii*: beta = −0.04, t (107) = −1.47, p = 0.14; amber-white: *M. schreibersii*: beta = −0.025, t (119) = −0.91, p = 0.36; *M. capaccinii*: beta = 0.02, t (107) = −1.12, p = 0.26).

3.2. Cave entrance experiment

Most bats emerged from the cave within 30 min, as evidenced by the clear drop in total acoustic activity 30 min after we saw the first bat emerging. Under natural dark-dark conditions, all three species preferred to emerge on the left side of the cave, resulting in lower relative acoustic activities already under the dark-dark treatment on the right side of 40%, 42% and 33% for *Rhinolophus* spp., *Myotis capaccinii*, *Miniopterus schreibersii*, respectively, compared to the left side (Fig. 4).

When the right, non-preferred side was lit with any of the three light colours (white, red, amber), the relative acoustic activity of all three bat species changed significantly to the left side by 16–38 percentage points (Fig. 4, Table 1). The highest shift of relative acoustic activity from right to the left was found for *Rhinolophus* spp. when the right side was lit with white light (increasing the relative acoustic activity on the left site by 38 percentage points (white light) compared to the dark-dark

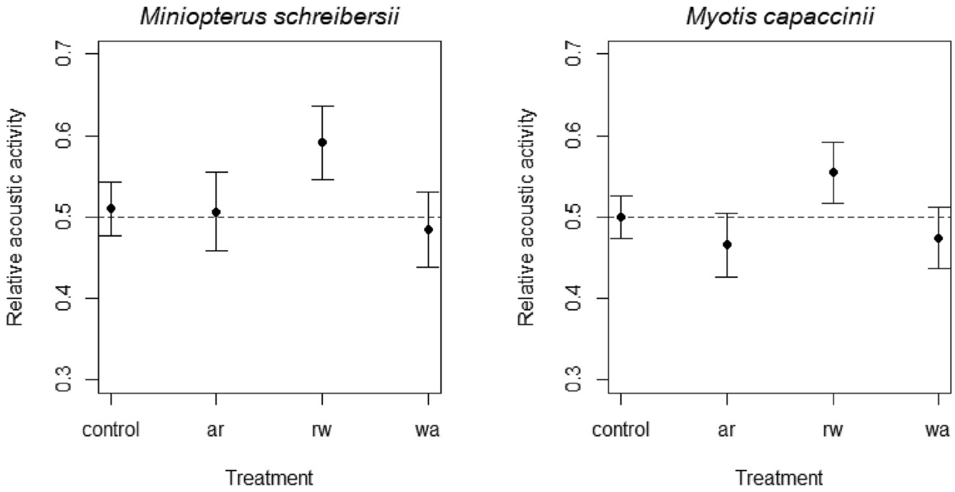

Fig. 3. Predicted relative acoustic activity (estimate and SE) of *M. schreibersii* and *M. capaccinii* in the flight room for different light combinations. Predicted relative acoustic activity is expressed relative to the light colour named first in the abbreviated treatment category code (a = amber, r = red and w = white). The dashed line at 50% indicates equal activity between both light colours, whereas values above the dashed line indicate a preference of the bats for the compartment with the light colour named first in the abbreviated treatment category code. The control treatment includes dark and same-light combinations.

