EFFECTS OF ARTIFICIAL LIGHT AT NIGHT ON ORGANISMS: FROM MECHANISMS TO FUNCTION

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PUBLISHED IN: Frontiers in Ecology and Evolution

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88976-111-1
DOI 10.3389/978-2-88976-111-1

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EFFECTS OF ARTIFICIAL LIGHT AT NIGHT ON ORGANISMS: FROM MECHANISMS TO FUNCTION

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Citation: Ríos-Chelén, A. A., Dominoni, D. M., Patricelli, G. L., Phillips, J. N., eds. (2022). Effects of Artificial Light at Night on Organisms: From Mechanisms to Function. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-111-1

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frontiers | Frontiers in Ecology and Evolution

EDITORIAL
published: 15 April 2022
doi: 10.3389/fevo.2022.896460

Editorial: Effects of Artificial Light at Night on Organisms: From Mechanisms to Function

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Keywords: ALAN, artificial light at night, organisms, proximate causes, ultimate causes, biodiversity, pollution

OPEN ACCESS

Edited and reviewed by:
Elise Huchard,
UMR5554 Institut des Sciences de l'Evolution de Montpellier (ISEM), France

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Specialty section:
This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *15 March 2022*
Accepted: *28 March 2022*
Published: *15 April 2022*

Citation:
Ríos-Chelén AA, Phillips JN, Patricelli GL and Dominoni DM (2022) Editorial: Effects of Artificial Light at Night on Organisms: From Mechanisms to Function. Front. Ecol. Evol. 10:896460. doi: 10.3389/fevo.2022.896460

Editorial on the Research Topic

Effects of Artificial Light at Night on Organisms: From Mechanisms to Function

Artificial light at night (hereafter, ALAN) has brought benefits to humans and was first perceived as a technological advancement that would increase the comfort and wellbeing of modern civilizations. However, it is now increasingly accepted by the scientific community that it also results in damaging effects to living organisms, including humans, and is an ever-increasing global pollutant that threatens biodiversity at multiple scales (Hölker et al., 2010; Swaddle et al., 2015; see below). Because ALAN reaches not only areas close to its sources, but also far away sites through sky-glow, it can have far-reaching impacts on organisms and ecosystems. Furthermore, its impact can potentially be seen at all levels of biodiversity from genes (e.g., Golden, 1995), individuals (e.g., Dominoni et al., 2013), populations and communities (e.g., Bennie et al., 2018), to ecosystems and landscapes (e.g., Perkin et al., 2011). ALAN affects both nocturnal and diurnal organisms (Rich and Longcore, 2006) by influencing gene expression (e.g., Gilmartin et al., 1990; Haim and Zubidat, 2015) and production of melatonin (van Geijlswijk et al., 2010), a crucial hormone regulating the sleep-awake cycle on which many animals depend (Ferguson et al., 2010). Indeed, ALAN effects have been documented on a wide variety of species, including unicellular organisms (Quraishi and Spencer, 1971), plants (Bennie et al., 2016), invertebrates (Van den Broeck et al., 2021), and vertebrates (Wilson et al., 2018).

In this Research Topic we aimed to compile studies that included a large variety of species and different approaches, which together address the mechanisms (e.g., hormonal changes) by which ALAN affect organisms and the possible biological consequences (e.g., in behavior, development, survival, reproductive success, population, and community changes). We have collected 12 papers representing state-of-the-art knowledge on the effects of ALAN on fishes (Mondal et al.), birds (van Dis et al.; Bani Assadi and Fraser; Kumar et al.; Rodríguez et al.), crickets and grass (Crump et al.), beetles (Kaunath and Eccard), other invertebrates (Hey et al.; Coetzee et al.), and rodents (Hoffmann et al.). We have collected both studies at the individual level and at the community level, as well as from proximate and ultimate perspectives.

At the individual level, Mondal et al. investigate the effect of different photoperiods on the expression of appetite-regulating hormones and enzymes in the zebrafish (*Danio rerio*), providing a mechanistic explanation for changes in feeding behavior in altered light conditions. van Dis et al. address the effects of different light wavelengths (white, green, red, and control) on the temperature of great tit (*Parus major*)'s nests, incubation behavior and a possible link with fitness. Bani Assadi and Fraser show that artificial light impacts nestlings' development and departure times from the nest, with green light having a lesser impact on chicks than white light. Kumar et al. show how exposure to ALAN at different periods during the night has different behavioral and physiological effects on a migratory songbird. Kaunath and Eccard investigate the attraction effects that ALAN can have on three genera of beetles (Coleoptera: Carabidae), and show a differential response associated with the level of light exposure to which these beetles are exposed in their habitat. Hoffmann et al. investigate the effects of ALAN on the nocturnal and subsequent diurnal foraging behavior of a small mammal, the bank vole (*Myodes glareolus*). The authors find changes in nocturnal foraging activity and link these effects to vole personalities; the study also shows that ALAN can have carry-over effects into daytime foraging behavior. Finally, Rodríguez et al. investigate the influence of ALAN on a well-known phenomenon in seabirds: bird fallouts, where migratory birds are grounded before reaching their destinations. Using GPS they address how ALAN may interact with intrinsic (e.g., down abundance, body mass, and body condition), and extrinsic factors (e.g., flight behavior) of Cory's shearwater *Calonectris borealis* fledglings, to predict their probability of fallouts.

At a community level, Hey et al. investigate the possible effects of ALAN on the structure and trophic interactions of an invertebrates-plant community, looking, among other aspects, at changes in abundance of secondary and tertiary consumers. On the other hand, Crump et al. study the effects of low-level ALAN and the action of an herbivore (crickets, *Acheta domesticus*) on growth rate and physiology of Kentucky bluegrass (*Poa pratensis*).

The Research Topic also provides a summary and a synthesis of research on ALAN, with two reviews and a perspective. Halfwerk and Jerem review the current evidence that ALAN affects animals in conjunction with another urban pollutant that normally co-occurs with ALAN: anthropogenic noise. In this way, they undertake a multisensory approach to investigate different possible outcomes, such as additive and interactive effects (i.e., antagonistic, synergistic, and emergent). The review by Coetzee et al. considers both mechanisms and functions to understand how artificial light can impact the behavior of disease vectors (mosquitoes) and thus the probability of disease propagation; the authors discuss how we may use light to mitigate the spread of these diseases. Hölker et al. summarize what are some of the most important questions that we need to answer if we are to reduce the damaging effects of ALAN on biodiversity. Although valuable steps have been put forward to understand how ALAN affects organisms at different levels of organization and scales, as discussed by Hölker et al., this is a relatively new field of research and more work is needed to better understand how ALAN impacts biodiversity in a broad sense, and what we can do to mitigate its effects. Hölker et al.'s discussion stems from a workshop on the effects of ALAN on biodiversity that took place during the first World Biodiversity Forum in Davos, Switzerland.

Though further work is needed to illuminate how the effects of ALAN scale across biological levels, this Research Topic gives insight into the mechanisms and consequences of ALAN at individual and community levels in a wide variety of taxa. This body of work combines studies from both basic and applied science perspectives, addresses the complexity of the possible outcomes that ALAN may have on biological systems, and discusses the next steps we should take in order to mitigate its effects.

AUTHOR CONTRIBUTIONS

AR-C conceived the Research Topic of this compilation and wrote the first draft of this editorial. All authors contributed equally in editing and improving this Editorial.

ACKNOWLEDGMENTS

We want to thank all the researchers and reviewers who contributed with their work to form this compilation, and the Frontiers team who opened the way for us to co-edit this Research Topic.

REFERENCES

Bennie, J., Davies, T. W., Cruse, D., and Gaston, K. J. (2016). Ecological effects of artificial light at night on wild plants. *J. Ecol.* 104, 611–620. doi: 10.1111/1365-2745.12551

Bennie, J., Davies, T. W., Cruse, D., Inger, R., and Gaston, K. J. (2018). Artificial light at night causes top-down and bottom-up trophic effects on invertebrate populations. *J. Appl. Ecol.* 55, 2698–2706. doi: 10.1111/1365-2664.13240

Dominoni, D., Quetting, M., and Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proc. R. Soc. B.* 280, 20123017. doi: 10.1098/rspb.2012.3017

Ferguson, S. A., Rajaratnam, S. M., and Dawson, D. (2010). Melatonin agonists and insomnia. *Expert Rev. Neurother.* 10, 305–318. doi: 10.1586/ern.10.1

Gilmartin, P. M., Sarokin, L., and Memelink, J., and Chua, N-H. (1990). Molecular light switches for plant genes. *Plant Cell* 2, 369–378. doi: 10.1105/tpc.2.5.369

Golden, S. S. (1995). Light-responsive gene expression in cyanobacteria. *J. Bacteriol.* 177, 1651–1654. doi: 10.1128/jb.177.7.1651-1654.1995

Haim, A., and Zubidat, A. E. (2015). Artificial light at night: melatonin as a mediator between the environment and epigenome. *Phil. Trans. R. Soc. B.* 370:20140121. doi: 10.1098/rstb.2014.0121

Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C. C., and Henckel, D. (2010). The dark side of light: a transdisciplinary research agenda for light pollution policy. *Ecol. Soc.* 15:13. doi: 10.5751/ES-03685-150413

Perkin, E. K., Hölker, F., Richardson, J. S., Sadler, J. P., Wolter, K., and Tockner, K. (2011). The influence of artificial light on stream and riparian ecosystems: questions, challenges, and perspectives. *Ecosphere* 2:122. doi: 10.1890/ES11-00241.1

Quraishi, F. O., and Spencer, C. P. (1971). Studies on the growth of some marine unicellular algae under different artificial light sources. *Mar. Biol.* 8, 60–65. doi: 10.1007/BF00349346

Rich, C., and Longcore, T. (2006). *Ecological Consequences of Artificial Night Lighting.* Washington, DC: Island Press.

Swaddle, J. P., Francis, C. D., Barber, J. R., Cooper, C. B., Kyba, C. C. M., Dominoni, D. D., et al. (2015). A framework to assess evolutionary responses to anthropogenic light and sound. *Tree* 30, 550–560. doi: 10.1016/j.tree.06, 009.

Van den Broeck, M., Cock, D., Van Dongen, R., and Matthysen, S. E. (2021). Blinded by the light: artificial light lowers mate attraction success in female glow-worms (*Lampyris noctiluca* L.). *Insects* 12:734. doi: 10.3390/insects12080734 van Geijlswijk, I. M., Korzilius, H. P., and Smits, M. G. (2010). The use of exogenous melatonin in delayed sleep phase disorder: a meta-analysis. *Sleep* 33, 1605–1614. doi: 10.1093/sleep/33.12.1605

Wilson, P., Thums, M., Pattiaratchi, C., Meekan, M., Pendoley, K., Fisher, R., et al. (2018). Artificial light disrupts the nearshore dispersal of neonate flatback turtles *Natator depressus*. *Mar. Ecol. Progr. Ser.* 600, 179–192. doi: 10.3354/meps12649

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 10 August 2021
doi: 10.3389/fevo.2021.676332

The Daily Pattern of Expression of Leptin and Ghrelin O-Acyl Transferase Under Various Lighting Schedules in the Whole Brain of Zebrafish (*Danio rerio*)

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Specialty section:
This article was submitted to
Urban Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *05 March 2021*
Accepted: *15 July 2021*
Published: *10 August 2021*

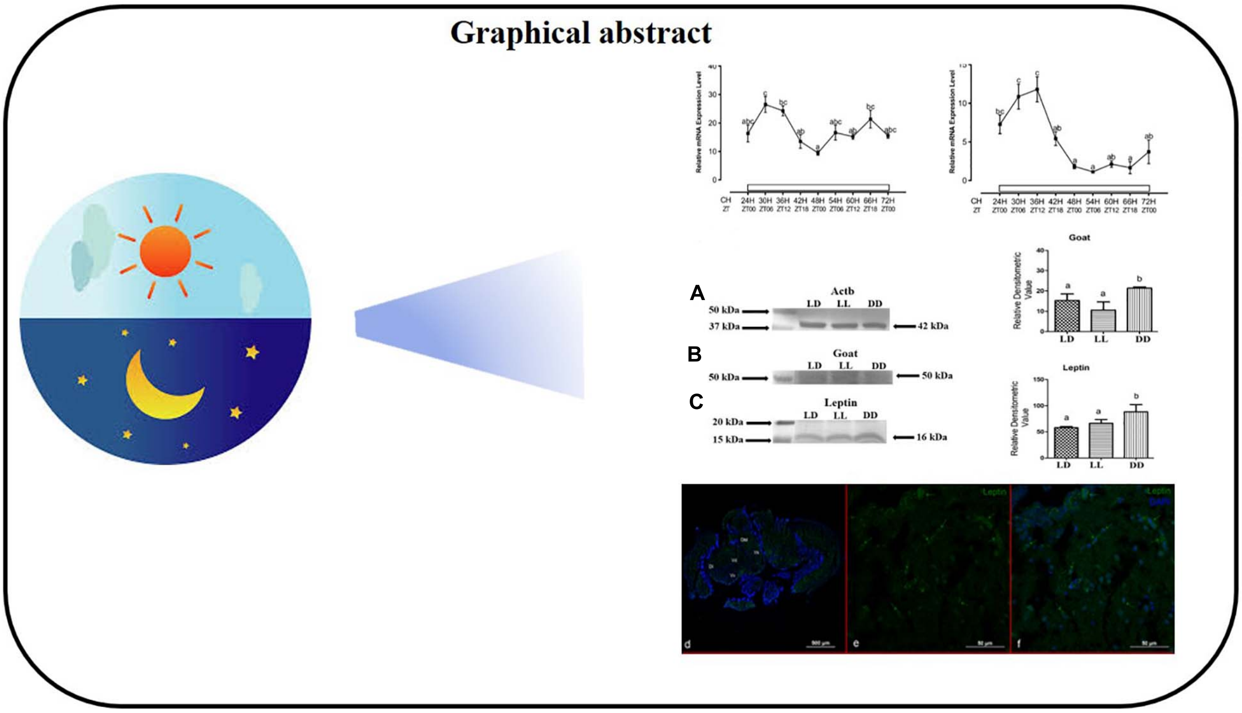
Citation:
Mondal G, Khan ZA, Devi SD, Labala RK and Chattoraj A (2021) The Daily Pattern of Expression of Leptin and Ghrelin O-Acyl Transferase Under Various Lighting Schedules in the Whole Brain of Zebrafish (Danio rerio). Front. Ecol. Evol. 9:676332. doi: 10.3389/fevo.2021.676332

The influence of Artificial Light at Night (ALAN) is a severe interference in the biological rhythm of organisms. Feeding dysregulation might be the main factor responsible for developing metabolic diseases. The molecular basis of these physiological dysregulations is yet to be elucidated. The effect of light on appetite-regulating signal in the brain of zebrafish is still unknown. Therefore, the present study aimed to find the daily mRNA expression profile of two significant appetite and energy-balancing peptide hormone (*leptin*) and enzyme (*goat*) in the brain in various illumination conditions, LD (12Light:12Dark), LL (24L), and DD (24D). Moreover, Ga-SI, the protein level of the two appetite-regulating peptides, and brain and serum melatonin are measured after 72 h of incubation under three different photic conditions. The immunohistochemical localization of the primary mediators of appetite regulators, leptin and goat, in the brain of zebrafish, under different photic conditions, is reported for the first time in zebrafish. The study revealed that in continuous light after 72 h of incubation, feeding (Ga-SI) is found the highest and the translational level of two appetite-related genes (*leptin* and *goat*) is the lowest compared to LD. In continuous dark, this relation becomes opposite. The daily variation of mRNA expression of *leptin* and *goat* in LD shows peak expression at the light and dark phase, respectively. This pattern was abolished in continuous conditions. Thus, our study suggests that the photoperiod (zeitgeber) may influence the expression pattern (daily variation) of appetite-regulating peptide hormone and enzyme in the brain of zebrafish.

Keywords: ALAN, melatonin, circadian clock, orexigenic, anorexigenic

INTRODUCTION

Energy balance is a critical process for the survival of organisms, maintained by a complex neuro-anatomical network between the central and peripheral nervous systems. Several hormones play an integral role in energy homeostasis to cope with varying external zeitgebers like food availability and environmental conditions. The energy-balancing hormone is produced by different tissues;



GRAPHICAL ABSTRACT | Light dark cycle influences the levels of Leptin and Ghrelin O-Acyl Transferase (GOAT).

among them, the brain plays a critical role to regulate energy homeostasis by secreting appetite-stimulating (orexigenic) and appetite-inhibiting (anorexigenic) endocrine signals according to the input of energy status from several central and peripheral neuroendocrine tissues in fish (Suzuki et al., 2010; Hoskins and Volkoff, 2012; Mondal et al., 2021a). Energy homeostasis depends on a proper balance between energy intake and expenditure involving the feeding behavior, which is regulated by various environmental conditions (light, temperature) (Volkoff et al., 2005; Valassi et al., 2008; Mondal et al., 2021a). Among different environmental signals, periodic changes in light and dark cycle are the major factors that can influence the energy-balancing system (Helwig et al., 2009). The relationship of leptin and ghrelin (major anorexigenic and orexigenic peptides, respectively) with different photic conditions is unknown.

Leptin, a peptide hormone, is mainly secreted by adipose tissue in mammals and first cloned in ob/ob mice (Zhang et al., 1994; Park and Ahima, 2015). In both mammals and fish, leptin regulates food intake and energy homeostasis (Copeland et al., 2011; Li, 2011). In fish, leptin is secreted from the liver, although it is known to express in the brain and other peripheral tissues (Huising et al., 2006; Frøiland et al., 2010; Denver et al., 2011; Tinoco et al., 2014). Leptin is reported to act as an anorectic signal or hormone; however, its regulation through overfeeding and fasting is dependent on the feeding regime, and it is species-specific (Rønnestad et al., 2010; Kobayashi et al., 2011; Tinoco et al., 2012). The daily expression pattern of leptin was described in rat and human adipose tissue (Xu et al., 1999; Gómez Abellán et al., 2011). In Atlantic salmon and goldfish, the daily changes of leptin mRNA expression were found in the brain, white muscle, belly flap, visceral adipose tissue, and liver (Moen and Finn, 2013; Tinoco et al., 2014). Daily variation in *leptin* expression was found under 12L:12D with scheduled feeding, but under 24L with a 12-h shift in feeding time; or fasting diminishes the rhythmic expression of *leptin* in goldfish (Tinoco et al., 2014). The expression of leptin in the brain and hepatic tissue is regulated differently in goldfish. The peak expression of hepatic leptin was found 9 h post-feeding (Tinoco et al., 2012). However, hypothalamic acrophase of leptin rhythm was noted before or around mealtime, and no postprandial changes were reported in goldfish (Tinoco et al., 2012).

Ghrelin is a 28-amino acid acylated peptide hormone largely produced in the gastric mucosa. Like mammals, ghrelin is found in many fish species and also increases food intake and thereby body weight (Kojima et al., 1999; Wren et al., 2001; Unniappan et al., 2002; Kaiya et al., 2008; Amole and Unniappan, 2009; Hatef et al., 2015). Ghrelin is encoded from preproghrelin mRNA, and for the functional form, it needs to be acylated at the third serine residue. Ghrelin O-acyltransferase (Goat), also known as the membrane-bound O-acyltransferase 4 (Mboat4), is mainly responsible for this acylation (Yang et al., 2008; Amole and Unniappan, 2009). In mice, the goat mRNA expression was found in ghrelin immunopositive cells (Sakata et al., 2009). Moreover, the similar expression profile of acylated ghrelin and *goat* or *mboat4* mRNA was also revealed in mammals (Stengel et al., 2010b). The goat expression is responsible for the availability of acylated ghrelin in teleost (Hatef et al., 2015). In mammals, the reduction in plasma goat follows a decrease in plasma acylated ghrelin, whereas the goat level in gut gives a minor increase in the same time (Stengel et al., 2010a). These findings support the idea that the tissue-specific synthesis of goat is responsible for the availability/indirect assessment of local acylated ghrelin. In mammals, goat expression is tissue-specific and mainly expressed in the stomach (Yang et al., 2008; Lim et al., 2011). Similarly, in zebrafish, the goat expression was observed in various tissues but primarily in the gut (Hatef et al., 2015; Mondal et al., 2021a). The presence of goat in the brain of mammals and zebrafish indicates a facilitative role in feeding regulation by local acylation of *preproghrelin*, coming from central and peripheral organs (Matsuda et al., 2006; Lim et al., 2011; Tinoco et al., 2014; Hatef et al., 2015). Besides that, it was reported that an increased level of plasma acylated ghrelin coincided with an elevation of hypothalamic and pituitary goat mRNA expression in fasted mice (Gahete et al., 2010). Alteration of goat mRNA expression in the brain of zebrafish was reported in various feeding conditions (Hatef et al., 2015).

These two factors, leptin and goat, are involved in central appetite regulation and show daily rhythm in fish and mammals (Tinoco et al., 2014; Blanco et al., 2016a, 2017). However, future studies are needed regarding the role of centrally synthesized leptin and goat, and the daily pattern of expression and effect of altered photic conditions on the expression in the brain of zebrafish with unaltered scheduled feeding.

The aim of this study was to investigate whether leptin and goat mRNA expression has any daily pattern in normal photic condition and if it can be affected by altered photoperiodic conditions with scheduled feeding. We examine the brain expression of leptin and goat mRNA up to 72 h in three different photoperiodic conditions. The localization and abundance of the leptin and goat protein in zebrafish brain were assessed by fluorescence immunohistochemistry and Western blot under different photic conditions. In addition, we also measured melatonin level and Gastrosomatic Index (Ga-SI) in various photic conditions. In this study, we tried to show that the altered photoperiod can change the pattern and level of expression of leptin and goat in the zebrafish brain.

MATERIALS AND METHODS

Animals and Housing

The second generation of wild-type zebrafish (*Danio rerio*) approximately 6–7 months old, with a body length of 4.0 ± 0.3 cm and a weight of 0.4 ± 0.15 g, was obtained from the zebrafish facility of IBSD, Imphal, Manipur, India. Fish were maintained in 50-L glass aquaria (30 fish/aquaria) under normal (12 h light:12 h dark) photic conditions (light intensity was fixed at 300 lux by a household fluorescent tube) (Khan et al., 2018). Everyday light was turned on in the morning (at 6:00 a.m.) and turned off in the evening (at 6:00 p.m.), maintained by a timer (Frontier Digital Timer, Taiwan) (Reed and Jennings, 2011). The adequate water temperature for zebrafish (28 ± 0.5°C) was maintained by using glass submersible aquarium immersion heaters (100 W, RS Electrical, India) placed in each aquarium. A biological filter (E-Jet, P.R.C) was used for aeration and recirculation of water. The pH, hardness, and other parameters of water were maintained under standard conditions (Westerfield, 2000) at our laboratory (Khan et al., 2016). Food was given thrice a day. At morning (9:00 am; ZT03) and midday (1:00 pm; ZT07) with commercial floating type small pellets (Perfect Companion Group Co. Ltd., Thailand). Live *Artemia nauplii* (cultured from Artemia cysts, Ocean Star International, United States) was given at the late afternoon (5:00 pm; ZT11). Fish care and study schedule were done by following international standards (Portaluppi et al., 2010). Ethical clearance was obtained from the Institutional Animals Ethical Committee as per the recommendations of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Experiment Designs and Sampling

The male zebrafish were randomly distributed into three experimental groups (27 fish/group): (i) standard photoperiod (12L:12D, LD), lights on at 6:00 a.m. and lights off at 6:00 p.m.; (ii) continuous light (24L, LL); and (iii) continuous dark (24D, DD). The temperature was set at 28 ± 0.5°C for all these groups. Food was given in the same composition three times in a day as earlier elaborated (9:00 am; ZT03, 1:00 pm; ZT07 and 5:00 pm; ZT11). Fish were kept for 24 h in each experimental condition before the start of sampling (Khan et al., 2016). The sampling process for all the photic conditions started from Clock Hour CH 24 [Day 2 (D2); ZT0/24, through Day 3 (D3) and continued up to CH 72 Day 4 (D4); ZT0/24], with an interval of 6 h (CH24/ZT0/24; CH30/ZT06; CH36/ZT12; CH42/ZT18; CH48/ZT0/24; CH54/ZT06; CH60/ZT12; CH66/ZT18 and CH72/ZT0/24) (Amaral and Johnston, 2012). At each time point, three zebrafish were taken in 0.1% Tricaine (Sigma-Aldrich, United States) solution and kept on ice for anesthesia. The brain was taken out by removing the cranium and quickly stored in TRIzol® (Ambion, Carlsbad, CA, United States) and frozen at −80°C before the total RNA extraction (Rajiv et al., 2016). Simultaneously, in an identical condition, three experimental groups (as mentioned above) were established, and after 72 h of incubation, 15 zebrafish were taken from each lighting condition (LD, LL, and DD; total 15 × 3 = 45 fish) in 0.1% Tricaine (Sigma-Aldrich, United States) solution and kept on ice for anesthesia. The body length and weight of each fish were measured before they were euthanized. Five fish (5 × 3 = 15 fish) were used for brain and serum melatonin enzyme-linked immunosorbent assay (ELISA), five fish (5 × 3 = 15 fish) were used for the Western blot analysis, and the remaining five fish (5 × 3 = 15 fish) were used for the immunohistochemistry and quantification of intestine content as a Ga-SI. Brains for Melatonin ELISA were collected in 0.1 M PBS (pH 7.4) (Yumnamcha et al., 2017) and those for Western blot were kept in radioimmunoprecipitation assay (RIPA) buffer (Sigma–Aldrich) with protease inhibitor (Sigma–Aldrich) at a 1:100 ratio at 4°C. For the immunohistochemical study and the quantification of intestine content as a Ga-SI, the whole brain and whole gut, respectively, of all five fish from each experimental group were kept in 4% PFA (mass/vol) diluted in

0.1 M phosphate buffer saline (PBS, pH 7.4) at 4°C (Amaral and Johnston, 2012; Hatef et al., 2015). The entire intestinal content of zebrafish is considered for the quantity of feeding as they do not have any stomach (Brugman, 2016). Samplings at dark were carried out in dim red light.

Quantitative RT-PCR Analysis

Gene expression analysis was performed in a StepOnePlus^{TM} Real-Time PCR System (Applied Biosystems®, Inc., ABI, United States) as previously described (Khan et al., 2018). Total RNA was extracted from the homogenized brain of zebrafish using the TRIzol® Reagent (Life Technologies, United States) method, and 5 μg of total RNA was treated with the DNA-free^{TM} Kit (Ambion®RNA by Life Technologies^{TM}, United States). Then, for cDNA synthesis, 1 μg of DNase-treated total RNA was reverse transcribed, using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems^{TM}, United States) in the ProFlex^{TM} Base PCR System (Applied Biosystems®, Inc., ABI, United States) by following the manufacturer's protocol. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was performed using Jumpstart SYBR Green/ROX qPCR Master Mix (Sigma-Aldrich, United States). Amplification was performed in technical triplicates for each sample, each of which contains 10 μl reaction volume of forward and reverse primers, JUMPstart qPCR Master Mix (Sigma-Aldrich, United States), and cDNA. Primers for this study were taken from the published data (Falcinelli et al., 2016; **Table 1**). The reaction condition for PCR was an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, annealing at 60°C for the 30 s, and extension at 72°C for 30 s. Melting curve analysis (Tm) confirmed single gene amplification by designated primers, and the 2% agarose gel shows a single band of the endpoint PCR product. The relative expression of the gene was calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) using *rpl13a* gene as a reference (Tang et al., 2007).

Brain Melatonin ELISA

Thirty milligrams of the brain (pooled from five fish) was used to measure the melatonin level in the whole brain from each experimental condition. The brain tissue was homogenized by sonication in PBS, and then sequential centrifugations were performed at 3,000 × *g* at 4°C for 20 min in a microcentrifuge to obtain a clear supernatant. Melatonin level was assayed in this clear supernatant by using a Fish Melatonin (MT) ELISA Kit (Gen Asia, China) (Yumnamcha et al., 2017; Dharmajyoti Devi et al., 2021a) according to the manufacturer's instruction. Absorbance was measured at 450 nm using a Multiskan spectrum reader (Thermo Fisher). The concentration of melatonin in the tissue was presented as pg per 100 mg (pg/100 mg) of tissue.

Serum Melatonin ELISA

Blood was collected according to the published protocol (Babaei et al., 2013; Khan et al., 2018) and was centrifuged at 13,700 × *g* at 4°C for 15 min. The supernatant (Serum) was collected and melatonin level was assayed using a Fish Melatonin (MT) ELISA Kit (Gen Asia, China) (Khan et al., 2018), following the manufacturer's protocol. Absorbance was taken at 450 nm in a Multiskan spectrum reader (Thermo Fisher). The concentration of melatonin in serum was presented as pg per ml of serum (pg/ml).

Immunohistochemical Study

The expression level of leptin and goat in zebrafish brain was studied by immunohistochemical staining. The brain tissue was washed twice in 0.1 M PBS and immersed in PBS, containing 30% sucrose at 4°C overnight and then embedded in Jung TISSUE FREEZING MEDIUM (Leica Microsystems; Nussloch, Germany) as described previously (Cruz et al., 2010; Dharmajyoti Devi et al., 2021b). A frozen section (12 μm) from the telencephalon region of the brain for goat and leptin immunoreaction was prepared with a Leica CM3050S cryostat microtome (Leica Biosystems; Nussloch, Germany). An earlier report on fish confirms that the goat immunoreactive cells are present in different encephalic areas including the telencephalon (Blanco et al., 2016a,b). Immunohistochemistry was conducted with some modification as described previously (Hanna et al., 2010). After blocking with 3% BSA (dissolved in 0.1 M PBS), the tissue section was incubated with primary rabbit polyclonal anti-leptin antibody (1:100 dilution; ab16227, Abcam, United Kingdom) and primary rabbit polyclonal anti-Ghrelin O-acyltransferase antibody (1:100 dilution; ab170690, Abcam, United Kingdom) at 4°C overnight. Subsequently, sections were incubated with Donkey Anti-Rabbit IgG (H+L) secondary antibody, Alexa Fluor® 488 (1:200, Invitrogen; United States) for leptin and Goat Anti-Rabbit IgG (H+L) secondary antibody, Alexa Fluor^{TM} 594 for goat (1:200, Invitrogen; United States) for 1 h at room temperature. Then, the slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen, United States) for nuclei staining. For negative control, a separate set of tissue sections were treated with secondary antibody only. The slides were cover slipped with Vectashield (H-1000; Vector Laboratories, Inc., Burlingame, California, United States) and observed under a Nikon A1 R HD25 confocal microscope (NIKON CORPORATION, Konan, Minato-ku, Tokyo, Japan). Fifteen slides (from five fish) in each experimental condition for each antibody (each slide containing six sections) were stained using the above protocol and analyzed. Only representative images of brain staining for leptin and goat are shown here. For the quantification of immunopositive cells in each experimental condition, first, the total number of cells immunoreactive for leptin and goat in LD, LL, and DD was separately counted in all sections that were prepared. To calculate the percentage in each experiment, the total number of cells under each category (LD/LL/DD) of staining was divided by the total number of immunoreactive cells for each antibody (leptin/goat) of all three experimental conditions (LD, LL, and DD). Then, the result was multiplied by 100 to obtain the percentage of cells in every experimental condition (Hatef et al., 2015).

Western Blot Analysis

The pooled brain tissue was homogenized by sonication using SONICS unit (Vibra cell^{TM}) and centrifuged at 30,000 rpm

TABLE 1 | List of Primer sequences used in Quantitative Realtime PCR (RT-PCR) analysis.

Gene	Name of Gene	Primer Sequence 5′-3′	Amplicon Size	Accession Number*
lepa	Leptin a	F: AGCTCTCCGCTCAACCTGTA R: CAGCGGGAATCTCTGGATAA	194	NM_001128576.1
mboat4	Membrane bound O-acyltransferase domain containing 4	F: CACCCTCAGCTGTTTTACCA R: GAATCCTCCCATCGCCAAAT	120	NM_001122944.1
rpl13a	Ribosomal protein L13a	F: TCTGGAGGACTGTTAGAGGTATGC R: AGACGGACAATCTTGAGAGCAG	148	NM_212784.1

F, forward; R, reverse.

**Accession Number is provided by the National Centre for Biotechnology Information, Bethesda, MD, United States.*

The primers were taken from the published data, references have been given in the text.

at 4°C for 20 min in a microcentrifuge. After collecting the supernatant, protein was estimated with the RC DC protein assay (Bio-Rad Laboratories) (Yumnamcha et al., 2017). Total protein was separated on 4–20% Mini-PROTEAN TGX^{TM} Precast Protein Gels (Cat. 4561094, BIO-RAD) and then transferred into polyvinylidene fluoride (Immobilon-PSQ Transfer Membrane, Merck Millipore, United States) (Chattoraj et al., 2008; Wang et al., 2018). For blocking the membranes, 5% non-fat milk in TBST buffer (50 mM Tris, 100 mM NaCl, and 0.1% Tween 20, pH 7.4) was used (Chattoraj et al., 2005). The membranes were incubated overnight with the primary antibodies, rabbit polyclonal anti-leptin antibody (1:1,000; ab16227, Abcam, United States), rabbit polyclonal anti-Ghrelin O-acyltransferase antibody (1:500 dilution; ab170690, Abcam, United States), and mouse monoclonal anti-β-actin (1:5,000 dilution; AM4302, Invitrogen, United States) at 4°C. Then, the membranes were incubated for 1 h at room temperature with alkaline phosphatase conjugated secondary antibody (1:10,000 dilution; Goat anti-rabbit and Goat anti-mouse for goat and actb, respectively, and 1:5,000 dilution; Goat anti-rabbit for leptin) (Sigma-Aldrich, United States). Finally, the membranes were developed with BCIP/NBT (Merck Millipore, United States). The intensity of the individual band of immunoblot was quantified by densitometry using ImageJ software (National Institutes of Health, Bethesda, MD; https://imagej.nih.gov/ij/), and values were normalized to β-actin abundance (Chattoraj et al., 2009).

Ga-SI Measurement

The collected gastrointestinal tract (in 4% PFA) was weighed (uniBloc analytical balance, Shimadzu) (Amaral and Johnston, 2012) and Ga-SI was calculated using the formula Ga-SI (%) = [Weight of gut (g)/Weight of fish (g)] × 100 (Biswas and Takeuchi, 2003).

Statistical Analysis

Statistical changes in the expression of genes, quantity of brain and serum melatonin, and Ga-SI at different time points in every group were determined by one-way ANOVA (SPSS 16.0 software; Macrovision Corporation Santa Carlo, California, United States) followed by Tukey's *post-hoc* test to compare the difference between the time points. $p < 0.05$ was considered as statistically significant.

RESULTS

Ga-SI Under Different Photic Conditions (LD/LL/DD)

After 72 h of incubation in three different photoperiodic conditions, the gut food content in terms of Ga-SI was significantly higher in continuous light, twice in LL, and lower by half in DD as compared to LD (**Figure 1**). Recently, higher activity and excitation rates after feeding in zebrafish larva were demonstrated due to exposure to continuous light (Kopp et al., 2018).

The Expression Pattern of Leptin and Goat mRNA in the Brain Under Different Photic Conditions (LD/LL/DD)

In this experiment, the mRNA expression patterns of two genes *leptin* and *goat* were studied in zebrafish brain under different illuminations.

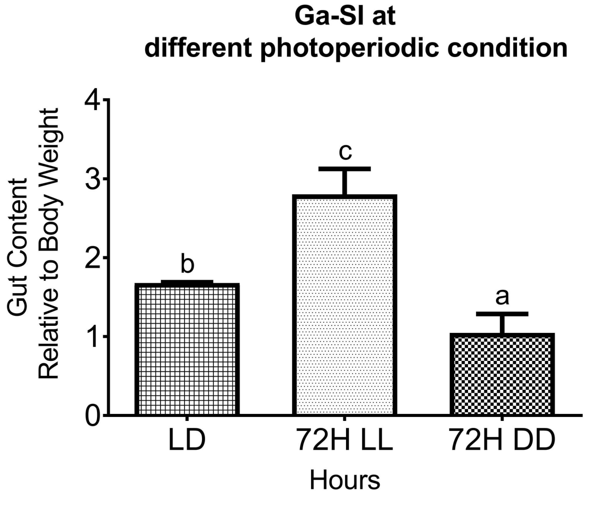


FIGURE 1 | Ga-SI (Gastrointestinal Somatic Index) in different photic conditions (LD, LL, and DD). Food intake as a Ga-SI (intestine food content relative to body mass) at different photoperiodic conditions (LD, LL, and DD) after 72 h of incubation. Results are expressed as mean ± SEM ($n = 5$). Different letters indicate significant differences evaluated by one-way ANOVA [$F(2,6) = 43.743$, $p = 0.000$] with the Tukey *post-hoc* test ($p < 0.05$).

The expression of *leptin* mRNA was in a particular pattern with a peak at ZT12 or the end of the light phase in LD in the brain (**Figure 2A**). A rhythmic pattern of *leptin* expression with a high level during the end of the light phase or daytime and a low level during night-time was also observed in goldfish brain and liver in normal photic conditions (Tinoco et al., 2014). In LL, the expression pattern of *leptin* was abolished after 2 days of exposure under continuous light as compared to LD (**Figure 2B**); nevertheless, in DD, the pattern was in-phase with LD (**Figure 2C**). Moreover, the mRNA expression was decreased up to 2.5-fold under constant conditions as compared to LD (**Figure 2**). The *goat* mRNA expression in zebrafish brain displays a night-time surge and remains constant, whereas a daytime decline was observed in normal photic condition (LD) with scheduled feeding (**Figure 3A**). In this regard, the daily pattern of *preproghrelin* expression in goldfish forebrain, hypothalamus, hindbrain, pituitary, and gastrointestinal tract during a 12L:12D photocycle shows significant rhythm with night-time acrophase (Sanchez-Bretano et al., 2015). In the continuous light condition (LL), the goat expression started to decrease from the second day (**Figure 3B**), whereas in the continuous dark condition (DD), the highest level of expression was observed at ZT06 D2 and then became lower, and finally a surge was detected at ZT18 D3 (**Figure 3C**). Moreover, under continuous photic conditions, the expression level was increased up to twofold from normal photic conditions (**Figure 3**).

Leptin and Goat Immunoreactivity in the Brain Under LD and Continuous Photic Conditions, LL and DD

A cross-section of the zebrafish brain through the telencephalon region identified leptin and goat immunoreactive cells. These were scattered in the lateral, medial, and lower parts of the telencephalon. No staining was found in negative controls stained with only secondary antibodies (**Supplementary Figures 1A,B**). Quantification of immunoreactivity cells reveals that leptin immunopositive cells were higher in DD (∼51%) and lower under LL conditions (∼15%) as compared to LD (∼34%) (**Figure 4**). In the case of the goat, the number of immunopositive cells was high in LD (∼41%) and DD (∼45%). On the contrary, immunoreactivity of goat decreased in LL (∼14%) (**Figure 5**).

Western Blot Analysis of Leptin and Goat in the Brain Under LD and Continuous Photic Conditions, LL and DD

We have found that the mRNA expression pattern of *leptin* and *goat* was changed in continuous photoperiodic conditions. From the immunohistochemical localization study, after 72 h of incubation in different photoperiodic conditions (LD, LL, and DD), it was found that the immunoreactive cells against leptin and goat were decreased in LL and increased in DD compared to LD in the brain of zebrafish. Similarly, Western blot analysis of leptin and goat with a corresponding band of 16 and 50 kd, respectively, showed an increase in DD, whereas no significant variation was found in LL compared to LD (**Figure 6**). There is no such report available in fish regarding expression at the

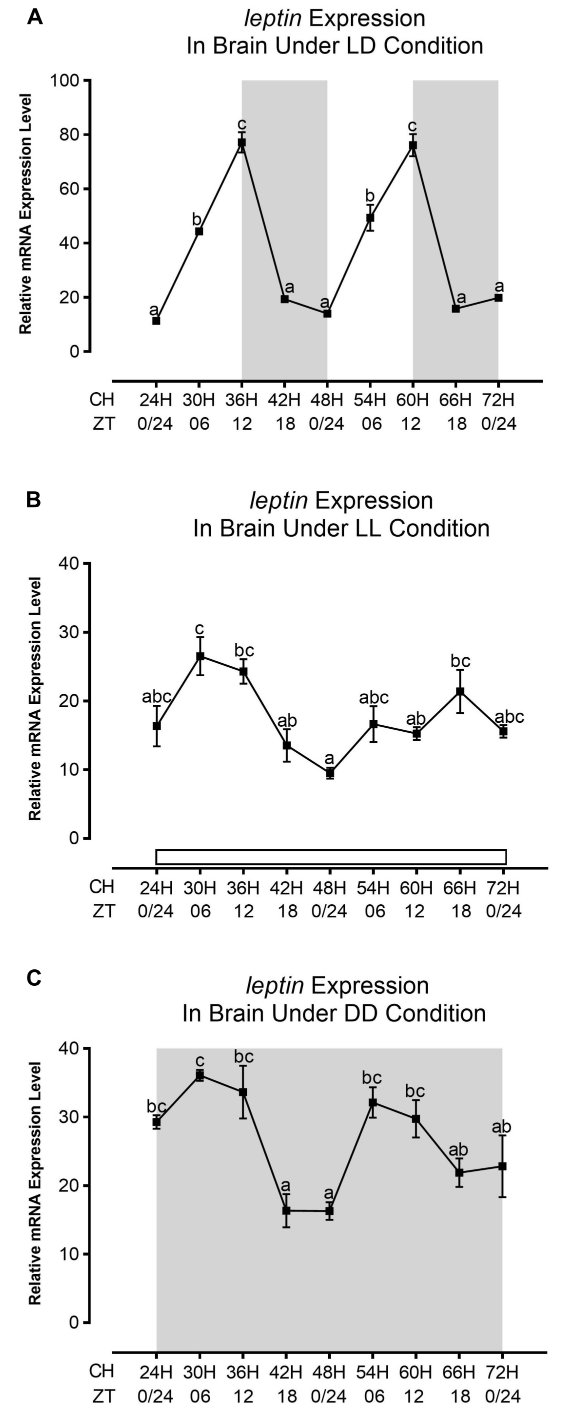


FIGURE 2 | Daily expression profile of *leptin* gene under different photic conditions (LD, LL, and DD) in the brain of zebrafish. The daily expression profile of *leptin* mRNA at **(A)** LD, **(B)** LL, and **(C)** DD. The relative quantification values in the graph are shown as the mean ± SEM ($n = 3$). Different letters indicate significant differences evaluated by one-way ANOVA [**(A)** $F(8,18) = 109.908$, $p = 0.000$, **(B)** $F(8,18) = 5.926$, $p = 0.001$, and **(C)** $F(8,18) = 8.012$, $p = 0.000$] followed by *post-hoc* Tukey's test ($p < 0.05$). The gray background resembles the dark phase and the white background is for the light phase. CH and ZT in the *x*-axis stand for Clock Hour and Zeitgeber Time, respectively.

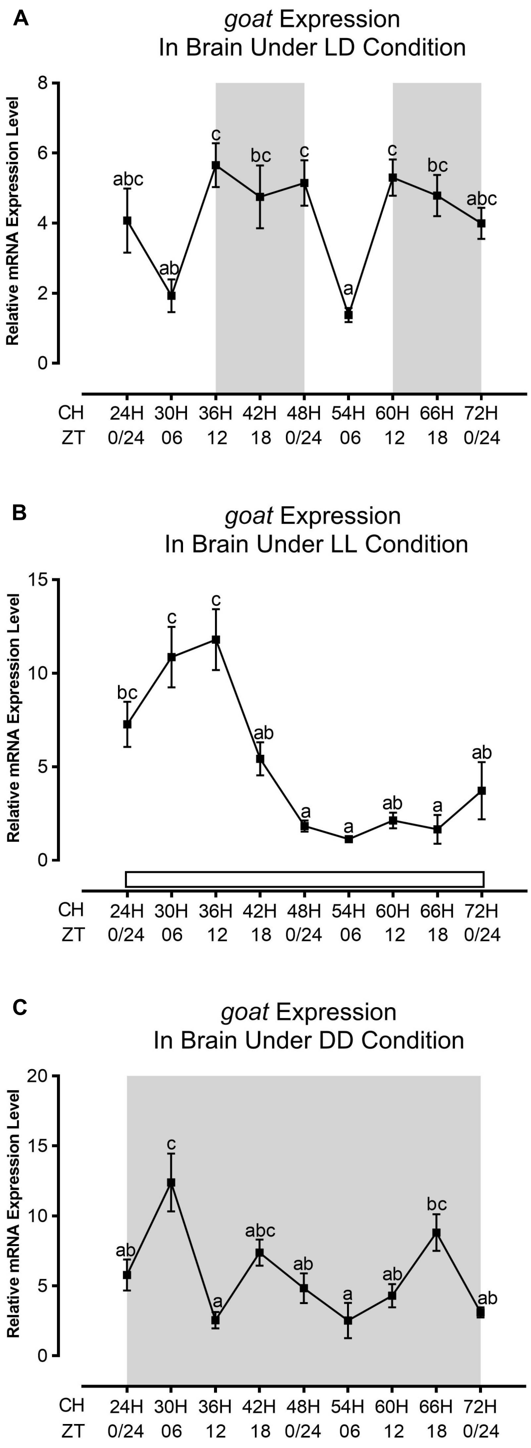


FIGURE 3 | Daily expression profile of *goat* gene under different photic conditions (LD, LL, and DD) in the brain of zebrafish. The daily expression profile of *goat* mRNA at **(A)** LD, **(B)** LL, and **(C)** DD. The relative quantification values in the graph are shown as the mean ± SEM ($n = 3$). Different letters indicate significant differences evaluated by one-way ANOVA [**(A)** $F(8,18) = 5.743$, $p = 0.001$, **(B)** $F(8,18) = 13.813$, $p = 0.000$, and **(C)** $F(8,18) = 8.109$, $p = 0.000$] followed by *post-hoc* Tukey's test ($p < 0.05$). The gray background resembles the dark phase and the white background is for the light phase. CH and ZT in the *x*-axis stand for Clock Hour and Zeitgeber Time, respectively.

translational level of leptin and goat in the brain, although it was demonstrated that zebrafish expression was increased under unfed conditions at the transcriptional level of goat in the brain and at the transcriptional and translational level of goat in the gut (Hatef et al., 2015).

Melatonin Profile in the Brain and Serum Under Different Photic Conditions (LD/LL/DD)

Melatonin, a chronobiotic molecule, is mainly synthesized in the pineal organ and retinal. This neuroendocrine hormone is the key signal of the vertebrate circadian clock, and it is related to many processes that have a common rhythmic expression such as food intake, metabolism, and glucose uptake (Reiter, 1991; Falcon et al., 2007; Cipolla-Neto et al., 2014; Mondal et al., 2021b). In this study, we have found a significantly higher level of melatonin in continuous dark. The level of melatonin in the brain was approximately 350 pg/100 mg of tissue and 410 pg/ml in serum in constant dark (24D; DD). However, the melatonin level was reduced under LL, and it was approximately 190 pg/100 mg of tissue in the brain and 250 pg/ml in serum in comparison to LD, which was about 280 pg/100 mg and 345 pg/ml of tissue in the brain and serum, respectively (**Supplementary Figure 2**). A similar type of elevation in the brain and serum melatonin in continuous dark and decline in continuous light was reported in zebrafish and common dentex (Pavlidis et al., 1999).

DISCUSSION

In few fish, the different photoperiod and feeding schedules demonstrated the daily expression pattern of leptin, preproghrelin, and ghrelin in the brain, liver, and gut (Tinoco et al., 2014; Mondal et al., 2021a). However, the localization of ghrelin and goat was detected only in the gut under similar conditions (Hatef et al., 2015; Sanchez-Bretano et al., 2015; Blanco et al., 2017). However, information on the expression pattern and cellular localization of leptin and goat (third peptide involved in ghrelinergic system) in the brain of zebrafish under different photoperiods with scheduled feeding is missing. These two peptide hormones and enzymes are mainly responsible for central appetite regulation and energy balance (Shlimun and Unniappan, 2011). The present study, for the first time, demonstrates the daily expression pattern of *leptin* and *goat* under different photoperiodic conditions in the brain of zebrafish along with the level of melatonin (in both brain and serum) and Ga-SI after 72 h of incubation under different photoperiods. According to the transcriptional expression profile under different photic conditions, the immunohistochemical localization and immunoblotting of leptin and goat after 72 h of incubation conclusively demonstrate the presence and level of leptin and the ghrelinergic system in the zebrafish brain. The brain goat mRNA increased following 3 days of fasting and remained the same up to 7 days in zebrafish (Hatef et al., 2015).