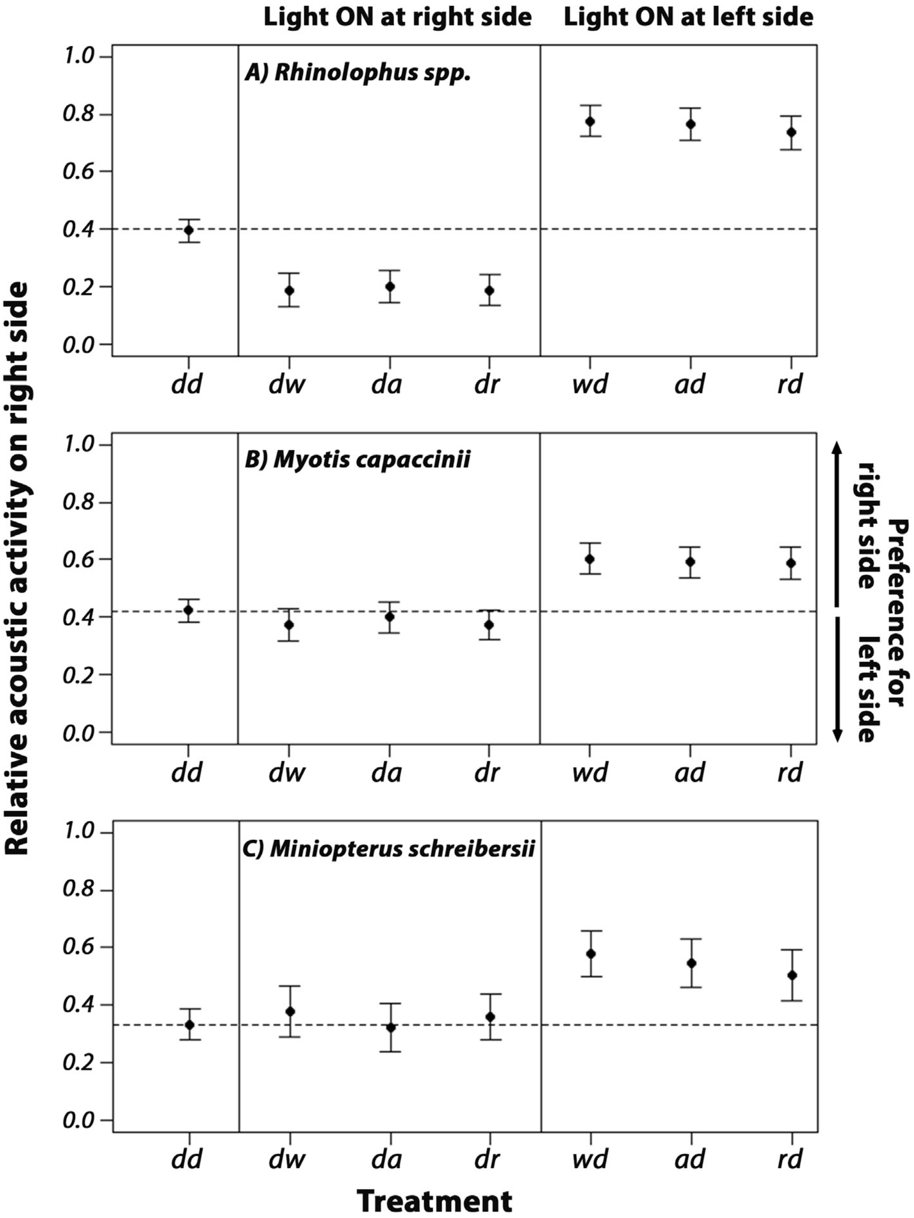

Fig. 4. Predicted relative acoustic activity of *Rhinolophus* spp., *M. capaccinii and M. schreibersii* (estimate and SE) at the cave entrance during the dark-dark control (first box) and under different treatments of one-sided lighting (second box = light on in the right compartment, third box = light on in the left compartment, d = dark, w = white, a = amber, r = red). Predicted relative acoustic activity is expressed relative to the right compartment. First and second letter of the abbreviated treatment category code indicate the light at the left and right compartment, respectively. The dashed line indicates the relative acoustic activity in the right compartment under the dark-dark treatment. Values above the dashed line indicate a preference of the bats for the light on the right side, which is named second in the abbreviated treatment category, and values below the dashed line indicate a preference for the light on the left side.

treatment (Fig. 4, Table 1, all $p < 0.001$)). *Myotis capaccinii* and *Miniopterus schreibersii* showed smaller shifts of 16–18 percentage points and 17–25 percentage points, respectively.

When the left, preferred side was lit with any of the three light colours, only *Rhinolophus* spp. significantly avoided the left side and emerged more from the non-preferred and unlit right side (decreasing the relative acoustic activity on the left site by 20–21 percentage points (Fig. 4, Table 1). In contrast, *Myotis capaccinii* and *Miniopterus schreibersii* did not significantly shift their activity to the non-preferred and unlit right side when the left, preferred side was lit (Fig. 4, Table 1).