In goldfish, a rhythmic pattern of expression of leptin and ghrelin in the brain has been reported in normal photoperiod and scheduled feeding (Tinoco et al., 2014; Sanchez-Bretano et al., 2015). Although feeding timing can affect the leptin

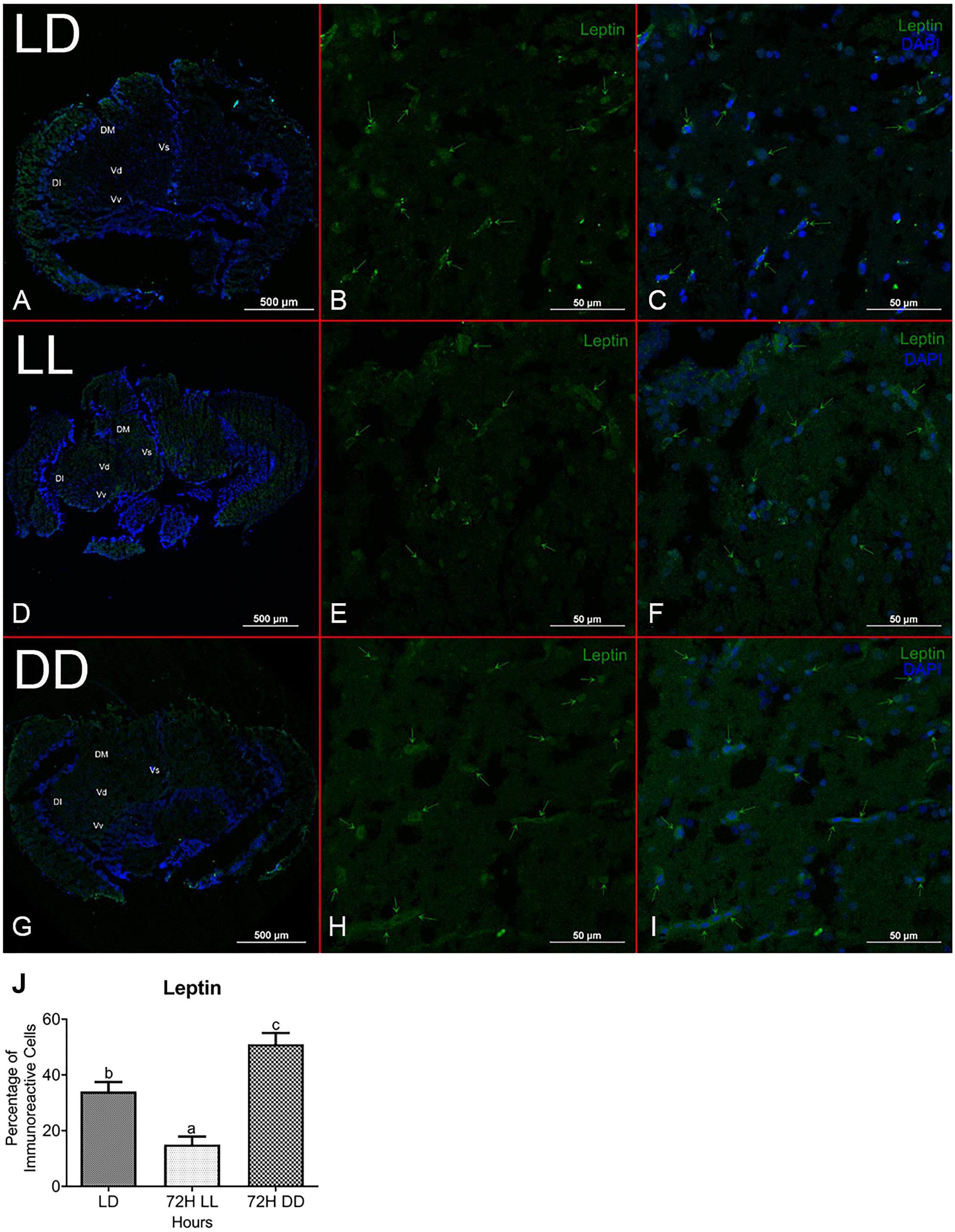


FIGURE 4 | Localization and characterization of Leptin in zebrafish brain under different photic conditions (LD, LL, and DD). The figure shows Leptin immunoreactivity cells in LD **(B)**, LL **(E)**, and DD **(H)** in the telencephalon region of the brain in zebrafish. Immunohistochemical localization shows Leptin immunoreactive cells (green arrow) in the dorsomedial, dorsolateral, and ventromedial zone of the telencephalon in the brain. The whole section of brain through telencephalic region **(A,D,G)**, Leptin-positive cells (green) **(B,E,H)**, and merge imaged with nuclear stain DAPI **(C,F,I)**. Relative abundance of Leptin immunopositive cells in the brain under different photic conditions. The relative quantification values in the graph are shown as the mean ± SEM ($n = 5$). Different letters indicate significant differences evaluated by one-way ANOVA [$F(2,6) = 26.536$, $p = 0.001$] followed by *post-hoc* Tukey's test ($p < 0.05$) **(J)**. For the percentage calculation method, please consult the section "Materials and Methods." Representative images were taken from multiple sections of five separate zebrafish brain in each experimental condition (Vs, supracommissural nucleus; Vd, dorsal nucleus; Vv, ventral nucleus of ventromedial telencephalon).

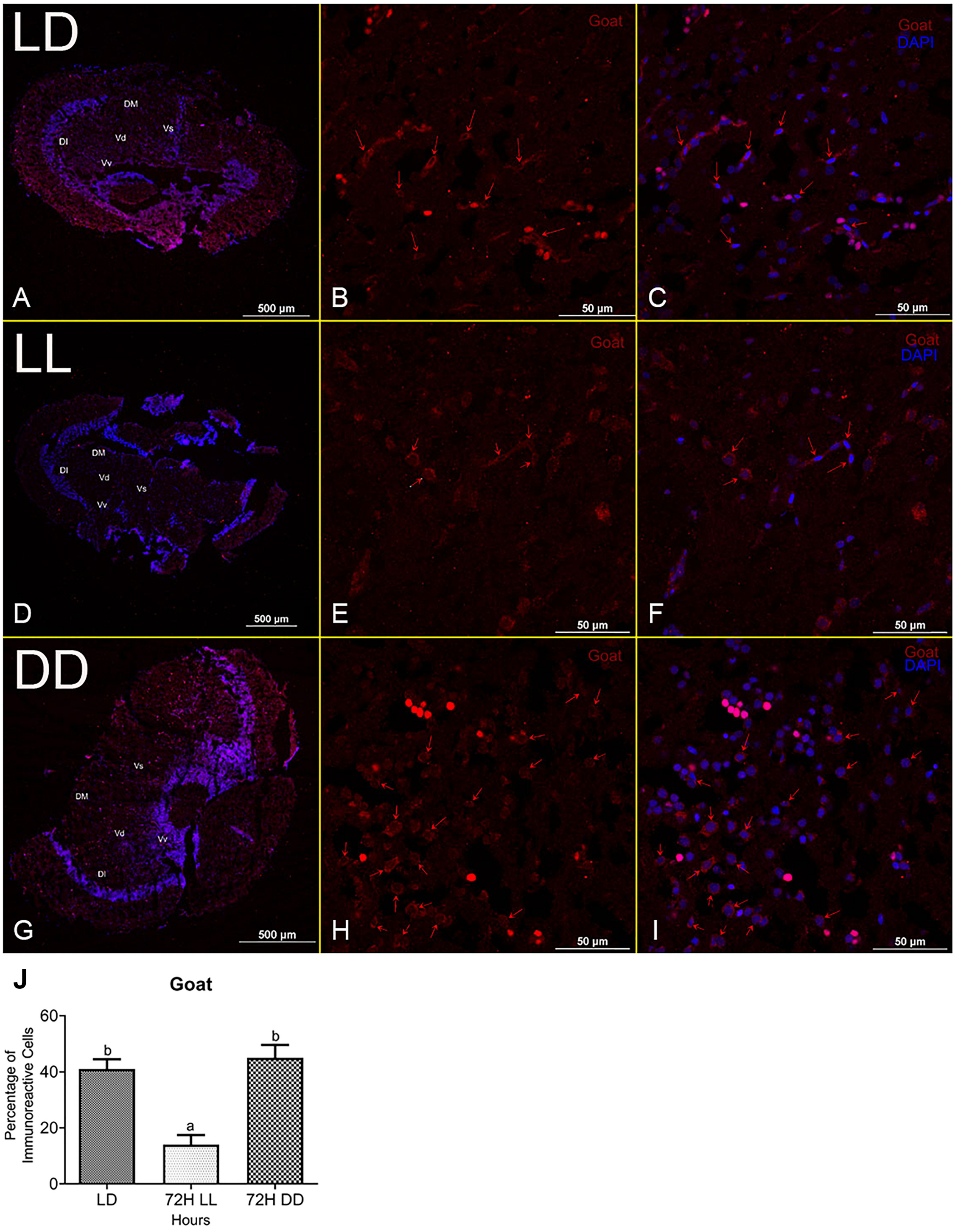


FIGURE 5 | Localization and characterization of Goat in zebrafish brain under different photic conditions (LD, LL, and DD). The figure shows Goat immunoreactivity cells in LD **(B)**, LL **(E)**, and DD **(H)** in the telencephalon region of the brain in zebrafish. Immunohistochemical localization shows Goat immunoreactive cells (red arrow) in the dorsomedial, dorsolateral, and ventromedial zone of the telencephalon in the brain. The whole section of the brain through the telencephalic region **(A,D,G)**, Goat-positive cells (red) **(B,E,H)**, and merge imaged with nuclear stain DAPI **(C,F,I)**. Relative abundance of Goat immunopositive cells in the brain under different photic conditions. The relative quantification values in the graph are shown as the mean ± SEM ($n = 5$). Different letters indicate significant differences evaluated by one-way ANOVA [$F(2,6) = 18.816$, $p = 0.003$] followed by *post-hoc* Tukey's test ($p < 0.05$) **(J)**. For the percentage calculation method, please consult the section "Materials and Methods." Representative images were taken from multiple sections of five separate zebrafish brain in each experimental condition (Vs, supracommissural nucleus; Vd, dorsal nucleus; Vv, ventral nucleus of ventromedial telencephalon).

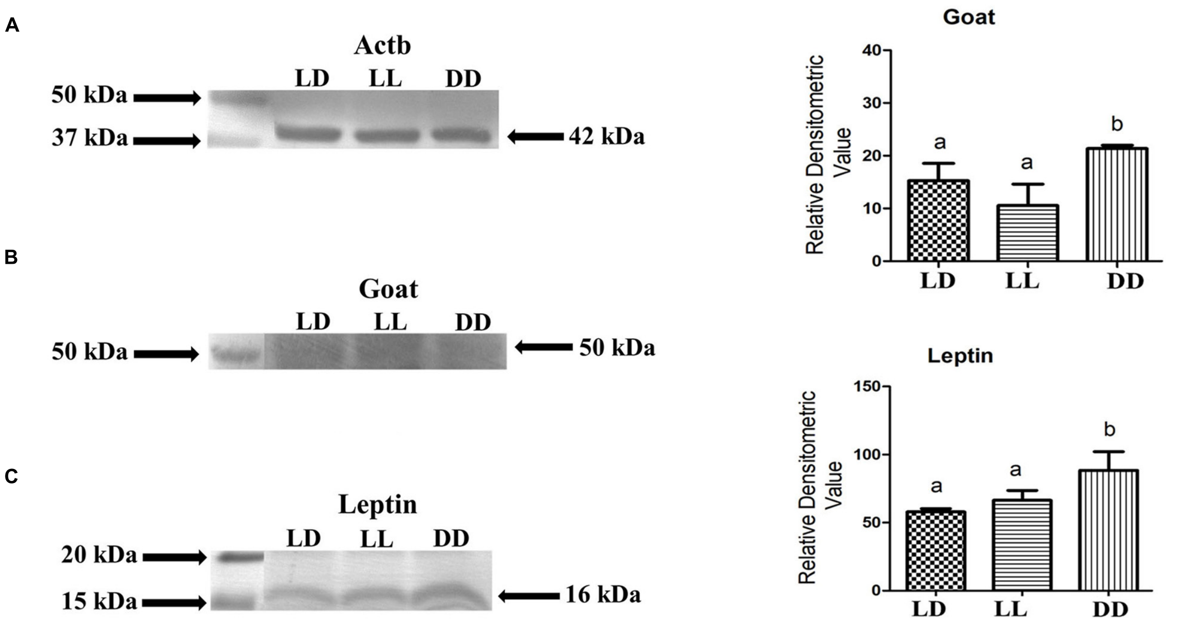


FIGURE 6 | Leptin and Goat proteins are expressed in zebrafish brain and are affected by different photic conditions. Representative immunoblot shows bands representing Actb corresponding to 42 kDa **(A)**, Goat corresponding to 50 kDa **(B)** and Leptin corresponding to 16 kDa **(C)**. Average relative protein expression levels from five zebrafish brain from each experimental condition were analyzed by densitometry analysis. One-way ANOVA [**(B)** $F(2,6) = 32.327$, $p = 0.001$, **(C)** $F(2,6) = 60.216$, $p = 0.000$] followed by *post-hoc* Tukey's test has been done for statistically significant relative quantification. Groups sharing a common letter show no significant difference ($p < 0.05$; $n = 5$ zebrafish).

and ghrelin expression in the brain, feeding itself cannot synchronize the rhythm. Moreover, the clock gene cannot control the rhythmic expression alone (Tinoco et al., 2014; Sanchez-Bretano et al., 2015; Blanco et al., 2017). So, environmental factors like light–dark cycle, feeding time, and endogenous oscillators such as clock genes, hormones, and food-derived metabolites are all involved in the daily rhythmic expression of these two important appetite-regulatory peptide hormones (Simon et al., 1998; Kalsbeek et al., 2001; Cuesta et al., 2009; Falcon et al., 2010; Tsang et al., 2014). The central appetite-controlling device is well conserved among vertebrates, which include many orexigenic (appetite-stimulating) and anorexigenic (appetite-inhibiting) neuropeptides and hormones in mammals, which are also evident in fish (Ronnestad et al., 2017). These neuropeptides and hormones are mainly controlled by leptin, insulin, and ghrelin (Page et al., 2020). The phenomenon of disruption of circadian rhythms and the development of obesity are established by many researchers proposing the possibilities of leptin-ghrelin resistance (Hernández Morante et al., 2020). The molecular mechanisms of leptin and ghrelin resistance are still under investigation. In this regard, the daily expression pattern of *leptin* and *ghrelin* was demonstrated in goldfish, but the effect of photic conditions on daily expression patterns in other fish, mammals, and other vertebrates is totally unknown. In this study, the daily mRNA expression pattern of these two-appetite regulators leptin and goat (indirect estimation for active form of ghrelin) in the brain of zebrafish (indirect estimation for active form of ghrelin) was demonstrated in different photic conditions (LD, LL, and DD) with scheduled feeding to avoid the masking effect of feeding. Leptin mRNA expression in the brain shows the highest peak at ZT12 or at the end of the light phase and lowest at the end of the dark phase (**Figure 2A**). Hypothalamic acrophase in *leptin* expression is known before or during mealtime in goldfish (Tinoco et al., 2014). Our study indicates that *leptin* expression peak coincides with the highest Ga-SI or at the time of the full meal. This supports the notion that an increase in leptin is not the postprandial response of the brain (Tinoco et al., 2014). As a result, an increase in circulating leptin is expected during night-time and can lower nocturnal activity. The lowest levels of circulating leptin is anticipated before the food-anticipatory activity during daytime in these animals (Vivas et al., 2011). In goldfish, the 24-h rhythm of leptin expression in hypothalamus is regained with scheduled feeding under 24 L and 24-h fasting conditions. However, in the peripheral organ, leptin does not perform similarly, establishing the different functions for central and peripheral leptin. On the other hand, under 24 L and 24-h fasting conditions with shifting feeding, leptin rhythm is abolished in both brain and peripheral organ (Tinoco et al., 2014). Unlikely, our data reveal the total depletion of leptin expression after 2 days of incubation in LL (**Figure 2B**), whereas in DD (**Figure 2C**), it was in the same phase as LD with scheduled feeding. In both continuous photic conditions, a 2.5-fold decrease in amplitude of expression of *leptin* in the brain was observed. Such type of results indicates the pleotropic nature of central leptin and further study is needed to determine the role of centrally synthesized leptin. The *goat* mRNA expression in the brain also displayed a daily variation with night-time surge in LD with the earlier said scheduled feeding (**Figure 3A**), and the same type of night-time peak of *preproghrelin* was found in the central and peripheral organ of goldfish (Sanchez-Bretano et al., 2015; Blanco et al., 2017). The amplitude in the expression level of *goat* in the brain was lower than that of *leptin* at LD, which is the same as earlier reported in zebrafish (Montalbano et al., 2018). Moreover, a twofold increase in expression level was detected under continuous photic conditions compared to normal photic conditions (**Figure 3**). Some studies on goldfish and mammals reported that the daily variation in expression of *leptin* and *goat* is driven by endogenous oscillations or circadian systems, which may involve clock genes, photoperiod, hormones, and different metabolites (Cuesta et al., 2009; Froy, 2010). In this regard, melatonin is a key component of the circadian system in vertebrates (Falcon et al., 2010). It was also reported that the 24-h ghrelinergic expression profile overlaps with melatonin, a key component of the circadian system in vertebrates, and melatonin and leptin act in similar intracellular signaling pathways (Falcon et al., 2010; Montalbano et al., 2018). The SCN controls melatonin synthesis in the pineal gland based on environmental photic information, the main clue for the melatonin level in both blood and cerebrospinal fluid in mammals (Simonneaux and Ribelayga, 2003). Melatonin is the hormonal mediator of photoperiodic information to the central nervous system in vertebrates, allowing the regulation of energy homeostasis through the establishment of a proper balance between energy intake and energy expenditure (Lopez-Olmeda et al., 2006; Falcon et al., 2007). This energy balance and food intake control by melatonin happens directly or indirectly due to modification of secretions of hormones involved in food intake control mainly by stimulating the anorexigenic and inhibiting the orexigenic signals (Lima-Cabello et al., 2014). In this communication, we have found an increase and a decrease in Ga-SI of zebrafish in continuous light, LL and continuous dark, DD, respectively (**Figure 1**), whereas brain and serum melatonin levels were observed as an indicator and show significantly low and high levels in continuous light and dark, respectively, compared to normal photoperiod (**Supplementary Figure 2**). Similar to mammals, melatonin is the primary neurohumoral output of the circadian system in zebrafish (Zhdanova, 2011). The involvement of melatonin in the regulation of appetite and food intake is reported by many authors (Pandi-Perumal et al., 2006; Piccinetti et al., 2010). In teleost fish, melatonin can reduce food intake and control energy balance by activating anorexigenic and inhibiting orexigenic peptides (Piccinetti et al., 2010). This finding again supports the involvement of melatonin in food intake. Melatonin may play a role as an intermediate between circadian disruption to the alteration in the transcript expression profile of appetite regulation-related genes in the brain such as *leptin* and *goat*. As a change is observed in *leptin* expression under altered photic conditions (**Figures 2B,C**) and in LL, a decline in expression of *goat* was observed from the second day (**Figure 3B**), but in DD, a high level of expression was observed throughout the experiment (**Figure 3C**). The present study and two previous studies from our laboratory (Yumnamcha et al., 2017; Khan et al., 2018) have indicated a significant change in melatonin level either in the central/peripheral organ or in serum under different photic conditions. From our immunohistochemistry study (**Figures 4**, **5**), it was found that the number of leptin immunoreactive cells is lower in LL and higher in DD in comparison to LD. This finding again supports the stimulating effect of melatonin on leptin (Piccinetti et al., 2010). The goat immunoreactivity was low in LL and high in DD, which further supports the finding that the goat mRNA increases after 3 days of fasting (Hatef et al., 2015) in zebrafish. To our knowledge, immunohistochemical localization of leptin in the brain of fish was not there, although in Wistar rats, leptin receptors were localized with anorexigenic peptides (Romanova et al., 2018), whereas *preproghrelin* was detected in goldfish hypothalamus (Kerbel and Unniappan, 2012) and co-localization of the ghrelinergic system with the orexigenic hormone was shown in hypothalamus of goldfish (Willesen et al., 1999). So, localization of leptin and goat in the zebrafish brain for the first time confirms the role of leptin and goat in feeding. Our immunoblot study also shows a similar type of expression with an immunohistochemical study for leptin and goat in zebrafish brain (**Figure 6**). The measurement of orexigenic and anorexigenic molecules/peptides in the serum will clarify the issue further in the future.

The balance in energy metabolism through food intake is the primary property of life. Feeding ensures daily activities, somatic growth, immune system development, reproductive investments, and so on. It has been elaborated that environmental factors (mainly photoperiod) and signals from the brain and peripheral organs give impetus to the central feeding center (in the brain) to regulate feeding and energy homeostasis (Volkoff, 2016). It is evident that molecules like leptin, ghrelin, and melatonin, responsible for feeding and energy homeostasis, are cyclically regulated by the circadian system (Bass and Takahashi, 2010; Gimble et al., 2011).

In zebrafish, the rhythmic secretion of melatonin depends on the pineal, although the brain may take part in this mechanism in the daily light–dark cycle (Falcon et al., 2010; Moore and Whitmore, 2014; Khan et al., 2016). This synchronization is also correct for tropical carp (Sanjita Devi et al., 2016). However, to date, it is evidenced that the pineal organ is mainly responsible for the serum melatonin in fish, like other vertebrates (Reiter, 1980; Khan et al., 2016; Rajiv et al., 2016, 2017; Sanjita Devi et al., 2016).

CONCLUSION

In conclusion, the expression of *leptin* and *goat* transcript shows a daily variation with a pattern that is disrupted by continuous illumination and change in Ga-SI. Melatonin may have a role in this feeding regulation (in LD) as well as feeding alteration due to constant photic conditions (LL and DD) through the circadian system. The expression at the protein level of leptin after 72 h of incubation in different photic conditions shows that leptin is higher in DD compared to LD and LL. It may be the result of an inducing effect of melatonin on leptin as melatonin was higher in DD. Ga-SI was highest in LL and lowest in DD compared to LD, which indicates leptin-induced feeding inhibition in DD. Leptin is mainly regulated through photic cues through melatonin as its expression changed under different photoperiodic conditions.

Similarly, the level of goat was lower in LL and higher in DD as Ga-SI was highest in LL and lowest in DD, indicating feeding regulation of goat expression in zebrafish brain. The further question whether continuous photic conditions can disrupt the circadian system remains, which further changed the melatonin level results in changes in Ga-SI. These changes in Ga-SI may cause alteration of expression of leptin and goat. Desynchronization of the abovementioned unit may lead to different lifestyle diseases like diabetes and obesity, which is a grave concern in the present-day life, where the anthropogenic sources of light (mainly LED) is increasing day by day (Ouyang et al., 2018; Berge et al., 2020; Gomes, 2020; Schroer et al., 2020). The use of artificial light at night desynchronizes the circadian or endogenous rhythm, which may affect this peptide expression through melatonin and other factors. This phenomenon of change of "proper signal at the proper time" can also influence shift workers. In this scenario, zebrafish can be an excellent tool for biomedical research in this changing environment. Modulating the expression and activity of leptin and goat (affecting the biological action of ghrelin) in the brain could be an excellent target to develop chronotherapeutic approaches to regulate energy intake and body weight in higher mammals in these changing modern societies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals, Govt. of India.

AUTHOR CONTRIBUTIONS

GM: acquisition of data, analysis/interpretation, statistical analysis, and drafting of the manuscript. ZK: critical analysis of the data, organization of figures, and preparation of the manuscript. SD, RL, and AC: concept/design, manuscript preparation, and critical review of the definitive version. All authors contributed to the article and approved the submitted version.

FUNDING

We would like to thank the Director, IBSD, India, and CSIR-JRF Programme to GM [09/1044(0003)/2016-EMR-I (20/12/2016)] for financial support.

ACKNOWLEDGMENTS

We would like to thank W. Rahul for the continuous support in the collection and maintenance of fish in the IBSD Zebrafish Facility, Imphal, Manipur, India. We would also like to thank the security personnel of the Institute for their support in this long experiment during holidays. We would also like to thank the reviewers for their suggestions. We would also like to thank every Indian taxpayer for their contribution toward the funding of this research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.676332/full#supplementary-material

REFERENCES

Amaral, I. P., and Johnston, I. A. (2012). Circadian expression of clock and putative clock-controlled genes in skeletal muscle of the zebrafish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R193–R206.

Amole, N., and Unniappan, S. (2009). Fasting induces preproghrelin mRNA expression in the brain and gut of zebrafish, *Danio rerio*. *Gen. Comp. Endocrinol.* 161, 133–137. doi: 10.1016/j.ygcen.2008.11.002

Babaei, F., Ramalingam, R., Tavendale, A., Liang, Y., Yan, L. S. K., Ajuh, P., et al. (2013). Novel blood collection method allows plasma proteome analysis from single zebrafish. *J. Proteome Res.* 12, 1580–1590. doi: 10.1021/pr3009226

Bass, J., and Takahashi, J. S. (2010). Circadian integration of metabolism and energetics. *Science* 330, 1349–1354. doi: 10.1126/science.1195027

Berge, J., Geoffroy, M., Daase, M., Cottier, F., Priou, P., Cohen, J. H., et al. (2020). Artificial light during the polar night disrupts Arctic fish and zooplankton behaviour down to 200 m depth. *Commun. Biol.* 3:102.

Biswas, A. K., and Takeuchi, T. (2003). Effects of photoperiod and feeding interval on food intake and growth rate of Nile tilapia *Oreochromis niloticus* L. *Fish. Sci.* 69, 1010–1016. doi: 10.1046/j.1444-2906.2003.00720.x

Blanco, A. M., Gómez-Boronat, M., Alonso-Gómez, ÁL., Yufa, R., Unniappan, S., Delgado, M. J., et al. (2017). Characterization of Ghrelin O-Acyltransferase (GOAT) in goldfish (*Carassius auratus*). *PLoS One* 12:e0171874. doi: 10.1371/journal.pone.0171874

Blanco, A. M., Gomez-Boronat, M., Redondo, I., Valenciano, A. I., and Delgado, M. J. (2016a). Periprandial changes and effects of short- and long-term fasting on ghrelin, GOAT, and ghrelin receptors in goldfish (*Carassius auratus*). *J. Comp. Physiol. B* 186, 727–738. doi: 10.1007/s00360-016-0986-0

Blanco, A. M., Sanchez-Bretano, A., Delgado, M. J., and Valenciano, A. I. (2016b). Brain mapping of ghrelin O-Acyltransferase in goldfish (*Carassius Auratus*): novel roles for the ghrelinergic system in fish? *Anat. Rec. (Hoboken)* 299, 748–758. doi: 10.1002/ar.23346

Brugman, S. (2016). The zebrafish as a model to study intestinal inflammation. *Dev. Comp. Immunol.* 64, 82–92. doi: 10.1016/j.dci.2016.02.020

Chattoraj, A., Bhattacharyya, S., Basu, D., Bhattacharya, S., Bhattacharya, S., and Maitra, S. K. (2005). Melatonin accelerates maturation inducing hormone (MIH): induced oocyte maturation in carps. *Gen. Comp. Endocrinol.* 140, 145–155. doi: 10.1016/j.ygcen.2004.10.013

Chattoraj, A., Seth, M., and Maitra, S. K. (2008). Influence of serotonin on the action of melatonin in MIH-induced meiotic resumption in the oocytes of carp *Catla catla*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 150, 301–306. doi: 10.1016/j.cbpa.2008.03.014

Chattoraj, A., Seth, M., and Maitra, S. K. (2009). Localization and dynamics of Mel(1a) melatonin receptor in the ovary of carp *Catla catla* in relation to serum melatonin levels. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 152, 327–333. doi: 10.1016/j.cbpa.2008.11.010

Cipolla-Neto, J., Amaral, F. G., Afeche, S. C., Tan, D. X., and Reiter, R. J. (2014). Melatonin, energy metabolism, and obesity: a review. *J. Pineal Res.* 56, 371–381. doi: 10.1111/jpi.12137

Copeland, D. L., Duff, R. J., Liu, Q., Prokop, J., and Londraville, R. L. (2011). Leptin in teleost fishes: an argument for comparative study. *Front. Physiol.* 2:26. doi: 10.3389/fphys.2011.00026

Cruz, S. A., Tseng, Y. C., Kaiya, H., and Hwang, P. P. (2010). Ghrelin affects carbohydrate-glycogen metabolism via insulin inhibition and glucagon stimulation in the zebrafish (*Danio rerio*) brain. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 156, 190–200. doi: 10.1016/j.cbpa.2010.01.019

Cuesta, M., Clesse, D., Pévet, P., and Challet, E. (2009). From daily behavior to hormonal and neurotransmitters rhythms: comparison between diurnal and nocturnal rat species. *Horm. Behav.* 55, 338–347. doi: 10.1016/j.yhbeh.2008.10.015

Denver, R. J., Bonett, R. M., and Boorse, G. C. (2011). Evolution of leptin structure and function. *Neuroendocrinology* 94, 21–38. doi: 10.1159/000328435

Dharmajyoti Devi, S., Mondal, G., Ahmad Khan, Z., Labala, R. K., Kumar Sarma, H., and Chattoraj, A. (2021a). Daily rhythmic expression patterns of melatonin bio-synthesizing genes in zebrafish (*Danio rerio*) testis in response to altered feeding condition. *J. Endocrinol. Reprod.* 24, 31–41.

Dharmajyoti Devi, S., Mondal, G., Khan, Z. A., Sarma, H. K., and Chattoraj, A. (2021b). Differential gene expression and immunohistochemical localization of the key melatonin biosynthesizing enzymes in the testis of zebrafish (*Danio rerio*). *Biol. Rhythm Res.* 1–17. doi: 10.1080/09291016.2021.1926078

Falcinelli, S., Rodiles, A., Unniappan, S., Picchietti, S., Gioacchini, G., Merrifield, D. L., et al. (2016). Probiotic treatment reduces appetite and glucose level in the zebrafish model. *Sci. Rep.* 6:18061.

Falcon, J., Besseau, L., Sauzet, S., and Boeuf, G. (2007). Melatonin effects on the hypothalamo-pituitary axis in fish. *Trends Endocrinol. Metab.* 18, 81–88. doi: 10.1016/j.tem.2007.01.002

Falcon, J., Migaud, H., Munoz-Cueto, J. A., and Carrillo, M. (2010). Current knowledge on the melatonin system in teleost fish. *Gen. Comp. Endocrinol.* 165, 469–482. doi: 10.1016/j.ygcen.2009.04.026

Frøiland, E., Murashita, K., Jørgensen, E. H., and Kurokawa, T. (2010). Leptin and ghrelin in anadromous *Arctic charr*: cloning and change in expressions during a seasonal feeding cycle. *Gen. Comp. Endocrinol.* 165, 136–143. doi: 10.1016/j.ygcen.2009.06.010

Froy, O. (2010). Metabolism and circadian rhythms–implications for obesity. *Endocr. Rev.* 31, 1–24. doi: 10.1210/er.2009-0014

Gahete, M. D., Córdoba-Chacón, J., Salvatori, R., Castaño, J. P., Kineman, R. D., and Luque, R. M. (2010). Metabolic regulation of ghrelin O-acyl transferase (GOAT) expression in the mouse hypothalamus, pituitary, and stomach. *Mol. Cell Endocrinol.* 317, 154–160. doi: 10.1016/j.mce.2009.12.023

Gimble, J. M., Sutton, G. M., Bunnell, B. A., Ptitsyn, A. A., and Floyd, Z. E. (2011). Prospective influences of circadian clocks in adipose tissue and metabolism. *Nat. Rev. Endocrinol.* 7, 98–107. doi: 10.1038/nrendo.2010.214

Gomes, D. G. E. (2020). Orb-weaving spiders are fewer but larger and catch more prey in lit bridge panels from a natural artificial light experiment. *PeerJ* 8:e8808. doi: 10.7717/peerj.8808

Gómez Abellán, P., Gómez Santos, C., Madrid, J. A., Milagro, F. I., Campion, J., Martínez, J. A., et al. (2011). Site-specific circadian expression of leptin and its receptor in human adipose tissue. *Nutr. Hosp.* 26, 1394–1401.

Hanna, R. N., Daly, S. C., Pang, Y., Anglade, I., Kah, O., Thomas, P., et al. (2010). Characterization and expression of the nuclear progestin receptor in zebrafish gonads and brain. *Biol. Reprod.* 82, 112–122. doi: 10.1095/biolreprod.109.078527

Hatef, A., Yufa, R., and Unniappan, S. (2015). Ghrelin O-Acyl transferase in zebrafish is an evolutionarily conserved peptide upregulated during calorie restriction. *Zebrafish* 12, 327–338. doi: 10.1089/zeb.2014.1062

Helwig, M., Archer, Z. A., Heldmaier, G., Tups, A., Mercer, J. G., and Klingenspor, M. (2009). Photoperiodic regulation of satiety mediating neuropeptides in the brainstem of the seasonal Siberian hamster (*Phodopus sungorus*). *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 195, 631–642. doi: 10.1007/s00359-009-0438-3

Hernández Morante, J. J., Díaz Soler, I., Muñoz, S. G. J., Sánchez, P. H., Barberá Ortega, D. M., Martínez, M. C., et al. (2020). Moderate weight loss modifies leptin and ghrelin synthesis rhythms but not the subjective sensations of appetite in obesity patients. *Nutrients* 12:916. doi: 10.3390/nu12040916

Hoskins, L. J., and Volkoff, H. (2012). The comparative endocrinology of feeding in fish: insights and challenges. *Gen. Comp. Endocrinol.* 176, 327–335. doi: 10.1016/j.ygcen.2011.12.025

Huising, M. O., Geven, E. J. W., Kruiswijk, C. P., Nabuurs, S. B., Stolte, E. H., Spanings, F. A. T., et al. (2006). Increased leptin expression in common Carp (*Cyprinus carpio*) after food intake but not after fasting or feeding to satiation. *Endocrinology* 147, 5786–5797. doi: 10.1210/en.2006-0824

Kaiya, H., Miyazato, M., Kangawa, K., Peter, R. E., and Unniappan, S. (2008). Ghrelin: a multifunctional hormone in non-mammalian vertebrates. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 149, 109–128. doi: 10.1016/j.cbpa.2007.12.004

Kalsbeek, A., Fliers, E., Romijn, J. A., La Fleur, S. E., Wortel, J., Bakker, O., et al. (2001). The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* 142, 2677–2685. doi: 10.1210/endo.142.6.8197

Kerbel, B., and Unniappan, S. (2012). Nesfatin-1 suppresses energy intake, co-localises ghrelin in the brain and gut, and alters ghrelin, cholecystokinin and orexin mRNA expression in goldfish. *J. Neuroendocrinol.* 24, 366–377. doi: 10.1111/j.1365-2826.2011.02246.x

Khan, Z. A., Labala, R. K., Yumnamcha, T., Devi, S. D., Mondal, G., Sanjita Devi, H., et al. (2018). Artificial Light at Night (ALAN), an alarm to ovarian physiology: a study of possible chronodisruption on zebrafish (*Danio rerio*). *Sci. Total Environ.* 628-629, 1407–1421. doi: 10.1016/j.scitotenv.2018.02.101

Khan, Z. A., Yumnamcha, T., Rajiv, C., Devi, H. S., Mondal, G., Devi, S. D., et al. (2016). Melatonin biosynthesizing enzyme genes and clock genes in ovary and whole brain of zebrafish (*Danio rerio*): Differential expression and a possible interplay. *Gen. Comp. Endocrinol.* 233, 16–31. doi: 10.1016/j.ygcen.2016.05.014

Kobayashi, Y., Quiniou, S., Booth, N. J., and Peterson, B. C. (2011). Expression of leptin-like peptide (LLP) mRNA in channel catfish (*Ictalurus punctatus*) is induced by exposure to *Edwardsiella ictaluri* but is independent of energy status. *Gen. Comp. Endocrinol.* 173, 411–418. doi: 10.1016/j.ygcen.2011.06.011

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402, 656–660. doi: 10.1038/45230

Kopp, R., Legler, J., and Legradi, J. (2018). Alterations in locomotor activity of feeding zebrafish larvae as a consequence of exposure to different environmental factors. *Environ. Sci. Pollut. Res. Int.* 25, 4085–4093. doi: 10.1007/s11356-016-6704-3

Li, M. D. (2011). Leptin and beyond: an odyssey to the central control of body weight. *Yale J. Biol. Med.* 84, 1–7.

Lim, C. T., Kola, B., Grossman, A., and Korbonits, M. (2011). The expression of ghrelin O-acyltransferase (GOAT) in human tissues. *Endocr. J.* 58, 707–710. doi: 10.1507/endocrj.k11e-117

Lima-Cabello, E., Diaz-Casado, M. E., Guerrero, J. A., Otalora, B. B., Escames, G., Lopez, L. C., et al. (2014). A review of the melatonin functions in zebrafish physiology. *J. Pineal Res.* 57, 1–9. doi: 10.1111/jpi.12149

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Lopez-Olmeda, J. F., Madrid, J. A., and Sanchez-Vazquez, F. J. (2006). Melatonin effects on food intake and activity rhythms in two fish species with different activity patterns: Diurnal (goldfish) and nocturnal (tench). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 144, 180–187. doi: 10.1016/j.cbpa.2006.02.031

Matsuda, K., Miura, T., Kaiya, H., Maruyama, K., Shimakura, S., Uchiyama, M., et al. (2006). Regulation of food intake by acyl and des-acyl ghrelins in the goldfish. *Peptides* 27, 2321–2325. doi: 10.1016/j.peptides.2006.03.028

Moen, A. G., and Finn, R. N. (2013). Short-term, but not long-term feed restriction causes differential expression of leptins in Atlantic salmon. *Gen. Comp. Endocrinol.* 183, 83–88. doi: 10.1016/j.ygcen.2012.09.027

Mondal, G., Dharmajyoti Devi, S., Ahmad Khan, Z., Kumar Labala, R., and Chattoraj, A. (2021a). Light, feeding and melatonin: an interplay in the appetite regulation in the gut of zebrafish (*Danio rerio*). *J. Endocrinol. Reprod.* 23, 81–97.

Mondal, G., Dharmajyoti Devi, S., Khan, Z. A., Yumnamcha, T., Rajiv, C., Sanjita Devi, H., et al. (2021b). The influence of feeding on the daily rhythm of mRNA expression on melatonin bio-synthesizing enzyme genes and clock associated genes in the zebrafish (*Danio rerio*) gut. *Biol. Rhythm Res.* 58, 1–18. doi: 10.1080/09291016.2021.1905989

Montalbano, G., Mania, M., Abbate, F., Navarra, M., Guerrera, M. C., Laura, R., et al. (2018). Melatonin treatment suppresses appetite genes and improves adipose tissue plasticity in diet-induced obese zebrafish. *Endocrine* 62, 381–393. doi: 10.1007/s12020-018-1653-x

Moore, H. A., and Whitmore, D. (2014). Circadian rhythmicity and light sensitivity of the zebrafish brain. *PLoS One* 9:e86176. doi: 10.1371/journal.pone.0086176

Ouyang, J. Q., Davies, S., and Dominoni, D. (2018). Hormonally mediated effects of artificial light at night on behavior and fitness: linking endocrine mechanisms with function. *J. Exp. Biol.* 221:jeb156893.

Page, A. J., Christie, S., Symonds, E., and Li, H. (2020). Circadian regulation of appetite and time restricted feeding. *Physiol. Behav.* 220:112873. doi: 10.1016/j.physbeh.2020.112873

Pandi-Perumal, S. R., Srinivasan, V., Maestroni, G. J., Cardinali, D. P., Poeggeler, B., and Hardeland, R. (2006). Melatonin: Nature's most versatile biological signal? *FEBS J.* 273, 2813–2838. doi: 10.1111/j.1742-4658.2006.05322.x

Park, H. K., and Ahima, R. S. (2015). Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. *Metabolism* 64, 24–34. doi: 10.1016/j.metabol.2014.08.004

Pavlidis, M., Greenwood, L., Paalavuo, M., Mölsä, H., and Laitinen, J. T. (1999). The effect of photoperiod on diel rhythms in serum melatonin, cortisol, glucose, and electrolytes in the common dentex, *Dentex dentex*. *Gen. Comp. Endocrinol.* 113, 240–250. doi: 10.1006/gcen.1998.7190

Piccinetti, C. C., Migliarini, B., Olivotto, I., Coletti, G., Amici, A., and Carnevali, O. (2010). Appetite regulation: the central role of melatonin in *Danio rerio*. *Horm. Behav.* 58, 780–785. doi: 10.1016/j.yhbeh.2010.07.013

Portaluppi, F., Smolensky, M. H., and Touitou, Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol. Int.* 27, 1911–1929. doi: 10.3109/07420528.2010.516381

Rajiv, C., Sanjita Devi, H., Mondal, G., Devi, S. D., Khan, Z. A., Yumnamcha, T., et al. (2016). Daily and seasonal expression profile of serum melatonin and its biosynthesizing enzyme genes (tph1, aanat1, aanat2, and hiomt) in pineal organ and retina: a study under natural environmental conditions in a tropical carp, *Catla catla*. *J. Exp. Zool. A Ecol. Genet. Physiol.* 325, 688–700. doi: 10.1002/jez.2061

Rajiv, C., Sanjita Devi, H., Mondal, G., Devi, S. D., Khan, Z. A., Yumnamcha, T., et al. (2017). Cloning, phylogenetic analysis and tissue distribution of melatonin bio-synthesizing enzyme genes (Tph1, Aanat1, Aanat2 and Hiomt) in a tropical carp, *Catla catla*. *Biol. Rhythm Res.* 48, 371–386. doi: 10.1080/09291016.2016.1263019

Reed, B., and Jennings, M. (2011). *Guidance on the Housing and Care of Zebrafish, Danio Rerio*. Southwater: Research Animals Department, Science Group, RSPCA.

Reiter, R. J. (1980). The pineal and its hormones in the control of reproduction in mammals. *Endocr. Rev.* 1, 109–131. doi: 10.1210/edrv-1-2-109

Reiter, R. J. (1991). Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr. Rev.* 12, 151–180. doi: 10.1210/edrv-12-2-151

Romanova, I. V., Derkach, K. V., Mikhrina, A. L., Sukhov, I. B., Mikhailova, E. V., and Shpakov, A. O. (2018). The leptin, dopamine and serotonin receptors in hypothalamic POMC-neurons of normal and obese rodents. *Neurochem. Res.* 43, 821–837. doi: 10.1007/s11064-018-2485-z

Ronnestad, I., Gomes, A. S., Murashita, K., Angotzi, R., Jonsson, E., and Volkoff, H. (2017). Appetite-controlling endocrine systems in teleosts. *Front. Endocrinol. (Lausanne)* 8:73. doi: 10.3389/fendo.2017.00073

Rønnestad, I., Nilsen, T. O., Murashita, K., Angotzi, A. R., Gamst Moen, A. G., Stefansson, S. O., et al. (2010). Leptin and leptin receptor genes in Atlantic salmon: cloning, phylogeny, tissue distribution and expression correlated to long-term feeding status. *Gen. Comp. Endocrinol.* 168, 55–70. doi: 10.1016/j.ygcen.2010.04.010

Sakata, I., Yang, J., Lee, C. E., Osborne-Lawrence, S., Rovinsky, S. A., Elmquist, J. K., et al. (2009). Colocalization of ghrelin O-acyltransferase and ghrelin in gastric mucosal cells. *Am. J. Physiol. Endocrinol. Metab.* 297, E134–E141.

Sanchez-Bretano, A., Blanco, A. M., Unniappan, S., Kah, O., Gueguen, M. M., Bertucci, J. I., et al. (2015). In situ localization and rhythmic expression of ghrelin and ghs-r1 ghrelin receptor in the brain and gastrointestinal tract of goldfish (*Carassius auratus*). *PLoS One* 10:e0141043. doi: 10.1371/journal.pone.0141043

Sanjita Devi, H., Rajiv, C., Mondal, G., Khan, Z. A., Dharmajyoti Devi, S., Yumnamcha, T., et al. (2016). Melatonin bio-synthesizing enzyme genes (Tph1, Aanat1, Aanat2 and Hiomt) and their temporal pattern of expression in brain and gut of a tropical carp in natural environmental conditions. *Cogent Biol.* 2:1230337. doi: 10.1080/23312025.2016.1230337

Schroer, S., Huggins, B. J., Azam, C., and Hölker, F. (2020). Working with inadequate tools: legislative shortcomings in protection against ecological effects of artificial light at night. *Sustainability* 12:2551. doi: 10.3390/su12062551

Shlimun, A., and Unniappan, S. (2011). Ghrelin o-acyl transferase: bridging ghrelin and energy homeostasis. *Int. J. Pept.* 2011:217957.

Simon, C., Gronfier, C., Schlienger, J. L., and Brandenberger, G. (1998). Circadian and ultradian variations of leptin in normal man under continuous enteral nutrition: relationship to sleep and body temperature. *J. Clin. Endocrinol. Metab.* 83, 1893–1899. doi: 10.1210/jcem.83.6.4864

Simonneaux, V., and Ribelayga, C. (2003). Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. *Pharmacol. Rev.* 55, 325–395. doi: 10.1124/pr.55.2.2

Stengel, A., Goebel, M., Wang, L., Reeve, J. R. Jr., Taché, Y., and Lambrecht, N. W. (2010a). Lipopolysaccharide differentially decreases plasma acyl and desacyl ghrelin levels in rats: potential role of the circulating ghrelin-acylating enzyme GOAT. *Peptides* 31, 1689–1696. doi: 10.1016/j.peptides.2010.06.015

Stengel, A., Goebel, M., Wang, L., Taché, Y., Sachs, G., and Lambrecht, N. W. (2010b). Differential distribution of ghrelin-O-acyltransferase (GOAT) immunoreactive cells in the mouse and rat gastric oxyntic mucosa. *Biochem. Biophys. Res. Commun.* 392, 67–71. doi: 10.1016/j.bbrc.2009.12.169

Suzuki, K., Simpson, K. A., Minnion, J. S., Shillito, J. C., and Bloom, S. R. (2010). The role of gut hormones and the hypothalamus in appetite regulation. *Endocr. J.* 57, 359–372. doi: 10.1507/endocrj.k10e-077

Tang, R., Dodd, A., Lai, D., Mcnabb, W. C., and Love, D. R. (2007). Validation of zebrafish (*Danio rerio*) reference genes for quantitative real-time RT-PCR normalization. *Acta Biochim. Biophys. Sin. (Shanghai)* 39, 384–390. doi: 10.1111/j.1745-7270.2007.00283.x

Tinoco, A. B., Nisembaum, L. G., De Pedro, N., Delgado, M. J., and Isorna, E. (2014). Leptin expression is rhythmic in brain and liver of goldfish (*Carassius auratus*). Role of feeding time. *Gen. Comp. Endocrinol.* 204, 239–247. doi: 10.1016/j.ygcen.2014.06.006

Tinoco, A. B., Nisembaum, L. G., Isorna, E., Delgado, M. J., and De Pedro, N. (2012). Leptins and leptin receptor expression in the goldfish (*Carassius auratus*). Regulation by food intake and fasting/overfeeding conditions. *Peptides* 34, 329–335. doi: 10.1016/j.peptides.2012.02.001

Tsang, A. H., Barclay, J. L., and Oster, H. (2014). Interactions between endocrine and circadian systems. *J. Mol. Endocrinol.* 52, R1–R16.

Unniappan, S., Lin, X., Cervini, L., Rivier, J., Kaiya, H., Kangawa, K., et al. (2002). Goldfish ghrelin: molecular characterization of the complementary deoxyribonucleic acid, partial gene structure and evidence for its stimulatory role in food intake. *Endocrinology* 143, 4143–4146. doi: 10.1210/en.2002-220644

Valassi, E., Scacchi, M., and Cavagnini, F. (2008). Neuroendocrine control of food intake. *Nutr. Metab. Cardiovasc. Dis.* 18, 158–168.

Vivas, Y., Azpeleta, C., Feliciano, A., Velarde, E., Isorna, E., Delgado, M. J., et al. (2011). Time-dependent effects of leptin on food intake and locomotor activity in goldfish. *Peptides* 32, 989–995. doi: 10.1016/j.peptides.2011.01.028

Volkoff, H. (2016). The neuroendocrine regulation of food intake in fish: a review of current knowledge. *Front. Neurosci.* 10:540. doi: 10.3389/fnins.2016.00540

Volkoff, H., Canosa, L. F., Unniappan, S., Cerda-Reverter, J. M., Bernier, N. J., Kelly, S. P., et al. (2005). Neuropeptides and the control of food intake in fish. *Gen. Comp. Endocrinol.* 142, 3–19.