Differences between light colours were small, however, red light shifted the activity somewhat less than amber and white light when the right, non-preferred side was lit (for *Rhinolophus* spp. only 34 compared to 37–38 percentage points; for *Myotis capaccinii* only 16 compared to 17–18 percentage points; for *Miniopterus schreibersii* only 17 compared to 21 and 25 percentage points; Fig. 4, Table 1).

Table 1
Results of the linear mixed effects model (estimates and SE) for the relative acoustic activity of three bat species at the cave entrance (*Rhinolophus* spp., *Myotis capaccinii* and *Miniopterus schreibersii*) under different treatments of one-sided lighting. Relative acoustic activity is calculated in relation to the right compartment of the cave entrance. First and second letter of the abbreviated treatment category code indicate the light at the left and right compartment, respectively (d = dark, w = white, a = amber, r = red). P-values <0.05 are highlighted in bold. Abbreviations: Treat. = treatment, Est. = estimate, SE = Standard error. Values for the lit treatments depict the change from the respective control (dd) condition value, whereby positive values indicate a preference for the right and negative values for the left side.

Lit side	Treat.	Rhinolophus spp.			*M. capaccinii*			M. schreibersii		
		Est.	SE	p-value	Est.	SE	p-value	Est.	SE	p-value
None	dd	0.40	0.02	< **0.001**	0.42	0.02	< **0.001**	0.33	0.03	< **0.001**
Right	dw	−0.21	0.03	< **0.001**	−0.05	0.03	0.14	0.04	0.05	0.43
Right	da	−0.20	0.03	< **0.001**	−0.03	0.03	0.43	−0.01	0.05	0.80
Right	dr	−0.21	0.03	< **0.001**	−0.05	0.03	0.11	0.03	0.05	0.61
Left	wd	0.38	0.03	< **0.001**	0.18	0.02	< **0.001**	0.25	0.05	< **0.001**
Left	ad	0.37	0.03	< **0.001**	0.17	0.03	< **0.001**	0.21	0.05	< **0.005**
Left	rd	0.34	0.03	< **0.001**	0.16	0.03	< **0.001**	0.17	0.05	< **0.01**

4. Discussion

The fundamental purpose of artificial lighting at night (ALAN) is to assist and please humans. With a steadily growing human population, ALAN is expanding into natural habitats with adverse effects on wildlife, particularly on nocturnal species such as bats (Rowse et al., 2016; Voigt et al., 2018). Hence, it is a central question for ecology and conservation whether we can mitigate the conflict between human desire to illuminate the night and the needs of nocturnal wildlife by selecting light colours that have least negative impact on wildlife, yet sufficient intensity to meet human visibility needs. Here, we tested in two dual-choice experiments the preference of three bat species for different light colours. We showed that captive *M. capaccinii* and *M. schreibersii* preferred red LED light to white LED light in a flight room, simulating the interior of a cave. The cave entrance experiment showed that all wild bats avoided LED illumination irrespective of colour, yet red light seems to have the least, but nonetheless a negative effect on emerging cave-roosting bats. The aversive behaviour towards red light calls for attention since this colour is widely assumed to have little effect on bats. It might be a good compromise for street illumination (Spoelstra et al., 2017) but should still be avoided for continuous illuminations around roosting locations.

While the negative effect of ALAN on bats has been extensively demonstrated (Stone et al., 2015a; b, Rowse et al., 2016), previous studies also suggested that bats might be more tolerant to artificial light of longer wavelengths compared to light in the spectrum visible to humans (shorter wavelengths up to UV light; Müller et al., 2009; Spoelstra et al., 2017; Zeale et al., 2018). Our red LED lights contained longer wavelengths than the amber and white LED lights (see Supplementary Material Figs. S1–3), and in both of our experiments, red LED light had the least negative effect on bat activity. Although amber LED light has a spectrum that is only shifted by 40 nm into the short-wave range compared to red light, it was still less tolerated by the bats compared to red. Hence, we confirm an increasing tolerance to lights with longer wavelengths also for our studied cave-dwelling bat species. The tolerance to red above white light was particularly obvious in the flight room experiment, where the bats could not escape the lighting and were forced to make a choice. While the bats could choose not to enter the lit area in the cave entrance experiment, red light caused the least negative effect on bat activity second to the unlit dark condition. Differences between light colours were small during the cave experiment. However, this is not too surprising given that in this experiment, we could not test light colours against each other but only against the dark treatment, which was still preferred above all lights of any colour, also above red and amber. While there is little research on bats' visual perception of light intensity for different light colours, it should be noted that bats might have perceived the three light colours as differently bright, thus explaining some of the observed differences.