Wang, X., Villar, V. A., Tiu, A., Upadhyay, K. K., and Cuevas, S. (2018). Dopamine D2 receptor up-regulates leptin and IL-6 in adipocytes. *J. Lipid Res.* 59, 607–614. doi: 10.1194/jlr.m081000

Westerfield, M. (2000). *The Zebrafish Book : A Guide for the Laboratory Use of Zebrafish (Danio rerio)*. Eugene, OR: University of Oregon Press.

Willesen, M. G., Kristensen, P., and Rømer, J. (1999). Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* 70, 306–316. doi: 10.1159/000054491

Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., et al. (2001). Ghrelin enhances appetite and increases food intake in humans. *J. Clin. Endocrinol. Metab.* 86:5992. doi: 10.1210/jcem.86.12.8111

Xu, B., Kalra, P. S., Farmerie, W. G., and Kalra, S. P. (1999). Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanocortin, and adipocyte leptin gene expression and secretion: effects of food restriction. *Endocrinology* 140, 2868–2875. doi: 10.1210/endo.140.6.6789

Yang, J., Brown, M. S., Liang, G., Grishin, N. V., and Goldstein, J. L. (2008). Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* 132, 387–396. doi: 10.1016/j.cell.2008.01.017

Yumnamcha, T., Khan, Z. A., Rajiv, C., Devi, S. D., Mondal, G., Sanjita Devi, H., et al. (2017). Interaction of melatonin and gonadotropin-inhibitory hormone on the zebrafish brain-pituitary-reproductive axis. *Mol. Reprod. Dev.* 84, 389–400. doi: 10.1002/mrd.22795

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372, 425–432. doi: 10.1038/372425a0

Zhdanova, I. V. (2011). Sleep and its regulation in zebrafish. *Rev. Neurosci.* 22, 27–36.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer TY, declared a past co-authorship with several of the authors GM, SD, ZK, RL, and AC.

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ORIGINAL RESEARCH
published: 20 September 2021
doi: 10.3389/fevo.2021.732959

Effects of Low-Level Artificial Light at Night on Kentucky Bluegrass and an Introduced Herbivore

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to
Urban Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: 29 June 2021
Accepted: 26 August 2021
Published: 20 September 2021

Citation:
Crump MC, Brown C,
Griffin-Nolan RJ, Angeloni L,
Lemoine NP and Seymoure BM
(2021) Effects of Low-Level Artificial
Light at Night on Kentucky Bluegrass
and an Introduced Herbivore.
Front. Ecol. Evol. 9:732959.
doi: 10.3389/fevo.2021.732959

Increasing evidence suggests that artificial light at night (ALAN) can negatively impact organisms. However, most studies examine the impacts of ALAN on a single species or under high levels of artificial light that are infrequent or unrealistic in urban environments. We currently have little information on how low levels of artificial light emanating from urban skyglow affect plants and their interactions with herbivores. We examined how short-term, low levels of ALAN affect grass and insects, including growth rate, photosynthesis, and stomatal conductance in grass, and foraging behavior and survival in crickets. We compared growth and leaf-level gas exchange of Kentucky Bluegrass (*Poa pratensis*) under low-levels of ALAN (0.3 lux) and starlight conditions (0.001 lux). Furthermore, each light treatment was divided into treatments with and without house crickets (*Acheta domesticus*). Without crickets present, bluegrass grown under ALAN for three weeks grew taller than plants grown under natural night light levels. In the fourth week when crickets were introduced, grass height decreased resulting in no measurable effects of light treatment. There were no measurable differences in grass physiology among treatments. Our results indicate that low levels of light resulting from skyglow affect plant growth initially. However, with herbivory, the effects of ALAN on grass may be inconsequential. Gaining an understanding of how ALAN affects plant-insect interactions is critical to predicting the ecological and evolutionary consequences of anthropogenic light pollution.

Keywords: photosynthesis, urban light, growth rate, crickets (Gryllidae), photobiology

INTRODUCTION

Artificial light at night (ALAN) is an anthropogenic pollutant that is increasing spatially by a rate of 2.2% per year (Kyba et al., 2017). Direct ALAN sources, such as streetlights, can lead to skyglow: the atmospheric scattered light that can propagate up to several hundred kilometers into the environment (Luginbuhl et al., 2009; Aubé, 2015). Skyglow results in light encroaching into natural areas where direct sources of light pollution are not present (Gaston et al., 2015; Garrett et al., 2020).

The study of ALAN as an anthropogenic pollutant is a relatively young field (Longcore and Rich, 2004; Seymoure, 2018; Dominoni et al., 2020; Sanders et al., 2021), with most studies conducted at relatively high levels of nocturnal light pollution (e.g., 10–100 lux) (Gaston et al., 2013) but see Alaasam et al. (2018); Sanders and Gaston (2018). For reference, a full moon could create ambient light levels of 0.3 lux on its brightest nights (Biberman et al., 1966; Kyba et al., 2017). These high light levels are representative of organisms functioning under direct light pollution, such as directly beneath a streetlight, whereas most urban environments exist at lower light levels due to skyglow (e.g., 0.1–1.0 lux), which can impact environments several hundred kilometers away from a direct light source (Gaston et al., 2013; Dominoni et al., 2014; Seymoure et al., 2019a). Therefore, examining the impacts of light pollution at high intensities, although informative, is not representative of artificial light conditions in urban habitats at night. It remains an open question as to whether low levels of skyglow illumination (0.001–0.3 lux) affects communities to the same extent as direct illumination.

The intensity and spectral composition of light depends upon the phase of the moon, season, and weather, all of which create necessary cues for organisms (Kyba et al., 2015; Spitschan et al., 2016; Seymoure et al., 2019b). Plants use light as a cue for almost every physiological process including, but not limited to, seedling development, photosynthesis, growth, and budding (Briggs and Christie, 2002; Takemiya et al., 2005; Bennie et al., 2016; Gaston et al., 2017; Singhal et al., 2018). In addition to powering the electron transport chain in thylakoid membranes, light intensity and direction impacts photosynthetic efficiency through phototropism (i.e., the movement of the plant toward sunlight; (Celaya and Liscum, 2005), chloroplast movements (Wada et al., 2003), and light-induced stomatal opening to optimize water-use efficiency (Dietrich et al., 2001). Periods of darkness are also important for plant metabolic processes, particularly stress recovery, including recovery from herbivory events (McNaughton, 1983; Singhal et al., 2018).

Increased levels of ALAN from urbanization are changing natural light regimes by increasing the intensity and duration of light available at night (Davies et al., 2013; Seymoure et al., 2019a; Buxton et al., 2020), potentially altering plant-herbivore interactions. For example, by masking natural night light levels, ALAN can mislead herbivores to be more active at night and disrupt plant-herbivore interactions and critical dark recovery periods for plants (Dominoni et al., 2020). Plants in light polluted environments may experience changes in pollination, photoreceptor signaling, phenology and flowering (Ffrench-Constant et al., 2016; Singhal et al., 2018), which can have ecological consequences for food web dynamics (Polis et al., 2004). However, little is known about how constant illumination at the level of urban light alters plant-insect interactions. ALAN has led to declines in population sizes of a diversity of insect species through its interference with insect development, movement, foraging, and reproductive success, which can alter trophic systems (Owens and Lewis, 2018; Owens et al., 2020).

Here we test whether short-term exposure to ALAN affects plant-insect interactions by modifying plant photobiology and growth rates. We exposed two common urban species—Kentucky bluegrass (*Poa pratensis*), a cool season common turfgrass (Weissman and Rentz, 1977; Read et al., 1999; Suplick-Ploense and Qian, 2005), and the house cricket (*Acheta domesticus*), a nocturnal herbivore—to starlight (0.001 lux) and realistic urban nighttime light levels (0.3 lux) (Dominoni et al., 2013; Alaasam et al., 2018; Seymoure et al., 2019a) in order to test the following hypotheses: (1) Low levels of ALAN affect plant physiology. We predicted that plants grown under urban light would have higher net photosynthesis and dark respiration, increased growth rates, and increased stomatal conductance compared to control plants grown under starlight conditions. (2) Herbivory interacts with ALAN to affect plant biomass. We predicted cricket herbivores would reduce the biomass and height of grass. However, as crickets are nocturnal foragers, we predicted they would consume less plant material under urban light than starlight conditions and have lower survival rates in urban light.

MATERIALS AND METHODS

Light Treatments

We used a CMP6050 growth chamber (BDR16, Version 4.06, Conviron, Winnipeg, Manitoba) set to a temperature of 22.2°C with light control to create artificial light environments (0.3 lux, hereafter "urban light") and natural new moon light environments (0.001 lux, hereafter "starlight") at night (Dominoni et al., 2013; Alaasam et al., 2018; Seymoure et al., 2019a; Jones et al., 2020). Daytime light levels were 135, 300 lux, which is similar to natural daytime lux levels. There were two different light types in the chamber—high pressure sodium and mercury vapor—placed in alternating positions on the ceiling of the chamber. This is standard for the CMP6050 growth chamber. These lights were stepped up and down to simulate dawn and dusk in the chamber. Standard LED lights of the chamber remained off to create more realistic and desired light levels. To create urban light levels within the chamber, we used 4 layers of filter gels over the light sources (Rosco E-Colour #211.9 Neutral Density Filter, Stamford, CT, United States) that attenuated 83% of light. To further attenuate light, 90% black shade cloth was placed over starlight treatments, and 22% white shade cloth was placed over urban light environments. Shade cloth and filter gels only effect the quantity of light, but not the quality of light in the chamber. These were constructed as square boxes and placed over the plant treatment groups using PVC pipe and shade cloth. We confirmed that light levels were approximately 0.3 lux and 0.001 lux using a highly sensitive spectroradiometer (StellarNet Silver Nova, Tampa Bay, FL, United States) with a cosine corrected irradiance probe affixed to a 1000-micron optical fiber (StellarNet, Tampa Bay, FL, United States). We checked irradiance measurements using SpectraWhiz software (StellarNet, Tampa Bay, FL, United States); due to the low light levels, we set integration time to approximately 20 s for the 0.3 lux measurements and 8 min for the 0.001 lux measurements. This confirmed that light levels throughout the enclosure were within one order of magnitude of the chosen light level for each treatment: 0.3 and 0.001 lux.

Experimental Design

On day 1, Kentucky bluegrass seeds were sown in 10 cm round pots ($n = 72$) containing Scotts Miracle-Gro soil and placed in the growth chamber under experimental light conditions. On day 21, we measured the tallest blade of grass, then weeded down the pots randomly, excluding the tallest blade of grass, until there were 25 shoots of grass remaining. Weeding to a standard number of shoots ensured that there were no differences in grass abundance among measurements prior to the experiment (Lemoine et al., 2018*)*. After the initial 21-day growth period, one randomly selected juvenile cricket, male or female, was placed in each of 36 designated cricket pots. Herbivory and light environments were examined using a 2 × 2 factorial design in which light treatment was factorially crossed with cricket treatment in a 28-day experiment. The four treatments were arranged in a block test pattern, as shown in **Figure 1**. Treatment groups included: (1) plants without crickets in urban light, (2) plants without crickets in starlight, (3) plants with crickets in urban light, and (4) plants with crickets in starlight ($n = 18$ per treatment). Nighttime lighting conditions were imposed in the middle of the day from the start of the experiment to ensure nighttime measurements could be taken during regular working hours. Lighting conditions were altered twice daily; we placed filter paper and shade cloth structures over the plants at 08:00 and removed them at 18:00 to create a 14:10 light:dark cycle typical of summer in the northern hemisphere. Blocks were rotated daily one position clockwise to account for spatial variation in light levels within the chamber, and generously watered at this time. Drierite (W.A. Hammond 23005, Xenia, OH, United States) was placed in two trays on opposite sides of the chamber to control humidity and prevent mold growth (Hammond, 1935).

Crickets were sourced as juveniles from a stock population from Premium Crickets (Winder, Georgia) in December 2018 as juveniles at a mean size of 1.9 cm, before the adult phase. From day 21 to 28, cricket survival was monitored daily (i.e., when light conditions were shifted) and categorized as alive or dead. All crickets were juveniles from day 21 to day 28 and thus we only report survival of juveniles. If a cricket was found dead, the cricket and its designated plant were removed from the experiment. Upon removal, we measured the height of the tallest blade of grass and recorded the length of time the plant/cricket spent in the chamber. We also cut and weighed aboveground biomass to determine wet and dry mass. On day 28, we removed all remaining plants from the experiment and recorded the final height of the tallest blade of grass. We calculated the average daily growth rate in week four (day 21 to day 28) to control for plants that were removed prematurely due to cricket death.

Gas Exchange Measurements

To assess the effect of light treatment on bluegrass physiology independent of herbivory, we measured leaf photosynthetic responses on day 19 before crickets were placed into pots. We measured leaf gas exchange in each light treatment using a LI-6400XT infrared gas analyzer with a leaf chamber fluorometer attached (Li-Cor Biosciences; Lincoln, NE, United States) following previously published methods with slight modifications (Lemoine et al., 2018). Plants were removed from the growth chamber temporarily for gas exchange measurements. The environmental conditions inside the leaf chamber were standardized across measurements; leaf temperature was maintained at 20°C, relative humidity was maintained between 40 and 50%, sample chamber flow rate was set to 200 μmol m^2 s^{-1}, and reference chamber CO_2 concentration was set to 400 ppm. Low flow settings are commonly used for small leaved grasses with low photosynthetic rates (Taylor, 2014). Leaf level gas exchange was measured under two light conditions: dark and low light (10 μmol m^2 s^{-1} (740 lux) photosynthetically active radiation; PAR). Gas exchange in the dark provides an estimation of leaf respiration. The low light level was the minimum amount of light provided by the Li-6400 light source;

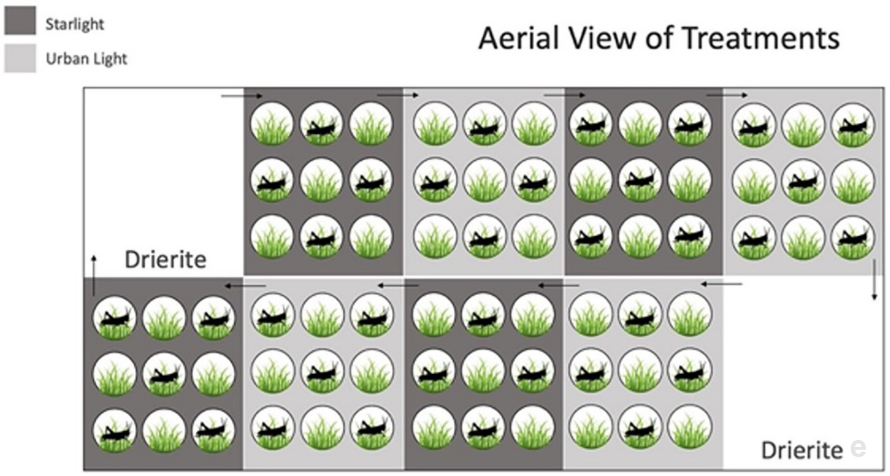

FIGURE 1 | Aerial view of treatment groups in the growth chamber after crickets were introduced (day 21–28). The treatment groups were arranged in a block test pattern with 4 blocks of urban light treatments and 4 blocks of starlight treatments, totaling 8 groups. Within each block, nine plants (every other one) had a cricket.

thus, we were unable to measure photosynthesis under the tested ALAN conditions imposed here (<10 umols, <740 lux), but instead measured whether treatments had an impact on plant photosynthetic responses to low levels of light. A newly emerged and fully expanded leaf from each individual (*n* = 10 individuals per treatment) was inserted into the leaf chamber. Prior to measurements, leaves were dark adapted for 2 h under a dark box that allowed no light to enter. Leaves were left in the chamber for 2–5 min to equilibrate to chamber conditions before gas exchange parameters (photosynthesis or respiration, and stomatal conductance) were recorded (average of three logged values taken in rapid succession). Steady-state fluorescence (Fs) was measured continuously before exposing plants to a saturating pulse of light (2,750 μmol m^{-2} s^{-1} of blue light or ~203,500 lux) (Thimijan and Heins, 1983) to measure maximum chlorophyll fluorescence. Light inside the chamber was then switched to the low light level (10 μmol m^2 s^{-1}). Once gas exchange reached stability, net photosynthetic rate and stomatal conductance were recorded, and a saturating pulse was applied to estimate photosystem II efficiency (ΦPSII): ΦPSII = (Fm′ − Fs)/Fm’ where Fm’ represents chlorophyll fluorescence under low light. As grass blades rarely filled the entire chamber, the measured leaf area was estimated using width and length, and photosynthetic parameters, which are based on the area of the chamber (6 cm^2), were adjusted accordingly.

Data Analysis

All statistical analyses were performed in R version 3.4.3 (R Development Core Team, 1999). We first confirmed that our data were normally distributed to enable the use of parametric tests. To test our first hypothesis that gas exchange increased under ALAN, we ran a MANOVA with net photosynthetic rate, stomatal conductance, dark respiration, and ΦPSII as response variables and with light treatment and block as explanatory variables (**Figure 2**). For our second hypothesis that light and cricket treatments would affect plant height, we modeled daily percent change in height between day 21 and day 28 using a two-way ANOVA with light treatment, cricket treatment, and block as explanatory variables (**Figure 3**). We then analyzed the data using two-way ANOVA, again with light treatment, cricket treatment, and block as explanatory variables, testing for an interaction between light treatment and cricket treatment. We also analyzed cricket survival using Kaplan-Meier analysis with the “survival” package in R (**Figure 4**) (Therneau and Lumley, 2009).

RESULTS

There was no difference in net photosynthesis, stomatal conductance, dark respiration, or ΦPSII between grass grown in the two light treatments (**Table 1**). On day 21, bluegrass grown in urban light was taller (mean = 5.35, sd = 1.02) than bluegrass grown in starlight (mean = 4.79, sd = 0.63, **Table 2**). However, the daily percent change in plant height from day 21 to day 28 was not significantly different between treatments (**Table 3**). The presence of crickets did affect plant height, whereby bluegrass with crickets present were shorter than bluegrass without crickets (**Table 3**).

Crickets in the urban light treatment had a 25.0% probability of survival, whereas crickets in the starlight treatment had a survival probability of 32.1%, but this difference was not statistically significant (Kaplan-Meier: *n* = 36, *p* = 0.37, **Figure 4**). There was no difference in survival due to sex (Kaplan-Meier: *n* = 36, *p* = 0.80, **Figure 4**).

DISCUSSION

Our study explored how short-term low levels of artificial light at night may affect immediate responses in plant photobiology and herbivore interactions. It is important to note that this study represents a brief novel environment akin to new lights being installed in an environment, and not long-term exposure. Contrary to our predictions, grass grown under low-level urban light conditions after 19 days did not have higher net photosynthetic rates than those grown under starlight, nor did stomatal conductance, dark respiration, or ΦPSII differ significantly between light treatments. However, plants under urban light conditions grew taller than plants grown under starlight conditions during the initial 21 days of growth before crickets were introduced. Additionally, we found no evidence that crickets under urban light consumed more plant matter than crickets in starlight treatments, and survival rates of crickets did not differ between treatments. The results from this study suggest that short-term exposure to low levels of ALAN may not have significant effects on grass photobiology but may affect plant height.

Studies investigating grass responses to higher levels but similar durations of ALAN illumination (e.g., 4 ± 1 μmol m^{-2} s^{-1} or 296 lux) found that plant photoreceptors were sensitive to small fluxes in light levels, which can change flowering phenology (Thimijan and Heins, 1983; Shin et al., 2010; Bennie et al., 2016). Many flowering plants require dark photoperiod signals to induce flowering (Bennie et al., 2016) such that light pulses, even one minute long, are enough to change their phenology (Parker et al., 1952; Singhal et al., 2018). The lower levels of light tested here were likely not bright enough to induce these changes in bluegrass and may have allowed bluegrass to properly detect photoperiod. Furthermore, plants often use nighttime darkness to repair damage from UV rays, suggesting the low levels of ALAN in our treatments may be dark enough for plants to continue to repair damaged cells and photoreceptors (Singhal et al., 2018). Moreover, net photosynthesis is a dynamic measurement that can vary within samples due to time and day (Miller et al., 1996) and our single measurement at the end of week 3 may not have captured treatment differences occurring at other times.

We found no difference in stomatal conductance or respiration between plants grown in urban light and starlight. Other studies of similar 4-6 week duration did note differences in stomatal density and stomatal opening and closing in the presence of ALAN at levels from 0.1 μmol m^{-2} s^{-1} to 1 μmol m^{-2} s^{-1} of blue and red light (Takemiya et al., 2005; Shimazaki et al., 2007). Another study found that yellow-poplar trees

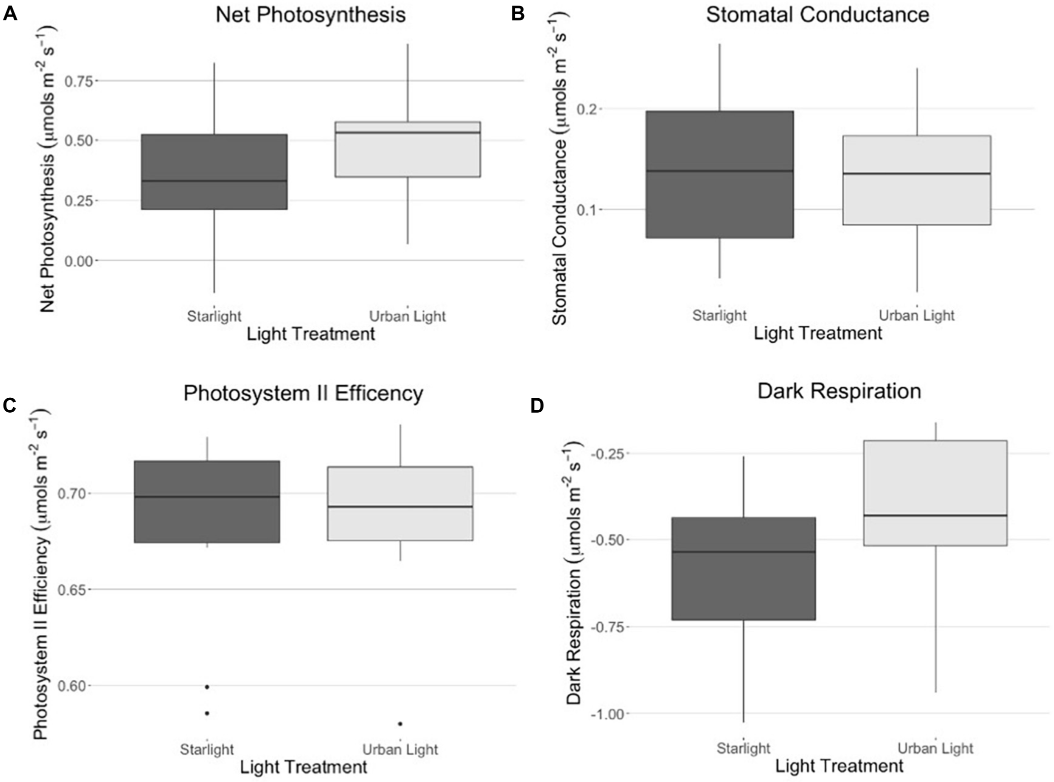

FIGURE 2 | (A) Net photosynthesis across light treatments, measured under low light conditions (10 µmols m^{-2} s^{-1} of light) and **(B)** stomatal conductance across light treatments. **(C)** Photosystem II efficiency is measured using a saturating pulse (ΦPSII): ΦPSII = (Fm' - Fs)/Fm' where Fm is chlorophyll fluorescence under low light. **(D)** Dark respiration measured in complete darkness. There were no differences in net photosynthesis, stomatal conductance, photosystem II efficiency, or dark respiration between light treatments.

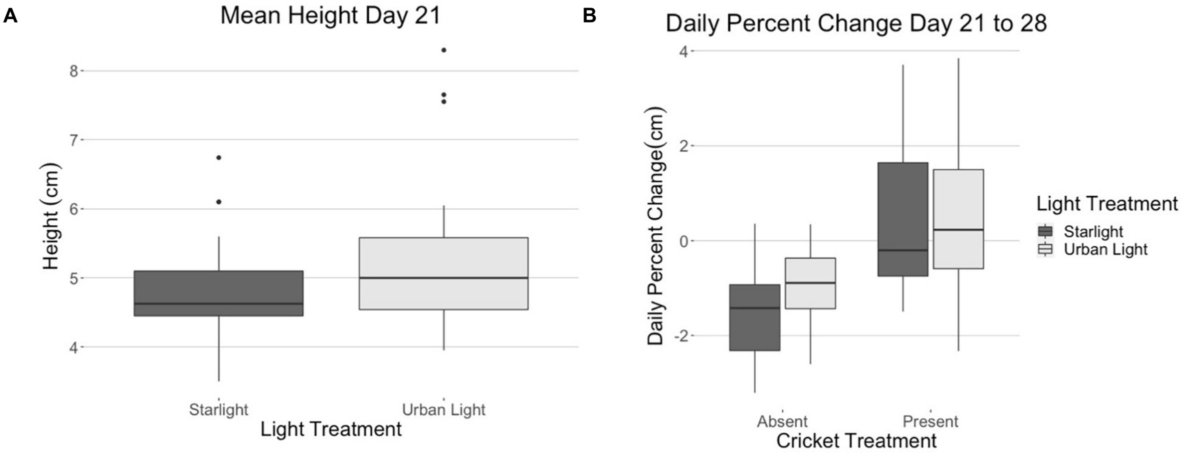

FIGURE 3 | (A) Bluegrass height at day 21 separated by light treatment when no crickets were present. Grass in urban light was taller than grass in starlight conditions. **(B)** Daily percent change in height of grass (change from day 21 to day 28 divided by the number of days in the chamber) separated by light treatment. There was no difference in daily percent change across light or cricket treatments.

exposed to ALAN (high pressure sodium lighting ranging from 82 lux to 4100 lux) for three years resulted in reduced nighttime stomatal conductance (Kwak et al., 2018). Given that we did not find any effects of ALAN on plant gas exchange, it is possible that our light levels were too low, or grass was not subjected to our light levels for a long enough duration, to induce such responses. Reduced chlorophyll and rubisco concentration has been observed in phytoplankton grown under low light levels (6.6

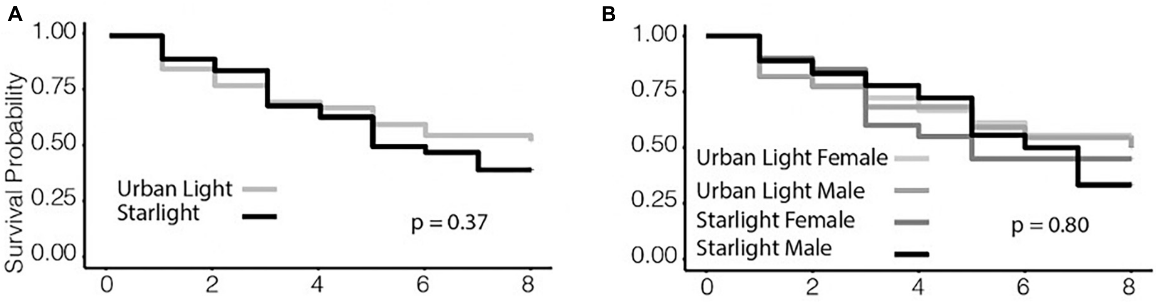

FIGURE 4 | Survival probability of crickets. **(A)** Survival probability of crickets under urban light and starlight treatments. **(B)** Survival probability of crickets under urban light and starlight treatments, split by sex in each treatment group. In both comparisons **(A,B)**, there were no differences in survival.

lux; Poulin et al., 2014), and light as low as 3.5 lux has induced flowering in tree species across the United Kingdom (Ffrench-Constant et al., 2016). We also observed no treatment effects on photosystem II efficiency despite other studies noting adverse reactions in these physiological responses to light pollution (Zhang and Reisner, 2019; Meravi and Prajapati, 2020). Kentucky Bluegrass might be more adaptable to changing light regimes given that it is commonly used as a turf grass selected for its resilience to drought and heat stress (Wang and Huang, 2004). We observed a faster growth rate for grasses grown under urban light conditions compared to starlight conditions before the introduction of an herbivore. Plant growth rate is determined by a variety of factors, including, but not limited to, photosynthetic rate, specific leaf area, leaf lifespan, leaf mass fraction, and nitrogen absorption rate (Campbell and Grime, 1989; Poorter et al., 1991; Osone et al., 2008). Although we found no difference in net photosynthetic rate between treatments, growth rate differences could have been due to greater allocation to leaf area in urban light (Poorter and Remkes, 1990) although we did not measure such attributes. Further, our ALAN levels of 0.3 lux, though extremely bright, still fall within the range of the natural lunar cycle, occurring during rare, very clear nights with full moons (Gaston et al., 2013); thus, bluegrass may have been well suited to handle the ALAN levels tested.

ALAN is known to alter photoperiod detection in multiple organisms (Bennie et al., 2016), and these changes in photoperiod can impact plant growth and flowering (Cathey and Campbell, 1975; Blanchard and Runkle, 2010; Basler and Körner, 2012; Craig and Runkle, 2016). Increased growth and biomass have been noted in *Poaceae* species when exposed to high levels of ALAN ranging from 0.349 to 1.145 μmols m^2 sec^{-1} from metal halide bulbs (Flowers and Gibson, 2018), which corresponds to approximately 24.78–81.30 lux (Thimijan and Heins, 1983). However, after introduction of the herbivore, we observed no physiological responses in Kentucky Bluegrass, including no change in biomass. Photoperiod detection may not have been disrupted at our lower levels of ALAN, or it may have caused undetectable or non-measured physiological responses.

While animals rely on plants as a food source and shelter, we found no evidence that short term, low-level light pollution would impact these typical interactions between plants and insects. Artificial light at the level of 0.3 lux was not enough to induce changes in the amount of plant matter consumed by crickets or their survival, but light pollution at higher levels for longer periods of time could modify these interactions

TABLE 1 | MANOVA table of the gas exchange results evaluating differences in photosynthesis, stomatal conductance in dark, stomatal conductance in light, fluorescence, and photosystem II efficiency (Urban light, *n* = 11; starlight, *n* = 11).

	df	Pillai	*f*	*p*
Treatment	1	0.18	0.45	0.83
Block	3	0.95	1.09	0.40
Residuals	17			

TABLE 2 | ANOVA table comparing mean grass height at day 21 across light treatments and blocks.

	Sum of squares	*df*	Mean square	F	*p*
Light treatment	3.50	1	3.50	5.63	0.021*
Block	7.87	6	1.31	2.11	0.064
Residuals	39.8	64	0.622		

**Indicates a significant response (Light treatment, urban light n = 36, starlight n = 36).*

TABLE 3 | ANOVA table showing the effects of light treatment, cricket treatment, and block (plus interactions between light and cricket treatment and cricket and block treatment) on daily percent change in grass height between day 21 and the end of the experiment.

	Sum of squares	*df*	Mean square	F	*p*
Light treatment	0.14	1	0.14	1.60	0.21
Cricket treatment	2.82	1	2.82	32.04	5.3×10^{-7}*
Block	0.85	6	0.14	1.62	0.16
Light: Cricket	0.002	1	0.002	0.023	0.88
Cricket: Block	0.90	6	0.15	1.70	0.14
Residuals	4.93	56	0.088		

**Indicates a significant response (Light treatment, urban light n = 36, starlight n = 36; Cricket treatment, present n = 36, absent, n = 36).*

(Gaston et al., 2013; Macgregor et al., 2015; Bennie et al., 2016; Knop et al., 2017).

Overall, our research detected few changes to plant physiology under short-term exposure to low levels of urban light, suggesting that low levels of ALAN may not be as harmful to community interactions as predicted, at least initially. With rapid increase in human development, new lights are being introduced to unlit environments. Our experimental conditions may be representative of environments recently exposed to ALAN, such as a new housing development or newly urbanized areas. Other studies conducted at high levels of ALAN suggest artificial light can induce large changes in physiology and community interactions (Longcore and Rich, 2004; Gaston et al., 2013; Seymoure et al., 2019a). There may be a threshold level and length of exposure at which artificial light becomes harmful, causing detrimental effects to individual and ecosystem function with additional increases in intensity and duration. Understanding and identifying this threshold would allow for more effective management of night skies and natural light conditions (Dominoni et al., 2020). With estimates suggesting two thirds of Key Biodiversity Areas experience ALAN (Seymoure et al., 2019a; Garrett et al., 2020), it is important to identify the level at which artificial light becomes harmful and how natural night skies can be managed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MC, CB, NL, and BS conceived and designed the experiment. MC, CB, LA, and BS received funding for the study. MC and CB ran the study and collected data. MC, CB, and RG-N measured plant physiology. MC, CB, RG-N, NL, and BS analyzed the data. MC and CB wrote the manuscript with revisions from RG-N, LA, NL, and BS. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported through a Zoological Lighting Institute Grants-In-Aid of Research grant awarded to MC and CB. MC was awarded a SEEDS grant to present this research at the 2019 meeting of the Ecological Society of America (ESA) where we received excellent feedback from the ESA community. Furthermore, this work was supported through the Colorado State University Honors Program. NPS NSNSD contributed fundings to cover publication costs.

ACKNOWLEDGMENTS

We are grateful for support from the Smith Lab, the Sound and Light Ecology Team at Colorado State University, and the Natural Sounds and Night Skies Division of the National Park Service. Jeremy White, Tammy Brenner, and Bob Meadows were foundational to the success of this study.

REFERENCES

Alaasam, V. J., Duncan, R., Casagrande, S., Davies, S., Sidher, A., Seymoure, B., et al. (2018). Light at night disrupts nocturnal rest and elevates glucocorticoids at cool color temperatures. *J. Exp. Zool. Ecol. Integr. Physiol.* 329, 465–472. doi: 10.1002/jez.2168

Aubé, M. (2015). Physical behaviour of anthropogenic light propagation into the nocturnal environment. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370:20140117. doi: 10.1098/rstb.2014.0117

Basler, D., and Körner, C. (2012). Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agric. For. Meteorol.* 165, 73–81. doi: 10.1016/j.agrformet.2012.06.001

Bennie, J., Davies, T. W., Cruse, D., and Gaston, K. J. (2016). Ecological effects of artificial light at night on wild plants. *J. Ecol.* 104, 611–620. doi: 10.1111/1365-2745.12551

Biberman, L. M., Dunkelman, L., Fickett, M. L., and Finke, R. G. (1966). *Levels of Nocturnal Illumination*. Alexandria: Institute for Defense Analyses.

Blanchard, M. G., and Runkle, E. S. (2010). Intermittent light from a rotating high-pressure sodium lamp promotes flowering of long-day plants. *HortScience* 45, 236–241. doi: 10.21273/hortsci.45.2.236

Briggs, W. R., and Christie, J. M. (2002). Phototropins 1 and 2: versatile plant blue-light receptors. *Trends Plant Sci.* 7, 204–210. doi: 10.1016/s1360-1385(02)02245-8

Buxton, R. T., Seymoure, B. M., White, J., Angeloni, L. M., Crooks, K. R., Fristrup, K., et al. (2020). The relationship between anthropogenic light and noise in U.S. national parks. *Landsc. Ecol.* 35, 1371–1384. doi: 10.1007/s10980-020-01020-w

Campbell, B. D. and Grime, J. P. (1989). A comparative study of plant responsiveness to the duration of episodes of mineral nutrient enrichment. *New Phytologist* 112, 261–267. doi: 10.1111/j.1469-8137.1989.tb02382.x

Cathey, H. M., and Campbell, L. E. (1975). Security lighting and its impact on the landscape. *J. Arboricult.* 1975, 181–187.

Celaya, R. B., and Liscum, E. (2005). Phototropins and associated signaling: providing the power of movement in higher plants. *Photochem. Photobiol.* 81, 73–80. doi: 10.1111/j.1751-1097.2005.tb01524.x

Craig, D. S., and Runkle, E. S. (2016). An intermediate phytochrome photoequilibria from night-interruption lighting optimally promotes flowering of several long-day plants. *Environ. Exp. Bot.* 121, 132–138. doi: 10.1016/j.envexpbot.2015.04.004

Davies, T. W., Bennie, J., Inger, R., and Gaston, K. J. (2013). Artificial light alters natural regimes of night-time sky brightness. *Sci. Rep.* 3:1722.

Dietrich, P., Sanders, D., and Hedrich, R. (2001). The role of ion channels in light-dependent stomatal opening. *J. Exp. Bot.* 52, 1959–1967. doi: 10.1093/jexbot/52.363.1959

Dominoni, D., Quetting, M., and Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proc. Biol. Sci.* 280:20123017. doi: 10.1098/rspb.2012.3017

Dominoni, D. M., Carmona-Wagner, E. O., Hofmann, M., Kranstauber, B., and Partecke, J. (2014). Individual-based measurements of light intensity provide new insights into the effects of artificial light at night on daily rhythms of urban-dwelling songbirds. *J. Anim. Ecol.* 83, 681–692. doi: 10.1111/1365-2656.12150

Dominoni, D. M., Halfwerk, W., Baird, E., Buxton, R. T., Fernández-Juricic, E., Fristrup, K. M., et al. (2020). Why conservation biology can benefit from sensory ecology. *Nat. Ecol. Evol.* 4, 502–511. doi: 10.1038/s41559-020-1135-4

Ffrench-Constant, R. H., Somers-Yeates, R., Bennie, J., Economou, T., Hodgson, D., Spalding, A., et al. (2016). Light pollution is associated with earlier tree budburst across the United Kingdom. *Proc. Biol. Sci.* 283:20160813. doi: 10.1098/rspb.2016.0813

Flowers, N. D., and Gibson, D. J. (2018). Quantified effects of artificial versus natural nighttime lighting on the Eurasian grasses: *Bothriochloa bladhii* (Poaceae) and *Bothriochloa ischaemum* (Poaceae) and the North American grasses: *Panicum virgatum* (Poaceae) and *Sorghastrum nutans* (Poaceae). *J. Torrey Bot. Soc.* 145, 147–155. doi: 10.3159/torrey-d-17-00038.1

Garrett, J. K., Donald, P. F., and Gaston, K. J. (2020). Skyglow extends into the world's Key biodiversity areas. *Anim. Conserv.* 23, 153–159. doi: 10.1111/acv.12480

Gaston, K. J., Bennie, J., Davies, T. W., and Hopkins, J. (2013). The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev. Camb. Philos. Soc.* 88, 912–927. doi: 10.1111/brv.12036

Gaston, K. J., Davies, T. W., Nedelec, S. L., and Holt, L. A. (2017). Impacts of artificial light at night on biological timings. *Annu. Rev. Ecol. Evol. Syst.* 48, 49–68. doi: 10.1146/annurev-ecolsys-110316-022745

Gaston, K. J., Duffy, J. P., and Bennie, J. (2015). Quantifying the erosion of natural darkness in the global protected area system. *Conserv. Biol.* 29, 1132–1141. doi: 10.1111/cobi.12462

Hammond, W. A. (1935). Use and regeneration of drierite. *J. Chem. Educ.* 12:445. doi: 10.1021/ed012p445

Jones, B. M., Seymoure, B. M., Comi, T. J., and Loew, E. R. (2020). Species and sex differences in eye morphometry and visual responsivity of two crepuscular sweat bee species (*Megalopta* spp., Hymenoptera: Halictidae). *Biol. J. Linn. Soc. Lond.* 130, 533–544. doi: 10.1093/biolinnean/blaa064

Knop, E., Zoller, L., Ryser, R., Gerpe, C., Hörler, M., and Fontaine, C. (2017). Artificial light at night as a new threat to pollination. *Nature* 548, 206–209. doi: 10.1038/nature23288

Kwak, M. J., Je, S. M., Cheng, H. C., Seo, S. M., Park, J. H., Baek, S. G., et al. (2018). Night light-adaptation strategies for photosynthetic apparatus in yellow-poplar (*Liriodendron tulipifera* L.) exposed to artificial night lighting. *For. Trees Livelihoods* 9:74. doi: 10.3390/f9020074

Kyba, C. C. M., Kuester, T., Sánchez de Miguel, A., Baugh, K., Jechow, A., Hölker, F., et al. (2017). Artificially lit surface of earth at night increasing in radiance and extent. *Sci. Adv.* 3:e1701528. doi: 10.1126/sciadv.1701528

Kyba, C. C. M., Tong, K. P., Bennie, J., Birriel, I., Birriel, J. J., Cool, A., et al. (2015). Worldwide variations in artificial skyglow. *Sci. Rep.* 5:8409.

Lemoine, N. P., Griffin-Nolan, R. J., Lock, A. D., and Knapp, A. K. (2018). Drought timing, not previous drought exposure, determines sensitivity of two shortgrass species to water stress. *Oecologia* 188, 965–975. doi: 10.1007/s00442-018-4265-5

Longcore, T., and Rich, C. (2004). Ecological light pollution. *Front. Ecol. Environ.* 2, 191–198.

Luginbuhl, C. B., Duriscoe, D. M., Moore, C. W., Richman, A., Wesley Lockwood, G., and Davis, D. R. (2009). From the ground Up II: sky glow and near-ground artificial light propagation in Flagstaff, Arizona. *PASP* 121:204. doi: 10.1086/597626

Macgregor, C. J., Pocock, M. J. O., Fox, R., and Evans, D. M. (2015). Pollination by nocturnal Lepidoptera, and the effects of light pollution: a review. *Ecol. Entomol.* 40, 187–198. doi: 10.1111/een.12174

McNaughton, S. J. (1983). Compensatory plant growth as a response to herbivory. *Oikos* 40, 329–336. doi: 10.2307/3544305

Meravi, N., and Prajapati, S. K. (2020). Effect street light pollution on the photosynthetic efficiency of different plants. *Biol. Rhythm Res.* 51, 67–75. doi: 10.1080/09291016.2018.1518206

Miller, D. P., Howell, G. S., and Flore, J. A. (1996). A whole-plant, open, gas-exchange system for measuring net photosynthesis of potted woody plants. *HortScience* 31, 944–946. doi: 10.21273/hortsci.31.6.944

Osone, Y., Ishida, A., and Tateno, M. (2008). Correlation between relative growth rate and specific leaf area requires associations of specific leaf area with nitrogen absorption rate of roots. *New Phytol.* 179, 417–427. doi: 10.1111/j.1469-8137.2008.02476.x

Owens, A. C. S., Cochard, P., Durrant, J., Farnworth, B., Perkin, E. K., and Seymoure, B. (2020). Light pollution is a driver of insect declines. *Biol. Conserv.* 241:108259. doi: 10.1016/j.biocon.2019.108259

Owens, A. C. S., and Lewis, S. M. (2018). The impact of artificial light at night on nocturnal insects: A review and synthesis. *Ecol. Evol.* 8, 11337–11358. doi: 10.1002/ece3.4557

Parker, M. V., Hendricks, S. B., Borthwick, H. A., and Jenner, C. E. (1952). Photoperiodic responses of plants and animals. *Nature* 169, 242–243. doi: 10.1111/j.1469-8137.2008.02681.x

Polis, G. A., Power, M. E., and Huxel, G. R. (2004). *Food Webs at the Landscape Level*. Chicago: University of Chicago Press.

Poorter, H., and Remkes, C. (1990). Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia* 83, 553–559. doi: 10.1007/bf00317209

Poorter, H., van der Werf, A., Atkin, O. K., and Lambers, H. (1991). Respiratory energy requirements of roots vary with the potential growth rate of a plant species. *Physiol. Plantarum* 83, 469–475. doi: 10.1034/j.1399-3054.1991.830321.x

Poulin, C., Bruyant, F., and Laprise, M. H. (2014). The impact of light pollution on diel changes in the photophysiology of *Microcystis aeruginosa*. *J. Plankton Res.* 36, 286–291. doi: 10.1093/plankt/fbt088

R Development Core Team. (1999). *The R Reference Manual: Base Package. Network Theory*. Boston, MA.

Read, J. C., Reinert, J. A., Colbaugh, P. F., and Knoop, W. E. (1999). Registration of "Reveille" hybrid bluegrass. *Crop Sci.* 39:590. doi: 10.2135/cropsci1999.0011183x003900020059x

Sanders, D., Frago, E., Kehoe, R., Patterson, C., and Gaston, K. J. (2021). A meta-analysis of biological impacts of artificial light at night. *Nat. Ecol. Evol.* 5, 74–81. doi: 10.1038/s41559-020-01322-x

Sanders, D., and Gaston, K. J. (2018). How ecological communities respond to artificial light at night. *J. Exp. Zool. Ecol. Integr. Physiol.* 329, 394–400. doi: 10.1002/jez.2157

Seymoure, B. M, Buxton, R., White, J., Linares, C., Fristrup, K., Crooks, K., et al. (2019a). *Anthropogenic light disrupts natural light cycles in critical conservation areas*. [preprint]. doi: 10.2139/ssrn.3439670

Seymoure, B. M., Linares, C., and White, J. (2019b). Connecting spectral radiometry of anthropogenic light sources to the visual ecology of organisms. *J. Zool.* 308, 93–110. doi: 10.1111/jzo.12656

Seymoure, B. M. (2018). Enlightening butterfly conservation efforts: The importance of natural lighting for butterfly behavioral ecology and conservation. *Insects* 9:22. doi: 10.3390/insects9010022

Shimazaki, K.-I., Doi, M., Assmann, S. M., and Kinoshita, T. (2007). Light regulation of stomatal movement. *Annu. Rev. Plant Biol.* 58, 219–247. doi: 10.1146/annurev.arplant.57.032905.105434

Shin, J. H., Jung, H. H., and Kim, K. S. (2010). Night interruption using Light Emitting Diodes (LEDs) promotes flowering of *Cyclamen persicum* in winter cultivation. *Horticult. Environ. Biotechnol.* 51, 391–395.

Singhal, R. K., Kumar, M., and Bose, B. (2018). Ecophysiological responses of artificial night light pollution in plants. *Russ. J. Plant Physiol.* 66, 190–202. doi: 10.1134/s1021443719020134

Spitschan, M., Aguirre, G. K., Brainard, D. H., and Sweeney, A. M. (2016). Variation of outdoor illumination as a function of solar elevation and light pollution. *Sci. Rep.* 6:26756.

Suplick-Ploense, M. R., and Qian, Y. (2005). Evapotranspiration, rooting characteristics, and dehydration avoidance: Comparisons between hybrid bluegrass and Kentucky bluegrass. *Int. Turfgrass Soc. Res. J.* 10, 891–898.

Takemiya, A., Inoue, S.-I., Doi, M., Kinoshita, T., and Shimazaki, K.-I. (2005). Phototropins promote plant growth in response to blue light in low light environments. *Plant Cell* 17, 1120–1127. doi: 10.1105/tpc.104.030049

Taylor, J. R. (2014). A simple inquiry-based lab for teaching osmosis. *Am. Biol. Teach.* 76, 265–269. doi: 10.1525/abt.2014.76.4.9

Therneau, T., and Lumley, T. (2009). *Survival: Survival Analysis, Including Penalised Likelihood. R Package Version 2. Vienna: R Foundation for Statistical Computing.*

Thimijan, R. W., and Heins, R. D. (1983). Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. *HortScience* 18, 818–822.

Wada, M., Kagawa, T., and Sato, Y. (2003). Chloroplast movement. *Annu. Rev. Plant Biol.* 54, 455–468.

Wang, Z., and Huang, B. (2004). Physiological recovery of Kentucky bluegrass from simultaneous drought and heat stress. *Crop Sci.* 44, 1729–1736. doi: 10.2135/cropsci2004.1729

Weissman, D. B., and Rentz, D. C. F. (1977). Feral house crickets *Acheta domesticus* (L.)(Orthoptera: Gryllidae) in southern Calif. *Entomol. News* 88, 246–248.

Zhang, J. Z., and Reisner, E. (2019). Advancing photosystem II photoelectrochemistry for semi-artificial photosynthesis. *Nat. Rev. Chem.* 4, 6–21. doi: 10.1038/s41570-019-0149-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

SYSTEMATIC REVIEW
published: 30 November 2021
doi: 10.3389/fevo.2021.765950

A Systematic Review of Research Investigating the Combined Ecological Impact of Anthropogenic Noise and Artificial Light at Night

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Levels of anthropogenic noise and artificial light at night (ALAN) are rapidly rising on a global scale. Both sensory pollutants are well known to affect animal behavior and physiology, which can lead to substantial ecological impacts. Most studies on noise or light pollution to date have focused on single stressor impacts, studying both pollutants in isolation despite their high spatial and temporal co-occurrence. However, few studies have addressed their combined impact, known as multisensory pollution, with the specific aim to assess whether the interaction between noise and light pollution leads to predictable, additive effects, or less predictable, synergistic or antagonistic effects. We carried out a systematic review of research investigating multisensory pollution and found 28 studies that simultaneously assessed the impact of anthropogenic noise and ALAN on animal function (e.g., behavior, morphology or life-history), physiology (e.g., stress, oxidative, or immune status), or population demography (e.g., abundance or species richness). Only fifteen of these studies specifically tested for possible interactive effects when both sensory pollutants were combined. Four out of eight experimental studies revealed a significant interaction effect, in contrast to only three out seven observational studies. We discuss the benefits and limitations of experimental vs. observational studies addressing multisensory pollution and call for more specific testing of the diverse ways in which noise and light pollution can interact to affect wildlife.

Keywords: multisensory pollution, anthropogenic noise, emergent properties, synergism, antagonism, artificial light at night (ALAN)

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Specialty section:
This article was submitted to
Urban Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *27 August 2021*
Accepted: *15 October 2021*
Published: *30 November 2021*

Citation:
Halfwerk W and Jerem P (2021) A Systematic Review of Research Investigating the Combined Ecological Impact of Anthropogenic Noise and Artificial Light at Night. Front. Ecol. Evol. 9:765950. doi: 10.3389/fevo.2021.765950

INTRODUCTION

The natural world is under threat due to a multitude of anthropogenic disturbances, including habitat destruction, climate change, pollution, and urbanization (Vitousek et al., 1997). Many of these human-induced environmental stressors covary in space and time making their combined impact difficult to predict (Crain et al., 2008; Piggott et al., 2015; Orr et al., 2020; Tekin et al., 2020). Artificial light at night (ALAN) and anthropogenic noise are two environmental stressors associated with urbanization, transport and industry, and are well known to influence biological processes ranging from individual physiology, reproduction and survival, to large scale processes occurring across whole ecosystems (Halfwerk and Slabbekoorn, 2015; Swaddle et al., 2015; Dominoni D.M. et al., 2020; Svechkina et al., 2020; Jerem and Mathews, 2021). High levels of anthropogenic noise and ALAN often co-occur at the same location and at the same time of day, depending on the latitude and time of year (Halfwerk and Slabbekoorn, 2015; Buxton et al., 2020; Dominoni D.M. et al., 2020). Despite this frequent covariance, the majority of studies have addressed their biological impact in isolation, ignoring potential interactive mechanisms (Piggott et al., 2015; Orr et al., 2020). Most reports therefore over- or underestimate effect sizes when these sensory pollutants are combined (Dominoni D. et al., 2020).

Our understanding of the combined impact of anthropogenic noise and ALAN can benefit from concepts and theory applied to situations involving other multiple stressors (Crain et al., 2008; Darling and Côté, 2008; Orr et al., 2020). Multi-stressor research has a long history in various fields of biology, ranging from eco-toxicological lab studies to global conservation modeling, and from aquatic to terrestrial study systems (Orr et al., 2020). In general, most of these studies acknowledge combined exposure to multiple stimuli or stressors can have functional, physiological or demographic impacts that cannot be understood when studying these stressors in isolation. The different research fields share little overlap in theory or nomenclature, however, which is why we adhere to the terms and definitions outlined by Piggott et al. (2015) and Orr et al. (2020) to study multi-stressor impacts. According to Orr et al. (2020), the simplest (and easiest to predict) effect of multisensory pollution is an additive effect, which occurs when the combined impact of noise and light pollution is similar to the sum of their parts (Orr et al., 2020). When the effect of anthropogenic noise is affected or modulated by exposure to light pollution and/or vice versa, we consider their combined impact to be interactive (**Figure 1**). The interactive impact can either result in less than the expected additive effect (antagonistic), or more than the expected effect (synergistic) (Orr et al., 2020). The most extreme form of interaction occurs where individual exposure to noise and light has no impact, but their combined exposure does—known as an emergent effect (Halfwerk and Slabbekoorn, 2015).

Researchers examining impacts of sensory pollutants have recently started paying greater attention to the combined and possible interactive effects of ALAN and anthropogenic noise (Casasole et al., 2017; McMahon et al., 2017; Raap et al., 2017; Dominoni D. et al., 2020; Wilson et al., 2021). To provide an overview of this work, we performed a systematic review with two specific aims. Firstly, to determine whether combined impacts deviate from additive expectations. And secondly, to evaluate which study systems and trait types are most commonly affected where any such deviations occur. We discuss whether our findings help identify conditions under which sensory pollutants should be studied in conjunction, or can be considered in isolation. Additionally, we highlight the most common pitfalls relating to observational and experimental methods and provide some thoughts on the design of future studies in this developing research field.

METHODS

Search Strategy

We used an "All Database" *Web of Science* search (Clarivate Analytics, 2021) to create our candidate study list. We first performed a search using the following terms (within which speech marks define phrases, and asterisks indicate truncation wildcards).

TS = ("anthropogenic noise*" OR "noise* pollution" OR "sound* pollution" OR "anthropogenic sound*") AND TS = ("light*" OR "light* pollution" OR "artificial light*" OR "artificial light at night" OR "ALAN").

Preliminary searches showed that including simple "noise*" and "sound*" terms returned an unworkably large number of articles for screening (∼30,000 when "noise*" was added, and ∼80,000 when both terms were included). Given the multiple potential meanings of both words, most of these articles would be beyond the scope of this review. Therefore, we only incorporated "noise" in our search term when included in phrases alongside the filtering words "anthropogenic" and "pollution." After screening all papers returned by our initial search (see below), we augmented our candidate study list with papers identified on *Web of Science* as citing articles from the initial search which met our screening criteria (and so likely focused on relevant topics). Our initial search and the subsequent identification of citing articles were carried out between 26 and 28th July, 2021.

Screening

We initially applied the inclusion criteria to all titles and abstracts. Full-texts were gathered when the title and abstract appeared to meet all criteria, or when there was insufficient information to form a judgment. The inclusion criteria were then re-applied to the full-texts to confirm eligibility. Our inclusion criteria were defined using a PICO framework (Frampton et al., 2017). Articles were included in our analyses if they presented data from primary research addressing the question "*What are the combined effects of anthropogenic noise and ALAN on non-human animals?*" We specified the PICO components for this question as: Population = non-human animals; Interventions = anthropogenic noise and ALAN; Comparators = absence and/or differing types/levels of anthropogenic noise and ALAN; Outcomes = functional, demographic or physiological effects. Accordingly, articles were considered eligible for inclusion if the research presented met each of the following criteria:

- Investigated populations of non-human animals
- Examined effects of both anthropogenic noise and ALAN
- Compared anthropogenic noise and ALAN with non-noise and -light controls, or different types/levels of anthropogenic noise and ALAN
- Assessed functional, demographic or physiological outcomes.

Data Extraction

For each included full-text, we first characterized experimental design as either observational or experimental. We then noted subject taxon, effect(s) and response traits assessed (categorized as physiological—e.g., effects on endocrine or immune systems, functional—e.g., behavior, morphology or life history, or demographic—e.g., abundance, population density, or spatial distribution). Additionally, we recorded whether statistically significant individual effects of ALAN and anthropogenic noise were observed, and whether any relationship was positive or negative. Finally, we assessed whether possible interactions

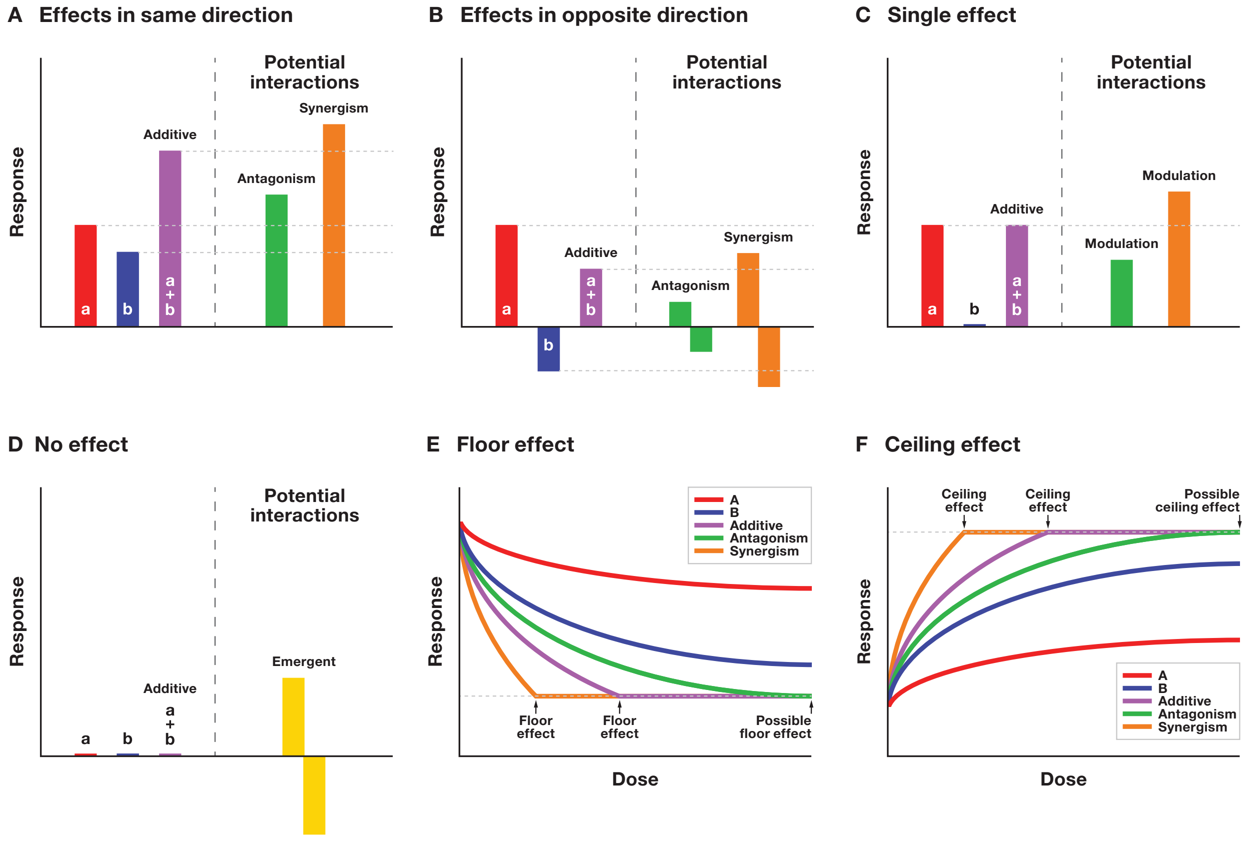

FIGURE 1 | Classification of different types of interactive effects of noise and light pollution. Various scenarios where noise and light pollution have either the same **(A)**, opposite **(B)**, no effect **(C,D)** or a dose-dependent effect **(E,F)** when exposed in isolation. The effect of noise and light can either add up (a + b) or interact and lead to a lower (antagonistic) or higher (synergistic) than expected effect when compared to the additive effect. **(A)** Scenario when the effect of noise and light pollution is in the same direction. **(B)** Scenario when noise and light have opposite effects. Antagonistic effects can be in either direction, as long as they are closer to zero compared to either one of the single effects or the additive effect. Synergistic effects should be either further away from zero compared to additive effects, or single effects, but not necessarily both. **(C)** Modulation scenario where one sensory pollutant has no effect on its own. Interactive effects are considered to be a modulation of the dominant pollutant by the other pollutant. The modulatory effect can either be lower (antagonistic) or higher (synergistic) than the additive effect, which is the same as the effect of the dominant pollutant. **(D)** Emergent scenario where both sensory pollutants have no effect on their own, whereas their combined exposure either has a positive or negative effect. **(E)** Illustration of the floor effect. Depending on the dose of pollutant **(A,B)**, their additive effect may reach a physical or energetic limit. Synergistic effects can only be assessed at low dosage levels, whereas at high dosages, antagonistic and additive effects may not be discernable. **(F)** Illustration of the ceiling effect.

between ALAN and noise were explicitly tested for, and if so, whether the interaction was statistically significant. We considered interaction testing to be explicit either when an interaction term was specified in statistical models, or where light, noise and combined light and noise groups were statistically compared with each other and a control group. We categorized interaction effects as significant when estimate 95% confidence intervals did not include zero. And, we classified significant interactive effects as either additive, antagonistic, synergistic or emergent according to the definitions set out in the Introduction and **Figure 1**, by comparing effect sizes in text, tables, or figures.

RESULTS

We identified 839 unique articles through our literature search, of which 28 met the inclusion criteria, and so were incorporated into our analyses (see **Supplementary Figure 1** for numbers of articles identified and screened at each stage).

Our analysis revealed that most multisensory pollution studies (21 out of 28) addressed effects of both noise and light pollution on birds (**Table 1**, **Figure 2A**, and **Supplementary Data Sheet**). In particular, on the timing of dawn singing (eight out of 21 studies), but also on a range of other functional, physiological and demographic traits, including breeding success, stress, immune and oxidative status, abundance, and species richness. Functional traits were studied almost twice as often as physiological and demographic traits combined (**Figure 2B**). Seven out of eight studies on dawn song were observational in nature and only one study explicitly tested whether noise and light exposure interacted. Across all study systems, more research reported observational data (20 out of 28 studies), than experimental (10 out of 28 studies). Only eight observational studies statistically tested for possible interactive effects of noise and light pollution (**Figure 2C**). Of these studies, four reported significant interactions. In contrast, eight out of ten experimental studies tested for interaction effects, with interactions detected in four instances. Two studies reported statistical interactions between noise and light without performing follow-up analyses to identify interaction effect type. For these studies we scored effect type from figures or effect sizes reported in tables or text (listed as "possible" effects in **Table 1**). The eight studies reporting interaction effects of noise and light (**Table 1**, also see Discussion for details) covered a range of characteristics, including physiological (e.g., haptoglobin and body temperature), functional (life history and behavior), and demographic (abundance) traits. Interactions detected included antagonistic, synergistic and emergent types, and one example of a floor effect. Of three experimental studies testing for but not reporting an interactive effect, two were carried out with groups of fish in the lab (**Table 1**).

DISCUSSION

We screened the literature for research that simultaneously assessed ecological impacts of noise and light pollution, to determine how frequently possible interactive impacts of both sensory pollutants were tested for. We found 15 of 28 studies that statistically tested for interactive effects of noise and light. Within this group, four out of eight observational studies and four out of eight experimental studies detected an interactive effect, which was either antagonistic, synergistic, or emergent.

Evidence of Interactive Effects

Six studies reported an antagonistic, or less-than expected effect of combined noise and light exposure, whereas four studies reported a synergistic, or more-than expected effect of noise and light pollution. Two studies found emergent effects, one observational, the other experimental. Ferraro et al. (2020) exposed free-living Western blue birds (S*ialia Mexicana)* to noise and light at their nests and tracked chick development. Chick fledging success, mass and size were influenced by noise and light exposure in different ways, suggesting emergent effects in some cases (**Supplementary Data Sheet**). For example, chicks grew shorter wing chords during combined exposure, but not during single exposure of noise and light treatments. However, emergent effects were not specifically tested for (Ferraro et al., 2020).

Some studies reported multiple types of interactive effects, depending on the trait that was measured, or the species that was studied (**Table 1**). Dominoni D. et al. (2020) scored overall activity of captive great tits and found light on its own increased activity at night, while noise on its own reduced activity during the day. When combined, the nighttime effect was enhanced, demonstrating a synergistic impact, whereas the daytime effect was reduced, revealing an antagonistic effect. Interestingly, these patterns were stronger in urban than forest birds. Wilson et al. (2021) related data from bird feeders throughout continental United States to modeled data on ALAN and anthropogenic noise. Using more than a million sightings from thousands of feeder stations they found an interactive effect of both pollutants on abundances for 50 out of 140 species. Noise and light had either an effect on abundances in the same or opposite direction (see also **Figure 1**), depending on bird species, and their combined effect was either antagonistic, synergistic, or emergent.

Finally, two experimental fish behavior studies suggested noise and light operate independently from each other (Shafiei-Sabet et al., 2016; Ginnaw et al., 2020). For example, fish actively avoided the loudspeaker and showed more freezing behavior during sound playback. Whereas, they spent more time in the upper water column in response to light treatment, but showed no avoidance or preference for the dark part of the tank (Shafiei-Sabet et al., 2016). Noise and light did not therefore interact. Although, any effect on individual behavior may have been overruled by group-level processes, as both studies focused on social fish during schooling formation.

Limitations and Potential Pitfalls Related to Study Design

Studies reporting antagonistic effects may suffer from ceiling or floor effects (**Figures 1E,F**), especially when working with numbers or percentages (Tekin et al., 2020). McMahon et al. (2017) for example, compared isolated effects of noise and light exposure to a control treatment and found a strong reduction in the number of parasitic midges approaching calling male frogs. The single effects were already so severe that their combined estimated additive effect should have resulted in a negative value for abundance. The reported significant interaction effect of noise and light might therefore result from statistical limitations, rather than a genuine underlying mechanism driving an antagonistic response. Likewise, an observational study on dawn song found birds started singing later than expected based on single effects of noise and light exposure (Nordt and Klenke, 2013), which could relate to energetic or physiological limits. In both cases, accounting for the dosages of noise and light might have provided insight into possible floor or ceiling effects.

Observational studies have the benefit of testing hypotheses under realistic field scenarios where sensory pollutants often vary in space and time in their intensity and composition, and covary with other important environmental variables, such as temperature, habitat structure and dietary composition. Potential pitfalls related to observational studies include: (i) insufficient statistical power to test for interactive effects; (ii) non-linear responses; (iii) biases toward single effect models; and (iv) unstandardized variables. Studies in which noise and light levels are correlated to other factors, such as food availability or temperature may require higher sample sizes to test for interactive effects, especially when these effects follow a non-linear function (e.g., when effects only start to occur after a certain threshold level is reached; **Figures 1F,G**). Furthermore, observational studies often use an information theoretic approach based on Aikaike values, as did all but one of the included observational studies that tested for interaction effects. Such methods penalize the number of terms added to candidate models, thus biasing single effect models over full models (especially for low sample sizes). And, environmental

TABLE 1 | Studies included in the systematic review of research investigating combined ecological impact of anthropogenic noise and artificial light at night.

Paper	Study type	Taxon	Effect assessed	Interaction tested for	Interaction significant	Interaction type
Willems et al. (2021)	Experimental	Mammal	Physiological + Functional	Yes	No	
Morelli et al. (2021)	Observational	Bird	Demographic	No		
Wilson et al. (2021)	Observational	Bird	Demographic	Yes	Yes	Abundance: mixed
Brunner et al. (2021)	Observational	Bird	Functional	No		
Issad et al. (2021)	Experimental	Mammal	Physiological + Functional	Yes	Yes	Body temperature rhythm: Robustness: Possibly synergistic Acrophase: Possibly synergistic
Marín-Gómez et al. (2020)	Observational	Bird	Demographic + Functional	No		
Dominoni D.M. et al. (2020)	Experimental	Bird	Functional	Yes	Yes	Total activity (all Birds)/Night-time activity (Urban Birds only): synergistic Daytime activity (Urban Birds only): antagonistic
Injaian et al. (2020)	Observational	Bird Reptile	Physiological	No		
Ferraro et al. (2020)	Experimental	Bird	Functional	Yes	Possibly	Nestling mass: possibly antagonistic Wing chord: possibly emergent Retrice: possibly emergent Tarsus length: possibly emergent
Senzaki et al. (2020)	Observational	Bird	Functional	No		
Ginnaw et al. (2020)	Experimental	Fish	Functional	Yes	No	
Sánchez-González et al. (2020)	Observational	Bird	Functional	No		
Stuart et al. (2019)	Observational	Bird	Functional	No		
Hennigar et al. (2019)	Experimental	Bird	Functional	Yes	Yes	Distance to disturbance (Swainson's thrush only): synergistic
Hanafi et al. (2019)	Observational	Bird	Functional	Yes	No	
Casasole et al. (2017)	Observational	Bird	Physiological	Yes	No	
McMahon et al. (2017)	Experimental + Observational	Invertebrate + Amphibian	Demographic	Yes	Yes	Midge abundance: antagonistic with floor effect
Raap et al. (2017)	Observational	Bird	Physiological	Yes	Yes	Haptoglobin: antagonistic
Ciach and Frohlich (2017)	Observational	Bird	Demographic	No		
Lee et al. (2017)	Observational	Bird	Demographic + Functional	No		
Dorado-Correa et al. (2016)	Observational	Bird	Functional	Yes	No	
Shafiei-Sabet et al. (2016)	Experimental	Fish	Functional	Yes	No	
Francis et al. (2015)	Observational	Mammal	Demographic + Functional	No		
Russ et al. (2015)	Observational	Bird	Physiological	Yes	No	
Da Silva et al. (2014)	Observational	Bird	Functional	No		
Nordt and Klenke (2013)	Observational	Bird	Functional	Yes	Yes	Dawn chorus onset: antagonistic
Arroyo-Solís et al. (2013)	Experimental + Observational	Bird	Functional	No		
Chan et al. (2010)	Experimental	Invertebrate	Functional	No		

factors are not always standardized and scaled. For example, only three of the eight included observational studies testing for interaction effects standardized/scaled environmental variables. Failure to standardize and scale potentially biases one factor over the other, which makes statistical testing for interaction terms difficult (Stuart et al., 2019).

Future observational studies should thus take environmental covariance into account, ideally carrying out some *a priori*

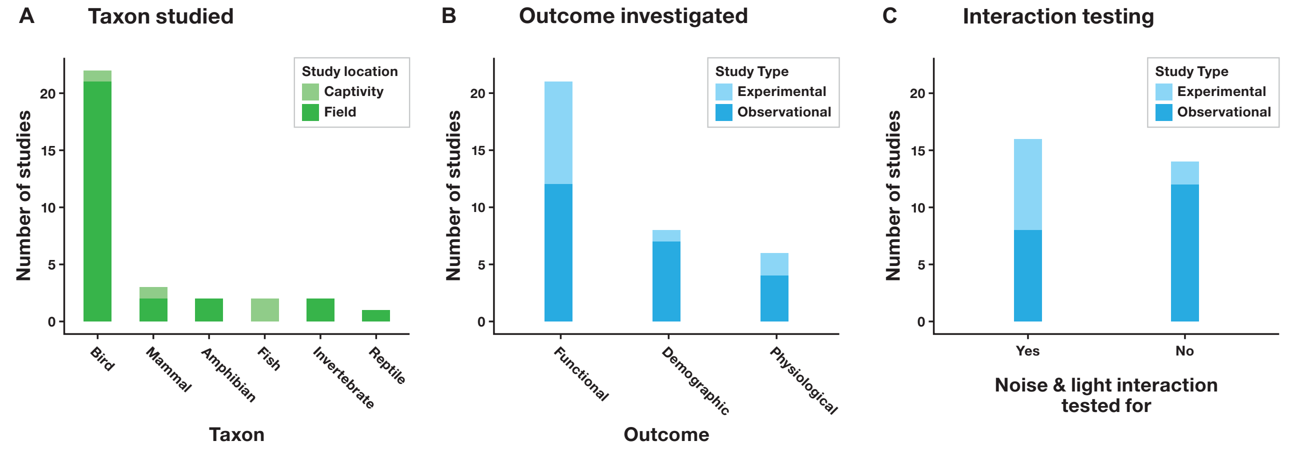

FIGURE 2 | (A) Taxa and **(B)** outcomes investigated in included studies, and **(C)** numbers of included studies where interaction effects were explicitly tested for.

modeling or power analyses using standardized variables to inform on the best sampling design and sample sizes required to test for the different type of interactive effects. Also, while information theoretic approaches can be informative, penalization of interactive terms should be taken into account in some way.

Experimental studies have the benefit of enabling randomized and balanced full-factorial designs, which control for any of the possible confounding effects that occur in observational studies. Experimental studies also allow testing for dose-dependent effects of single vs. combined pollutants, commonly used in eco-toxicological studies, but not in other disciplines. Notably, of all included experimental studies only one (McMahon et al., 2017) included more than a single dosage level. While experimental studies are often easier to carry out in the laboratory, most lab-based studies are limited in terms of how generalizable their findings are to field conditions, as any interactive impact can be context-dependent. For example, under optimal conditions such as *ad libitum* food, provided in many captive bird and rodent studies (including the two experimental captive avian/rodent studies identified in this review), mechanisms necessary for interactive multi-stressor effects may not be in place. Therefore, although experimental studies might be better suited to demonstrate interactive effects of multisensory pollutants when compared to observational studies, care must be taken with their interpretation. In that sense, field experiments—ideally carried out across multiple (breeding) seasons—seem to provide the best design for studying impacts of noise and light pollution under realistic ecological conditions, while controlling for confounding effects.

Developing Theory on Underlying Mechanisms of Multisensory Pollution

The newly emerged field of multisensory pollution is in dire need of theories regarding the underlying mechanisms to improve our predictions of combined effects of anthropogenic noise and ALAN. Such mechanisms appear likely to differ across levels of biological organization. At an organismal level, sensory pollutants can alter an individual's physiology and behavior through multiple perceptual mechanisms. Both noise and light can mask important signals and cues, distract animals from challenging cognitive tasks, or lead to misidentification of sensory pollutants as relevant natural signals or cues (Dominoni D.M. et al., 2020). Theory on multisensory pollution at the individual level could therefore usefully concentrate on situations where both pollutants influence the same perceptual mechanism and/or whether the same type of response is observed (e.g., both pollutants influencing hormone concentrations, or specific behavior such as dawn song).

At the community-level, biotic interactions and associated positive or negative feedback loops will strongly determine the outcome of single as well as combined effects of sensory pollutants. Species experiencing a direct negative impact of either high levels of noise or light (or both) on their behavior and/or physiology may simultaneously indirectly profit from impacts on their predators, prey or competitors. Blue tits (*Cyanistes caeruleus*) for example, benefit by occupying noisy nest boxes that are abandoned by their larger great tit (*Parus major*) competitor (Halfwerk et al., 2016). Theories on population-level or community-level impacts of multisensory pollution may therefore benefit from approaches developed for other multi-stressor impacts, which aim to predict when combined impacts are either synergistic or antagonistic (Bulleri et al., 2018; Griffith et al., 2019; Orr et al., 2020).

In conclusion, our bibliographic analysis revealed few studies have specifically addressed combined impact of anthropogenic noise and ALAN, despite the fact that these sensory pollutants often co-occur, especially in urbanized areas. This small number of studies limits conclusions that can be drawn with respect to whether noise and light pollution should be considered in conjunction, or can be studied on their own, for a given context and/or study system. Consequently, we call for more dedicated observational and experimental work on multisensory pollution, necessarily based on theoretical understanding of the underlying mechanisms through which interactive impacts may arise, and using appropriate experimental designs. Such approaches will greatly improve our understanding of the risks presented by sensory pollution, and provide greater predictive power to identify the most urgent conservation issues, and design the most cost-effective mitigation measures.

AUTHOR CONTRIBUTIONS

WH and PJ conceived the idea and wrote the manuscript. PJ carried out the literature search.

FUNDING

This study was supported by the ERC-Starting Grant—CITISENSE (802460).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.765950/full#supplementary-material

Supplementary Figure 1 | Flow chart of the systematic review process detailing numbers of studies identified at each stage.

Supplementary Data Sheet | Data for each stage of the systematic review process, comprising separate spreadsheets for the initial Web of Science search, included studies from the initial Web of Science search, articles citing the included studies (with and without duplicates removed), and the final list of all included studies.

REFERENCES

Arroyo-Solís, A., Castillo, J. M., Figueroa, E., López-Sánchez, J. L., and Slabbekoorn, H. (2013). Experimental evidence for an impact of anthropogenic noise on dawn chorus timing in Urban Birds. *J. Avian Biol.* 44, 1–9. doi: 10.1111/j.1600-048X.2012.05796.x

Brunner, N., Kühleitner, M., and Renner-Martin, K. (2021). Bertalanffy-Pütter models for avian growth. *PLoS One* 16:e0250515. doi: 10.1371/journal.pone.0250515

Bulleri, F., Eriksson, B. K., Queirós, A., Airoldi, L., Arenas, F., Arvanitidis, C., et al. (2018). Harnessing positive species interactions as a tool against climate-driven loss of coastal biodiversity. *PLoS Biol.* 16:e2006852. doi: 10.1371/journal.pbio.2006852

Buxton, R. T., Seymoure, B. M., White, J., Angeloni, L. M., Crooks, K. R., Fristrup, K., et al. (2020). The relationship between anthropogenic light and noise in US national parks. *Landsc. Ecol.* 35, 1371–1384.

Casasole, G., Raap, T., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., et al. (2017). Neither artificial light at night, anthropogenic noise nor distance from roads are associated with oxidative status of nestlings in an urban population of songbirds. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 210, 14–21. doi: 10.1016/j.cbpa.2017.05.003

Chan, A. A. Y.-H., Giraldo-Perez, P., Smith, S., and Blumstein, D. T. (2010). Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. *Biol. Lett*. 6, 458–461. doi: 10.1098/rsbl.2009.1081

Ciach, M., and Fröhlich , A. (2017). Habitat type, food resources, noise and light explain the species composition, abundance and stability of a winter bird assemblage in an urban environment. *Urban Ecosyst*. 20, 547–559. doi: 10.1007/s11252-016-0613-6

Crain, C. M., Kroeker, K., and Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol. Lett.* 11, 1304–1315. doi: 10.1111/j.1461-0248.2008.01253.x

Da Silva, A., Samplonius, J. M., Schlicht, E., Valcu, M., and Kempenaers, B. (2014). Artificial night lighting rather than traffic noise affects the daily timing of dawn and dusk singing in common European songbirds. *Behav. Ecol*. 25, 1037–1047. doi: 10.1093/beheco/aru103

Darling, E. S., and Côté, I. M. (2008). Quantifying the evidence for ecological synergies. *Ecol. Lett.* 11, 1278–1286. doi: 10.1111/j.1461-0248.2008.01243.x

Dominoni, D., Smit, J. A., Visser, M. E., and Halfwerk, W. (2020). Multisensory pollution: artificial light at night and anthropogenic noise have interactive effects on activity patterns of great tits (Parus major). *Environ. Pollut.* 256:113314. doi: 10.1016/j.envpol.2019.113314

Dominoni, D. M., Halfwerk, W., Baird, E., Buxton, R. T., Fernández-Juricic, E., Fristrup, K. M., et al. (2020). Why conservation biology can benefit from sensory ecology. *Nat. Ecol. Evol.* 4, 502–511. doi: 10.1038/s41559-020-1135-4

Dorado-Correa, A. M., Rodríguez-Rocha, M., and Brumm, H. (2016). Anthropogenic noise, but not artificial light levels predicts song behaviour in an equatorial bird. *R. Soc. Open Sci*. 3:160231. doi: 10.1098/rsos.160231

Ferraro, D. M., Le, M.-L. T., and Francis, C. D. (2020). Combined effect of anthropogenic noise and artificial night lighting negatively affect Western Bluebird chick development. *Condor* 122:duaa037.

Frampton, G. K., Livoreil, B., and Petrokofsky, G. (2017). Eligibility screening in evidence synthesis of environmental management topics. *Environ. Evid.* 6, 1–13. doi: 10.1097/00587875-200609000-00001

Francis, M. J., Spooner, P. G., and Matthews, A. (2015). The influence of urban encroachment on squirrel gliders (*Petaurus norfolcensis*): effects of road density, light and noise. *Wildlife Res.* 42, 324–333. doi: 10.1071/WR14182

Ginnaw, G. M., Davidson, I. K., Harding, H. R., Simpson, S. D., Roberts, N. W., Radford, A. N., et al. (2020). Effects of multiple stressors on fish shoal collective motion are independent and vary with shoaling metric. *Anim. Behav.* 168, 7–17. doi: 10.1016/j.anbehav.2020.07.024

Griffith, G. P., Strutton, P. G., Semmens, J. M., and Fulton, E. A. (2019). Identifying important species that amplify or mitigate the interactive effects of human impacts on marine food webs. *Conserv. Biol.* 33, 403–412. doi: 10.1111/cobi.13202

Halfwerk, W., Both, C., and Slabbekoorn, H. (2016). Long-term nestbox noise experiments reveal an impact on nest-site selection but not on reproduction. *Behav. Ecol.* 27, 1592–1600.

Halfwerk, W., and Slabbekoorn, H. (2015). Pollution going multimodal: the complex impact of the human-altered sensory environment on animal perception and performance. *Biol. Lett.* 11:e20141051. doi: 10.1098/rsbl.2014.1051

Hanafi, S., Chong, L. P., Maruthaveeran, S., and Yeong, K. L. (2019). Vocal response of oriental magpie robin (*Copsychus saularis*) to urban environmental factors in peninsular Malaysia. *Sains Malaysiana*. 48, 2061–2069. doi: 10.17576/jsm-2019-4810-01

Hennigar, B., Ethier, J. P., and Wilson, D. R. (2019). Experimental traffic noise attracts birds during the breeding season. *Behav. Ecol.* 30, 1591–1601. doi: 10.1093/beheco/arz123

Injaian, A. S., Francis, C. D., Ouyang, J. Q., Dominoni, D. M., Donald, J. W., Fuxjager, M. J., et al. (2020). Baseline and stress-induced corticosterone levels across birds and reptiles do not reflect urbanization levels. *Conserv. Physiol.* 8:coz110. doi: 10.1093/conphys/coz110

Issad, S. M., Benhafri, N., El Allali, K., Farsi, H., Ouali-Hassenaoui, S., and Dekar-Madoui, A. (2021). Effects of prolonged night-time light exposure and traffic noise on the behavior and body temperature rhythmicity of the wild desert rodent, *Gerbillus tarabuli*. *Chronobiol. Int*. 38, 415–425. doi: 10.1080/07420528.2020.1862858

Jerem, P., and Mathews, F. (2021). Trends and knowledge gaps in field research investigating effects of anthropogenic noise. *Conserv. Biol.* 35, 115–129.

Lee, J. G. H., Macgregor-Fors, I., and Yeh, P. J. (2017). Sunrise in the city: disentangling drivers of the avian dawn chorus onset in urban greenspaces. *J. Avian Biol*. 48, 955–964. doi: 10.1111/jav.01042

Marín-Gómez, O. H., García-Arroyo, M., Sánchez-Sarria, C. E., Sosa-López, J. R., Santiago-Alarcon, D., and Macgregor-Fors, I. (2020). Nightlife in the city: drivers of the occurrence and vocal activity of a tropical owl. *Avian Res*. 11:9. doi: 10.1186/s40657-020-00197-7

McMahon, T. A., Rohr, J. R., and Bernal, X. E. (2017). Light and noise pollution interact to disrupt interspecific interactions. *Ecology* 98, 1290–1299. doi: 10.1002/ecy.1770

Morelli, F., Benedetti, Y., Ibáñez-Álamo, J. D., Tryjanowski, P., Jokimäki, J., Kaisanlahti-Jokimäki, M.-L., et al. (2021). Effects of Urbanization on taxonomic, functional and phylogenetic avian diversity in Europe. *Sci. Total Environ*. 795:148874. doi: 10.1016/j.scitotenv.2021.148874

Nordt, A., and Klenke, R. (2013). Sleepless in town–drivers of the temporal shift in dawn song in urban European blackbirds. *PLoS One* 8:e71476. doi: 10.1371/journal.pone.0071476

Orr, J. A., Vinebrooke, R. D., Jackson, M. C., Kroeker, K. J., Kordas, R. L., Mantyka-Pringle, C., et al. (2020). Towards a unified study of multiple stressors: divisions and common goals across research disciplines. *Proc. R. Soc. B* 287:20200421. doi: 10.1098/rspb.2020.0421

Piggott, J. J., Townsend, C. R., and Matthaei, C. D. (2015). Reconceptualizing synergism and antagonism among multiple stressors. *Ecol. Evol.* 5, 1538–1547. doi: 10.1002/ece3.1465

Raap, T., Pinxten, R., Casasole, G., Dehnhard, N., and Eens, M. (2017). Ambient anthropogenic noise but not light is associated with the ecophysiology of free-living songbird nestlings. *Sci. Rep.* 7:2754.

Russ, A., Reitemeier, S., Weissmann, A., Gottschalk, J., Einspanier, A., and Klenke, R. (2015). Seasonal and urban effects on the endocrinology of a wild passerine. *Ecol. Evol*. 5, 5698–5710. doi: 10.1002/ece3.1820

Sánchez-González, K., Aguirre-Obando, O. A., and Ríos-Chelén, A. A. (2020). Urbanization levels are associated with the start of the dawn chorus in vermilion flycatchers in Colombia. *Ethol. Ecol. Evol*. 33, 377–393. doi: 10.1080/03949370.2020.1837963

Senzaki, M., Barber, J. R., Phillips, J. N., Carter, N. H., Cooper, C. B., Ditmer, M. A., et al. (2020). Sensory pollutants alter bird phenology and fitness across a continent. *Naure* 587, 605–609. doi: 10.1038/s41586-020-2903-7

Shafiei-Sabet, S., Van Dooren, D., and Slabbekoorn, H. (2016). Son et lumiere: sound and light effects on spatial distribution and swimming behavior in captive zebrafish. *Environ. Pollut.* 212, 480–488. doi: 10.1016/j.envpol.2016.02.046

Stuart, C. J., Grabarczyk, E. E., Vonhof, M. J., and Gill, S. A. (2019). Social factors, not anthropogenic noise or artificial light, influence onset of dawn singing in a common songbird. *Auk* 136:ukz045. doi: 10.1093/auk/ukz045

Svechkina, A., Portnov, B. A., and Trop, T. (2020). The impact of artificial light at night on human and ecosystem health: a systematic literature review. *Landsc. Ecol.* 35, 1725–1742. doi: 10.1007/s10980-020-01053-1

Swaddle, J. P., Francis, C. D., Barber, J. R., Cooper, C. B., Kyba, C. C., Dominoni, D. M., et al. (2015). A framework to assess evolutionary responses to anthropogenic light and sound. *Trends Ecol. Evol.* 30, 550–560. doi: 10.1016/j.tree.2015.06.009

Tekin, E., Diamant, E. S., Cruz-Loya, M., Enriquez, V., Singh, N., Savage, V. M., et al. (2020). Using a newly introduced framework to measure ecological stressor interactions. *Ecol. Lett.* 23, 1391–1403. doi: 10.1111/ele.13533

Vitousek, P. M., Mooney, H. A., Lubchenco, J., and Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science* 277, 494–499. doi: 10.1126/science.277.5325.494

Willems, J. S., Phillips, J. N., Vosbigian, R. A., Villablanca, F. X., and Francis, C. D. (2021). Night lighting and anthropogenic noise alter the activity and body condition of pinyon mice (*Peromyscus truei*). *Ecosphere* 12:e03388. doi: 10.1002/ecs2.3388

Wilson, A. A., Ditmer, M. A., Barber, J. R., Carter, N. H., Miller, E. T., Tyrrell, L. P., et al. (2021). Artificial night light and anthropogenic noise interact to influence bird abundance over a continental scale. *Glob. Chang. Biol.* 27, 3987–4004. doi: 10.1111/gcb.15663

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 11 October 2021
doi: 10.3389/fevo.2021.728377

Color of Artificial Light at Night Affects Incubation Behavior in the Great Tit, *Parus major*

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Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *21 June 2021*
Accepted: *21 September 2021*
Published: *11 October 2021*

Citation:
van Dis NE, Spoelstra K,
Visser ME and Dominoni DM (2021)
Color of Artificial Light at Night Affects
Incubation Behavior in the Great Tit,
Parus major.
Front. Ecol. Evol. 9:728377.
doi: 10.3389/fevo.2021.728377

Artificial light at night (ALAN) has been recognized as a biodiversity threat due to the drastic effects it can have on many organisms. In wild birds, artificial illumination alters many natural behaviors that are important for fitness, including chick provisioning. Although incubation is a key determinant of the early developmental environment, studies into the effects of ALAN on bird incubation behavior are lacking. We measured nest temperature in nest boxes of great tits during the incubation period in two consecutive years. Nest boxes were located in eight previously dark field sites that have been experimentally illuminated since 2012 with white, green, or red light, or were left dark. We tested if light treatment affected mean nest temperature, number of times birds leave the nest (off-bout frequency), and off-bout duration during the incubation period. Subsequently, we investigated if incubation behavior is related to fitness. We found that birds incubating in the white light during a cold, early spring had lower mean nest temperatures at the end of incubation, both during the day and during the night, compared to birds in the green light. Moreover, birds incubating in white light took fewer off-bouts, but off-bouts were on average longer. The opposite was true for birds breeding in the green light. Low incubation temperatures and few but long off-bouts can have severe consequences for developing embryos. In our study, eggs from birds that took on average few off-bouts needed more incubation days to hatch compared to eggs from birds that took many off-bouts. Nevertheless, we found no clear fitness effects of light treatment or incubation behavior on the number of hatchlings or hatchling weight. Our results add to the growing body of literature that shows that effects of ALAN can be subtle, can differ due to the spectral composition of light, and can be year-dependent. These subtle alterations of natural behaviors might not have severe fitness consequences in the short-term. However, in the long term they could add up, negatively affecting parent condition and survival as well as offspring recruitment, especially in urban environments where more environmental pollutants are present.

Keywords: light pollution, incubation, spectral composition, *Parus major*, fitness, ALAN

INTRODUCTION

Artificial light at night (ALAN) is considered a polluter of natural environments due to the profound effects it has on wildlife (Hölker et al., 2010). Organisms use natural light and dark cycles as a cue to time important biological processes, and possess endogenous circannual and circadian biological clocks synchronized to these cycles (Dunlap, 1999). Light is thus an important physiological signal, providing ALAN with ample targets to affect physiological processes and behavior (Falcón et al., 2020). To effectively mitigate negative effects of light pollution, we need to understand how ALAN affects different species, and how light intensity and light spectrum modulate these effects (Gaston et al., 2015).

Relatively few studies have assessed the effects of ALAN on parental behavior, while such effects could have important implications for fitness as the early developmental environment can have long-term effects on the behavior and physiology of offspring (De Kogel, 1997; van Oers et al., 2015). In birds, ALAN can affect nestling condition, with experimentally exposed nestlings having lower body mass (Raap et al., 2016a) and increased immune responses (Raap et al., 2016b; Saini et al., 2019). Nestling condition might be further affected through parental behavior, as ALAN can increase parental provisioning rates (Titulaer et al., 2012), but this is not always observed (Welbers et al., 2017).

ALAN could also affect the developmental environment through effects on parental condition. Birds breeding under white and red light were found to have higher baseline stress levels, and birds with higher baseline stress levels tended to produce fewer fledglings (Ouyang et al., 2015). Additionally, birds have been found to be more active at night under white light, increasing their sleep debt (Ouyang et al., 2017), although cavity breeders might be shielded from such negative effects on sleep (Raap et al., 2018).

Negative effects of ALAN on parental condition could affect incubation behavior. Parents in poor condition are expected to be less effective incubators, as they need to leave the nest more often for self-maintenance (DuRant et al., 2013). For example, lower ambient temperatures result in parents taking longer off-bouts, presumably due to higher energetic demands (Schöll et al., 2020). This can have important implications for developing embryos, as leaving the nest can result in considerable reductions in nest temperature (Greeney, 2009). Regular cooling periods can lower embryo mass and reduce yolk reserves (Olson et al., 2006), and can adversely affect incubation period length and hatching success (Olson et al., 2006; Nord and Nilsson, 2011). Decreased nest attendance is especially detrimental late in incubation, as thermal tolerance of embryos decreases with age, to which parents respond by taking more frequent but shorter off-bouts (Cooper and Voss, 2013). Parental condition during incubation can also indirectly affect offspring, as parents with increased stress levels produce eggs with higher corticosterone concentrations, which affects their hatching success as well as offspring condition (Saino et al., 2005). In captivity, constant light pollution can interrupt incubation in commercially bred turkey hens (Proudman and Opel, 1981). However, studies that investigate the effects of ALAN on incubation behavior in wild birds are lacking.

Here, we investigate if ALAN of different spectra affects incubation behavior. In two consecutive years, we monitored the incubation of great tits breeding in nest boxes that were spread over eight previously dark field sites, which have been experimentally illuminated since 2012 with white, green, or red light, or were left dark. For each nest box, we recorded general life-history data including hatching date and number of hatchlings, and measured mean nest temperature, off-bout frequency, and off-bout duration per day during the incubation period. Subsequently, we investigated if incubation behavior related to offspring condition and fitness. We expected different colors of ALAN to affect incubation behavior and fitness to different extents. Several studies have shown that the response of birds to ALAN is often wavelength-specific (de Jong et al., 2017; Alaasam et al., 2018; Aulsebrook et al., 2020), likely because the photoreceptors involved in several physiological processes, such as reproduction and hormone production, are more sensitive to certain wavelengths (Rani and Kumar, 2000; Hunt et al., 2009; Grubisic et al., 2019). Specifically, we predicted ALAN to negatively affect incubation in at least the white light treatment compared to the dark as previous research shows that white light can negatively affect bird condition (Ouyang et al., 2015, 2017). We therefore expected birds in the white light to take longer off-bouts and have lower incubation temperatures. As incubation is a key determinant of the prenatal environment, we furthermore expected incubation behavior to affect life-history traits: we expected birds that showed suboptimal incubation behavior (e.g., low nest temperatures) to have a longer incubation length and/or lower hatching success.

MATERIALS AND METHODS

Experimental Set-Up

To investigate the effects of ALAN, we made use of a standardized experimental field set-up in which eight previously dark forest edges have been illuminated from sunset to sunrise since 2012 (see for details Spoelstra et al., 2015). In short, each field site consists of four forest edge transects with five lampposts, emitting either ClearField red, Fortimo white, or ClearSky green LED light (Philips, Amsterdam, the Netherlands), or no light, with the order of transects randomly assigned at the start of the experiment. As ALAN is meant for civil use, light intensities were standardized to lux (8.2 ± 0.3 lux measured directly under the lamp at ground level). All three light colors have a full color spectrum, but with a different composition: green lamps emit more short and less long wavelengths, while red lights emit less short and more long wavelengths. In each transect, nine nest boxes were placed in the forest edge around the lamp post in a standardized pattern, differing in the distance to the nearest light post (36 nest boxes per field site, see for details de Jong et al., 2015).

Incubation Behavior Data Collection

In two consecutive breeding seasons (2016 and 2017), we measured nest temperature and life-history traits for great tits breeding in the nest boxes around the lamp posts at the eight different field sites. Temperature loggers (Maxim Integrated, DS1922L-F5#, United Kingdom) were covered with panty hose and attached to a piece of wire to prevent birds from removing it from the nest. Loggers were then placed in the nest cup alongside the eggs, either just before or during incubation (**Figure 1**), with similar sample size distributions between light treatments for when during incubation loggers were placed. Loggers recorded nest temperature every 2 min at a resolution of 0.0625°C and were read out in the field every 4–5 days, until removed completely on the day of hatching of the first egg. Read-out lasted on average 4 min, and if females were present at read-out and subsequently left, they returned on average after 6–8 min (estimated from nest temperature data, see next section). At two sites, we also recorded ambient temperature on the outside of a nest box with temperature loggers. Ambient temperature data were supplemented with hourly average temperatures downloaded from a nearby weather station (Royal Dutch Meteorological Institute (KNMI) stations Hoogeveen and Deelen).

We checked each nest on average twice a week and recorded lay date of the first egg, clutch size, hatch date, and the number of hatchlings. In 2016, for a subset of the nests, chicks were also weighed 2 days after hatching as a measure of chick condition. Incubation length was calculated from the life-history data, assuming great tits lay one egg a day from the first lay date onward and start incubation on the day the last egg was laid. As clutch size was noted down at every nest check, we corrected incubation length for any observed lay delays.

For each nest box, nest temperature data from the last 13 days before hatching were used for the analysis of incubation behavior, as this is the average incubation length of great tits (Van Balen, 1973). We included boxes for which we measured at least the last 3 days of incubation in the analysis, as decreased nest attendance is especially detrimental late in incubation (Cooper and Voss, 2013). Boxes for which the last 3 days of incubation were missing were excluded from the analysis (2017 $N = 2$). Boxes that were abandoned (2016 $N = 10$, 2017 $N = 5$) or predated (2017 $N = 1$) before hatching were also excluded. One other box was excluded from analysis, because eggs were unfertilized and did not hatch (2016 $N = 1$), and one box was excluded because of misplacement of the temperature logger, which only recorded ambient temperature (2016 $N = 1$). **Table 1** reports sample sizes of boxes included in the analysis for each treatment in each year. Sample sizes showed similar distributions between light treatments for when during the season and for when during incubation nests were sampled (**Supplementary Figures 1, 2**).

FIGURE 1 | Example of temperature logger placed in the nest cup just before or during incubation. Loggers were covered with panty hose and attached to a piece of wire to prevent birds from removing it from the nest.