Rhinolophus species are among the most light-averse bat species (Stone et al., 2012; Zeale et al., 2018), which both our experiments confirm. Both rhinolophids (*R. euryale* and *R. mehelyi*) significantly avoided the lit compartment at the cave entrance. Additionally, both species did not fly under any lit conditions in our flight room experiment, for which reason we had to exclude them from this experiment. The effect of ALAN on *Rhinolophus hipposideros* is increasingly well documented, including avoidance of white and red light while commuting (Stone et al., 2012; Zeale et al., 2018) and potentially becoming outcompeted during foraging by more light-tolerant species (Arlettaz et al., 2000). Our study adds clear evidence for the negative impact of light on rhinolophids during the previously untested behavioural contexts of roosting and emergence from their day roosts.

Our experiment investigated only short-term effects of light on the behaviour of cave-dwelling bats. However, the cumulative and long-term effects might be severe or even detrimental for complete colonies, as documented for house-dwelling bats (Boldogh et al., 2007; Rydell et al., 2017). Although bats might habituate to low levels of artificial lights at the entrance of their roost, bats in illuminated buildings were smaller and lighter compared to bats in unlit buildings (Boldogh et al., 2007) or not emerging the roost at all for nights as found for several *Myotis myotis* colonies in Germany (pers. comm. Kugelschafter). Hence, installing LED lighting systems at caves as well as at other potential bat roosts like the facades of historical buildings or churches throughout Europe is highly questionable from the perspective of bat conservation (Rydell et al., 2017). Additionally, it would be commendable to establish dark corridors outside of roosting sites. This would reduce the ever-increasing fragmentation of bat habitats by anthropogenic and illuminated landscapes (Hale et al., 2015) and may improve the connectivity of adjacent habitats. If these dark bridges cannot be realised where artificial lighting is unavoidable, red LED light should be chosen for illumination. Moreover, a time-limited lighting schedule and/or the use of human-activated motion-detectors could reduce the time of lighting to times of human activity. The strategic placement of trees and hedges could be another measure to mitigate the negative effect of light on bats by providing darker areas (Mathews et al., 2015). Planting trees or hedges parallel to illuminated paths creates a separation between lit and dark parts, similar to the felt-divider used in our cave experiment. Alternatively, placing artificial lights next to single big trees again creates a shadow next to the illuminated path behind the tree. Finally, lights should be placed close to the ground to reduce light scatter and should be equipped with shields to direct the light only to the area where it is needed by humans, preventing light penetration back- and upwards into the habitat of nocturnal wildlife.

5. Conclusions

LED light of all colours affected all bat species in this study negatively, with horseshoe bats being the most photophobic species. Hence, their roosting and commuting sites should be particularly well protected from the impact of artificial light. Our results also confirm that bats react differently to lights of different colours. Red light had the least negative effect on the studied cave-dwelling bat species. Considering the applicability for outdoor lighting such as street lamps, red LED lights might present a compromise for a bat-friendly nightly illumination. However, it should be noted that red light still reduced the acoustic activity and thus the number of emerging individuals at roost entrances, as shown here and by Downs et al. (2003). Hence, we emphasise the need to maintain an adequate amount of areas without any nightly illumination plus creating dark shadowed corridors in illuminated areas within the habitats of extremely light-averse species like horseshoe bats. The illumination of bat roosts should be avoided under all circumstances. Overall, it remains a challenge to find light of a specific spectrum that has no effect on nocturnal organisms at all. Given the multifaceted mechanisms of light-induced effects on nature, upcoming technologies like LED lighting require a lot more research before large-scale installations. This is particularly important since low economic costs of LED light cause rebounding effects, most likely causing an even sharper increase in light pollution over time than with conventional lighting (Falchi et al., 2011).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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