Statistical Analyses

Incubation Behavior

To analyze the recorded nest temperatures, we summarized the data for each nest into five incubation behavior parameters per incubation day measured. These parameters and their respective models were:

1. Mean day nest temperature	(Linear Mixed Model (LMM))
2. Mean night nest temperature	(LMM)
3. Mean variation in temperature at night	(log transformed LMM)
4. Mean off-bout frequency	(Poisson Generalized LMM (GLMM))
5. Mean off-bout duration	(log transformed LMM)

Mean day and night nest temperature were chosen to reflect average incubation effort, mean variation in temperature at night to investigate ALAN effects related to restlessness at night (e.g., Ouyang et al., 2017), and mean off-bout frequency and duration to investigate ALAN effects related to food requirements and availability (e.g., Welbers et al., 2017). Incubation days with data gaps larger than 1 h due to temperature logger malfunctioning were excluded from analysis (86 days out of 1,118 for 63 boxes), as such gaps would exert a large influence on daily means. In total, we included 1,032 incubation days for 103 boxes in the analysis (see **Table 1** for sample sizes per treatment).

TABLE 1 | Number of nest boxes per light treatment per year included in the analysis.

Treatment	Year	Box count	Mean nr days	±SD
DARK	2016	14	9.71	3.00
GREEN	2016	14	9.71	2.79
RED	2016	17	9.65	3.14
WHITE	2016	16	9.88	2.73
DARK	2017	11	10.55	2.94
GREEN	2017	10	10.00	3.23
RED	2017	8	9.50	3.16
WHITE	2017	13	11.23	2.92

For each light treatment per year, we report the mean ± SD number of incubation days per nest for which nest temperature was recorded.

Off-bout frequency and off-bout duration were inferred from the data with a running mean algorithm. This off-bout detection algorithm fits a running mean with a time window of 2 h through the nest temperature data (see **Supplementary Figure 3** for an example). If the temperature dropped below the running mean, the start of an off-bout was detected, with the end of the off-bout detected when the minimum temperature was reached and the subsequent temperature measures increased again. Each off-bout's start, minimum temperature, and duration were recorded, from which mean off-bout frequency and mean off-bout duration could be calculated for each nest per incubation day measured. The distributions of detected off-bouts were similar between treatments (**Supplementary Figure 4**) and showed a large peak for off-bouts with a very short duration and/or small drop in temperature (i.e., off-bout surface defined as the product of the difference from the running mean times off-bout duration). As these are most likely not true off-bouts, we excluded off-bouts with a duration < 5 min and a surface < 1.25 from the analysis, based on the peaks in the respective histograms (**Supplementary Figure 5**). We only analyzed off-bouts that occurred between sunrise and sunset as the majority of the drops below the running mean during the night are a result of much more stable incubation temperatures.

For each incubation parameter, we fit a linear or generalized linear mixed model (LMM or GLMM, see above) with R version 4.02. (R Core Team, 2020) packages lme4 and nlme (Bates et al., 2015; Pinheiro et al., 2021). Ambient temperature, clutch size, and Julian hatch date nested in year were included as covariates. All covariates were centered before inclusion in the model. As fixed effects, we used light treatment as a factor with year specific treatment levels, in interaction with (1) distance to lamp post, (2) incubation day, and (3) incubation day squared. Incubation day was defined as day until hatching, with hatching at day 0. Because effects of artificial illumination are often year-dependent (e.g., Dominoni et al., 2020), we chose year specific treatment levels to avoid having to fit three-way interactions. Interaction terms with $P > 0.05$ were dropped from the final model to be able to then test for the main effect of light treatment. Field site and nest box ID were included as random effects, to account for multiple incubation days per nest box and shared field site between nest boxes.

For three out of four LMM models, we used variance models because linear model assumptions were violated (see above, R package nlme). Variance decreased with higher nest temperatures, following the natural increase in incubation effort as eggs get closer to hatching (Haftorn, 1981). To account for this variance heterogeneity, we modeled variance by incubation day which resulted in models with more homogenous residual distributions and lower Akaike information criterion (AIC) values (dAIC > 10 compared to non-variance model). Mean variation in night nest temperature and mean off-bout duration were log transformed to meet model assumptions. Residuals from the Poisson GLMM for off-bout frequency were not overdispersed.

We performed *post-hoc* tests for models whose ANOVA indicated that light treatment was a significant explainer ($\alpha = 0.05$). All *post-hoc* treatment comparisons were performed within breeding season year and were corrected for multiple comparisons with the Tukey method, using R package emmeans (Lenth, 2020). When the interaction between incubation day and treatment was significant, we first performed *post-hoc* tests to compare slopes between treatments. We then performed a broken-stick analysis to divide the incubation period into two phases: early and late incubation. This way we could find the period before hatching when great tits have reached their maximum incubation effort (Haftorn, 1981). Subsequently, treatment effects were compared for early and late incubation separately within year. For our results, we considered significant comparisons ($P < 0.05$) and trends ($P < 0.10$).

Fitness Data

For each incubation parameter, we calculated per box the mean of the incubation days included in the analyses described above. We then tested if incubation behavior was correlated with incubation length in days and fitness, measured as number of hatchlings. For a subset of boxes from 2016, we also tested for effects on chick weight 2 days after hatching and on nest variation in chick weight at day two (i.e., standard deviation of chick weight in grams per nest). For the chick weight analysis, we had 12 nest boxes for the red light treatment and 9 boxes for each of the other light treatments.

For each fitness measure, we fit a linear mixed model (LMM) with year specific light treatment levels, and 1 day time-incubation effort parameter (off-bout frequency) and one night time-incubation effort parameter (mean night nest temperature) included as fixed effects as we expect ALAN to affect day and night behavior via different routes (Spoelstra et al., 2015). To avoid multicollinearity, we did not include the other incubation effort parameters (mean day nest temperature, variation in night nest temperature, and off-bout duration). For the analysis of number of hatchlings and hatch weight, incubation length was also included as fixed effect. The same centered covariates were included as in the analysis of incubation behavior (see above). Field site was included as a random factor. For models with light treatment as a significant explainer, we fit the same model with all fixed effects except light treatment, and then tested for the effect of light treatment on the residuals of these models. These models were more informative of the actual light treatment effects on fitness, as treatment effects are not only tested for the average value of the covariates, but the dependent variable is corrected for the covariates before testing. All *post-hoc* treatment comparisons were performed within breeding season year.

RESULTS

Mean Day and Night Nest Temperature

Artificial light at night only affected nest temperature in 2017 (**Figure 2**, **Supplementary Figure 6**, and **Supplementary Tables 1, 2**), and the effects depended on light color, incubation day, incubation day squared, and year (**Supplementary Figure 7**). The fitted models explained a large proportion of the variance in nest temperature (day mean conditional R squared (R^2c) = 0.79, night mean R^2c = 0.67). Our broken stick analysis indicated

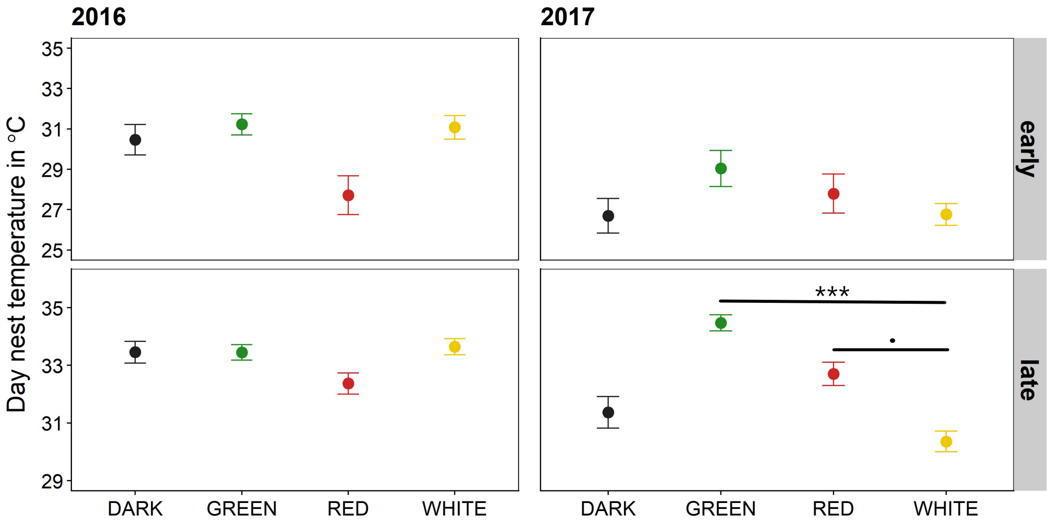

FIGURE 2 | Average day nest temperature in early and late incubation. Raw nest temperature means +-SE are depicted for each artificial light treatment (2016: dark and green *N* = 14, red *N* = 17, white *N* = 16; 2017: dark *N* = 11, green *N* = 10, red *N* = 8, white *N* = 13 nest boxes). Treatments only differed from each other in the breeding season of 2017 late in incubation (bottom right panel): the average day nest temperature was significantly lower in in the white treatment compared to the green treatment (estimate = −5.04°C, t.ratio = −4.48, $P < 0.001$), with a trend in the same direction compared to the red treatment (estimate = −3.47°C, t.ratio = −3.00, $P = 0.06$). The same results were found for mean night nest temperatures (**Supplementary Figure 6** and **Supplementary Table 2**). ***$P < 0.001$, ∘$P < 0.10$.

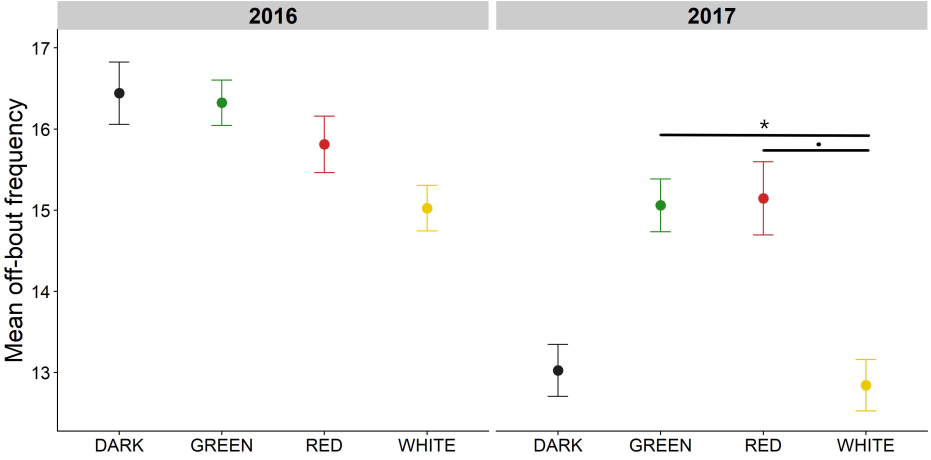

FIGURE 3 | Mean off-bout frequency (number of off-bouts per incubation day). Raw means +-SE are depicted for each artificial light treatment in two breeding seasons. Treatments only differed from each other in the breeding season of 2017 (right panel): females in the white treatment took significantly fewer off-bouts compared to the green treatment (estimate = −2.1, z.ratio = −2.90, $P = 0.04$), with a trend in the same direction compared to the red treatment (estimate = −2, z.ratio = −2.59, $P = 0.08$). The effect was independent of incubation period. *$P < 0.05$, ∘$P < 0.10$.

that birds reached their maximum nest temperature 8 days before hatching of the eggs (day mean break point = −8.39 and night mean break point = −7.70), which we then used to split the incubation period in early and late phases. During the day, birds in the white treatment had a significantly lower mean nest temperature toward the end of incubation compared to birds breeding in the green treatment [estimate = −5.04°C, $t(77) = -4.48$, $P < 0.001$], with a similar trend compared to the red treatment [estimate = −3.47°C, $t(77) = -3.00$, $P = 0.06$] but not compared to the dark [estimate = −2.57°C, $t(77) = -2.19$, $P = 0.34$]. Mean day and mean night nest temperature were highly correlated [$r(1{,}030) = 0.76$, $P < 0.001$], and we found the same effects of light treatment on mean night nest temperatures in 2017 (**Supplementary Figure 6** and **Supplementary Table 2**). We found no effects of light at night on variation in night nest temperature [$F(7) = 0.67$, $P = 0.70$, **Supplementary Table 3**].

Off-Bout Frequency and Duration

Artificial light at night only affected mean off-bout frequency in 2017 (**Figure 3** and **Supplementary Table 4**): birds in the white light treatment took significantly fewer off-bouts compared to the green treatment [estimate = −2.12, z(Inf) = −2.90,

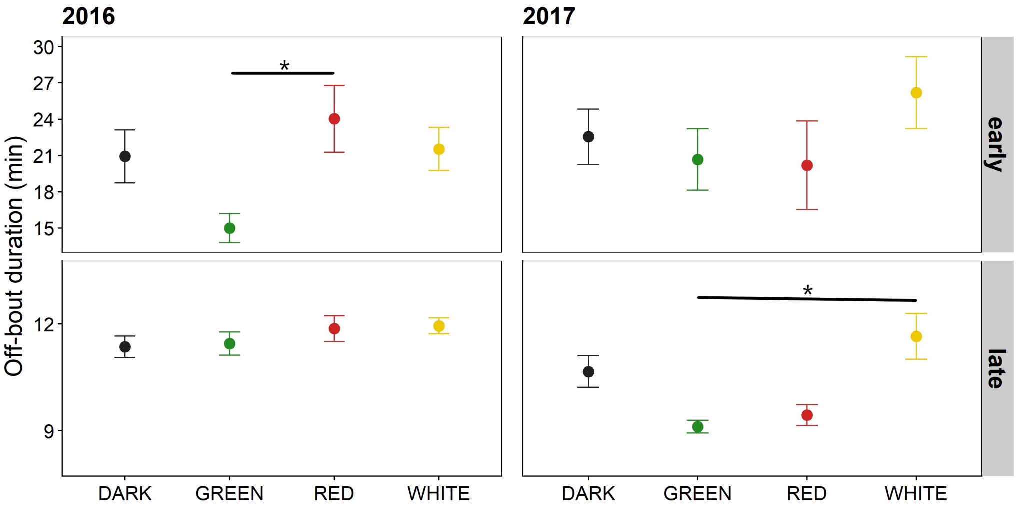

FIGURE 4 | Mean off-bout duration in early and late incubation. Raw means for off-bout duration in minutes ± SE are depicted for each treatment. In the breeding season of 2016, females in the green light treatment had significantly shorter off-bouts compared to the red treatment (top left panel; estimate = −6.52 min, t.ratio = −3.43, *P* = 0.02). While this effect disappeared in late incubation in 2016, it became more pronounced in 2017 (bottom right panel). Specifically, in late incubation females had significantly shorter off-bouts in the green treatment compared to the white treatment (estimate = −2.63 min, t.ratio = −3.65, *P* = 0.009). **P* < 0.05.

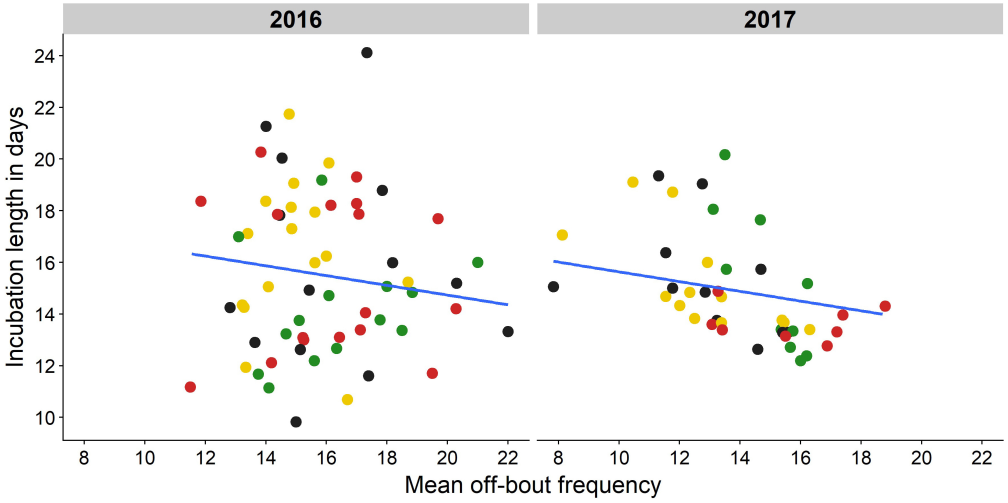

FIGURE 5 | Relation between off-bout frequency and incubation length in days. Mean off-bout frequency during the incubation period is shown for each monitored nest in relation to incubation length in days (green: green light, red: red light, yellow: white light, black: no light). The line shows the significant negative relationship as predicted by a linear mixed model (estimate = −0.19 days, SE = 0.12, *F* = 5.47, *P* = 0.02), with a shorter incubation length when females took on average more off-bouts on incubation days. Artificial light treatment did not affect incubation length (*P* = 0.60) nor was it affected by average night nest temperature (estimate = −0.12 days, SE = 0.09, *F* = 1.37, *P* = 0.24, **Supplementary Tables 6, 7**).

P = 0.04], with a similar trend compared to the red treatment [estimate = −2, z(Inf) = −2.59, *P* = 0.08], but not compared to the birds in the dark [estimate = −0.27, z(Inf) = −0.38, *P* = 1.00]. Although off-bout frequency increased over the course of incubation ($P < 0.001$), the slope was not modulated by light treatment (interaction terms $P > 0.05$ dropped from final model, model fit R^2c delta = 0.40).

Off-bout duration decreased over the course of incubation and was significantly affected by ALAN with the effects depending on light color and incubation day (**Figure 4**, interaction terms $P < 0.001$, model fit $R^2c = 0.85$, **Supplementary Table 5**). A broken stick analysis indicated that birds reached their minimum mean off-bout duration 9 days before hatching of the eggs (break point = 9.43). When comparing treatments in early and late incubation, we found that 2016 birds breeding in the green light took significantly shorter off-bouts compared to the red treatment in early incubation [estimate = −6.52 min, *t*(77) = −3.42, *P* = 0.02]. However, these differences disappeared toward the end of incubation. This is in contrast to 2017, where birds in the green light took significantly shorter off-bouts

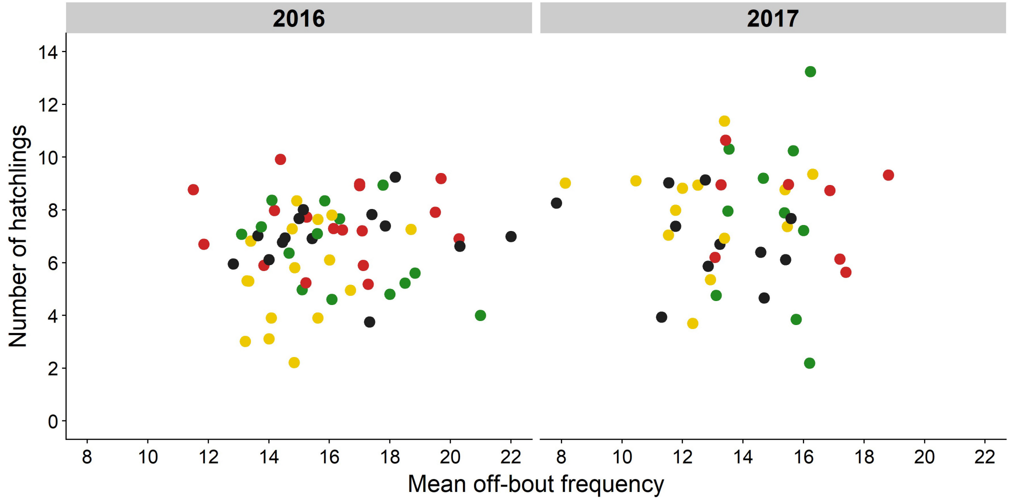

FIGURE 6 | No relation between off-bout frequency and number of hatchlings. Mean off-bout frequency during the incubation period is shown for each monitored nest in relation to number of hatchlings (green: green light, red: red light, yellow: white light, black: no light). Number of hatchlings could not be explained by off-bout frequency, average night nest temperature, or incubation length ($P > 0.05$, **Supplementary Table 8**).

compared to birds in the white light only late in the incubation period [estimate = −2.63 min, $t(77) = -3.65$, $P = 0.009$]. Mean off-bout frequency correlated non-linearly to off-bout duration [**Supplementary Figure 8**, Generalized Additive Model (GAM) effective degrees of freedom (edf) = 7.85, $F(9) = 387.6$, $P < 0.001$]: birds that took very few off-bouts, tended to take very long off-bouts, while birds that took more off-bouts only stayed away from the nest for a short time. Mean off-bout frequency was also non-linearly correlated to mean absolute temperature [**Supplementary Figure 9**, GAM edf = 4.87, $F(9) = 90.53$, $P < 0.001$], with an increase in off-bout frequency relating to an increase in mean day nest temperature, but with diminishing returns.

Fitness Effects

Length of the incubation period was not affected by light at night [$F(7) = 0.78$, $P = 0.60$] nor was it related to mean night nest temperature [$F(1) = 1.37$, $P = 0.24$], but it was related to off-bout frequency [$F(1) = 5.47$, $P = 0.02$, **Supplementary Tables 6, 7**). Incubation length and off-bout frequency were negatively correlated (**Figure 5**, estimate = −0.19 days, SE = 0.12, **Supplementary Table 6**): eggs from birds that took on average more off-bouts hatched after a shorter period of incubation. Most of the variance in length of the incubation period, however, remained unexplained (model fit $R^2c = 0.31$).

We did not find any effects of incubation effort on number of hatchlings (**Figure 6**, mean night temp: $P = 0.51$, off-bout frequency: $P = 0.60$, model fit $R^2c = 0.59$, **Supplementary Tables 8–10**), on chick hatching weight (mean night temp: $P = 0.92$, off-bout frequency: $P = 0.86$, incubation length: $P = 0.71$, model fit $R^2c = 0.10$, **Supplementary Table 11**), nor on variation in chick hatching weight (mean night temp: $P = 0.63$, off-bout frequency: $P = 0.61$, incubation length: $P = 0.13$, model fit $R^2c = 0.45$, **Supplementary Table 12**). Light at night did not affect chick hatching weight [$F(3) = 0.84$, $P = 0.48$), while we did find treatment effects on variation in chick hatching weight [$F(3) = 3.08$, $P = 0.04$, **Supplementary Tables 12, 13**): in 2016, nests from green light transects showed significantly less within-nest variation in chick hatching weight compared to nests from red light transects [estimate = −0.29, $t(35) = -2.99$, $P = 0.02$, **Supplementary Table 14**].

In 2016, birds in white light had significantly less hatchlings compared to the red light treatment [estimate = −1.51, $t(95) = -3.52$, $P = 0.03$], with a similar trend compared to the dark [$t(95) = -1.39$, $P = 0.10$, **Supplementary Table 10**]. However, this was only the case when considering the subset of nests for which we measured incubation behavior, since the effect disappeared when including all other nests for which we had hatchling data. An overview figure with the mean fitness measures per treatment can be found in (**Supplementary Figure 10**).

DISCUSSION

Incubation largely determines the early developmental trajectory in birds (DuRant et al., 2013), but until now no studies have investigated how ALAN affects this key parental behavior. We investigated if ALAN of different spectra affects incubation behavior, and consequently how incubation behavior relates to fitness in two consecutive years. As expected, we found that white light affected incubation behavior of birds, but only when compared to birds in the green light in one of the two breeding seasons: these birds took fewer off-bouts, and late in incubation they took longer off-bouts and had lower nest temperatures. In our study, birds that took fewer off-bouts had a longer incubation length, but we did not find a relationship between incubation behavior and hatchling weight or hatchling number.

The observed lower incubation temperatures and longer off-bout durations in the white light treatment group in 2017 could indicate that these females had increased energy demands. This could be due to the effects of ALAN on body condition, as light pollution can affect immune responses in birds (Kernbach et al., 2018) and spectra combining long- and short-wavelengths can increase night-time activity and corticosterone levels in captive birds (Alaasam et al., 2018). Similarly, free-living birds nesting in white and close to red light have higher corticosterone levels (Ouyang et al., 2015). Another possibility is that male feeding is affected by white ALAN, for example because of sleep deprivation (Ouyang et al., 2017; Aulsebrook et al., 2020). Female blue tits that are fed more by males tend to have shorter off-bouts (Bambini et al., 2019). In our study, males subjected to white light might have fed females less leading to the observed longer off-bouts in this treatment group.

Contrary to our expectations, we only find differences for birds breeding in white light compared to green light, and to some extent compared to red light, but not compared to the dark control. Whereas birds in the green light had on average high incubation temperatures and took many short off-bouts already from the start of the incubation period, birds in the dark control areas had more intermediate incubation parameters in 2017. It might be that areas illuminated with green artificial light have become high quality habitats, as food abundance seems to be higher in green and white light areas at least during the chick feeding phase (Welbers et al., 2017). This could mean that foraging is more successful and leads to shorter off-bouts. However, a potential increase in energy demands in the white light, as discussed above, might counteract the positive effects of higher food abundance for birds breeding in the white light areas.

At our field sites, previously dark forest has been experimentally illuminated since 2012. In this setting it is not possible to control for settlement differences, although previous work did not find differences in breeding density between light treatments nor settlement preference for a particular light color in surviving birds (de Jong et al., 2015). When given the choice in the lab, wild birds preferred to sleep under green light as opposed to white light or darkness (Ulgezen et al., 2019). Thus, the differences we found between birds breeding in the white light compared to the green light could also be due to individual differences with high quality individuals nesting more in green lit areas as opposed to white lit areas.

Although we find some negative effects of white ALAN on incubation, this was only observed in one of the two breeding seasons. Such year-to-year variation in the effects of ALAN has been observed before (de Jong et al., 2015; Dominoni et al., 2020) and highlights the need for long-term monitoring (Spoelstra et al., 2015). When we compare the two monitored breeding seasons, the season of 2017 started earlier and the weather conditions during the incubation period were much colder compared to 2016. Cold weather conditions are thought to increase the energy demands of incubating females (Schöll et al., 2020), which perhaps aggravated the subtle effects of white ALAN, causing females in the white treatment group to have suboptimal incubation in this year. However, we will need more than 2 years of data to test if this is really the case.

Earlier in the season, off-bouts can result in greater incubation temperature drops (Bentzen et al., 2010), and the onset of full incubation tends to start later (Haftorn, 1981), indicating that long off-bouts could have been more stressful for developing embryos in 2017. Nevertheless, incubation behavior was not related to hatchling number in either year or to hatchling weight in 2016. This is in contrast with previous studies, which found decreased hatching success with lower incubation temperatures (Nord and Nilsson, 2011) and decreased embryo mass (Olson et al., 2006). However, in these studies eggs were incubated experimentally in the laboratory, which may have resulted in greater temperature differences between groups than those we observe in our wild bird populations. Moreover, small alterations in incubation can already affect offspring phenotype (DuRant et al., 2013), such as metabolic rate (Nord and Nilsson, 2011) and immune response (DuRant et al., 2012), which we did not measure here.

In both years, off-bout frequency was related to incubation length, with a shorter incubation period when birds took many off-bouts. In our study, birds that took many off-bouts tended to take shorter off-bouts, which keeps incubation temperatures more stable (Cooper and Voss, 2013), and could reduce the incubation period (Olson et al., 2006). For parents, a longer incubation length is undesirable as it is related to reduced adult survival, immunity, and future reproductive success, as well as increased risk of predation (reviewed in DuRant et al., 2013). Although we did not find significant differences in incubation length between the light treatments, birds in the white light took fewer off-bouts that were longer in 2017 compared to birds breeding in the green light. Future studies should investigate if this can lead to reduced adult survival and future reproduction for birds breeding under white light.

Our study finds spectrum dependent effects of ALAN on avian incubation behavior. The effects we found were subtle, did not affect offspring fitness in terms of hatchling number and weight, and were only observed in a cold, early season. This highlights the need for long-term monitoring to further map the conditions under which light pollution is harmful to breeding birds, to be able to successfully mitigate such effects. The subtlety of the effects indicates that future studies should investigate not only direct fitness consequences such as chick survival, but also investigate offspring phenotype and offspring recruitment. The fact that we only observe effects in a cold but not a warmer spring could indicate that ALAN at best becomes harmful when more stressors are present, which is an important consideration in urban environments. Moreover, we find effects on incubation behavior of cavity breeders, which are only exposed to very low levels of ALAN. This suggests that the potential effects of ALAN on open-cup breeders might be much stronger, but this is currently an overlooked research area.

DATA AVAILABILITY STATEMENT

The datasets and scripts used in the final analysis in this study can be found in Dryad (van Dis et al., 2021) at https://doi.org/10.5061/dryad.73n5tb2xq.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Experimentation Committee KNAW, with the protocol number "NIOO 14.05 addendum2".

AUTHOR CONTRIBUTIONS

ND, KS, MV, and DD designed the study. ND and DD collected the data. ND and KS analyzed the data. ND wrote the manuscript. All authors read, commented on, and approved the final version of the manuscript.

FUNDING

This research was supported by the Dutch Technology Foundation STW, which was part of the Netherlands Organization for Scientific Research (NWO) and which was partly funded by the Ministry of Economic Affairs. The project was supported by the Philips and the Nederlandse Aardolie Maatschappij (NAM). Additional funding was obtained by an NWO Open Competition grant ("Aging in the light," 260-25310) to DD and MV.

ACKNOWLEDGMENTS

We thank Dutch nature conservation organizations and terrain owners for allowing us to test the effects of experimental artificial lighting on their terrain: Staatsbosbeheer, Natuurmonumenten, the Dutch Ministry of Defence, Het Drentse Landschap, and the Municipality of Ede. We also thank Anouk Welbers, Anne Dijkzeul, Jamie Kalla, and Chiel Boom for their assistance in the field and Hugo Loning and Pablo Capilla-Lasheras for their help with scripting in R. We thank the two reviewers for their constructive comments to improve the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.728377/full#supplementary-material

REFERENCES

Alaasam, V. J., Duncan, R., Casagrande, S., Davies, S., Sidher, A., Seymoure, B., et al. (2018). Light at night disrupts nocturnal rest and elevates glucocorticoids at cool color temperatures. *J. Exp. Zool. Part A* 329, 465–472. doi: 10.1002/jez.2168

Aulsebrook, A. E., Connelly, F., Johnsson, R. D., Jones, T. M., Mulder, R. A., Hall, M. L., et al. (2020). White and amber light at night disrupt sleep physiology in birds. *Curr. Biol.* 30, 3657–3663.e5. doi: 10.1016/j.cub.2020.06.085

Bambini, G., Schlicht, E., and Kempenaers, B. (2019). Patterns of female nest attendance and male feeding throughout the incubation period in Blue Tits *Cyanistes caeruleus*. *IBIS* 161, 50–65. doi: 10.1111/ibi.12614

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Bentzen, R. L., Powell, A. N., Phillips, L. M., and Suydam, R. S. (2010). Incubation behavior of king eiders on the coastal plain of northern Alaska. *Polar Biol.* 33, 1075–1082. doi: 10.1007/s00300-010-0787-y

Cooper, C. B., and Voss, M. A. (2013). Avian incubation patterns reflect temporal changes in developing clutches. *PLoS One* 8:e65521. doi: 10.1371/journal.pone.0065521

de Jong, M., Caro, S. P., Gienapp, P., Spoelstra, K., and Visser, M. E. (2017). Early birds by light at night: effects of light color and intensity on daily activity patterns in blue tits. *J. Biol. Rhythms* 32, 323–333. doi: 10.1177/0748730417719168

de Jong, M., Ouyang, J. Q., Da Silva, A., Grunsven, R. H. A., Van, Kempenaers, B., et al. (2015). Effects of nocturnal illumination on life-history decisions and fitness in two wild songbird species. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370, 1–8. doi: 10.1098/rstb.2014.0128

De Kogel, C. H. (1997). Long-term effects of brood size manipulation on morphological development and sex-specific mortality of offspring. *J. Anim. Ecol.* 66:167. doi: 10.2307/6019

Dominoni, D. M., Kjellberg Jensen, J., Jong, M., Visser, M. E., and Spoelstra, K. (2020). Artificial light at night, in interaction with spring temperature, modulates timing of reproduction in a passerine bird. *Ecol. Appl.* 30, e2062. doi: 10.1002/eap.2062

Dunlap, J. C. (1999). Molecular bases for circadian clocks. *Cell* 96, 271–290. doi: 10.1016/S0092-8674(00)80566-8

DuRant, S. E., Hopkins, W. A., Hawley, D. M., and Hepp, G. R. (2012). Incubation temperature affects multiple measures of immunocompetence in young wood ducks (*Aix Sponsa*). *Biol. Lett.* 8, 108–111. doi: 10.1098/rsbl.2011.0735

DuRant, S. E., Hopkins, W. A., Hepp, G. R., and Walters, J. R. (2013). Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biol. Rev.* 88, 499–509. doi: 10.1111/brv.12015

Falcón, J., Torriglia, A., Attia, D., Viénot, F., Gronfier, C., Behar-Cohen, F., et al. (2020). Exposure to artificial light at night and the consequences for flora, fauna, and ecosystems. *Front. Neurosci.* 14:602796. doi: 10.3389/fnins.2020.602796

Gaston, K. J., Visser, M. E., and Hölker, F. (2015). The biological impacts of artificial light at night: the research challenge. *Philos. Trans. B* 370:20140133. doi: 10.1098/rstb.2014.0133

Greeney, H. F. (2009). Unusual incubation rhythms the Spotted Barbtail, *Premnoplex brunnescens*. *J. Ornithol.* 150, 529–535. doi: 10.1007/s10336-009-0372-8

Grubisic, M., Haim, A., Bhusal, P., Dominoni, D. M., Gabriel, K. M. A., Jechow, A., et al. (2019). Light pollution, circadian photoreception, and melatonin in vertebrates. *Sustainability* 11:6400. doi: 10.3390/su11226400

Haftorn, S. (1981). Incubation during the egg-laying period in relation to clutch-size and other aspects of reproduction in the great tit parus major. *Ornis Scand.* 12, 169–185.

Hölker, F., Wolter, C., Perkin, E. K., and Tockner, K. (2010). Light pollution as a biodiversity threat. *Trends Ecol. Evol.* 25, 681–682. doi: 10.1016/j.tree.2010.09.007

Hunt, D. M., Carvalho, L. S., Cowing, J. A., and Davies, W. L. (2009). Evolution and spectral tuning of visual pigments in birds and mammals. *Philos. Trans. R. Soc. B* 364, 2941–2955. doi: 10.1098/rstb.2009.0044

Kernbach, M. E., Miller, J. M., Hall, R. J., Unnasch, T. R., Burkett-Cadena, N. D., and Martin, L. B. (2018). Light pollution increases west nile virus competence in a ubiquitous passerine reservoir species. *BioRxiv* [Preprint] doi: 10.1101/269209

Lenth, R. (2020). *emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.1*. Available online at: https://cran.r-project.org/package=emmeans (accessed October 23, 2020).

Nord, A., and Nilsson, J. -Å (2011). Incubation temperature affects growth and energy metabolism in blue tit nestlings. *Am. Nat.* 178, 639–651. doi: 10.1086/662172

Olson, C. R., Vleck, C. M., and Vleck, D. (2006). Periodic cooling of bird eggs reduces embryonic growth efficiency. *Physiol. Biochem. Zool.* 79, 927–936.

Ouyang, J. Q., de Jong, M., Hau, M., Visser, M. E., van Grunsven, R. H. A., and Spoelstra, K. (2015). Stressful colours: corticosterone concentrations in a free-living songbird vary with the spectral composition of experimental illumination. *Biol. Lett.* 11:20150517. doi: 10.1098/rsbl.2015.0517

Ouyang, J. Q., de Jong, M., van Grunsven, R. H. A., Matson, K. D., Haussmann, M. F., Meerlo, P., et al. (2017). Restless roosts: light pollution affects behavior, sleep, and physiology in a free-living songbird. *Glob. Change Biol.* 23, 4987–4994. doi: 10.1111/gcb.13756

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and Team, R. C. (2021). *nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-152*. Available online at: from https://cran.r-project.org/package=nlme (accessed February 26, 2021).

Proudman, J. A., and Opel, H. (1981). Turkey prolactin: validation of a radioimmunoassay and measurement of changes associated with broodiness. *Biol. Reprod.* 25, 573–580.

R Core Team (2020). *R: A Language And Environment For Statistical Computing*. Available online at: https://www.r-project.org/ (accessed October 23, 2020).

Raap, T., Casasole, G., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., et al. (2016a). Artificial light at night affects body mass but not oxidative status in free-living nestling songbirds: an experimental study. *Sci. Rep.* 6:35626. doi: 10.1038/srep35626

Raap, T., Casasole, G., Pinxten, R., and Eens, M. (2016b). Early life exposure to artificial light at night affects the physiological condition: an experimental study on the ecophysiology of free-living nestling songbirds. *Environ. Pollut.* 218, 909–914. doi: 10.1016/j.envpol.2016.08.024

Raap, T., Pinxten, R., and Eens, M. (2018). Cavities shield birds from effects of artificial light at night on sleep. *J. Exp. Zool. Part A* 329, 449–456. doi: 10.1002/jez.2174

Rani, S., and Kumar, V. (2000). Phasic response of the photoperiodic clock to wavelength and intensity of light in the redheaded bunting, *Emberiza bruniceps*. *Physiol. Behav.* 69, 277–283. doi: 10.1016/S0031-9384(99)00247-4

Saini, C., Hutton, P., Gao, S., Simpson, R. K., Giraudeau, M., Sepp, T., et al. (2019). Exposure to artificial light at night increases innate immune activity during development in a precocial bird. *Comp. Biochem. Physiol. Part A* 233, 84–88. doi: 10.1016/j.cbpa.2019.04.002

Saino, N., Romano, M., Ferrari, R. P., Martinelli, R., and Møller, A. P. (2005). Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. *J. Exp. Zool. Part A* 303, 998–1006. doi: 10.1002/jez.a.224

Schöll, E. M., Aparisi, M. P., and Hille, S. M. (2020). Diurnal patterns of ambient temperature but not precipitation influence incubation behavior in Great Tits. *J. Ornithol.* 161, 529–538. doi: 10.1007/s10336-019-01737-9

Spoelstra, K., van Grunsven, R. H. A., Donners, M., Gienapp, P., Huigens, M. E., Slaterus, R., et al. (2015). Experimental illumination of natural habitat–an experimental set-up to assess the direct and indirect ecological consequences of artificial light of different spectral composition. *Philos. Trans. R. Soc. B* 370:20140129. doi: 10.1098/rstb.2014.0129

Titulaer, M., Spoelstra, K., Lange, C. Y. M. J. G., and Visser, M. E. (2012). Activity patterns during food provisioning are affected by artificial light in free living great tits (*Parus major*). *PLoS One* 7:e37377. doi: 10.1371/journal.pone.0037377

Ulgezen, Z. N., Käpylä, T., Meerlo, P., Spoelstra, K., Visser, M. E., and Dominoni, D. M. (2019). The preference and costs of sleeping under light at night in forest and urban great tits. *Proc. R. Soc. B* 286:20190872. doi: 10.1098/rspb.2019.0872

Van Balen, H. (1973). A comparative study of the breeding ecology of the great tit Parus major in different habitats. *Ardea* 61, 1–93.

van Dis, N. E., Spoelstra, K., Visser, M. E., and Dominoni, D. M. (2021). Color of artificial light at night affects incubation behavior in the great tit, Parus major. *Dryad, Dataset* doi: 10.5061/dryad.73n5tb2xq

van Oers, K., Kohn, G., Hinde, C., and Naguib, M. (2015). Parental food provisioning is related to nestling stress response in wild great tit nestlings: implications for the development of personality. *Front. Zool.* 12(Suppl 1):S10. doi: 10.1186/1742-9994-12-S1-S10

Welbers, A. A. M. H., van Dis, N. E., Kolvoort, A. M., Ouyang, J., Visser, M. E., Spoelstra, K., et al. (2017). Artificial light at night reduces daily energy expenditure in breeding great tits (*Parus major*). *Front. Ecol. Evol.* 5:55. doi: 10.3389/fevo.2017.00055

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 20 October 2021
doi: 10.3389/fevo.2021.748983

Artificial Light at Night Impacts the Litter Layer Invertebrate Community With No Cascading Effects on Litter Breakdown

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Artificial light at night (ALAN) can impact the trophic structure of assemblages of ground-dwelling invertebrates, and changes in such assemblages can affect decomposition in terrestrial systems due to the various functional roles of these invertebrates, including microbial grazing, comminution of litter, and predation of other invertebrates, that can directly or indirectly affect plant-litter breakdown. Despite this, we are unaware of any studies that have evaluated the effects of ALAN on the breakdown of plant litter in a terrestrial ecosystem. We sought to answer whether ALAN affects litter breakdown via its effects on a community of ground-dwelling arthropods using two field experiments. In one experiment, we manipulated the presence of ALAN and the size classes of soil invertebrates that could enter mesh bags containing plant litter (litterbags). We found that the rate of plant-litter breakdown increased with the mesh size of litterbags but was unaffected by presence of ALAN. In a second field experiment carried out to examine the effects of ALAN on the trophic structure of litter-layer invertebrate communities, while controlling for potential effects of ALAN on vegetation, we again found that ALAN did not affect litter breakdown despite the fact that ALAN increased the abundances of secondary and tertiary consumers. Our finding that larger assemblages of ground-dwelling secondary and tertiary consumer invertebrates under ALAN did not slow litter breakdown through increased top-down control of detritivores suggests ALAN may disrupt predator-prey interactions in litter-layer communities.

Keywords: light pollution, ecosystem function, trophic structure, trophic cascade, grassland

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Specialty section:
This article was submitted to
Urban Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *28 July 2021*
Accepted: *30 September 2021*
Published: *20 October 2021*

Citation:
Hey MH, Epstein HE and Haynes KJ (2021) Artificial Light at Night Impacts the Litter Layer Invertebrate Community With No Cascading Effects on Litter Breakdown.
Front. Ecol. Evol. 9:748983.
doi: 10.3389/fevo.2021.748983

INTRODUCTION

Artificial light at night (hereafter ALAN) is a widespread sensory pollutant which currently affects nearly a quarter of the terrestrial surface of our planet (Gaston et al., 2014; Falchi et al., 2016, 2019; Kyba et al., 2017). It is widely considered to have extensive ecological consequences across levels of biological organization ranging from the organism (e.g., physiology and behavior) to the ecosystem (Longcore and Rich, 2004; Gaston et al., 2013, 2014). Most research documenting effects of ALAN has occurred at the organismal and population levels (Longcore and Rich, 2004; Hölker et al., 2010; Gaston et al., 2015; Sanders and Gaston, 2018). Perhaps the best evidence that ALAN affects higher levels of organization are studies that have shown effects on multi-trophic structure or dynamics (Meyer and Sullivan, 2013; Bennie et al., 2018a; Grenis and Murphy, 2019; Sullivan et al., 2019). At the community level there is increasing recognition of the potential for ALAN to disrupt pollination (Knop et al., 2017; Macgregor et al., 2017; Giavi et al., 2020) as well as invertebrate food-web dynamics (Bennie et al., 2018a; Sanders et al., 2018; Maggi et al., 2019). However, effects of ALAN on the ecosystem processes of nutrient transfer and decomposition are largely unknown.

Decomposition is a critical ecosystem process driving nutrient transfer from dead organic matter to plant-available forms, which in turn can affect plant growth and carbon fixation (Bardgett, 2005). The structure and trophic dynamics of ground-dwelling invertebrate assemblages have profound effects on the decomposition of organic matter in terrestrial ecosystems (Moran et al., 1996; Heneghan et al., 1998; Schmitz, 2009; Hawlena et al., 2012; Tonin et al., 2018). For example, the presence of predatory invertebrates can slow decomposition by limiting the activity of detritivores, which break down plant material in the litter layer (Kajak, 1995; Lawrence and Wise, 2000; Schmitz et al., 2010; Hawlena et al., 2012). The opposite has also been found, wherein the absence of predatory invertebrates slows decomposition, which could be attributed to competition among prey invertebrates in the litter layer (Lawrence and Wise, 2004; Melguizo-Ruiz et al., 2020). Artificial light at night is known to impact the composition of invertebrate assemblages (Meyer and Sullivan, 2013; Davies et al., 2017; Manfrin et al., 2017; Desouhant et al., 2019) and their trophic dynamics (Sanders et al., 2015, 2018; Bennie et al., 2018a; Manfrin et al., 2018; Sanders and Gaston, 2018). Multiple studies have documented that ground-dwelling invertebrate assemblages under ALAN have higher abundances of predators such as arachnids and carabid beetles than those found in areas that are dark at night (Davies et al., 2012, 2017; Manfrin et al., 2017; McMunn et al., 2019; Willmott et al., 2019). The attraction of predators to ALAN-affected areas has been predicted to lead to increased top-down control (Sanders and Gaston, 2018). Given that (a) large ground-dwelling predatory invertebrates are capable of initiating trophic cascades (Moran et al., 1996; Schmitz, 2007, 2009; Hawlena et al., 2012) and that (b) greater numbers of these are observed in light-polluted conditions (Wolff, 1982; Davies et al., 2012, 2017; Holzhauer et al., 2015), ALAN may elicit indirect effects on decomposition by increasing the strength of top-down control over detritivores. Despite the observed effects of ALAN on trophic structure of ground-dwelling invertebrates (Davies et al., 2012, 2017; Meyer and Sullivan, 2013; Manfrin et al., 2017), the effects of ALAN on decomposition of organic matter in terrestrial systems are poorly understood.

Three interacting factors most strongly influence the rate of decomposition in terrestrial systems: abiotic conditions, litter nutritional quality (primarily nitrogen content), and the composition of soil fauna and microorganisms (Swift et al., 1979; Wardle et al., 2004; Hättenschwiler et al., 2005; García-Palacios et al., 2016). In the litter-layer food web there are typically up to three levels of consumers: primary, secondary, and tertiary. Primary consumers, including microorganisms (bacteria and fungi) and invertebrate detritivores, feed directly on dead plant matter. Primary consumers drive most terrestrial decomposition (Swift et al., 1979; McGuire and Treseder, 2010). Detritivores enhance the activity of bacteria and fungi by fragmenting plant material, thereby increasing attackable surface area, and by depositing frass (Vossbrinck et al., 1979; Beare et al., 1992; Coleman et al., 2004). Secondary consumers, in contrast, can slow the breakdown of plant matter through their consumption of bacteria, fungi, or detritivores. In systems where decomposition is primarily driven by bacterial or fungal pathways, secondary consumers which regulate bacterial and fungal populations include protozoa, nematodes, and mites (Santos et al., 1981; Ruess and Ferris, 2004). Tertiary consumers can enhance the rate of decomposition by releasing microbial decomposers from predation from secondary consumers (Hedlund and Ohrn, 2000; Lawrence and Wise, 2004), and can also slow decomposition by exerting top-down effects on detritivores (Kajak, 1995; Lawrence and Wise, 2000; Hawlena et al., 2012).

We conducted two studies to explore the effects of ALAN on the breakdown of plant litter in a temperate grassland ecosystem through its effects on litter-layer fauna. First, to test for potential effects of ALAN on decomposition mediated by multitrophic interactions (e.g., trophic cascades), we carried out a field experiment in which we manipulated the presence/absence of ALAN and the size classes of soil fauna. We did this by quantifying rates of litter breakdown, or proportion decomposed over time, within litterbags of three different mesh sizes in plots that were exposed to ALAN or ambient light levels at night. Exclusion of soil organisms of different body sizes from plant litter has previously been used successfully to draw conclusions about how subsets of the invertebrate community belonging to different size classes influence decomposition (Vossbrinck et al., 1979; Setälä et al., 1996; Bradford et al., 2002). As litterbag mesh size is increased, the assemblages of invertebrates that establish within the litterbags increase in maximum organism body size, species richness (Bradford et al., 2002; Cole et al., 2006), abundance and diversity of secondary and tertiary consumers, and functional complexity (Setälä et al., 1996; Bradford et al., 2002; Smith and Bradford, 2003). A second field experiment was carried out to test effects of ALAN alone on the trophic composition of soil arthropods and plant litter breakdown, while controlling for potential effects of ALAN on vegetation (Bennie et al., 2016, 2018b; Grenis and Murphy, 2019), which could potentially affect invertebrate assemblages or litter decomposition. We manipulated the presence/absence of ALAN (as above), while controlling for potential effects of ALAN on above-ground vegetation by mowing all plots prior to the experiment. To characterize effects of ALAN alone on invertebrate trophic structure, we used litterbags of a single large mesh size. Based on previous findings documenting higher abundances of ground-dwelling predatory invertebrates under ALAN compared with unlit areas (e.g., Davies et al., 2012, 2017), and that tertiary consumers can slow decomposition (Kajak, 1995; Lawrence and Wise, 2000; Hawlena et al., 2012), we predicted that ALAN would reduce rates of litter breakdown indirectly via increased top-down control of primary consumers by secondary and/or tertiary consumers.

MATERIALS AND METHODS

Experimental Design

We conducted two experiments at Blandy Experimental Farm (BEF) in Boyce, Virginia, United States (39.0640°N, 78.0652°W). In the fall of 2017, we conducted an experiment to test the independent and interacting effects of ALAN and invertebrate size classes on decomposition of plant litter. The experiment was carried out in eight replicate plots, half exposed to ALAN and half receiving no added ALAN. Within each plot, we measured the decomposition of litter within mesh bags (litterbags), using litterbags with three different mesh sizes (0.1, 2, and 4 mm) to manipulate the size classes of fauna that could gain access to the litter. In the fall of 2018, we carried out a second experiment to for two primary reasons: (1) to control for potential effects of ALAN on vegetation (Bennie et al., 2016, 2018b; Grenis and Murphy, 2019), which could potentially affect invertebrate assemblages or litter decomposition, and (2) to test effects of ALAN on the trophic composition of soil arthropods. This experiment was carried out in ten replicate plots, five with ALAN added and five with no ALAN added. To control for potential effects of ALAN on vegetation, we minimized differences in vegetation height and biomass by mowing all plots prior to the start of the experiment. To examine the effects of ALAN on arthropod trophic composition, we deployed litterbags in the second experiment that would allow us to recover larger numbers of arthropods than we could in the previous experiment. This was accomplished by using litterbags that were larger, contained more litter, and had only the largest mesh size (4 mm).

Experiment 1: Untangling Effects of Artificial Light at Night and Invertebrate Size Classes on Decomposition

This experiment was carried out in the Native Plant Meadow at BEF. Dominant vegetation in the meadow consisted of warm season, C_4 grasses including switchgrass (*Panicum virgatum*), Indian grass (*Sorghastrum nutans*), and big bluestem (*Andropogon gerardii*). Controlled burning is the primary management practice used to prevent succession of the meadow from grassland to forest. The most recent controlled burn prior to the experiment was carried out in the spring of 2017.

The experiment was carried out in eight 20-m diameter circular plots arranged in a paired design. Within each of four pairs of plots, one plot was randomly assigned to receive artificial light at night (hereafter ALAN plot). The other plot received no addition of ALAN, and thus only was lit with ambient diurnal sunlight, moonlight, and starlight (hereafter ambient-light plot). There were no barriers to restrict movement of invertebrates into, or out of, a plot. Within a pair, plots had a minimum distance of 10-m between edges, and pairs were a minimum of 20-m apart. Each plot represented a replicate. Each ALAN plot was illuminated from dusk to dawn by four broad-spectrum (4922 K) 12 W LED (Bullet®, RAB Lighting Inc., Northvale, New Jersey, United States) floodlights. The emission spectrum of this LED model is provided in the electronic (**Supplementary Figure 1**). Each LED floodlight was attached to the top of a 3 m post in the center of the plot. All floodlights were aimed downward, with a slight deflection of 25° outward toward the edge of the plot. To hold physical structure constant across treatments, we installed identical posts without floodlights at the center of ambient-light plots. The plots were originally established in 2015 (Firebaugh and Haynes, 2016), and ALAN was manipulated throughout the summers of 2015 and 2016. For this study, ALAN was manipulated continuously beginning in the spring (April) through the fall including the study period, which ran from August through October 2017.

At the end of the growing season (August 17, 2017), we placed mesh bags (litterbags) containing litter within each plot. The litterbags were placed 1 m from the central post. At this distance from the central post, we measured nighttime light intensity 1 m above the ground to minimize the blocking of light by the vegetation canopy, recording intensities of 193.16 ± 5.0 lux (mean ± 1 SD) in the ALAN plots and 0.014 ± 0.012 lux in the ambient-light plots. The light levels in our plots were well within the range for canopy level Illuminance reported by Bennie et al. (2016) which extends from 30 to 1200 lux depending on vertical distance from the light source in question. Lower values have been reported elsewhere in the literature (Bennie et al., 2016; Grenis and Murphy, 2019), and we assume that the light levels reaching the litter layer were significantly lower and more variable than at 1 m above the ground.

We set out litterbags in groups of three, with one bag of each of the three mesh sizes per group. Within a group, we arranged litterbags so that none overlaid the other, and all were staked down to maintain contact with the soil surface. We placed six groups in each plot, with the direction of each group relative to the central pole chosen haphazardly.

To obtain standardized litter for the experiment, we grew switchgrass, *Panicum virgatum*, in a greenhouse at BEF during the summer of 2017. For a detailed description of the methods of grass propagation and growth (see **Supplementary Material Methods**). We harvested green leaf material on August 12, 2017, by trimming blades to the collar, and then oven dried it at 40°C for ~72 h. Prior to placing the leaf material into the litterbags, all leaf material was intermixed to maximize homogeneity among samples. We placed 0.72 ± 0.25 g of dried leaf material into each litterbag (W × L, 9 × 9 cm). The edges of the bags were sealed using a heat sealer.

To explore which invertebrates mediated ALAN-induced changes in decomposition, we manipulated the size classes of fauna that could gain access to the litter by using litterbags with three different mesh sizes: 0.1, 2, and 4 mm. These mesh sizes exclude (in order) all macrofauna and mesofauna, all macrofauna, and some macrofauna (Setälä et al., 1996; Bradford et al., 2002; Smith and Bradford, 2003). Litter-inhabiting organisms with body sizes < 0.1 mm include bacteria, fungi, protozoa, and nematoda (Wallwork, 1970; Swift et al., 1979; Wall and Moore, 1999); these organisms directly (and indirectly in the case of protozoa and nematoda) effectuate nutrient cycling (McGuire and Treseder, 2010). Mesofauna (body size between 0.1 and 2 mm, Wallwork, 1970; Swift et al., 1979) include (but are not limited to) Collembolans (springtails), Acari (mites), Isoptera (termites) in addition to larval organisms (Wall and Moore, 1999;

Cole et al., 2006). Mesofauna are responsible for modification of the microbial community, comminution of litter, and in some instances predation of other invertebrates (Swift et al., 1979; Vossbrinck et al., 1979; Scheu and Setälä, 2002). Macrofauna (body size > 2 mm, Wallwork, 1970; Swift et al., 1979) include Araneae, Hymenoptera, and Coleoptera (larvae and adults), along with larvae and nymphs from other orders. Litter-dwelling macrofauna are responsible for comminution of litter and predation of secondary and tertiary invertebrates (Scheu and Setälä, 2002; Briones, 2014).

We expected that primary and secondary consumer microorganisms would gain access into the litterbags with the 0.1 mm mesh, but that arthropod tertiary consumers, along with arthropod secondary consumers whose body sizes exceeded 0.1 mm in diameter, would be excluded (Wallwork, 1970; Swift et al., 1979). We expected that primary, secondary, and tertiary consumers would all gain access into the litterbags with the 2 and 4 mm mesh sizes, but that fewer tertiary consumers would be excluded from the 4-mm mesh size litterbags than from the 2-mm mesh size litterbags (Wallwork, 1970; Swift et al., 1979; Bradford et al., 2002; Cole et al., 2006).

A small but growing body of literature highlights some of the effects of ALAN on plants in roadside or semi-natural conditions. ALAN from street-lighting has direct and indirect effects on the plant community and its herbivores (Bennie et al., 2018a,b; Grenis and Murphy, 2019). We therefore considered it plausible that ALAN-induced changes in plant biomass could affect the rate of plant litter decomposition. For example, increased density of standing senesced vegetation could attract detritivores or affect the litter-layer microclimate. To test for potential effects of ALAN in our experimental plots on plant growth, we estimated the density of aboveground grass biomass (g/m^2) in each plot from the mean biomass harvested from five 0.3-m^2 quadrats placed randomly within each plot. The biomass was harvested in early August, 2017, dried for 5 days at 55°C, and subsequently weighed.

Experiment 2: Effects of Artificial Light at Night on Decomposition and Litter-Layer Invertebrates While Controlling Aboveground Vegetation

Because ALAN can affect vegetation (Bennie et al., 2016, 2018b; Grenis and Murphy, 2019), which could potentially affect invertebrate assemblages or litter decomposition, we carried out a second field experiment in the fall of 2018 in a different set of plots at BEF. Like in the first experiment (carried out in 2017), we measured rates of plant litter breakdown in litterbags; however, in the second experiment we took steps to minimize differences across plots in the structure and biomass of aboveground vegetation via two means. First, the plots used in the 2018 experiment were not exposed to ALAN during the 2018 growing season; in the prior experiment, ALAN was manipulated throughout the growing season as well as during the fall decomposition experiment. In 2018, ALAN was not manipulated until August 16, 2 days before litterbags were placed in the plots. Second, we mowed all plots 2 days prior to the start of the 2018 experiment to further minimize differences in aboveground vegetation structure and biomass across plots.

This experiment was carried out in ten 1-m diameter field plots that were arrayed in a grid pattern with 5 m between adjacent plots. Half of the plots were selected at random to receive ALAN, while the other half received ambient light only. As with our first study, each plot represented a replicate. A plot was exposed to ALAN by one broad-spectrum 12 W LED (same model as in Experiment 1) floodlight, which was positioned on the underside of the horizontal arm of a light post at a height of 3 m and aimed directly downward over the center of the plot. Identical light posts were established for all plots (both ALAN-plots and ambient-light plots). There were no barriers to prevent movement in or out of plots. In this experiment, we were able to measure nighttime light intensity at ground level (the top of the litter layer) because the vegetation was mowed. Nighttime light intensity in the ALAN plots was 126.8 ± 7.32 lux (mean ± 1 SD) and 0.4 ± 0.21 lux in the ambient-light plots.

In this experiment, we intended to use plant litter that closely resembled litter occurring in the grasslands at our study site. Thus, we collected senesced leaves in August 2018 from standing C_4 grasses in the BEF Native Plant Meadow (> 50 m from sources of ALAN). The nitrogen content of the collected leaf litter was 1.02 ± 0.33 (% of total mass mean ± SD). The leaf litter was dried at 50°C for 5 days, homogenized, and then placed into litterbags.

To increase our ability to characterize effects of ALAN on invertebrate trophic structure, we took two steps to increase the numbers of invertebrates captured. First, we used a mesh size (4 mm) that excluded only large macrofauna. Second, we used larger litterbags (W × L, 10 × 20 cm) containing more litter material (3.0 ± 0.05 g) than in the 2017 experiment (0.72 ± 0.25 g). The edges of the bags were sealed using a heat sealer, and all litterbags were staked down to maintain contact with the ground surface when deployed in the plots.

Data Collection and Analysis

Experiment 1: Untangling Effects of Artificial Light at Night and Invertebrate Size Classes on Decomposition

To examine the effects of ALAN on litter breakdown over time, we retrieved half of the litterbags from each plot after 31 days, and the remaining half after 61 days. Immediately after retrieval, we then removed invertebrates from the litterbags using Tulgren extraction carried out over 24 h. Following extraction of the invertebrates, we dried the litter at 50°C for 24 h and removed any residual soil or debris by hand. We estimated the proportion of litter broken down, or decomposed, as (1–$\text{mass}_{final}/\text{mass}_{initial}$). We then pulverized the litter samples into fine powder using a ball mill (Cianflone Scientific LLC, Pittsburgh, PA, United States) and performed combustion analysis to determine final nitrogen content (Flash 2000 Elemental Analyzer, Thermo Fisher Scientific^{TM}, Hampton NH, United States). To obtain initial litter nitrogen content, 0.3 g sub-samples of the litter placed into each litterbag were collected for combustion analysis.

Statistical analyses were run using the mean of response variables because plots were considered true replicates in our study. We tested the effects of ALAN and litterbag mesh size on the mean proportion of litter broken down and the nitrogen content of remaining litter within each plot using linear mixed effects (LME) models. The fixed effects in the LME models were ALAN, mesh size, their interaction, time in the field, and aboveground grass biomass. The random effects of plot pairs were modeled using random intercepts. Mean change in litter nitrogen was transformed using a power transformation (x^3) to improve normality of residuals (Williamson and Gaston, 1999). The LME models were fitted using the "lmer" package (Bates et al., 2015) implemented in the program R (R Core Team, 2018). *Post hoc* pairwise comparisons based on least-squares means with p-values adjusted using the Tukey method were carried out using the R package "emmeans" (Lenth et al., 2019). In this experiment, we did not recover a sufficient number of invertebrates from the litterbags to explore how their abundances were affected by the experimental manipulations.

Experiment 2: Effects of Artificial Light at Night on Decomposition and Litter-Layer Invertebrates While Controlling Aboveground Vegetation

We deployed six litterbags in each of the plots on August 18, 2018, and collected them 116 days later. Upon retrieval from the field, invertebrates were extracted following the method used in Experiment 1. We identified invertebrates to order or family level, whichever was needed to determine their trophic position (primary consumers, secondary consumers, or secondary/tertiary consumers). We estimated the proportion of litter decomposed and nitrogen content using the same procedures described in Experiment 1.

We tested the effects of ALAN on the mean proportion of litter broken down ($1 - \text{mass}_{final}/\text{mass}_{initial}$) and mean nitrogen content of remaining litter using one-way ANOVAs. We examined the effect of ALAN on the ground-dwelling invertebrate community in two ways. First, we tested for the effect of ALAN on the mean total number of invertebrates identified using a one-way ANOVA. Total number of invertebrates was $\log(x + 1)$ transformed to reduce heterogeneity of variance. Second, we investigated the effect of ALAN on the three trophic groups present in our invertebrate community: primary consumers, secondary consumers, and secondary/tertiary consumers. Because of non-independence of potentially interacting trophic levels, we tested for a multivariate effect of ALAN on abundances of the three trophic groups using MANOVA. In the event of a significant multivariate effect, we tested the effects of ALAN on each of the trophic groups using univariate ANOVA (Quinn and Keough, 2002). Prior to these tests, the abundances of each trophic group were Box-Cox transformed to improve normality (Olivier and Norberg, 2010). We ran this test using the "manova" function in the "stats" package.

Given the proximity of the experimental plots (5 m between adjacent plots), it is possible that some litter-layer invertebrates may have visited litterbags in more than one experimental plot. This could potentially cause non-independence of the measures of invertebrate abundance from different plots. Through trophic interactions, non-independence in invertebrate abundance could translate into non-independence in measures of litter breakdown. We addressed these possibilities by testing for spatial autocorrelation in the residuals from the MANOVA and each ANOVA model (Ver Hoef and Cressie, 1993). Moran's I tests implemented in the "ape" package (Paradis and Schliep, 2019) showed there was no significant spatial autocorrelation in the residuals from any of the models.

RESULTS

Untangling Effects of Artificial Light at Night and Invertebrate Size Classes on Decomposition

Mean daily precipitation during this experiment (August 17to October 17) was 3.18 mm. This is 11% higher than mean daily precipitation for this range of dates over the previous 30 years (1986–2016) at our study site.

We observed a marginally significant interactive effect of ALAN and mesh size on the proportion of litter broken down ($P = 0.06$, **Table 1**), potentially reflecting a stronger effect of ALAN on the proportion of litter broken down or removed in litterbags with the largest mesh size than with the intermediate and smallest mesh sizes (14, 8, and 6% higher under ALAN than under ambient light after 60 days, respectively, **Figure 1**). The proportion of material broken down differed significantly between litterbag mesh sizes ($p < 0.001$, **Table 1**), with the least loss of litter with the mesh size that excluded all but microorganisms (smallest mesh size) and the most breakdown in the mesh that excluded only large macrofauna (largest mesh size; **Figure 1**). After 60 days, there was a 20% difference in proportion broken down in the largest mesh size compared with the smallest mesh size. The proportion of leaf litter broken down tended to be higher under ALAN than under ambient light; however, this difference was not statistically significant ($P = 0.07$, **Table 1** and **Figure 1**). There was no significant effect of aboveground grass biomass on the proportion of litter that broke down ($P = 0.14$, **Table 1**).

Mean initial nitrogen content the litter for this experiment was 3.36 ± 0.32 (mean ± SE, % of total mass) and mean nitrogen content of the litter remaining after 30 and 60 days was 3.25 ± 0.46%. We found no evidence that the nitrogen content of the remaining litter was affected by any of our experimental factors (**Table 2** and **Figure 2**).

Experiment 2: Effects of Artificial Light at Night on Decomposition and Litter-Layer Invertebrates While Controlling Aboveground Vegetation

During the 120-day period of this experiment, mean daily rainfall was 4.75 mm. This is approximately 78% greater than during the previous 30 years.

In the second experiment, we did not observe an effect of ALAN on the proportion of leaf litter that broke down after 120 days [$F_{(1,\ 8)} = 0.648$, $P = 0.44$]. The nitrogen content of the remaining litter was also not significantly affected by ALAN [$F_{(1,\ 8)} = 1.544$, $P = 0.249$].

We recovered and identified 348 invertebrates belonging to 7 orders from the experimental litterbags

TABLE 1 | Results of a linear-mixed-effects model to investigate the interactive effects of artificial light at night (ALAN) and litterbag mesh size on litter breakdown.

Source of variation	S.S.	M.S.	Numerator DF	Denominator DF	*F*	*P*
ALAN	0.01	0.01	1	6.17	4.81	0.07
Mesh size	0.27	0.13	2	33.89	63.21	<0.001***
ANPP	0.01	0.01	1	4.32	3.33	0.14
Time	0.12	0.12	1	33.89	55.68	<0.001***
ALAN × Mesh size	0.01	0.01	2	33.89	3.12	0.06

*Proportion broken down was calculated as (1-Final/Initial Mass). *** Significant at the a = 0.001 confidence level. Other variables included in the model include aboveground net primary production (ANPP) and length of time in the field (time).*

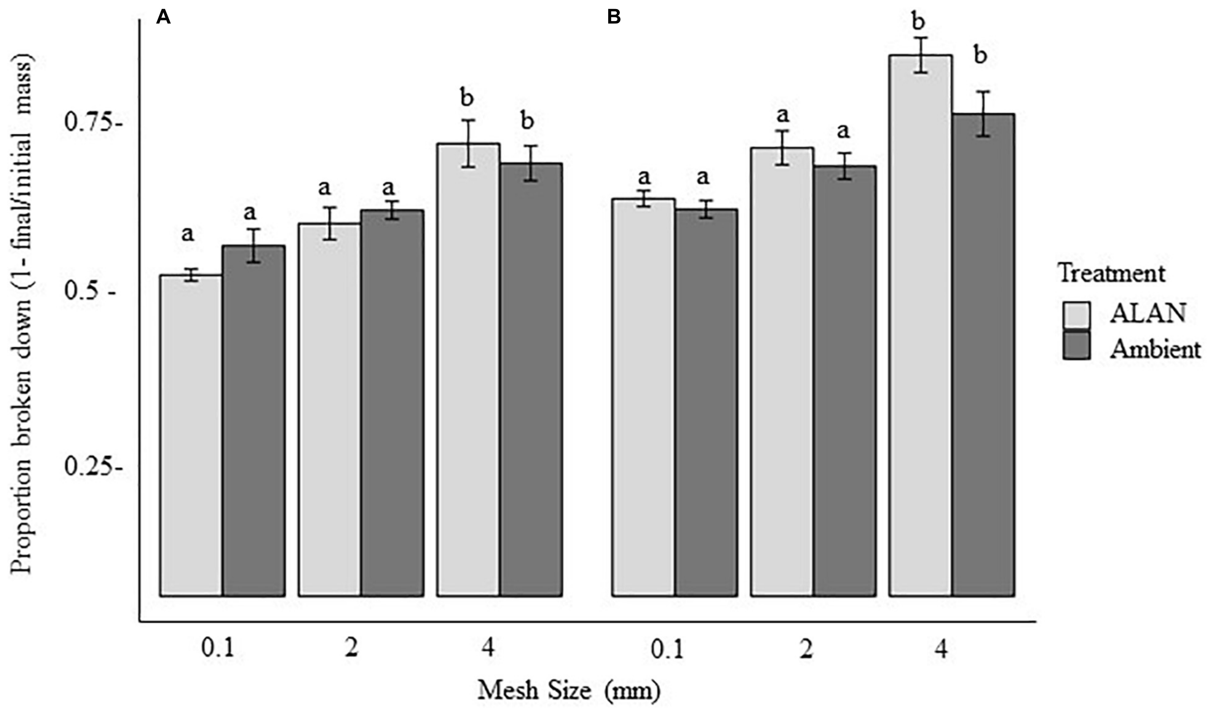

FIGURE 1 | Effect of litterbag mesh size and artificial light at night (ALAN) on the proportion of leaf litter lost (mean ± SE) after **(A)** 30 days or **(B)** 60 days in the field. Means significantly different (within the 30- or 60-day exposure times) are marked by different letters ($P < 0.05$, based on Tukey least-squares means comparisons).

TABLE 2 | Results of a linear-mixed-effects model to investigate the interactive effects of artificial light at night (ALAN) and litterbag mesh size on remaining nitrogen (%) in litter.

Source of variation	S.S.	M.S.	Numerator DF	Denominator DF	*F*	*P*
ALAN	344.24	344.24	1	8.78	3.12	0.11
Mesh size	607.92	303.96	2	37.98	2.76	0.08
ANPP	5.95	5.95	1	2.89	0.05	0.83
Time	91.95	91.95	1	37.98	0.83	0.37
ALAN × Mesh size	90.83	45.42	2	37.98	0.41	0.67

Other variables included in the model include aboveground net primary production (ANPP) and length of time in the field (time).

(**Supplementary Figure 2** and **Supplementary Table 1**). On average there were 65% more invertebrates in litterbags exposed to ALAN compared to ambient light [$F_{(1,\ 8)} = 12.33$, $P = 0.008$, **Figure 3**]. There was a significant multivariate effect of ALAN on the abundances of primary, secondary, and secondary/tertiary consumers (Pillai's trace = 0.75, $F = 6.025$, $P = 0.031$). Litterbags exposed to ALAN contained 4.6 times more secondary consumers [$F_{(1,\ 8)} = 6.688$, $P = 0.032$] and 3.5 times as many secondary/tertiary consumers [$F_{(1,\ 8} = 5.563$, $P = 0.045$] than litterbags exposed only to ambient light. In contrast, there was

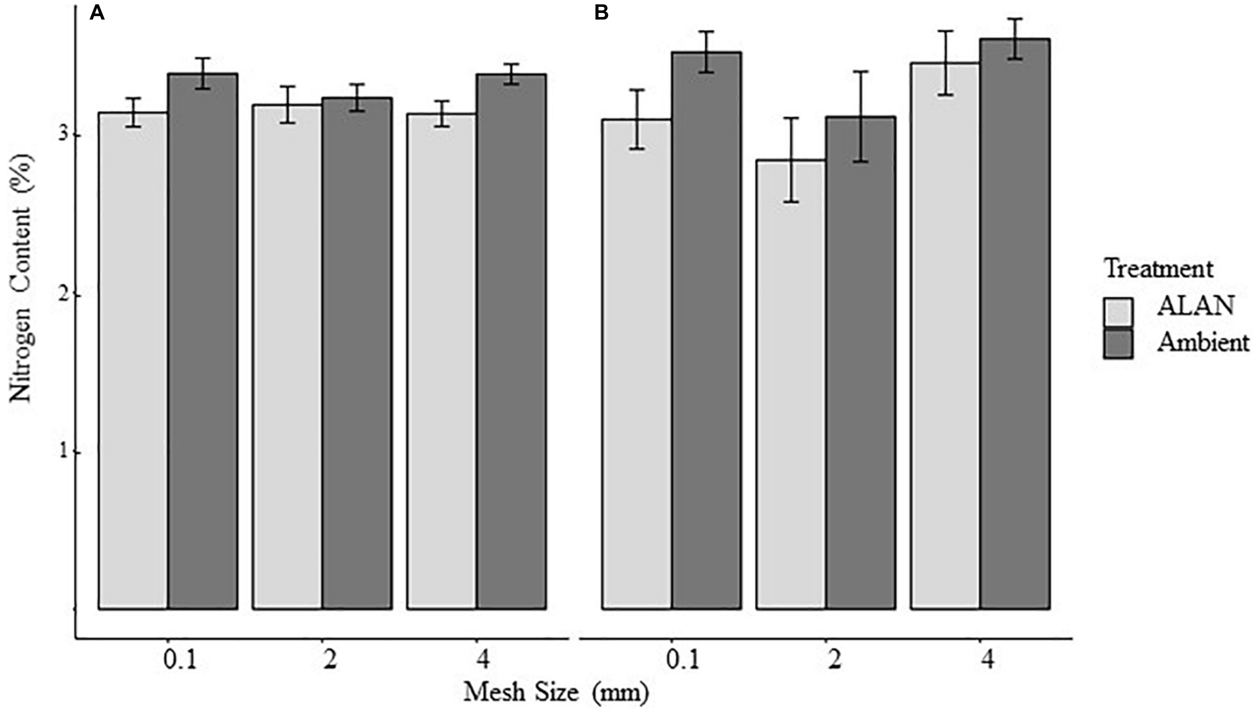

FIGURE 2 | Effect of litterbag mesh size and artificial light at night (ALAN) on the nitrogen content of litter retrieved from the field after **(A)** 30 days or **(B)** 60 days. Nitrogen is expressed as percent of tissue (mean ± SE).

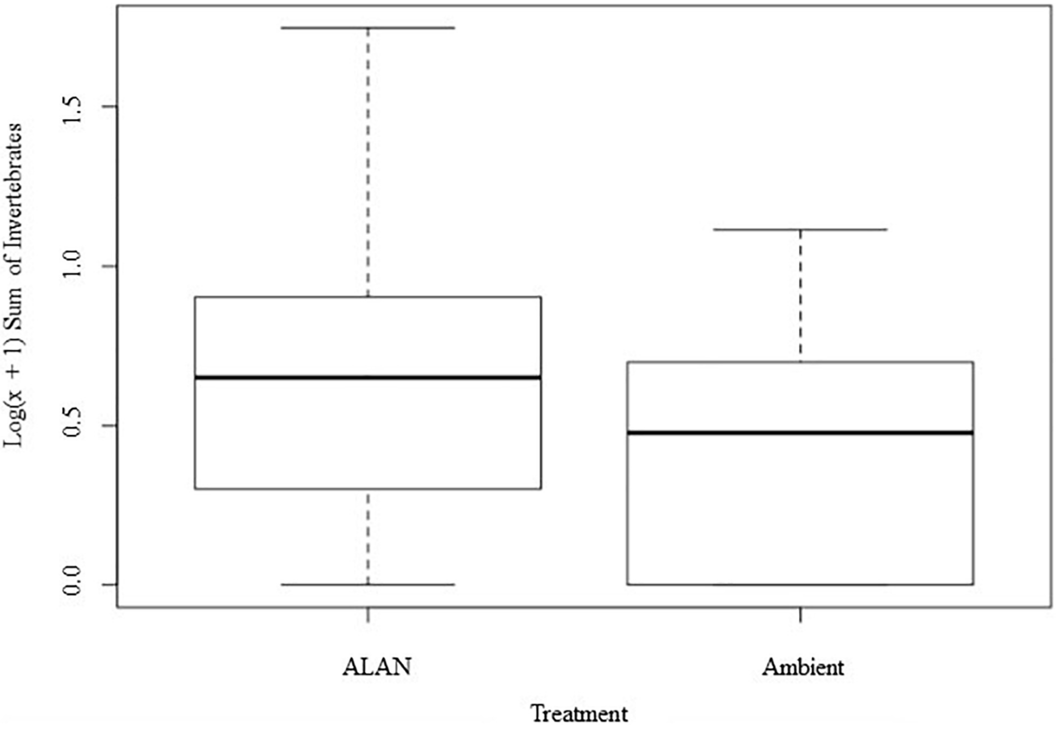

FIGURE 3 | Effect of artificial light at night (ALAN) on log-transformed number of invertebrates recovered and identified from litterbags for each light treatment. Data are represented in a box and whisker plot indicating the median, interquartile range, and minimum and maximum values.

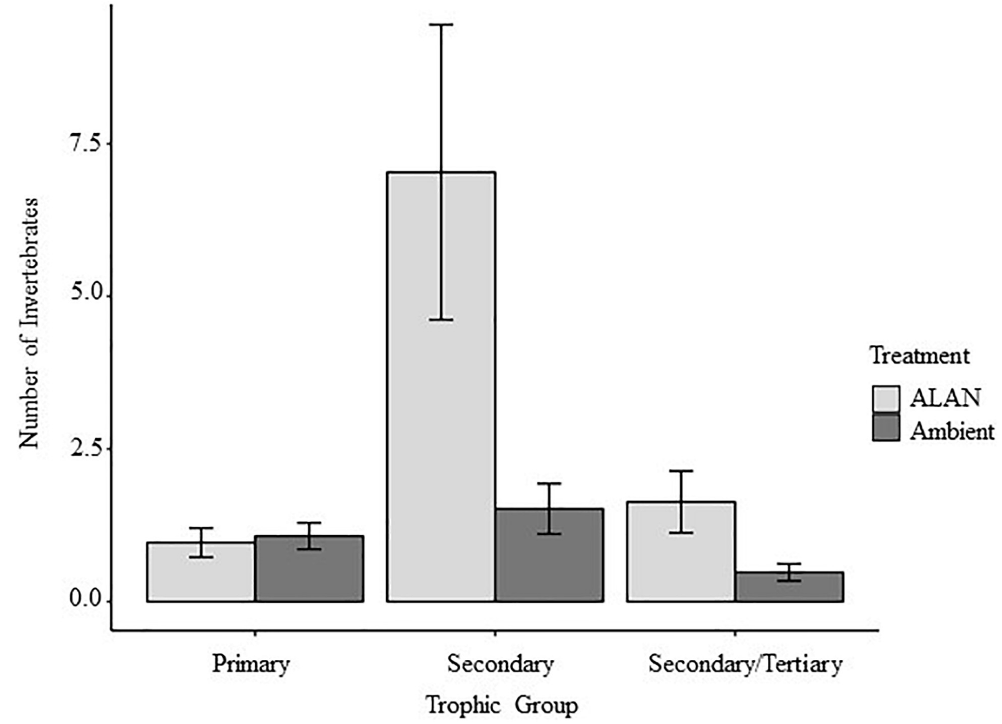

FIGURE 4 | Effects artificial light at night (ALAN) on numbers of invertebrate primary consumers, secondary consumers, and secondary/tertiary consumers (mean ± SE) recovered from litterbags.

no significant effect of ALAN on the abundance of primary consumers [$F_{(1,\,8)} = 0.134$, $P = 0.724$].

DISCUSSION

To our knowledge, this is the first study to evaluate the effects of ALAN on the breakdown of plant litter in a terrestrial ecosystem. However, a recent study showed that ALAN-induced changes in microbial communities can impact the decomposition of plant litter in polluted streams (Pu et al., 2019). Consistent with prior research (Bradford et al., 2002), we found that the rate of breakdown of leaf litter within litterbags increased with mesh size (**Table 1** and **Figure 1**). The phenomenon of greater litter breakdown with larger mesh sizes is thought to be caused by biotic factors including increased litter shredding and removal by detritivores as well as abiotic factors such as light, moisture, and temperature (Lecerf, 2017). We also observed that the proportion of leaf litter broken down was, on average, 11% higher under ALAN compared with ambient-lit plots in 2017 (although this effect was not significant, $P = 0.07$; **Figure 1**). The proportion of litter broken down was 10% higher, on average, after 60 days in 2017 than after 120 days in 2018. This is likely the result of the higher nutritional quality of the litter used in 2017 (3.36 ± 0.32%N) than in 2018 (1.02 ± 0.33%N). Litter of poor nutritional quality, or having lower nitrogen content, decomposes slowly compared to nutrient-rich litter, which is favored by detritivores (Smith and Bradford, 2003; Hessen et al., 2004). The lower amounts of breakdown in 2018 may have reduced our ability to detect potential effects of ALAN on litter breakdown in our second experiment. Lower foliar nitrogen has been reported for C_4 plants (like those used in both of our experiments) compared with C_3 plants (Sage and Pearcy, 1987). It is possible that ALAN may be more likely to affect the breakdown of nutrient-rich than nutrient-poor plant tissues, but further research is needed to resolve this question.

We detected greater abundances of invertebrates in litterbags exposed to ALAN compared with ambient light in our 2018 experiment ($P = 0.008$, **Figure 3**). Consistent with previous research on effects of ALAN on trophic structure within ground-dwelling arthropods (Davies et al., 2012, 2017), we found that ALAN increased the abundances of invertebrate secondary and/or tertiary consumers but had no effect on the abundance of primary consumers (**Figure 4**). Addition of ALAN may have slightly increased the rate at which plant litter decomposed (**Table 1** and **Figure 1**); however, there was no significant effect of ALAN on litter breakdown ($P = 0.07$). Thus, our prediction that ALAN would reduce rates of litter breakdown indirectly via increased top-down control of primary consumers by secondary and/or tertiary consumers was not supported. This prediction may have been based on an oversimplified view of food-web dynamics in the litter layer. Greater local abundance of predators can sometimes lead to increased intraguild predation, releasing primary consumers from top-down control (Finke and Denno, 2005). This might explain why litterbags with larger mesh sizes tend to have higher abundance, diversity, and food web complexity of litter-dwelling fauna, but rates of plant litter decomposition tend to be high despite the presence of secondary and tertiary consumers (Swift et al., 1979; Vossbrinck et al., 1979; Bradford et al., 2002; Bokhorst and Wardle, 2013; Liu et al., 2019). Higher intraguild predation under ALAN could potentially explain the lack of an effect of ALAN on primary consumer abundance and litter decomposition in our study. However, experimental approaches that allow direct control over food web structure, combined with manipulations of ALAN, will likely be needed to fully understand how ALAN alters predator-prey interactions among soil fauna.

This work confirms the findings of others (Davies et al., 2012, 2017) that ALAN leads to higher local densities of ground-dwelling predaceous invertebrates. However, effects of ALAN on secondary and tertiary consumer invertebrates may differ by habitat type or depending on the taxa present. For example, Manfrin et al. (2017) found that, in a riparian environment, in contrast to the grassland ecosystem studied here, ALAN increased abundances of Araneae but decreased abundances of carabid beetles. Contrary to our prediction, we found that increased abundance of arthropod secondary/tertiary consumers under ALAN did not suppress the breakdown of plant litter. This indicates that detritivores in the litter layer may not be subjected to increased predation under ALAN. Further work is needed to elucidate the activities of predaceous ground-dwelling invertebrates under ALAN, for example by investigating potential increases in intraguild predation. In addition, trophic dynamics under point sources of ALAN takes place within a matrix of darker habitats. Although ALAN is known to influence the movement behaviors of many arthropod taxa (Degen et al., 2016; Manfrin et al., 2017; Duarte et al., 2019), little is known about how ALAN affects the net attraction of litter layer invertebrates. Determining how ALAN impacts net spatial fluxes of invertebrates of different trophic ranks will be critical for understanding the consequences of ALAN for ecosystem dynamics across landscapes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MH, HE, and KH conceived, designed the experiments, revised, and edited the manuscript. MH performed the experiments and wrote the initial manuscript. MH and KH analyzed the data. All authors contributed to the article and approved the submitted version.

FUNDING

Financial support for this research was provided by a fellowship supported by the Foundation of the State Arboretum of Virginia.

ACKNOWLEDGMENTS

We would like to recognize the following individuals for their contributions to this work: Alex Oliver, Brandon Fox, Dennis Heflin, Aaron Mills, Meg Miller, Ariel Firebaugh, and the BEF Administration and Staff.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.748983/full#supplementary-material

REFERENCES

Bardgett, R. (2005). *The Biology of Soil: A Community and Ecosystem Approach*. Oxford: OUP.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Beare, M. H., Parmelee, R. W., Hendrix, P. F., Cheng, W., Coleman, D. C., and Crossley, D. A. Jr. (1992). Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecol. Monogr.* 62, 569–591. doi: 10.2307/2937317

Bennie, J., Davies, T. W., Cruse, D., and Gaston, K. J. (2016). Ecological effects of artificial light at night on wild plants. *J. Ecol.* 104, 611–620. doi: 10.1111/1365-2745.12551

Bennie, J., Davies, T. W., Cruse, D., Inger, R., and Gaston, K. J. (2018a). Artificial light at night causes top-down and bottom-up trophic effects on invertebrate populations. *J. Appl. Ecol.* 55, 2698–2706. doi: 10.1111/1365-2664.13240

Bennie, J., Davies, T. W., Cruse, D., Bell, F., and Gaston, K. J. (2018b). Artificial light at night alters grassland vegetation species composition and phenology. *J. Appl. Ecol.* 55, 442–450. doi: 10.1111/1365-2664.12927

Bokhorst, S., and Wardle, D. A. (2013). Microclimate within litter bags of different mesh size: implications for the "arthropod effect"on litter decomposition. *Soil Biol. Biochem.* 58, 147–152.

Bradford, M. A., Tordoff, G. M., Eggers, T., Hefin Jones, T., and Newington, J. E. (2002). Microbiota, fauna, and mesh size interactions in litter decomposition. *Oikos* 99, 317–323. doi: 10.1034/j.1600-0706.2002.990212.x

Briones, M. J. I. (2014). Soil fauna and soil functions: a jigsaw puzzle. *Front. Environ. Sci. Eng. China* 2:7. doi: 10.3389/fenvs.2014.00007

Cole, L., Bradford, M. A., Shaw, P. J. A., and Bardgett, R. D. (2006). The abundance, richness and functional role of soil meso- and macrofauna in temperate grassland—a case study. *Appl. Soil Ecol.* 33, 186–198. doi: 10.1016/j.apsoil.2005.11.003

Coleman, D. C., Crossley, D. A., and Hendrix, P. F. (2004). "Soil food webs: detritivory and microbivory in soils," in *Fundamentals of Soil Ecology*, eds D. C. Coleman, D. A. Crossley, and P. F. Hendrix (Cambridge, MA: Academic Press), 227–246. doi: 10.1016/b978-012179726-3/50007-1

Davies, T. W., Bennie, J., Cruse, D., Blumgart, D., Inger, R., and Gaston, K. J. (2017). Multiple night-time light-emitting diode lighting strategies impact grassland invertebrate assemblages. *Glob. Change Biol.* 23, 2641–2648.

Davies, T. W., Bennie, J., and Gaston, K. J. (2012). Street lighting changes the composition of invertebrate communities. *Biol. Lett.* 8, 764–767. doi: 10.1098/rsbl.2012.0216

Degen, T., Mitesser, O., Perkin, E. K., Weiß, N.-S., Oehlert, M., Mattig, E., et al. (2016). Street lighting: sex-independent impacts on moth movement. *J. Anim. Ecol.* 85, 1352–1360. doi: 10.1111/1365-2656.12540

Desouhant, E., Gomes, E., Mondy, N., and Amat, I. (2019). Mechanistic, ecological, and evolutionary consequences of artificial light at night for insects: review and prospective. *Entomol. Exp. Appl.* 167, 37–58. doi: 10.1111/eea.12754

Duarte, C., Quintanilla-Ahumada, D., Anguita, C., Manríquez, P. H., Widdicombe, S., Pulgar, J., et al. (2019). Artificial light pollution at night (ALAN) disrupts the distribution and circadian rhythm of a sandy beach isopod. *Environ. Pollut.* 248, 565–573. doi: 10.1016/j.envpol.2019.02.037

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C. M., Elvidge, C. D., Baugh, K., et al. (2016). The new world atlas of artificial night sky brightness. *Sci. Adv.* 2:e1600377. doi: 10.1126/sciadv.1600377

Falchi, F., Furgoni, R., Gallaway, T. A., Rybnikova, N. A., Portnov, B. A., Baugh, K., et al. (2019). Light pollution in USA and Europe: the good, the bad and the ugly. *J. Environ. Manage.* 248:109227. doi: 10.1016/j.jenvman.2019.06.128

Finke, D. L., and Denno, R. F. (2005). Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecol. Lett.* 8, 1299–1306. doi: 10.1111/j.1461-0248.2005.00832.x

Firebaugh, A., and Haynes, K. J. (2016). Experimental tests of light-pollution impacts on nocturnal insect courtship and dispersal. *Oecologia* 182, 1203–1211. doi: 10.1007/s00442-016-3723-1

García-Palacios, P., Ashley Shaw, E., Wall, D. H., and Hättenschwiler, S. (2016). Temporal dynamics of biotic and abiotic drivers of litter decomposition. *Ecol. Lett.* 19, 554–563. doi: 10.1111/ele.12590

Gaston, K. J., Bennie, J., Davies, T. W., and Hopkins, J. (2013). The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev.* 88, 912–927. doi: 10.1111/brv.12036

Gaston, K. J., Duffy, J. P., Gaston, S., Bennie, J., and Davies, T. W. (2014). Human alteration of natural light cycles: causes and ecological consequences. *Oecologia* 176, 917–931. doi: 10.1007/s00442-014-3088-2

Gaston, K. J., Visser, M. E., and Hölker, F. (2015). The biological impacts of artificial light at night: the research challenge. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370:20140133. doi: 10.1098/rstb.2014.0133

Giavi, S., Blösch, S., Schuster, G., and Knop, E. (2020). Artificial light at night can modify ecosystem functioning beyond the lit area. *Sci. Rep.* 10:11870. doi: 10.1038/s41598-020-68667-y

Grenis, K., and Murphy, S. M. (2019). Direct and indirect effects of light pollution on the performance of an herbivorous insect. *Insect Sci.* 26, 770–776. doi: 10.1111/1744-7917.12574

Hättenschwiler, S., Tiunov, A. V., and Scheu, S. (2005). Biodiversity and litter decomposition in terrestrial ecosystems. *Annu. Rev. Ecol. Evol. Syst.* 36, 191–218. doi: 10.1146/annurev.ecolsys.36.112904.151932

Hawlena, D., Strickland, M. S., Bradford, M. A., and Schmitz, O. J. (2012). Fear of predation slows plant-litter decomposition. *Science* 336, 1434–1438. doi: 10.1126/science.1220097

Hedlund, K., and Ohrn, M. S. (2000). Tritrophic interactions in a soil community enhance decomposition rates. *Oikos* 88, 585–591. doi: 10.1034/j.1600-0706.2000.880315.x

Heneghan, L., Coleman, D. C., Zou, X., Crossley, D. A., and Haines, B. L. (1998). Soil microarthropod community structure and litter decomposition dynamics: a study of tropical and temperate sites. *Appl. Soil Ecol.* 9, 33–38. doi: 10.1016/S0929-1393(98)00050-X

Hessen, D. O., Ågren, G. I., Anderson, T. R., Elser, J. J., and de Ruiter, P. C. (2004). Carbon sequestration in ecosystems: the role of stoichiometry. *Ecology* 85, 1179–1192. doi: 10.1890/02-0251

Hölker, F., Wolter, C., Perkin, E. K., and Tockner, K. (2010). Light pollution as a biodiversity threat. *Trends Ecol. Evol.* 25, 681–682. doi: 10.1016/j.tree.2010.09.007

Holzhauer, S. I. J., Franke, S., Kyba, C. C. M., Manfrin, A., Klenke, R., Voigt, C. C., et al. (2015). Out of the dark: establishing a large-scale field experiment to assess the effects of artificial light at night on species and food webs. *Sustain. Sci. Pract. Policy* 7, 15593–15616. doi: 10.3390/su71115593

Kajak, A. (1995). The role of soil predators in decomposition processes. *Eur. J. Entomol.* 92, 573–580.

Knop, E., Zoller, L., Ryser, R., Gerpe, C., Hörler, M., and Fontaine, C. (2017). Artificial light at night as a new threat to pollination. *Nature* 548, 206–209. doi: 10.1038/nature23288

Kyba, C. C. M., Kuester, T., de Miguel, A. S., Baugh, K., Jechow, A., Hölker, F., et al. (2017). Artificially lit surface of Earth at night increasing in radiance and extent. *Sci. Adv.* 3:e1701528. doi: 10.1126/sciadv.1701528

Lawrence, K. L., and Wise, D. H. (2000). Spider predation on forest-floor Collembola and evidence for indirect effects on decomposition. *Pedobiologia* 44, 33–39. doi: 10.1078/S0031-4056(04)70026-8

Lawrence, K. L., and Wise, D. H. (2004). Unexpected indirect effect of spiders on the rate of litter disappearance in a deciduous forest. *Pedobiologia* 48, 149–157. doi: 10.1016/j.pedobi.2003.11.001

Lecerf, A. (2017). Methods for estimating the effect of litterbag mesh size on decomposition. *Ecol. Model.* 362, 65–68. doi: 10.1016/j.ecolmodel.2017.08.011

Lenth, R., Singmann, H., Love, J., Buerkner, P., and Herve, M. (2019). *Package 'emmeans': Estimated Marginal Means, Aka Least-Squares Means. Version 1.4. 3.01. CRAN.*

Liu, Y., Wang, L., He, R., Chen, Y., Xu, Z., Tan, B., et al. (2019). Higher soil fauna abundance accelerates litter carbon release across an alpine forest-tundra ecotone. *Sci. Rep.* 9:10561. doi: 10.1038/s41598-019-47072-0

Longcore, T., and Rich, C. (2004). Ecological light pollution. *Front. Ecol. Environ.* 2, 191–198. doi: 10.1890/1540-92952004002[0191:ELP]2.0.CO;2

Macgregor, C. J., Evans, D. M., Fox, R., and Pocock, M. J. O. (2017). The dark side of street lighting: impacts on moths and evidence for the disruption of nocturnal pollen transport. *Glob. Change Biol.* 23, 697–707. doi: 10.1111/gcb.13371

Maggi, E., Bongiorni, L., Fontanini, D., Capocchi, A., Dal Bello, M., Giacomelli, A., et al. (2019). Artificial light at night erases positive interactions across trophic levels. *Funct. Ecol.* 895:201. doi: 10.1111/1365-2435.13485

Manfrin, A., Lehmann, D., van Grunsven, R. H. A., Larsen, S., Syväranta, J., Wharton, G., et al. (2018). Dietary changes in predators and scavengers in a nocturnally illuminated riparian ecosystem. *Oikos* 127, 960–969. doi: 10.1111/oik.04696

Manfrin, A., Singer, G., Larsen, S., Weiß, N., van Grunsven, R. H. A., Weiß, N.-S., et al. (2017). Artificial light at night affects organism flux across ecosystem boundaries and drives community structure in the recipient ecosystem. *Front. Environ. Sci. Eng. China* 5:61. doi: 10.3389/fenvs.2017.00061

McGuire, K. L., and Treseder, K. K. (2010). Microbial communities and their relevance for ecosystem models: decomposition as a case study. *Soil Biol. Biochem.* 42, 529–535. doi: 10.1016/j.soilbio.2009.11.016

McMunn, M. S., Yang, L. H., Ansalmo, A., Bucknam, K., Claret, M., Clay, C., et al. (2019). Artificial light increases local predator abundance, predation rates, and herbivory. *Environ. Entomol.* 48, 1331–1339.

Melguizo-Ruiz, N., Jiménez-Navarro, G., De Mas, E., Pato, J., Scheu, S., Austin, A. T., et al. (2020). Field exclusion of large soil predators impacts lower trophic levels and decreases leaf-litter decomposition in dry forests. *J. Anim. Ecol.* 89, 334–346. doi: 10.1111/1365-2656.13101

Meyer, L. A., and Sullivan, S. M. P. (2013). Bright lights, big city: influences of ecological light pollution on reciprocal stream–riparian invertebrate fluxes. *Ecol. Appl.* 23, 1322–1330. doi: 10.1890/12-2007.1

Moran, M. D., Rooney, T. P., and Hurd, L. E. (1996). Top-down cascade from a bitrophic predator in an old-field community. *Ecology* 77, 2219–2227. doi: 10.2307/2265715

Olivier, J., and Norberg, M. M. (2010). Positively skewed data: revisiting the box-cox power transformation. *Int. J. Psychol. Res.* 3, 68–95.

Paradis, E., and Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35, 526–528.

Pu, G., Zeng, D., Mo, L., He, W., Zhou, L., Huang, K., et al. (2019). Does artificial light at night change the impact of silver nanoparticles on microbial decomposers and leaf litter decomposition in streams? *Environ. Sci. Nano* 6, 1728–1739. doi: 10.1039/C9EN00081J

Quinn, G. P., and Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press.

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Ruess, L., and Ferris, H. (2004). Decomposition pathways and successional changes. *Nematol. Monogr. Perspect.* 2, 547–556.

Sage, R. F., and Pearcy, R. W. (1987). The nitrogen use efficiency of C3 and C4 plants: II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus*. *Plant Physiol.* 84, 959–963.

Sanders, D., and Gaston, K. J. (2018). How ecological communities respond to artificial light at night. *J. Exp. Zool. A Ecol. Integr. Physiol.* 329, 394–400. doi: 10.1002/jez.2157

Sanders, D., Kehoe, R., Cruse, D., van Veen, F. J. F., and Gaston, K. J. (2018). Low levels of artificial light at night strengthen top-down control in insect food web. *Curr. Biol.* 28, 2474–2478.e3. doi: 10.1016/j.cub.2018.05.078

Sanders, D., Kehoe, R., Tiley, K., Bennie, J., Cruse, D., Davies, T. W., et al. (2015). Artificial nighttime light changes aphid-parasitoid population dynamics. *Sci. Rep.* 5:15232. doi: 10.1038/srep15232

Santos, P. F., Phillips, J., and Whitford, W. G. (1981). The role of mites and nematodes in early stages of buried litter decomposition in a desert. *Ecology* 62, 664–669. doi: 10.2307/1937734

Scheu, S., and Setälä, H. (2002). "Multitrophic interactions in decomposer food," in *Multitrophic Level Interactions*, eds T. Tscharntke, and B. A. Hawkins (Cambridge: Cambridge University Press), 223.

Schmitz, O. J. (2007). Predator diversity and trophic interactions. *Ecology* 88, 2415–2426.

Schmitz, O. J. (2009). Effects of predator functional diversity on grassland ecosystem function. *Ecology* 90, 2339–2345.

Schmitz, O. J., Hawlena, D., and Trussell, G. C. (2010). Predator control of ecosystem nutrient dynamics. *Ecol. Lett.* 13, 1199–1209. doi: 10.1111/j.1461-0248.2010.01511.x

Setälä, H., Marshall, V. G., and Trofymow, J. A. (1996). Influence of body size of soil fauna on litter decomposition and 15N uptake by poplar in a pot trial. *Soil Biol. Biochem.* 28, 1661–1675. doi: 10.1016/s0038-0717(96)00252-0

Smith, V. C., and Bradford, M. A. (2003). Litter quality impacts on grassland litter decomposition are differently dependent on soil fauna across time. *Appl. Soil Ecol.* 24, 197–203. doi: 10.1016/S0929-1393(03)00094-5

Sullivan, S. M. P., Hossler, K., and Meyer, L. A. (2019). Artificial lighting at night alters aquatic-riparian invertebrate food webs. *Ecol. Appl.* 29:e01821. doi: 10.1002/eap.1821

Swift, M. J., Heal, O. W., Anderson, J. M., and Anderson, J. M. (1979). *Decomposition in Terrestrial Ecosystems*. Berkeley, CA: University of California Press.

Tonin, A. M., Pozo, J., Monroy, S., Basaguren, A., Pérez, J., Gonçalves, J. F. Jr., et al. (2018). Interactions between large and small detritivores influence how biodiversity impacts litter decomposition. *J. Anim. Ecol.* 87, 1465–1474. doi: 10.1111/1365-2656.12876

Ver Hoef, J. M., and Cressie, N. (1993). "Spatial statistics: analysis of field experiments," in *Design and Analysis of Ecological Experiments*, eds S. M. Scheiner, and J. Gurevitch (New York, NY: Chapman and Hall), 319–341.

Vossbrinck, C. R., Coleman, D. C., and Woolley, T. A. (1979). Abiotic and biotic factors in litter decomposition in a sermiarid grassland. *Ecology* 60, 265–271. doi: 10.2307/1937654

Wall, D. H., and Moore, J. C. (1999). Interactions underground: soil biodiversity, mutualism, and ecosystem processes. *Bioscience* 49, 109–117. doi: 10.2307/1313536

Wallwork, J. A. (1970). *Ecology of Soil Animals*. London: McGraw-Hill.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H., and Wall, D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science* 304, 1629–1633. doi: 10.1126/science.1094875

Williamson, M., and Gaston, K. J. (1999). A simple transformation for sets of range sizes. *Ecography* 22, 674–680. doi: 10.1111/j.1600-0587.1999.tb00516.x

Willmott, N. J., Henneken, J., Elgar, M. A., and Jones, T. M. (2019). Guiding lights: foraging responses of juvenile nocturnal orb-web spiders to the presence of artificial light at night. *Ethology* 125, 289–297. doi: 10.1111/eth.12852

Wolff, R. J. (1982). Nocturnal activity under artificial lights by the jumping spider Sitticus fasciger. *Peckhamia* 2:32.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
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ORIGINAL RESEARCH
published: 21 October 2021
doi: 10.3389/fevo.2021.735112

The Influence of Different Light Wavelengths of Anthropogenic Light at Night on Nestling Development and the Timing of Post-fledge Movements in a Migratory Songbird

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *02 July 2021*
Accepted: *01 October 2021*
Published: *21 October 2021*

Citation:
Bani Assadi S and Fraser KC (2021) The Influence of Different Light Wavelengths of Anthropogenic Light at Night on Nestling Development and the Timing of Post-fledge Movements in a Migratory Songbird. Front. Ecol. Evol. 9:735112. doi: 10.3389/fevo.2021.735112

Many different aspects of an animal's lifecycle such as its behavior, patterns of hormone activity, and internal clock time, can be affected by anthropogenic light at night (ALAN). Exposing an organism to ALAN during its early life could also have an impact on its development. Since photoperiod can trigger or schedule the migration timing of long-distance migratory birds, there is great potential for anthropogenic light to interact with photoperiod to affect timing. However, very little has been investigated regarding the impacts of ALAN on post-hatching development and migration timing. We investigated the impact of ALAN during nestling development in a long-distance migratory songbird to determine the potential impact on the timing of post-breeding movements in the wild. We experimentally manipulated the light by using programmable lighting, in the nest boxes of free-living nestlings of purple martin (*Progne subis*) in Manitoba, Canada. We exposed two groups of developing nestlings, from hatch to fledge date, to green or white LED lights (5 lux) during the night. We also included a control group that experienced natural, ambient light at night. We found that some adults abandoned their nests shortly after starting the experiment (4 of 15 nests in the white light treatment). For the nests that remained active, nestlings exposed to the white light treatment had higher weights (at day 20 or 22), later fledge dates (1.54 ± 0.37, 95% CI 0.80–2.28), and later colony departure date (2.84 ± 1.00, 95% CI 0.88–4.81), than young of the control group. Moreover, nestlings of both white and green light groups had longer nesting duration than nestlings of the control group. This study demonstrates the impact of ALAN on the development of post-breeding movement timing in nestlings of wild migratory birds. However, our results also indicate that green light may have less of an impact as compared to white light.

Keywords: light pollution, artificial light at night, phenotypic plasticity, post-breeding movements, migration timing, ontogenetic effect

INTRODUCTION

With more than 50% of the global human population inhabiting cities (Nations, 2012), anthropogenic light at night (ALAN), or light pollution, has become one of the problems of urban sprawl for its impact on local environments (Rich and Longcore, 2005; Chepesiuk, 2009; Dominoni D. M., 2015). It is hypothesized that artificial light can be perceived as an extension of photoperiod in birds (Farner, 1964), where photoperiod can have a strong role in synchronizing internal clock time with seasonal rhythm (Berson et al., 2002). A study by Dominoni and Partecke (2015) on European blackbird (*Turdus merula*) showed that the impacts of light pollution on a bird's physiology and seasonal activities are comparable to the influence of longer photoperiods. Therefore, light pollution through its impact on internal clock time which controls many physiological processes (Foster and Kreitzmann, 2004), could impact different aspects of many animal's lifecycles, such as their natural behavior, and patterns of hormone activity (Rich and Longcore, 2005). Previous studies have revealed an impact of ALAN on the timing of activities such as the timing of singing of songbirds (e.g., Miller, 2006; Kempenaers et al., 2010; Da Silva et al., 2015), the timing of reproductive maturity (e.g, Dominoni and Partecke, 2015), and molt (Dominoni D. et al., 2013). For example, in European blackbird, urban light pollution (0.3 lux) resulted in an advance in their physiological phenotypes, where ALAN contributed to advances in the onset of reproductive development by 26 days (Dominoni D. et al., 2013). In general, and across the annual cycle, changes in timing as a result of exposure to ALAN may have negative fitness consequences, particularly if birds become mismatched with the timing of key resources needed for migration or breeding (Visser and Gienapp, 2019).

There is great potential for anthropogenic light to interact with natural photoperiod to influence perceived photoperiod and affect migration timing which could have fitness consequences (De Jong et al., 2015). However, there have been few studies on the impact of ALAN on the migration timing of animals, particularly birds. For example, Riley et al. (2013) found delays in the dispersal of Atlantic salmon (*Salmo salar*) fry who were exposed to ALAN. Also, Smith et al. (2021) showed that adult purple martins (*Progne subis*) who experienced ALAN for more than 10 nights, initiated spring migration 8 days earlier than others who experienced natural darkness. This advance in timing was not compensated for during migration and birds experiencing ALAN that had left earlier also arrived at their breeding grounds 8 days earlier, suggesting the potential for mismatch between bird timing and the availability of resources in early spring. Further studies with other species and at different times of year are now required to further improve our understanding of the impact of ALAN on migration timing. Further, to our knowledge, there has been no study that has investigated the ontogenetic effects of light pollution on movement timing of long-distance migratory songbirds.

In this study, we used purple martin which is a gregarious long-distance Nearctic-Neotropical migratory songbird that journeys 10–20,000 km annually between breeding sites across eastern North American and overwintering locations in South America (Fraser et al., 2012; Neufeld et al., 2021). Considering the potential interaction between light pollution and photoperiod that may impact timing, the objectives of this study were to determine the ontogenetic effects of ALAN during nesting on the subsequent development of post-breeding movement timing in young birds. For this purpose, we experimentally exposed free-living nestlings to artificial light. To also examine whether different spectra of light have different impacts, we used both white and green lights (long and near short wavelength) in our experiment. The different impacts of green versus white light in a lab study on daily rhythms of blue tit (*Cyanistes caeruleus*) (De Jong et al., 2017), provided context for our investigation of the impacts of different wavelengths on the timing of post-breeding movements in the wild.

We subsequently tracked individuals as they fledged using an automated telemetry system (Taylor et al., 2017) to allow us to determine the responses of their timing to the simulated light pollution. We predicted that the light treatments would have some impacts on physiology as well as on timing, which could be measured through their weight and departure timing. We predicted that nestlings of the experimental groups would have later fledge dates and post-fledge movement timing in comparison with the control group. As songbirds may perceive light pollution as similar to longer day length (Dominoni and Partecke, 2015), we expected that birds in the experimental groups would have later timing of post-breeding movements. This is because a longer day length at the study latitude would simulate an earlier calendar date, as days get shorter through the nesting period. It was also expected that development and weight gain in birds experiencing the light treatments would be slower than those in the control group, as they may be more active under constant light at night. Regarding the study results of De Jong et al. (2017) that both white and green light at an intensity of 5 lux had the same influence on daily rhythms of blue tit (*C. caeruleus*), we predicted the influence of both spectra of lights would be the same.

MATERIALS AND METHODS

This study was conducted at two purple martin colonies in southern Manitoba, located just south of Winnipeg (49.7348° N, 97.1301° W) and at Altona (49.126748°, –97.570463°). Purple martins are dependent upon human-supplied houses for nesting, which have multiple nest boxes per housing unit (Brown et al., 2021). We used five purple martin houses for our study. Four of these houses had a total of 14 nest boxes each and the fifth house had 32 nest boxes. We used 33 nest boxes from the location south of Winnipeg and 14 nest boxes from the location at Altona in our experiment. To investigate the impact of ALAN on the timing of post-breeding movements of juveniles, the light within purple martin nest boxes was manipulated during dark hours. Light-emitting diodes (LED) in two colors (white and green) were attached to the ceiling of each nest box before nestlings hatched. Light emitted by the LEDs was directed downward toward the nests (Raap et al., 2016b,a). To control the time that lights turned on and off, LEDs were connected to an Arduino unit (a circuit board that can be programmed with associated software to set the light schedules) and a real-time clock mounted on a circuitboard. The LEDs were programmed to turn on at sunset, stay on during the entire night, and turn off at sunrise of the next day (**Supplementary Figure 1**). The experimental period began 3–4 days before the hatch date of each nest and continued until the fledge date. The nest boxes were divided into three different experimental groups: controls (no light, dark), treatment group 1 (white light), and treatment group 2 (green light). Light intensity for both treatment groups was set at 5 lux. The dim, 5 lux was chosen to align with De Jong et al. (2017), where in the lab there was no measured difference in the impact between green and white light at this intensity. Therefore, this provided us with the opportunity to compare results and investigate impacts on the timing of post-breeding movements on a migratory species in the wild. In total, each of the control and green light groups included 16 nest boxes each, and the white light group included 15 nest boxes. To determine the fledge date and colony departure date of young, we used the Motus Wildlife Tracking System,[1] which is a continent-wide automated radio-telemetry array of receiver stations (Taylor et al., 2017). At each of our research colonies, we installed a Motus receiver, within 8–70 m of the cavities. We randomly selected individuals (3–5 where available and of adequate weight for tagging) from each nest box and equipped them with individually coded radio nanotags (NTQB2-3-2 Lotek Inc.) (0.62 g, 12 × 6 × 5 mm in length, width, and height, respectively) using a leg-loop harness design (Rappole and Tipton, 1991; Streby et al., 2015) made of black elastic sewing thread (∼0.5 mm). Tag deployment was conducted when nestlings were near fledging at the age of 20–22 days (post-hatch). At the time of tagging, the weight of each nestling was recorded by using a digital scale with a resolution of 0.01 g. The weight of a tag and harness was less than 3% of the weight of the juveniles (∼54.27 grams) (the average weight of nestlings). Each nanotag emitted a signal every 29 s and had a battery life of approximately 367 days.[2] After turning on the lights, 4 of the nests in the white light group were abandoned. In total, 61, 55, and 49 tags were deployed on nestlings from nests that remained active in each of the control, green and white light groups, respectively.

The fledge date and colony departure date were determined by using a combination of variation in signal strength of each nanotag and complementary nest checks every other day. The date of fledging was determined when after a constant signal fluctuation (indicating the tagged bird is in the nest at a constant distance from the receiver), we observed a great fluctuation in signal strength which indicates fledging from the cavity. After this great fluctuation of signal strength, we observed repetition of this pattern which shows the fledged bird was rapidly changing position in relation to the receiver. Colony departure date was determined according to the fading signals of the tagged birds and the last detections of the tagged bird at the colony site (**Supplementary Figure 2**). Biologically unrealistic false positive detections were omitted (e.g., from distant receivers pre-fledge). Where tag signals ceased (e.g., owing to tag malfunction, predation, or another unknown cause) or in cases where a constant signal indicated a tag had fallen off or a bird had died, data were removed from further analysis.

All data collection procedures and experiments were conducted in accordance with the guidelines of the University of Manitoba's Animal Care Committee who have approved this project [Animal Care Protocol Number F18-031/1(AC11388)].

Data Analysis

For examining the effect of ALAN on the timing of fledge date of juveniles and their nesting duration linear mixed-effects models (LMMs) were fit by REML using the "lme4" package (Bates et al., 2014). The variables of weight (gram), first egg date, treatment (green or white light), and the number of nestmates were assigned as fixed effects and cavity ID and colony as the random effects. As only three nests had a second-year parent and all other adults were after-second year, the age of parents was not included in the analyses. To investigate the impact of ALAN on the duration (days) at the colony (hatch to departure) and colony departure date, we used LMMs with the same variables of first egg date, treatment (green or white light), and the number of nestmates as fixed and cavity ID and colony as random effects, except for the weight of the young as this was not possible to measure after their fledging. Preliminary investigation using likelihood ratio tests revealed that the random effect of the colony was not significant in the models (fledge date: $\chi^2 = 0$, $P = 1$, duration in the nest: $\chi^2 = 0$, $P = 1$; colony departure date: $\chi^2 = 0$, $P = 0.99$; duration at the colony: $\chi^2 = 0$, $P = 1$), and the models only converged with random effect of cavity ID. Therefore, to meet model parsimony, the factor of colony was omitted from further analysis. The distribution of residuals of each model was assessed to meet the assumption of normality and equality of variance (Zuur et al., 2010). The collinearity of variables in each model was assessed, which was less than 2 for all variables. To run the possible candidate models from the full model, Akaike Information Criteria corrected for small sample size (AICc) was used (Burnham and Anderson, 2002) through the package "MuMIn" (Barton, 2019). The best model among the competitive models with ΔAICc < 2 was selected (**Supplementary Tables 1–3**) according to the highest value of the marginal R^2 (variance explained by the fixed effects) and the conditional R^2 (variance explained by the whole model) (Barton, 2019). ANCOVA was used to test the interaction of treatments (categorical variable) with first egg date (continuous variable) for both dependent factors, fledge date and colony departure date (McDonald, 2014). One-way ANOVA was used to investigate whether the mean weight of juveniles is different among different groups (white light, green light, and control), and where applicable, to explore the differences among weight means of three groups, the package "lmerTest" (Kuznetsova et al., 2017) was used to run Tukey HSD tests for *post hoc* analyses.

The survival rate of young of each nest box was calculated by dividing the number of fledged young by the number of hatched nestlings of each nest box. Due to the non-normal distribution of data, a Kruskal-Wallis test was used to compare the survivability

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[1] www.motus.org

[2] www.Lotek.com

rate among three groups (white light, green light, and control groups). All analyses were conducted in R version 3.6.3 (R Core Team, 2020).

RESULTS

We tracked the fledge date of 61, 47, and 46 individuals of the control, green light and white light groups, respectively. Among these tagged nestlings, we were able to track the colony departure dates of 45 individuals from the control group, and 20 and 33 individuals of the green and white light groups, respectively.

Nesting Duration and Fledge Date

The average nesting duration of the control, green light and white light groups were (mean ± SEM) 28.16 ± 0.15, 29 ± 0.15, and 30.11 ± 0.23 days, respectively. Nestlings exposed to green light and white light spent (estimate ± SE) 0.78 ± 0.32 (95% CI 0.14–1.41) and (estimate ± SE) 1.67 ± 0.34 (95% CI 1.00–2.34) days, respectively, longer in the nest than those nestlings who experienced natural darkness during the night (**Table 1**). Moreover, one additional nestmate and a 1-gram increase in weight resulted in nesting duration that was longer by (estimate ± SE) 0.30 ± 0.13 (95% CI 0.03–0.57) and (estimate ± SE) 0.04 ± 0.02 (95% CI 0.00–0.08) days, respectively (**Table 1**).

Overall, fledge dates ranged from 12 July to 3 August. Average fledge dates of control, green light and white light groups were dates 21 July (mean ± SEM) (203 ± 0. 59), 24 July (206 ± 0.65), and 25 July (207.39 ± 0.52), respectively. Nestlings exposed to white light fledged (estimate ± SE) 1.54 ± 0.37 (95% CI 0.80–2.28) days later than nestlings of the control group (**Table 1**). There was not a significant difference between the fledge dates of nestlings of the green light group and the control group. Moreover, first egg dates that were one day later resulted in nestling fledge dates that were (estimate ± SE) 1.01 ± 0.03 (95% CI 0.93–1.08) days (**Figure 1A** and **Table 1**) later. One more nestmates in a cavity delayed fledge date about (estimate ± SE) 0.31 ± 0.14 (95% CI 0.02–0.60) days (**Table 1**). The results of ANCOVA showed the effect of first egg date on the fledge date is independent of the treatments or different colors of ALAN and it is assumed the slopes are similar (**Figure 1A** and **Supplementary Table 4**).

Duration at the Colony and Colony Departure Date

The best model of duration at the colony did not include any of the fixed effects as influential factors (**Table 1**). Nestlings who experienced white light at night departed the colony (estimate ± SE) 2.84 ± 1.00 (95% CI 0.88–4.81) days later than those of the non-treatment group (**Table 1**). The results of ANCOVA showed the effect of first egg date on the colony departure date was similar among the different treatments of ALAN (**Figure 1B** and **Supplementary Table 4**). Moreover, one day delay in first egg date resulted in nestlings departing the colony (estimate ± SE) 0.94 ± 0.12 (95% CI 0.71–1.18) days later (**Figure 1B** and **Table 1**).

Weight

The average weight of nestlings of the control, green light and white light groups at day 20–22 were (mean ± SEM) 53.50 ± 0.63, 53.85 ± 0.65, and 56.10 ± 0.92, respectively (**Figure 2**). The differences of the mean weight of nestlings among groups were significant ($DF = 2$, $F = 3.27$, $p = 0.04$). The Tukey *post hoc* test showed the mean weight of nestlings of the white light group was significantly more than those in the control group (estimate ± SE) (2.6 ± 1.06, $p = 0.03$) when they were 20–22 days old. There was no significant difference between the weight of nestlings of the green light group and the weight of nestlings of both the control and white light groups.

Survivability Rate

Three of 16 control group nests and 4/16 green light group nests did not fledge completely with 6 and 9 nestlings lost in each group, respectively. In the white light group, 4 out of 15 nests were abandoned at the beginning of the experiment and 2 out of the remaining 11 nests did not fledge completely. This resulted in a total of 10 nestlings lost from the white light group. There was not a significant difference in the nest survivability rate between the treatment groups, (white and green light groups) and the control group, nor between the two treatment groups ($\chi^2 = 0.17$, $p = 0.91$). The actual numbers of fledged young were 79, 74 and 49 from the control, green light, and white light groups, respectively. Nestlings that disappeared between nest checks were not found and may have been taken by avian predators (Brown et al., 2021), as predator guards would prevent terrestrial predators from accessing the nest boxes.

DISCUSSION

In this study, we demonstrate for the first time that ALAN impacts the timing of the post-breeding movements of juveniles of a long-distance migratory songbird. We examined the ALAN impacts of different spectra of light (white and green lights at 5 lux) on the duration of nesting and timing of fledge and post-breeding movements. We found that the effects of different spectra differed for the post-breeding movement timing of juvenile purple martins. Our results reveal that exposing nestlings to white light with an intensity of 5 lux during the night, resulted in later fledging and colony departure as compared to nestlings who experienced either green light or natural darkness. Thus, our data reveal important effects of ALAN on timing but that these differ by spectra of light. We found that green light with an intensity of 5 lux did not influence the timing of the post-breeding movement of young purple martins. However, nestlings exposed to either white or green light had a longer nesting duration than nestlings of the control group (that experienced natural darkness).

Evidence to date suggests that ALAN can influence the perception of photoperiod by birds (De Jong et al., 2015), influencing their internal clock time and consequently impacting biological functions and fitness (Farner, 1964; Dominoni D. M., 2015). Previous studies which investigated the impact of light pollution on circadian rhythms of songbirds revealed that it can

TABLE 1 | The best models of the linear mixed-effects analysis of effects of ALAN, weight, first egg date, number of nestmates on nesting period, fledge date, and effects of ALAN, first egg date, nestmate numbers on duration of staying at the colony and colony departure date.

Model		Estimate ± Std. error	95% CI (lower)	95% CI (upper)	AICc	mR^2/cR^2
Fledge date	**Fixed effects**					
	Treatment (green light)	0.54 ± 0.36	–0.15	1.25		
	Treatment (white light)*	1.54 ± 0.37	0.80	2.28	544.4	0.90/0.92
	First egg date*	1.01 ± 0.03	0.93	1.08		
	Number of nestmates*	0.31 ± 0.14	0.80	2.28		
	Random effect	**Variance**	**Std. Dev.**	**% Variance**		
	Cavity ID	0.48	0.69	24.61		
Duration in the nest (days)	**Fixed effects**					
	Treatment (green light)*	0.78 ± 0.32	0.14	1.41		
	Treatment (white light)*	1.67 ± 0.34	1.00	2.35	529.5	0.28/0.43
	Weight*	0.04 ± 0.02	0.00	0.08		
	Number of nestmates*	0.30 ± 0.13	0.03	0.57		
	Random effect	**Variance**	**Std. dev.**	**% Variance**		
	Cavity ID	0.37	0.61	21.14		
Colony departure date	**Fixed effects**					
	Treatment (green light)	0.54 ± 1.07	–1.57	2.65		
	Treatment (white light)*	2.84 ± 1.00	0.88	4.81	504.2	0.57/0.71
	First egg date*	0.94 **±** 0.12	0.70	1.18		
	Random effect	**Variance**	**Std. dev.**	**% Variance**		
	Cavity ID	3.21	1.79	32.44		
Duration at the colony (days)	**Fixed effects**					
	Null				472.4	0/0.34
	Random effect	**Variance**	**Std. dev.**	**% Variance**		
	Cavity ID	1.72	1.31	36.69		

The cavity ID is considered as a random effect.
** The significant factor.*
mR^2: marginal R^2, cR^2: conditional R^2.

cause a phase shift in their circadian rhythm (Gaston et al., 2013) and advance or delay the onset and offset of their daily activities such as singing (e.g., Kempenaers et al., 2010; Da Silva et al., 2014), foraging activity (Russ et al., 2015), and timing of reproduction (Kempenaers et al., 2010; De Jong et al., 2015). Moreover, egg-laying date is influenced by day length as one of the important zeitgebers (Lambrechts et al., 1997; Da Silva et al., 2015). De Jong et al. (2015) found when the temperature was low in spring in comparison with a warmer spring, light pollution at night was perceived as a longer photoperiod by great tit (*Parus major*) which changed the onset of egg-laying.

In addition to changes in the timing of nesting activities due to ALAN, a study by Smith et al. (2021), demonstrated the advancement of spring migration departure of adult purple martins who experienced light pollution during their overwintering period. One of the reasons posed for this advance was that ALAN led to a perception of a longer day length which mimicked a later calendar date, causing earlier development of reproductive organs (Smith et al., 2021). In this study, we showed an ontogenetic effect of ALAN at breeding sites on nestlings, which resulted in later post-breeding movements. This delay could be due to the impact of ALAN in the nest on the growth rate of nestlings, where birds exposed to ALAN are heavier leading to a delay in timing. An earlier study that compared the weights of nestlings exposed to white light (3 lux) with controls found similar results where ALAN-exposed nestlings gained more weight than those in the control group (Gagné, 2019). Previous studies revealed that a typical pattern of weight gain and loss in nestling purple martins is for them to lose weight as they approach fledge date, possibly to achieve a weight more suited to fledging and first flights (Allen and Nice, 1952; Dellinger and Rogillio, 1991; Gagné, 2019). White light may influence this natural pattern, changing the metabolism or the proportion of rest and active periods of the nestlings and increasing begging for food (Raap et al., 2016c) which could consequently prolong the duration of feeding by adults. This aligns with the results of a study by Titulaer et al. (2012) that showed an increase in the feeding rate of great tit females when nestlings were exposed to ALAN (10 lux) while they were between 9 and 16 days old. However, exposing free-living great tit nestlings to artificial light at night (3 lux), even for two nights, during their development led to substantial impacts on their physiological condition (Raap et al., 2016b) and increased their activity levels which resulted in the nestlings having no weight gain for the two nights of the experimental treatment (Raap et al., 2016a). Our results align with these earlier studies as we found that the weight of

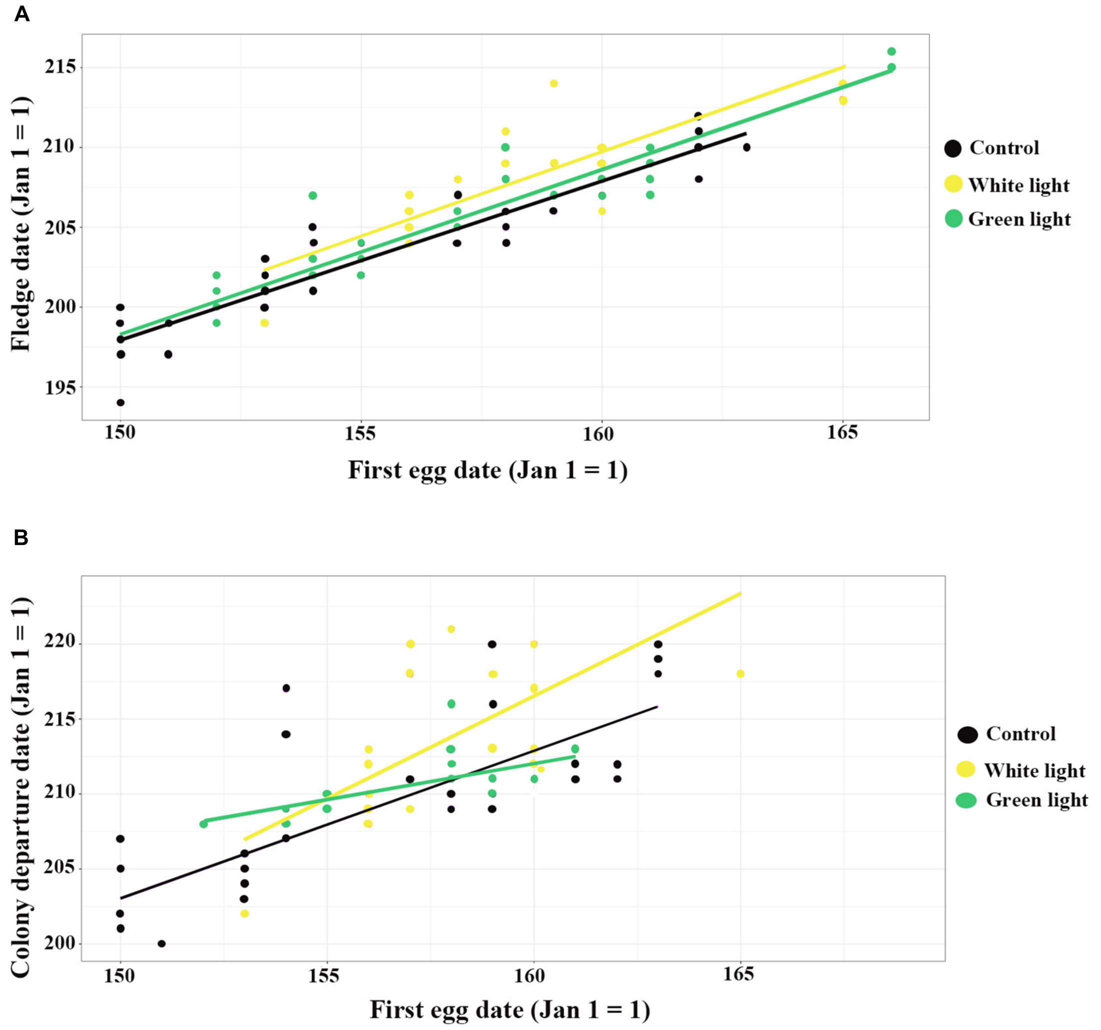

FIGURE 1 | The influence of ALAN (white and green lights) on fledge date and colony departure date, **(A)** shows the correlation between first egg date and fledge date; **(B)** shows the correlation between first egg date and colony departure date. Yellow = white light, Green: green light, and Black = control.

nestlings of the white light group was significantly more than the nestlings of the control group. We infer that given that nestling martins tend to lose weight just before fledging, that the higher weight of white light exposed nestlings may have led to the later fledge dates that we observed. However, we found no significant difference in weight between individuals in the green group and the other two groups.

While the ontogenetic effect of photoperiod on circadian rhythms of mammals has been demonstrated (e.g., Ciarleglio et al., 2011), there is scant information about the ontogenetic effects of daylength on avian migration timing (Knudsen et al., 2011). This is particularly so for the potential ontogenetic effects of light pollution. The ontogenetic effect of hatch date on spring migration timing of pied flycatcher and spring arrival time of Arctic terns (*Sterna paradisea*) has been suggested by Møller et al. (2009) and Both (2010), respectively. Moreover, in a previous study, we found phenotypic plasticity of post-breeding movement timing of young purple martin to an experimentally extended day length during their nesting (Bani Assadi and Fraser, 2021). In our study, the longer nesting period and later fledge date of nestlings exposed to the white light treatment, could potentially indicate a plasticity of post-breeding movements timing of nestlings to the ontogenetic effects of light during the nesting period, with carry-over effects on the timing of their colony departure date. How long these timing effects may last across the rest of the annual cycle, and how they trade-off these delays in timing in the next stage of their life cycle, are important areas of investigation for future research. Delays in timing may be compensated for during fall migration, or during the winter. In wood thrushes, fall migration may mitigate the carry-over effect of late-breeding timing (Stutchbury et al., 2011; Catry et al., 2013) and Gow et al. (2019) found that stationary periods during the non-breeding season in tree swallows may act as a timing reset period, removing carry-over effects on timing from the breeding season and fall migration. However, some impacts on timing may have longer-lasting effects, particularly

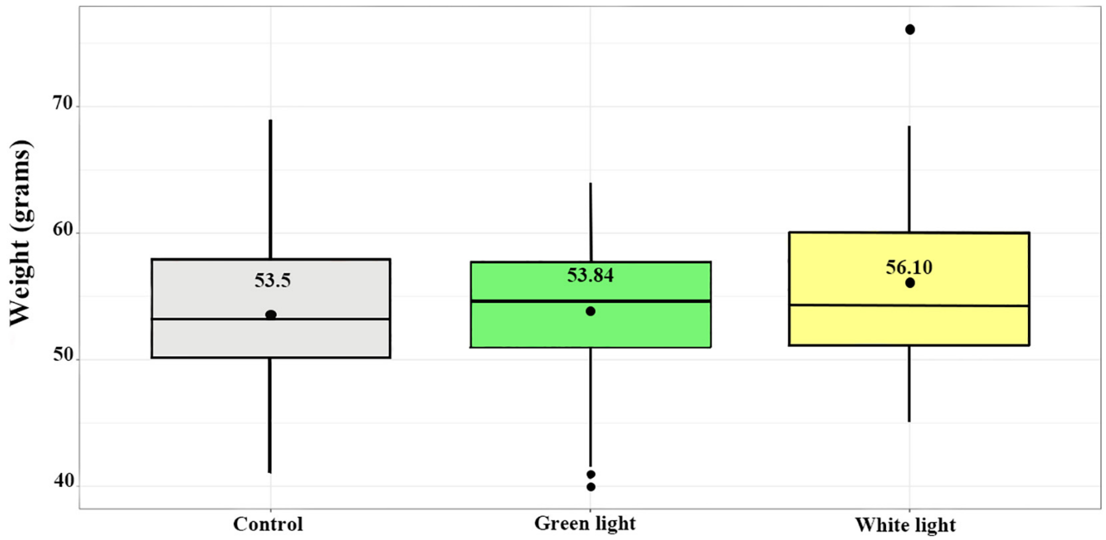

FIGURE 2 | Weights of young in the treatment groups (white and green lights) and control group. Boxes extend to upper and lower quartiles; the line indicates the median and the black point at the middle of the boxes indicates the mean. Whiskers extend to maximum and minimum values; outliers are indicated by filled points.

on juvenile birds. Ouwehand et al. (2017) showed that the carry-over effect of an experimentally delayed hatch for juvenile pied flycatchers extended to spring arrival date back at breeding sites in the following year. While we did not track the timing of our experimental birds to the subsequent spring, based upon the results of Ouwehand et al. (2017), we would expect to observe a carry-over effect of ALAN on their spring arrival date.

Similar to a study of De Jong et al. (2015), which showed a lack of impact of ALAN on the survivability rate of great tits nestlings, we did not detect a difference in the survivability rate (fledging) of the young among our three groups (white light, green light, and controls). However, previous studies have revealed some non-lethal but negative impacts of ALAN *via* increases in stress hormones (Ouyang et al., 2015) and decreases in melatonin levels (Dominoni D. M. et al., 2013). For example, a field study by Raap et al. (2016b) showed that exposing great tit nestlings to ALAN (0.3 lux) for two nights when they were 13 days old caused a deterioration of their immunity and health condition *via* a decrease in melatonin and an increase in oxidative stress and stress hormones. Melatonin is secreted by the pineal gland at night and plays an important role in maintaining the circadian rhythm (Raap et al., 2015). A disruption in circadian rhythm can impact several immune responses (Arjona et al., 2012). In our study, despite the lack of influence of two spectra of lights (white and green) on the survivability rate of young, we cannot rule out more subtle effects on their health condition and how this may influence migration. This would therefore be an important future research avenue, as the impact of ALAN on complex neuroendocrine functions, and how this may differ as they traverse different environments, is unknown (Haldar and Singh, 2001).

How different wavelengths of light may impact the behavior and physiology of birds have yielded mixed results across studies, species and time of year. For example, in a field study, Ouyang et al. (2015) showed that the concentration of stress hormone in great tits was greater when nests were closer to white lights in comparison with individuals with nests near green lights (8.2 ± 0.3 lux). Moreover, wavelengths around the blue spectrum have been demonstrated to be more influential on the reproductive physiology of birds (Dominoni D. M., 2015) and laying date (De Jong et al., 2015) than other spectra of light. In our field study, white light of higher intensity (5 lux) was influential on post-breeding movement timing of wild young purple martin, while green light with the same intensity did not have any impact. In contrast with our findings, a laboratory study by De Jong et al. (2017) revealed that at low intensity (0.5 and 1.5 lux), the daily rhythm of blue tits (*C. caeruleus*) was more disturbed under white and red lights than green light. However, they found that at a higher range of intensities (5 lux), both white and green lights had the same negative impact on the circadian rhythms of a blue tit. In another study De Jong et al. (2015), found that the lay date of great tit was influenced by white and green lights at night (8.2 ± 0.3 lux out of the nest, but 0.05 lux in the nests), however, pied flycatchers' lay date was not impacted by ALAN (De Jong et al., 2015). In contrast with this result, a study by Poot et al. (2008), showed an influence of the long-wavelength spectrum (red and white lights) where it caused nocturnal migratory birds to be disoriented during flight. In general, the greater influence of white light at low intensity across studies could be due to its greater penetration of the skull as compared to green light, where it may have a corresponding impact on photoreceptors (Hartwig and van Veen, 1979).

In addition to the impacts of our experimental treatments, we found that other factors also impacted timing as expected. For example, we found that first egg date was an influential factor in the timing of fledge date and colony departure date. The number of nestmates also impacted fledge date, which aligns with the results of Wagner et al. (1996), where an increase in the number of nestlings of purple martins increases competition for food and therefore they require more time to reach the optimal body condition for fledging. Among the zeitgebers that may influence timing, photoperiod is expected to play the largest role in synchronizing internal clock time (Gwinner, 1996; Åkesson and Helm, 2020). Therefore, it was expected that longer day lengths experienced by birds that hatched earlier would induce them to have earlier fledge dates (Coppack and Pulido, 2004). However, our investigation did not show any interactions between the experimental light treatments and first egg date.

CONCLUSION

This study demonstrated the ontogenetic effects of white ALAN during the nesting period on the timing of post-breeding movements of juvenile birds. However, we did not find any significant difference in the timing of post-breeding movements for birds that were exposed to green ALAN as compared to controls. The potential for negative carryover effects on other stages of the annual cycle (Norevik et al., 2017) or whether the carryover effects of light pollution on migration timing are compensated for during migration or during the overwintering period (Senner et al., 2014; Briedis et al., 2018; Gow et al., 2019) requires further investigation. The negative impact of white light on the circadian or circannual rhythm of young migratory songbirds that we demonstrate, and the lack of influence of green light leads to the recommendation of using a shorter wavelength (green light) for illuminating places that are close to breeding sites.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by University of Manitoba's Animal Care Committee [Animal Care Protocol Number F18 031/1(AC11388)].

AUTHOR CONTRIBUTIONS

KF designed, planned, and supervised the research, provided funding, and edited the manuscript. SB planned and performed the research, analyzed the data, and wrote the manuscript.

FUNDING

Funding and other supports were provided by John R. Evans Leaders Fund, Canadian Foundation for Innovation, Research Manitoba, the Natural Sciences and Engineering Research Council's Discovery Grant Program, the Connie Holland Bird Study Fund, and the University of Manitoba.

ACKNOWLEDGMENTS

We thank Gail Davoren, Colin Garroway, and Saman Muthukumarana for helpful comments on earlier drafts of this manuscript. We thank Leanne Neufeld for her assistance with fieldwork. We especially thank purple martin colony managers Alan Enns, Paul and Maxine Clifton for their patience and assistance with this research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.735112/full#supplementary-material

Supplementary Figure 1 | Schematic of the ALAN treatment. 5 lux of artificial white or green light were turned on at sunset and off at sunrise.

Supplementary Figure 2 | The panel shows detection data from a Motus receiver station for an individual bird. The colors represent various antennae at the station. Signal strength shows fledging date and time for hatch-year purple martin (first stage of post-fledging movement), with the initiation of fledging on July 24th, at 21:16 GMT and departure from the colony (second stage of post-fledging movement) on July 28th, at 12:00 GMT. The blank spaces demonstrate the moments that the bird left the vicinity of the tower. The signal strength before fledge date shows the time that the bird spent in the cavity.

REFERENCES

Åkesson, S., and Helm, B. (2020). Endogenous programs and flexibility in bird migration. *Front. Ecol. Evol.* 8:78. doi: 10.3389/fevo.2020.00078

Allen, R. W., and Nice, M. M. (1952). A study of the breeding biology of the Purple Martin (*Progne subis*). *Am. Mid. Nat.* 47, 606–665. doi: 10.2307/2422034

Arjona, A., Silver, A. C., Walker, W. E., and Fikrig, E. (2012). Immunity's fourth dimension: approaching the circadian–immune connection. *Trends Immunol.* 33, 607–612. doi: 10.1016/j.it.2012.08.007

Bani Assadi, S., and Fraser, K. C. (2021). Experimental manipulation of photoperiod influences migration timing in a wild, long-distance migratory songbird. *Proc. R. Soc. B.* 288:20211474. doi: 10.1098/rspb.2021.1474

Barton, K. (2019). *MuMIn: Multi-Model Inference, R Package Version 1.42.1*. Available online at: https://CRAN.R-project.org/package=MuMIn

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting linear mixed-effects models using lme4. *arXiv* [Preprint]. arXiv:1406.5823

Berson, D. M., Dunn, F. A., and Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295, 1070–1073. doi: 10.1126/science.1067262

Both, C. (2010). Flexibility oftiming ofavian migration to climate change masked by environmental constraints en route. *Curr. Biol.* 20, 243–248. doi: 10.1016/j.cub.2009.11.074

Briedis, M., Krist, M., Král, M., Voigt, C. C., and Adamík, P. (2018). Linking events throughout the annual cycle in a migratory bird-non-breeding period buffers accumulation of carry-over effects. *Behav. Ecol. Sociobiol.* 72:33.

Brown, C. R., Airola, D. A., and Tarof, S. (2021). *Purple Martin (Progne subis), version 2.0. In Birds of the World*, ed. P. G. Rodewald (Ithaca, NY: The Cornell Lab of Ornithology).

Burnham, K. P., and Anderson, D. R. (2002). *A Practical Information-Theoretic Approach. Model Selection And Multimodel Inference*. Vol. 10, New York, NY: Springer.

Catry, P., Dias, M. P., Phillips, R. A., and Granadeiro, J. P. (2013). Carry-over effects from breeding modulate the annual cycle of a long-distance migrant: an experimental demonstration. *Ecology* 94, 1230–1235. doi: 10.1890/12-2177.1

Chepesiuk, R. (2009). Missing the dark: health effects of light pollution. *Environ. Health Perspect.* 117, A20–A27.

Ciarleglio, C. M., Axley, J. C., Strauss, B. R., Gamble, K. L., and McMahon, D. G. (2011). Perinatal photoperiod imprints the clock. *Nat. Neurosci.* 14, 25–27.

Coppack, T., and Pulido, F. (2004). Photoperiodic response and the adaptability of avian life cycles to environmental change. *Adv. Ecol. Res.* 35, 131–150. doi: 10.1016/s0065-2504(04)35007-5

Da Silva, A., Samplonius, J. M., Schlicht, E., Valcu, M., and Kempenaers, B. (2014). Artificial night lighting rather than traffic noise affects the daily timing of dawn and dusk singing in common European songbirds. *Behav. Ecol.* 25, 1037–1047. doi: 10.1093/beheco/aru103

Da Silva, A., Valcu, M., and Kempenaers, B. (2015). Light pollution alters the phenology of dawn and dusk singing in common European songbirds. *Phil. Trans. R. Soc. B Biol. Sci.* 370:20140126. doi: 10.1098/rstb.2014.0126

De Jong, M., Caro, S. P., Gienapp, P., Spoelstra, K., and Visser, M. E. (2017). Early birds by light at night: effects of light color and intensity on daily activity patterns in blue tits. *J. Biol. Rhythms* 32, 323–333. doi: 10.1177/0748730417719168

De Jong, M., Ouyang, J. Q., Da Silva, A., van Grunsven, R. H., Kempenaers, B., Visser, M. E., et al. (2015). Effects of nocturnal illumination on life-history decisions and fitness in two wild songbird species. *Phil. Trans. R. Soc. B Biol. Sci.* 370:20140128. doi: 10.1098/rstb.2014.0128

Dellinger, T. B., and Rogillio, C. (1991). The graphic development of baby Purple Martins. *Purple Martin Update* 3, 6–11.

Dominoni, D. M. (2015). The effects of light pollution on biological rhythms of birds: an integrated, mechanistic perspective. *J. Ornithol.* 156, 409–418. doi: 10.1007/s10336-015-1196-3

Dominoni, D. M., and Partecke, J. (2015). Does light pollution alter daylength? A test using light loggers on free-ranging European blackbirds (*Turdus merula*). *Phil. Trans. R. Soc. B Biol. Sci.* 370:20140118. doi: 10.1098/rstb.2014.0118

Dominoni, D., Quetting, M., and Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proc. R. Soc. B Biol. Sci.* 280:20123017. doi: 10.1098/rspb.2012.3017

Dominoni, D. M., Goymann, W., Helm, B., and Partecke, J. (2013). Urban-like night illumination reduces melatonin release in European blackbirds (*Turdus merula*): implications of city life for biological time-keeping of songbirds. *Front. Zool.* 10:1–11. doi: 10.1186/1742-9994-10-60

Farner, D. S. (1964). The photoperiodic control of reproductive cycles in birds. *Am. Sci.* 52, 137–156.

Fraser, K. C., Stutchbury, B. J., Silverio, C., Kramer, P. M., Barrow, J., Newstead, D., et al. (2012). Continent-wide tracking to determine migratory connectivity and tropical habitat associations of a declining aerial insectivore. *Proc. R. Soc. B Biol. Sci.* 279, 4901–4906. doi: 10.1098/rspb.2012.2207

Foster, R. G., and Kreitzmann, L. (2004). *Rhythms of Life: The Biological Clocks That Control the Daily Lives of Every Living Thing*. New Haven, NH: Yale University Press.

Gagné, M. (2019). *The Effect Of Artificial Light At Night (Alan) On The Growth And Migration Timing Of Purple Martins (Progne Subis)*. Unpublished honours Thesis, University of Manitoba, Winnipeg.

Gaston, K. J., Bennie, J., Davies, T. W., and Hopkins, J. (2013). The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev.* 88, 912–927. doi: 10.1111/brv.12036

Gow, E. A., Burke, L., Winkler, D. W., Knight, S. M., Bradley, D. W., Clark, R. G., et al. (2019). A range-wide domino effect and resetting of the annual cycle in a migratory songbird. *Proc. R. Soc. B Biol. Sci.* 286:20181916. doi: 10.1098/rspb.2018.1916

Gwinner, E. (1996). Circadian and circannual programmes in avian migration. *J. Exp. Biol.* 199, 39–48. doi: 10.1242/jeb.199.1.39

Haldar, C., and Singh, S. S. (2001). Seasonal changes in melatonin and immunological adaptations in birds. *J. Endocrinol. Reprod.* 5, 13–24.

Hartwig, H. G., and van Veen, T. (1979). Spectral characteristics of visible radiation penetrating into the brain and stimulating extraretinal photoreceptors. *J. Comp. Physiol.* 130, 277–282. doi: 10.1007/bf00614615

Kempenaers, B., Borgström, P., Loës, P., Schlicht, E., and Valcu, M. (2010). Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds. *Curr. Biol.* 20, 1735–1739. doi: 10.1016/j.cub.2010.08.028

Knudsen, E., Lindén, A., Both, C., Jonzén, N., Pulido, F., Saino, N., et al. (2011). Challenging claims in the study of migratory birds and climate change. *Biol. Rev.* 86, 928–946.

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest Package: tests in linear mixed effects models. *J. Stat. Softw.* 82, 1–26. doi: 10.18637/jss.v082.i13

Lambrechts, M. M., Blondel, J., Maistre, M., and Perret, P. (1997). A single response mechanism is responsible for evolutionary adaptive variation in a bird's laying date. *PNAS* 94, 5153–5155. doi: 10.1073/pnas.94.10.5153

McDonald, J. H. (2014). *Handbook of Biological Statistics*, 3rd Edn. Baltimore, MD: Sparky House Publishing.

Miller, M. W. (2006). Apparent effects of light pollution on singing behavior of American robins. *Condor* 108, 130–139. doi: 10.1650/0010-5422(2006)108[0130:aeolpo]2.0.co;2

Møller, A. P., Flensted-Jensen, E., and Mardal, W. (2009). Adjustment of the annual cycle to climatic change in a long-lived migratory bird species. *Curr. Zool.* 55, 92–101. doi: 10.1093/czoolo/55.2.92

Nations, U. (2012). *World Urbanization Prospects: The 2014 Revision*, CD-ROM Edn. New York, NY: DESA.

Neufeld, L. R., Muthukumarana, S., Fischer, J. D., Ray, J. D., Siegrist, J., and Fraser, K. C. (2021). Breeding latitude is associated with the timing of nesting and migration around the annual calendar among Purple Martin (*Progne subis*) populations. *J. Ornithol.* 18, 1–16.

Norevik, G., Åkesson, S., and Hedenström, A. (2017). Migration strategies and annual space-use in an Afro-Palaearctic aerial insectivore–the European nightjar Caprimulgus europaeus. *J. Avian Biol.* 48, 738–747.

Ouwehand, J., Burger, C., and Both, C. (2017). Shifts in hatch dates do not provide pied flycatchers with a rapid ontogenetic route to adjust offspring time schedules to climate change. *Funct. Ecol.* 31, 2087–2097. doi: 10.1111/1365-2435.12940

Ouyang, J. Q., de Jong, M., Hau, M., Visser, M. E., van Grunsven, R. H., and Spoelstra, K. (2015). Stressful colours: corticosterone concentrations in a free-living songbird vary with the spectral composition of experimental illumination. *Biol. Lett.* 11:20150517. doi: 10.1098/rsbl.2015.0517

Poot, H., Ens, B. J., de Vries, H., Donners, M. A., Wernand, M. R., and Marquenie, J. M. (2008). Green light for nocturnally migrating birds. *Ecol. Soc.* 13:47.

R Core Team (2020). *R: A Language And Environment For Statistical Computing.* R Foundation for Statistical Computing. Vienna, Austria.

Raap, T., Casasole, G., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., et al. (2016a). Artificial light at night affects body mass but not oxidative status in free-living nestling songbirds: an experimental study. *Sci. Rep.* 6, 1–8.

Raap, T., Casasole, G., Pinxten, R., and Eens, M. (2016b). Early life exposure to artificial light at night affects the physiological condition: an experimental study on the ecophysiology of free-living nestling songbirds. *Environ. Pollut.* 218, 909–914. doi: 10.1016/j.envpol.2016.08.024

Raap, T., Pinxten, R., and Eens, M. (2016c). Artificial light at night disrupts sleep in female great tits (*Parus major*) during the nestling period, and is followed by a sleep rebound. *Environ. Pollut.* 215, 125–134. doi: 10.1016/j.envpol.2016.04.100

Raap, T., Pinxten, R., and Eens, M. (2015). Light pollution disrupts sleep in free-living animals. *Sci. Rep.* 5, 1–8.

Rappole, J. H., and Tipton, A. R. (1991). New harness design for attachment of radio transmitters to small passerines (Nuevo Diseño de Arnés para Atar Transmisores a Passeriformes Pequeños). *J. Field Ornithol.* 62, 335–337.

Rich, C., and Longcore, T. (2005). *Ecological Consequences of Artificial Night Lighting*. Washington, DC: Island Press.

Riley, W. D., Davison, P. I., Maxwell, D. L., and Bendall, B. (2013). Street lighting delays and disrupts the dispersal of Atlantic salmon (*Salmo salar*) fry. *Biol. Conserv.* 158, 140–146. doi: 10.1016/j.biocon.2012.09.022

Russ, A., Reitemeier, S., Weissmann, A., Gottschalk, J., Einspanier, A., and Klenke, R. (2015). Seasonal and urban effects on the endocrinology of a wild passerine. *Ecol. Evol.* 5, 5698–5710. doi: 10.1002/ece3.1820

Senner, N. R., Hochachka, W. M., Fox, J. W., and Afanasyev, V. (2014). An exception to the rule: carry-over effects do not accumulate in a long-distance migratory bird. *PLoS One* 9:e86588. doi: 10.1371/journal.pone.0086588

Smith, R. A., Gagné, M., and Fraser, K. C. (2021). Pre-migration artificial light at night advances the spring migration timing of a trans-hemispheric migratory songbird. *Environ. Pollut*. 269:116136. doi: 10.1016/j.envpol.2020.116136

Streby, H. M., McAllister, T. L., Peterson, S. M., Kramer, G. R., Lehman, J. A., and Andersen, D. E. (2015). Minimizing marker mass and handling time when attaching radio-transmitters and geolocators to small songbirds. *Condor* 117, 249–255. doi: 10.1650/condor-14-182.1

Stutchbury, B. J., Gow, E. A., Done, T., MacPherson, M., Fox, J. W., and Afanasyev, V. (2011). Effects of post-breeding moult and energetic condition on timing of songbird migration into the tropics. *Proc. R. Soc. B Biol. Sci*. 278, 131–137. doi: 10.1098/rspb.2010.1220

Taylor, P., Crewe, T., Mackenzie, S., Lepage, D., Aubry, Y., Crysler, Z., et al. (2017). The motus wildlife tracking system: a collaborative research network to enhance the understanding of wildlife movement. *Avian Conserv. Ecol.* 18:8.

Titulaer, M., Spoelstra, K., Lange, C. Y. M. J. G., and Visser, M. E. (2012). Activity patterns during food provisioning are affected by artificial light in free living great tits (*Parus Major*). *PLoS One* 7, 5–8. doi: 10.1371/journal.pone.0037377

Visser, M. E., and Gienapp, P. (2019). Evolutionary and demographic consequences of phenological mismatches. *Nat. Ecol. Evol.* 3, 879–885. doi: 10.1038/s41559-019-0880-8

Wagner, R. H., Schug, M. D., and Morton, E. S. (1996). Condition-dependent control of paternity by female purple martins: implications for coloniality. *Behav. Ecol. Sociobiol.* 38, 379–389. doi: 10.1007/s002650050255

Zuur, A. F., Ieno, E. N., and Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol*. 1, 3–14. doi: 10.1111/j.2041-210x.2009.00001.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 08 November 2021
doi: 10.3389/fevo.2021.751072

Impact of Light at Night Is Phase Dependent: A Study on Migratory Redheaded Bunting (*Emberiza bruniceps*)

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Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *31 July 2021*
Accepted: *06 October 2021*
Published: *08 November 2021*

Citation:
Kumar J, Malik S, Bhardwaj SK and Rani S (2021) Impact of Light at Night Is Phase Dependent: A Study on Migratory Redheaded Bunting (Emberiza bruniceps). Front. Ecol. Evol. 9:751072. doi: 10.3389/fevo.2021.751072

Artificial light at night (LAN) alters the physiology and behavior of an organism; however, very little is known about phase-dependent effects of LAN, particularly, in night migratory songbirds. Therefore, in this study, we investigated whether the effects of LAN on daily activity and photoperiodic responses in the Palearctic Indian migratory songbird, redheaded buntings (*Emberiza bruniceps*), is dependent on the different phases of the night. Male buntings maintained under short photoperiod (8L:16D; L = 100 lux, $D < 0.1$ lux) in individual activity cages were exposed to LAN (2 lux) for 6 weeks either in 4 h bin given at the different phases of 16 h night (early, mid, or late at ZT 08–12, ZT 14–18, or ZT 20–24, respectively; n = 9 each group) or throughout 16 h night (all night light, n = 6, ZT 08–24, the time of lights ON was considered as *Zeitgeber* time 0, ZT 0). A group (n = 6) with no LAN served as control. The results showed that LAN at the different phases of night induced differential effects as shown by an intense activity during the night, altered melatonin and temperature rhythms, and showed an increase in body mass and body fattening, food intake, and gonadal size. Midnight light exposure has a greater impact on migration and reproduction linked phenotypes, which is similar to the ones that received light throughout the night. The highlights of this study are that (i) LAN impacts day-night activity behavior, (ii) its continuity with the day alters the perception of day length, (iii) birds showed differential sensitivity to LAN in a phase-dependent manner, (iv) the direction of placing LAN affects the daily responses, e.g., LAN in the early night was “accepted” as extended dusk but the late night was considered as early dawn, and (v) midnight LAN was most effective and induced similar responses as continuous LAN. Overall, LAN induces long day responses in short days and shows differential sensitivity of the different phases of the night toward the light. This information may be valuable in adopting a part-night lighting approach to help reduce the physiological burden, such as early migration and reproduction, of artificial lighting on the nocturnal migrants.

Keywords: bunting, light at night, melatonin, migratory, photoperiodic response

INTRODUCTION

In nature, the day-night cycles are provided by bright sunlight and dim moon and starlight, respectively. These cycles are very consistent with no interannual variations and act as the most reliable cue to regulate daily and seasonal responses in the animals. However, the advent of artificial light and its use at night owing to various anthropogenic activities, such as industries, call centers, and the operation of a long-distance flight, have disrupted the consistent pattern of light:dark cycle. The artificial light at night (LAN) is of relatively high intensity than natural light, therefore, may alter the perception of day length (Kumar et al., 2018). Also, it is rich in the blue portion of the light spectrum, which could be perceived as the day (Navara and Nelson, 2007; Gaston et al., 2013; Yadav et al., 2015). Because the light synchronizes avian circadian and seasonal functions (Berson et al., 2002; Foster and Kreitzmann, 2004), changes in the light environment at night are likely to affect the expression of several biological functions. The artificial LAN is linked with changes in the locomotor activity and feeding behavior in both vertebrates (Fonken et al., 2010; Santos et al., 2010; Polak et al., 2011; Titulaer et al., 2012; Dominoni et al., 2014) and invertebrates (Moore et al., 2000; Eisenbeis and Hanel, 2009). The ecological consequences of increasing LAN are well-documented in different birds and mammals (Rich and Longcore, 2006; Navara and Nelson, 2007; Dominoni et al., 2014).

The effect of LAN seems to be more prominent in migratory birds because most of them are nocturnal migrants. The bird migration represents enduring flights in time and space that may range from non-stop trans-hemispheric flights to days- or months-long journeys (Conklin et al., 2017). Any disruption in this flight may induce harmful effects at individual and population levels (Alerstam and Lindström, 1990; Liechti, 2006; Hewson et al., 2016; Kelly et al., 2016; Cohen et al., 2017). Several studies have shown the role of different environmental factors in regulating nocturnal flight (Alerstam and Lindström, 1990; Richardson, 1990; Marra et al., 2005; Liechti, 2006; Kelly et al., 2016), including its orientation and navigation at night (Hiscock et al., 2016; Mouritsen et al., 2016). Because visual cues are important for the night flight, it is possible that brighter night affects abilities of birds to orient and navigate (Cochran and Graber, 1958; Day et al., 2015; Vincze et al., 2015) during a migration. It may also interfere with the geomagnetic compass, which tells the birds about the spatial maps (Kishkinev et al., 2015; Hiscock et al., 2016; Mouritsen et al., 2016).

Recent studies have shown the physiological mechanisms and underlying effects of LAN on daily and seasonal responses in birds. It is being observed that LAN disrupts their circadian rhythms and changes their flight behavior, which may lead to disorientation. It may alter their reproductive physiology (Larkin and Frase, 1988; Wiltschko et al., 1993; Bruderer et al., 1999; Dominoni et al., 2013c,d; Dominoni and Partecke, 2015; Raap et al., 2015; de Jong et al., 2016). The sensitivity of the circadian system to LAN may influence the overall physiology and behavior, making it vulnerable to the circadian disruption of the melatonin profile, metabolic functions, and other hormonally driven systems. Melatonin is produced in the dark, it tracks the night length and "informs" the animal about the time of the day and/or year. It helps in the synchronization of the circadian clock to the light:dark (L:D) cycle and is shown to be required for the persistence of circadian rhythms in an aperiodic environment (Gwinner et al., 1997; Gwinner and Brandstaetter, 2001; Rani et al., 2005a). Besides being a "read out" of physiological day and night, melatonin exhibits a phase relationship with the daily rhythms in cortisol, body temperature, and sleep (Gwinner et al., 1997; Arendt and Skene, 2005). Thus, any alteration in the external environment may alter this relationship (Yadav et al., 2015). Thus, a 24-h melatonin rhythm acts as a strong marker to assess the impact of LAN on daily rhythms.

Although several studies have shown the effect of LAN on daily and seasonal behaviors, there are still gaps, e.g., most of the studies have been done on resident bird species and almost none has demonstrated the differential sensitivity of the different phases of the night toward LAN. In this light, we chose a migratory species (redheaded bunting; *Emberiza bruniceps*) to conduct this study. Buntings proved to be a good model system for such studies due to the following reasons (i) they are long-distance latitudinal migrants representing the Palearctic Indian migratory system, (ii) show distinct life history stages (photosensitive overwintering, spring migration, photostimulated breeding, and autumn migration) in their annual cycle, (iii) in a laboratory condition under captivity, on exposure to increasing day lengths they exhibit nighttime migratory restlessness (*Zugunruhe*), and (iv) they are long-day breeders, and an increasing photoperiod of ≥12 h per day stimulates migratory and reproductive phenotypes present in them. Thus, an obvious question would be to study the effect of LAN using a non-stimulatory photoperiod (e.g., 8L:16D). The expectation is that if the birds are sensing the LAN, they would respond to it, as if, they have been exposed to long stimulatory day lengths. In view of this, we aimed to investigate the effect of LAN on circadian (daily activity rest pattern, body temperature, and melatonin rhythm) and seasonal (changes in migration and reproduction-linked phenologies) responses in migratory buntings and also to study the differential sensitivity of the different phases of the night toward LAN.

MATERIALS AND METHODS

Animals and Housing

This study was done on Palearctic Indian migratory songbirds, the redheaded bunting (*E. bruniceps*). They migrate two times a year between their breeding (∼40°N; in South-Eastern Europe, Asia Minor, Palestine, Syria, Upper Mesopotamia, and Persia) and wintering grounds (∼25°N; spreading throughout western and central India, chiefly in Rajasthan, Madhya Pradesh, Gujarat, Maharashtra, and Karnataka) representing autumn and spring migration, respectively. They come to their wintering grounds in the autumn (late July and early August) and return to their breeding grounds in late March and early April (prevailing day length ≥ 12 h light per day). It is estimated that these species spend a total of about 96 days (48 days each way), covering a migratory journey of about 7,000 km in both the ways and of

~90 days at breeding grounds; the remaining almost half of the year they spend at their wintering grounds (Ali and Ripley, 1974). Buntings are long-day breeders, and in captivity, the day length of ≥12 h light per day induces hyperphagia, body mass gain, fat deposition, gonadal recrudescence, and the nighttime migratory restlessness, *Zugunruhe* (Jain and Kumar, 1995).

Adult male buntings were captured in mid-February 2015 from the overwintering flocks near Lucknow (25°N) using the mist net. They were brought to the laboratory and acclimatized in an outdoor aviary (size = 3 m × 2.5 m × 2.5 m) for a week under a natural day length condition. Thereafter, they were transferred to an indoor aviary under short day length (8L:16D; L = ~100 lux and D ≤ 0.1 lux, closer to dark) and constant temperature (22°C ± 2°C) conditions. Food (seeds of *Setaria italica*) and water were available *ad libitum* and replenished during the light phase.

Experiment

The buntings (*n* = 39) maintained in an indoor aviary were transferred to individual activity cages (60 cm × 35 cm × 45 cm) placed singly in a light-tight photoperiodic chamber (75 cm × 50 cm × 70 cm). After more than a week under 8L:16D, the birds were given LAN (2 lux) for 6 weeks either in 4 h bin given at the different phases of 16 h night (early, mid, or late at ZT 08–12, ZT 14–18, or ZT 20–24, respectively; *n* = 9 each group) or throughout night (all night, *n* = 6, ZT 08–24, the time of lights ON was considered as *Zeitgeber* time 0, ZT 0). A group (*n* = 6) with no LAN served as control. The light:dark cycles were given by compact fluorescent lamps (CFL; 14 W, 230 V, Phillips, India). Desired light intensity was obtained by covering the CFL with a black paper sheet having small holes on the side facing the roof so that a bird received a diffused light from the light source.

We measured migratory and reproductive phenotypes, such as locomotor activity and change in food intake, body mass, body fattening, and gonadal size, along with the body temperature and melatonin levels.

Locomotor Activity

To collect the general activity of the birds, each cage was equipped with an IR motion sensor (Conrad Electronic, Hirschau, Germany, Haustier PIR-Melder). The activity was collected and analyzed by Chronobiology kit; Stanford Software Systems, Stanford, CA, United States (Malik et al., 2004). The activity records (actograms) were double plotted, and the hourly activity counts for 24 h were calculated for the selected duration of an experiment. The activities in the second week and last week of LAN exposure were considered as beginning and the end, respectively. The daily activity profile was plotted as mean (±SEM).

Measurement of Plasma Melatonin

To measure the plasma melatonin levels, blood samples from the birds of different groups (*n* = 5 each) were taken four times of the day (ZT 04, mid-day; ZT 10, early night; ZT 16, midnight; and ZT 22, late light) covering the midpoints of day and night, and of all the LAN phases, to make the data comparable. Blood was taken by puncturing the brachial wing vein. To avoid any stress to the birds, sampling was scattered over a period of 13 days (days 25–37 of night light treatment). All birds were bled for all the time points. Nighttime samples were collected in a dim green light (500 nm) from a KL1500 cold light source focused on the wing vein for not more than half-a-minute. This had shown no effect on the nocturnal melatonin secretion (Cassone et al., 2008). Each time about 200 μl of blood was collected by heparinized capillaries in the vials and immediately centrifuged at 3,000 rpm for 10 min to collect the plasma, which was stored at −20°C until assayed for melatonin.

Plasma melatonin assay was done by ELISA using a specific melatonin kit (product no. RE54021, IBL International GmbH, Hamburg, Germany). This assay was previously standardized and used for the measurement of plasma melatonin in several studies (Lahiri et al., 2004; Terzieva et al., 2009), including the studies from our laboratory (Singh et al., 2012; Yadav et al., 2015; Kumar et al., 2018). Melatonin was extracted and assayed from each plasma sample as per the protocol and instructions from the manufacturer. Briefly, 100 μl of experimental samples, standards, and controls were methanol extracted and dried by a vacuum concentrator. The obtained pellet was reconstituted in 150 μl of double-distilled water. Then, 50 μl of each reconstituted sample volume and 50 μl melatonin antiserum (rabbit, polyclonal) were incubated in a 96-well plate at 4°C for 20 h. Thereafter, 150 μl of a freshly prepared enzyme conjugate was added and incubated for 2 h at room temperature with continuous shaking (500 rpm). Then, 200 μl of a freshly prepared p-nitrophenyl phosphate (PNPP) substrate solution was added to each well and incubated for another 40 min. The reaction was stopped by adding 50 μl of PNPP stop solution to each well, and optical density (OD) was measured at 405 nm, using 650 nm wavelength as a reference. The ODs of standard samples were plotted to make a standard curve, and the concentration of melatonin in experimental samples was calculated with a reference to values in the standard curve. Individual values of each bird were used to calculate mean ± SEM melatonin levels (pg/ml) for a group. The analytical sensitivity (limit of detection) of the assay was 1.6 pg/ml, and the % CV for intra- and inter-assay was 7.69, 7.33, 6.59, and 9.67, respectively.

Body Temperature

The body temperature in each group was measured by a quick shot IR ThermoScan, India (Model: EXP-01B) from the flank area of the birds, just before the blood sampling at ZT 04, 10, 16, and 22.

Measurement of Physiological Parameters

Body mass was recorded using a top pan balance up to an accuracy of 0.1 g. The food intake (g)/bird/day was measured as described by Kumar et al. (2001). It was calculated as the difference between the food supplied and food recovered after 24 h. Body fattening (fat deposition in furcular, scapular, and abdominal areas) was assessed as the fat score on a scale of 0–5, where 0 represents no fat and 5 represents the maximum deposition of fat (Kumar et al., 2001). The testis size was measured by laparotomy performed under general anesthesia

(Kumar et al., 2002) using the formula 4/3 π ab^2, where a and b denote one-half of long (length) and short (width) axes, respectively (Kumar et al., 2002). The physiological parameters were measured before the LAN treatment (baseline data) and in the sixth week of LAN treatment, and a change was plotted as mean (±SEM).

Statistics

Data on activity profile, plasma melatonin, body temperature, and physiological parameters (food intake, body mass, body fattening, and testis volume) were plotted as mean ± SEM while the actograms given are of a representative bird in each treatment. We used a one-way ANOVA with or without a repeated measure (RM) (one-way RM ANOVA and one-way ANOVA, respectively) followed by the Newman-Keuls multiple comparison *post hoc* test to determine the effect of LAN on plasma melatonin levels and body temperature within the group and between the groups. We also used a one-way ANOVA without an RM (one-way ANOVA) followed by the Newman-Keuls multiple comparison *post hoc* test to determine the effect of LAN on physiological parameters. A significance was considered at $p < 0.05$. All data analyses were done using GraphPad Prism software version 5.0 (San Diego, CA, United States).

RESULTS

The results showed that LAN had an impact on both circadian and seasonal responses in a phase-dependent manner (**Figures 1–3**).

Locomotor Activity Behavior

Buntings are diurnal birds, therefore, their activity is restricted only within a day time of 8L:16D; however, during LAN treatment the daytime activity either extended into the dark (early night group) or start early (late night group). The buntings in the control group (8L:16D) showed no nighttime activity, but in the all night group (ZT 08–24), they developed a prominent nighttime activity. The groups that received LAN for 4 h at the different phases of night evoked different responses. In the "early night" group, the light given at ZT 08–12, delayed the end but in the "late night" group light at ZT 20–24 advanced the onset of the day. Contrarily, birds in the "midnight" group (ZT 14–18) showed a different pattern of activity in the night. In these birds, initially, the activity was restricted to 8 h day and 4 h segment of LAN but one time the intense nighttime activity; *Zugunruhe*, developed, the activity during LAN was compromised. This period of less activity was flanked by an intense nighttime activity (**Figure 1C**). Besides, the birds in the "late night" group (ZT 20–24) showed a mixed response. All the birds (100%) of this group were active during 8 h day, but 55.56% of the birds were active in a 4-h LAN segment too. Birds under the different phases of LAN developed an intense nighttime activity. In early and late night groups, 44.44% of birds and in midnight and all night groups, 100% of birds developed an intense nighttime activity, respectively.

Plasma Melatonin and Body Temperature Rhythm

Figure 2 shows the plasma melatonin levels and body temperature in different groups experiencing LAN at the different

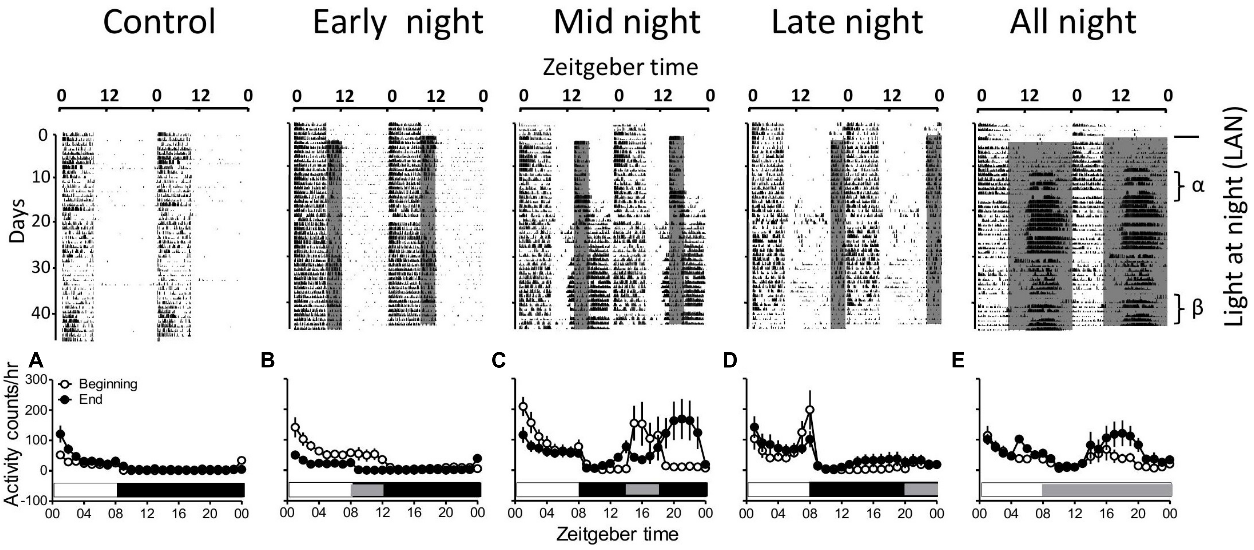

FIGURE 1 | Double plotted representative actograms showing the impact of light at night (LAN) in migratory redheaded bunting (*Emberiza bruniceps*). Birds, which are maintained under non-stimulatory short days (8L:16D; L = 100 lux, $D < 0.1$ lux), were given LAN (∼2 lux) either in 4 h bin placed at different phases of 16 h night (early, mid, or late at ZT08–12, ZT14–18, or ZT20–24 respectively; $n = 9$ each group) or throughout the night (all night, $n = 6$, ZT 08–24). A group ($n = 6$) with no LAN served as control. Note that early night and late night LAN evoke a response similar to dusk and dawn, respectively. Mid night LAN shows the fragmentation of activity or rest during the night, whichever applicable. In general, all the groups except the control show long day effects. The lower panel **(A–E)** shows a 24-h activity profile in the beginning (α) and end (β) of LAN treatment. Shaded and dark areas in the figure represent the LAN and dark phases of the night, respectively.

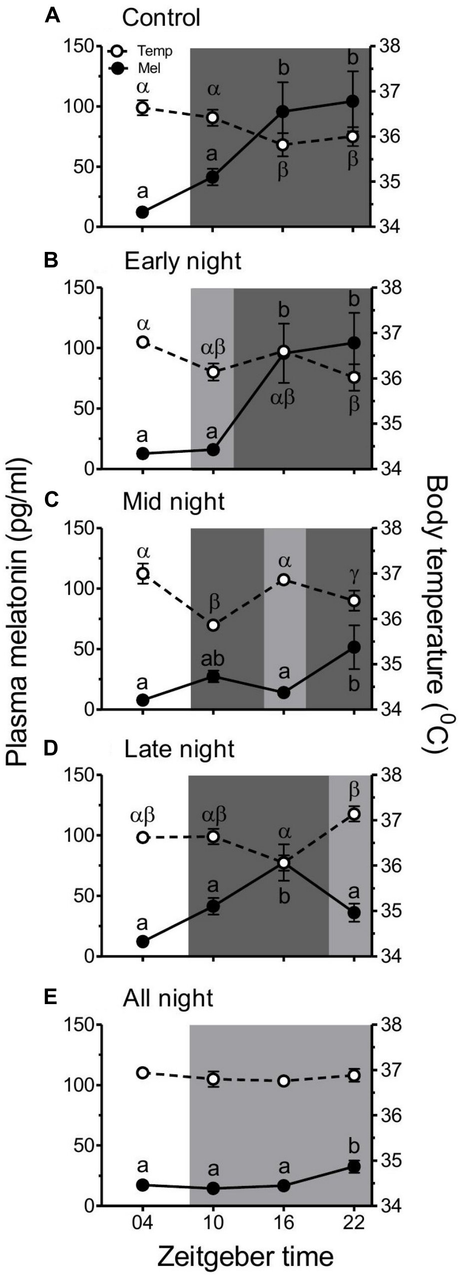

FIGURE 2 | An effect of LAN (~2 lux) on plasma melatonin (pg/ml) and body temperature (°C). Birds exposed to different light at night (LAN) treatments **(A–E)** were sampled for plasma melatonin levels and scanned for body temperature during mid-day and middle of each LAN treatment (ZT 04, 10, 16, and 22). English or Greek alphabets on the point symbol (Mean ± SEM, $n = 5$) indicate a statistical analysis for melatonin and temperature, respectively. Similar alphabets indicate no difference, whereas different alphabets indicate a significant difference ($p < 0.05$; the Newman–Keuls multiple comparison *post hoc* test) between ZTs. It is noted that melatonin and temperature rhythms are antiphasic and LAN alters their shape of the curve.

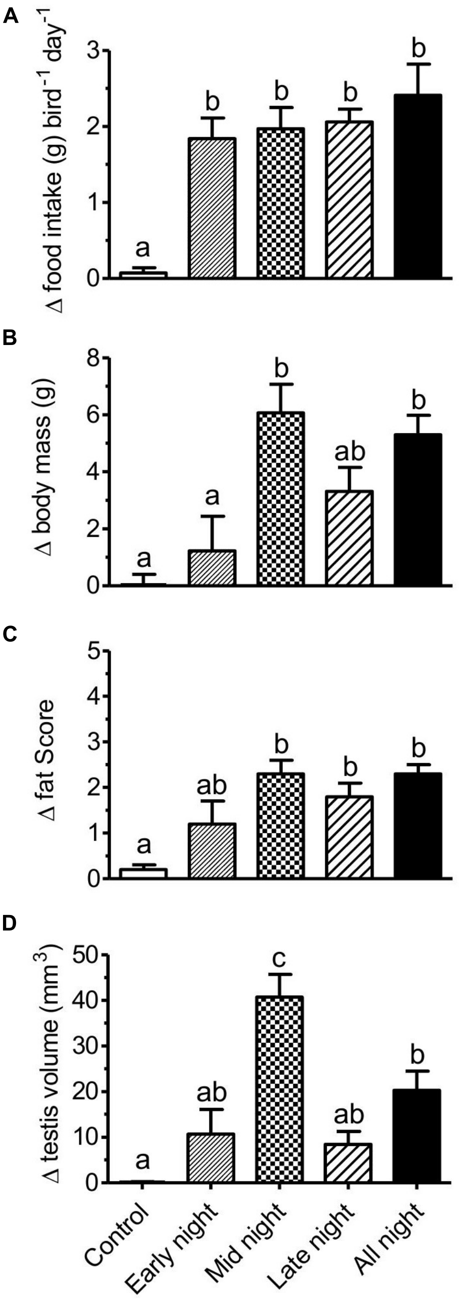

FIGURE 3 | An effect of light at night (LAN) (2 lux) exposure on seasonal responses in migratory redheaded bunting (*Emberiza bruniceps*). Changes (mean ± SEM) in **(A)** food intake, **(B)** body mass, **(C)** body fattening, and **(D)** testis volume under different LAN treatments. Similar alphabets on a bar indicate no difference, whereas different alphabets indicate a significant difference ($p < 0.05$; the Newman–Keuls multiple comparison *post hoc* test) between groups. It is noted that midnight LAN behaves similar to all night LAN but with greater intensity.

phases of the night. LAN provided at the different phases of night altered their melatonin and temperature profiles. In general, the melatonin levels showed a rhythmic pattern in all the groups (control group: $F_{3,\ 12} = 8.827$, $p = 0.0023$; early night group: $F_{3,\ 12} = 10.51$, $p = 0.0011$; midnight group: $F_{3,\ 12} = 4.394$, $p = 0.0264$; late night group: $F_{3,\ 12} = 10.10$, $p = 0.0013$; all night group: $F_{3,\ 12} = 5.009$, $p = 0.0177$; one-way RM ANOVA;

Figures 2A–E). Levels were minimum during the midday (ZT 04) and significantly elevated during the night except in the period of 4 h LAN when the melatonin was significantly lower and similar to day time levels (**Figures 2A–D**). Contrarily, melatonin levels in the all night group (**Figure 2E**) did not show a high amplitude rhythm. Levels were low throughout the night except at the end (ZT 22) when it became significantly high (all night group: $F_{3,\ 12} = 5.009$, $p = 0.0177$; one-way RM ANOVA; **Figure 2E**).

Body temperature also followed a rhythmic pattern as that of melatonin although in antiphase (control group: $F_{3,\ 12} = 3.526$, $p = 0.0487$; early night group: $F_{3,\ 12} = 5.013$, $p = 0.0176$; midnight group: $F_{3,\ 12} = 19.60$, $p < 0.0001$; late night group: $F_{3,\ 12} = 7.254$, $p = 0.0049$; all night group: $F_{3,\ 12} = 0.3900$, $p = 0.7624$; one-way RM ANOVA; **Figures 2A–E**). In general, the temperature was high during day (ZT 04) and at the 4-h LAN phase. Birds exposed to the all night group showed no change in their body temperature throughout 24 h (**Figure 2E**).

Change in Migration and Reproduction-Linked Phenotypes

Light at night-induced photoperiodic physiological responses, such as food intake, body mass, body fattening, and testis recrudescence, are shown in **Figure 3**. All groups (except control) showed the effects of LAN, the responses were dependent on the different phases of LAN exposure. The change in food intake is significantly high in buntings of all the four LAN-treated groups in comparison to the birds of the control group (change in food intake: $F_{4,\ 34} = 9.654$, $p < 0.0001$; one-way ANOVA; **Figure 3A**). A change in body mass is high in the birds of LAN-exposed groups but midnight and all night groups have a significant difference with control and early night groups (change in body mass: $F_{4,\ 34} = 6.683$, $p = 0.0004$; one-way ANOVA; **Figure 3B**). A change in the fat score is significantly high in midnight, late night, and all night groups in comparison to the birds of the control group (a change in the fat score: $F_{4,\ 34} = 5.871$, $p = 0.0011$; one-way ANOVA; **Figure 3C**). Testis recrudescence takes place in all the four LAN-treated groups, but the change in testis volume is significantly higher in the birds of midnight group with respect to the rest of the groups (a change in the testicular volume: $F_{4,\ 34} = 13.18$, $p < 0.0001$; one-way ANOVA; **Figure 3D**).

DISCUSSION

The results show that exposure to LAN even for a small duration of 4 h at an intensity of ∼2 lux induces photoperiodic responses in bunting maintained under short days, which is otherwise a non-stimulatory photoperiod for them. Irrespective of time, early night, midnight, or late night, the 4 h LAN could induce migratory restlessness in all the groups along with hyperphagia, an increase in body fattening, and the testis size. It is established if they are held captive in a caged condition and not allowed to fly (migrate) during their migratory season, they show a strong motivation to migrate, generally known as migratory restlessness, *Zugunruhe* (Gwinner, 1990).

The results thus suggest that the short-duration LAN was able to change the perception of day length in bunting. The change in the perception could be due to a subjective interpretation of different light intensities (Kumar et al., 1992, 2007; Foster and Kreitzmann, 2004; Longcore and Rich, 2004; Dominoni et al., 2013a) or a changed melatonin profile (Cassone et al., 2008). In black-headed bunting exposed to a combination of the bright and dim day:night (LD) cycle of 10 and 2 lux light intensities, respectively, the dim light period was perceived as night (Kumar et al., 1992). Previous studies on stonechats and baya weaver when exposed to the dim:bright LD also showed a change in the perception of day length and accordingly changed their melatonin profile (Kumar et al., 2007; Singh et al., 2012).

In this study, the birds were experiencing the three grades of light intensities, such as bright (100 lux) in 8L, dim (2 lux) in 4 h LAN, and close to dark (0.1 lux) at the night, but the transition of light intensities from the day to night was different in different groups. For example, in the early night group, the transition was from bright to dim to dark, in the midnight group, from bright to dark interrupted by dim, in the late night group, bright to dark to dim, and in all night group, from bright to dim. These transitions could have re-entrained the endogenous rhythm of photoperiodic photosensitivity as it is evident from their differential responses. A maximum response in the midnight group suggests that birds interpreted it as a lighting regimen of 18L:6D (8L + 6D + 4 h LAN), which is strongly stimulatory (Singh et al., 2002). There were a moderate response in the early night and late night groups, which suggests that birds interpreted it as a lighting regimen of 12L:12D (8L + 4 h LAN or 4 h LAN + 8L), which is a marginally inductive photoperiod for bunting. In a previous study on bunting (Singh et al., 2002), similar observations were made. The bunting exposed to 1L:8D:1L:14D and 1L:8D:10L:5D seemed to be re-entrained, and they interpreted the abovementioned lighting regimen as 10L:14D and 19L:5D (Singh et al., 2002).

There are a few studies on redheaded bunting (*E. bruniceps*), which are suggestive of the photoperiod-dependent effects on seasonal responses such as body fattening and testicular growth (Rani et al., 2005b). When exposed to increasing photoperiods such as 11.5L:12.5D, 12L:12D, 12.5L:11.5D, 13L:11D, 14L:10D, and 18L:6D, the buntings responded in a photoperiod-dependent manner and underwent growth and regression cycle under photoperiods ≥ 12 h per day (Rani et al., 2005b). A previous study on Japanese quail has also established a relationship between the length of the photoperiod and the rate of the photoperiodic induction. The rate of testicular growth was slower under 12L:12D as compared to 13L:11D, 14L:10D, 16L:8D, and 20L:4D photoperiods, which showed a maximum growth (Follett and Maung, 1978). The light intensity is suggestive of affecting the photoperiodic response as observed in the case of European starlings (*Sturnus vulgaris*). The starlings exposed to an 18L:6D photoperiod at 3-, 13-, 45-, and 108-lux light intensities responded differently, and it appeared as if they were exposed to different photoperiods such as 11L:13D, 13L:11D, 16L:8D, and 18L:6D, respectively (Bentley et al., 1998). Similarly, the buntings kept under a stimulatory photoperiod of 13L:11D and at the intensities of 50, 100, 400, 800, and 1,000 lux also showed an intensity-dependent photoperiodic induction (Misra et al., 2004).

In our study, the onset and end of a daytime activity were affected by the phase of LAN. The birds, which received 4 h LAN in the early night, extended their daytime activity into the night, and the ones, which received at late night, showed an early onset of morning activity, which extended their nighttime activity into the day. In midnight and all night groups, the onset of morning activity was not affected by LAN. Our results are slightly different from those on male great tits, which on exposure to 1.6 lux light intensity throughout the night advanced the onset of their morning activity (Raap et al., 2015). Also, in our study, the LAN accelerated a gonadal response in midnight and all night groups, similar to that observed in blackbirds where the LAN advanced the gonadal growth almost a month earlier than birds kept under dark nights (Dominoni et al., 2013c).

Light at night also seems to have a strong effect on song timing in some early singing species such as European robin (*Erithacus rubecola*), American robin (*Turdus migratorius*), Common blackbird (*Turdus merula*), and Great tit (*Parus major*) (Miller, 2006; Fuller et al., 2007; Kempenaers et al., 2010; Da Silva et al., 2014; Dominoni et al., 2014). The effect ranges from the previous onset of dawn song to singing in the middle of the night (Fuller et al., 2007; Da Silva et al., 2014; Dominoni et al., 2014). Although we have not measured the song behavior in our birds, we have shown a 24 h profile of their locomotor activity.

In our study, we also observed that LAN affected the rhythm of melatonin secretion. In general, the melatonin levels were low during the day and high at night, however, in the groups exposed to 4 h LAN, the level of melatonin was significantly low at the time of LAN, which seemed to be "perceived" as day. This altered the shape of the melatonin profile in different groups. The amplitude of melatonin was the highest in the early night and late night groups but was the lowest in midnight and all night groups (having a maximum gonadal response) that received light throughout the night. Thus, these results may be helpful in understanding the control of seasonal processes in birds experiencing high light intensity at night such as the urban birds. Although a direct role of melatonin in the regulation of seasonal responses in birds is debatable (Dawson et al., 2001), there is a possibility that reduced melatonin levels during the night must have advanced the onset of reproductive functions by changing the perception of day length longer than the actual one. Our speculation is based on a study on European starlings, which showed that the exogenous administration of melatonin reduces the volume of song control nuclei, HVC, and area X (Bentley et al., 1999). High melatonin levels upregulate GnIH in the brain and the gonads, which suppresses the hypothalamo–hypophyseal–gonadal axis and maintains the small gonadal size during the prebreeding stage (Ubuka et al., 2005; McGuire et al., 2011). LAN may also affect the physiology of birds by reducing the ratio between the day and night light intensity, which would not only lower the difference between melatonin levels during the day and nighttime but also advance the onset of *Zugunruhe* (Roenneberg et al., 2003; Singh et al., 2012). Lower melatonin levels during the night as a consequence of LAN may reduce the degree of self-sustainment of the circadian clock and allows an alternative temporal activity such as *Zugunruhe* in migratory birds (Fusani and Gwinner, 2005) or the appearance of activity during the night in a resident Indian weaver bird (Singh et al., 2012). Such a prediction is supported by a recent study on urban and rural blackbirds, which showed that possibly due to the night light, urban birds have faster (advanced) but weaker circadian clocks than their rural counterparts (Dominoni et al., 2013b).

CONCLUSION

To sum up, our results show that even under non-stimulatory short days, the low intensity (∼2 lux) night light could induce migratory and reproductive phenotypes. Also, the effect of LAN was phase dependent. These findings may have ecological implications the birds under short days are still in their recovery phase and, when exposed to LAN, they displayed an activity for ∼12 h in all the light treatments. Thus, 4-h LAN of ∼2 lux light intensity could alter the perception of day length, thereby advancing their migratory and reproductive activities. Our results also suggest that the LAN interruptions of 4 h during midnight have a greater impact on circadian and seasonal responses as compared to other groups where the LAN was given in continuation with the day. Hence, this information may be valuable in adopting a part-night lighting approach to help reduce the physiological burden, such as early migration and reproduction, of artificial lighting on the nocturnal migrants.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Ethics Committee, University of Lucknow, Lucknow, India (LU/ZOOL/SR/SM/Ph.D.JK/11-2014).

AUTHOR CONTRIBUTIONS

SR conceptualized and designed this study. JK and SM carried out the experiments. JK, SM, SB, and SR participated in the data analysis. JK, SM, and SR were involved in writing the manuscript. All authors gave final approval for publication.

FUNDING

Financial support from the Department of Biotechnology, New Delhi, India (BT/PR4984/MED/30/752/2012) and also from the PROTSAHAN scheme of the University of Lucknow, is gratefully acknowledged. JK is a recipient of the UGC Rajiv Gandhi National Fellowship (201112-RGNF-SC-UTT-11960).

REFERENCES

Alerstam, T., and Lindström, A. (1990). "Optimal bird migration: the relative importance of time, energy, and safety," in *Bird Migration: Physiology and Ecophysiology*, ed. E. Gwinner (Berlin: Springer), 331–351. doi: 10.1007/978-3-642-74542-3_22

Ali, S., and Ripley, S. D. (1974). *Handbook of the Birds of India and Pakistan*, Vol. 10. New York, NY: Oxford University Press.

Arendt, J., and Skene, D. J. (2005). Melatonin as a chronobiotic. *Sleep Med. Rev.* 9, 25–39. doi: 10.1016/j.smrv.2004.05.002

Bentley, G. E., Goldsmith, A. R., Dawson, A., Briggs, C., and Pemberton, M. (1998). Decreased light intensity alters the perception of day length by male European starlings (*Sturnus vulgaris*). *J. Biol. Rhythms* 13, 148–158. doi: 10.1177/074873098128999998

Bentley, G. E., Van't Hof, T. J., and Ball, G. F. (1999). Seasonal neuroplasticity in the songbird telencephalon: a role for melatonin. *Proc. Natl. Acad. Sci. U.S.A.* 96, 4674–4679. doi: 10.1073/pnas.96.8.4674

Berson, D. M., Dunn, F. A., and Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295, 1070–1073. doi: 10.1126/science.1067262

Bruderer, B., Peter, D., and Steuri, T. (1999). Behaviour of migrating birds exposed to X-band radar and a bright light beam. *J. Exp. Biol.* 202(Pt 9), 1015–1022. doi: 10.1242/jeb.202.9.1015

Cassone, V. M., Bartell, P. A., Earnest, B. J., and Kumar, V. (2008). Duration of melatonin regulates seasonal changes in song control nuclei of the house sparrow (*Passer domesticus*): independence from gonads and circadian entrainment. *J. Biol. Rhythms* 23, 49–58. doi: 10.1177/0748730407311110

Cochran, W. W., and Graber, R. R. (1958). Attraction of nocturnal migrants by lights on a television tower. *Wilson. Bull.* 70, 378–380.

Cohen, E. B., Barrow, W. C., Buler, J., and Deppe, J. (2017). How do en route events around the Gulf of Mexico influence migratory landbird populations? *Condor* 119, 327–343. doi: 10.1650/condor-17-20.1

Conklin, J. R., Senner, N. R., Battley, P. F., and Piersma, T. (2017). Extreme migration and the individual quality spectrum. *J. Avian. Biol.* 48, 19–36. doi: 10.1111/jav.01316

Da Silva, A., Samplonius, J. M., Schlicht, E., Valcu, M., and Kempenaers, B. (2014). Artificial night lighting rather than traffic noise affects the daily timing of dawn and dusk singing in common European songbirds. *Behav. Ecol.* 25, 1037–1047. doi: 10.1093/beheco/aru103

Dawson, A., King, V. M., Bentley, G. E., and Ball, G. F. (2001). Photoperiodic control of seasonality in birds. *J. Biol. Rhythms* 16, 365–380.

Day, R. H., Rose, J. R., Prichard, A. K., and Streever, B. (2015). Effects of gas flaring on the behavior of night-migrating birds at an artificial oil-production Island, Arctic Alaska. *Arctic* 68, 367–379.

de Jong, M., Ouyang, J. Q., van Grunsven, R. H. A., Visser, M. E., and Spoelstra, K. (2016). Do wild great tits avoid exposure to light at night? *PLoS One* 11:e0157357. doi: 10.1371/journal.pone.0157357

Dominoni, D. M., Carmona-Wagner, E. O., Hofmann, M., Kranstauber, B., and Partecke, J. (2014). Individual-based measurements of light intensity provide new insights into the effects of artificial light at night on daily rhythms of urban-dwelling songbirds. *J. Anim. Ecol.* 83, 681–692. doi: 10.1111/1365-2656.12150

Dominoni, D. M., and Partecke, J. (2015). Does light pollution alter day length? A test using light loggers on free-ranging European blackbirds (Turdus merula). *Philos. T. Roy. Soc. B*. 370:20140118. doi: 10.1098/rstb.2014.0118

Dominoni, D. M., Quetting, M., and Partecke, J. (2013c). Artificial light at night advances avian reproductive physiology. *Proc. R. Soc. Lond. B* 280:20123017. doi: 10.1098/rspb.2012.3017

Dominoni, D. M., Quetting, M., and Partecke, J. (2013d). Long term effects of chronic light pollution on seasonal functions of European blackbirds (*Turdus merula*). *PLoS One* 8:e85069. doi: 10.1371/journal.pone.0085069

Dominoni, D. M., Goymann, W., Helm, B., and Partecke, J. (2013a). Urban-like night illumination reduces melatonin release in European blackbirds (*Turdus merula*): implications of city life for biological time-keeping of songbirds. *Front. Zool.* 10:60. doi: 10.1186/1742-9994-10-60

Dominoni, D. M., Helm, B., Lehmann, M., Dowse, H. B., and Partecke, J. (2013b). Clocks for the city: circadian differences between forest and city songbirds. *Proc. R. Soc. B* 280:20130593. doi: 10.1098/rspb.2013.0593

Eisenbeis, G., and Hanel, A. (2009). "Light pollution and the impact of artificial night lighting on insects," in *Ecology of Cities and Towns*, eds M. J. McDonnell, A. K. Hahs, and J. H. Breuste (Cambridge: Cambridge University Press), 243–263. doi: 10.1017/cbo9780511609763.016

Follett, B. K., and Maung, S. L. (1978). Rate of testicular maturation, in relation to gonadotrophin and testosterone levels, in quail exposed to various artificial photoperiods and to natural daylengths. *J. Endocrinol*. 78, 267–280. doi: 10.1677/joe.0.0780267

Fonken, L. K., Workman, J. L., Walton, J. C., Weil, Z. M., Morris, J. S., Haim, A., et al. (2010). Light at night increases body mass by shifting the time of food intake. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18664–18669. doi: 10.1073/pnas.1008734107

Foster, R. G., and Kreitzmann, L. (2004). *Rhythms of Life: The Biological Clocks that Control the Daily Lives of Every Living Thing*. New Haven, CT: Yale University Press.

Fuller, R. A., Warren, P. H., and Gaston, K. J. (2007). Daytime noise predicts nocturnal singing in urban robins. *Biol. Lett.* 3, 368–370. doi: 10.1098/rsbl.2007.0134

Fusani, L., and Gwinner, E. (2005). Melatonin and nocturnal migration. *Ann. N. Y. Acad. Sci.* 1046, 264–270. doi: 10.1196/annals.1343.024

Gaston, K. J., Bennie, J., Davies, T. W., and Hopkins, J. (2013). The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev. Camb. Philos. Soc.* 88, 912–927. doi: 10.1111/brv.12036

Gwinner, E. (1990). *Bird Migration: Physiology and Ecophysiology*. Heidelberg: Springer-Verlag. doi: 10.1007/978-3-642-74542-3

Gwinner, E., and Brandstaetter, R. (2001). Complex bird clocks. *Philos. Trans. R. Soc. Lond. B Biol. Sci* 356, 1801–1810. doi: 10.1098/rstb.2001.0959

Gwinner, E., Hau, M., and Heigl, S. (1997). Melatonin: generation and modulation of avian circadian systems. *Brain Res. Bull.* 44, 439–444. doi: 10.1016/S0361-9230(97)00224-4

Hewson, C. M., Thorup, K., Pearce-Higgins, J. W., and Atkinson, P. W. (2016). Population decline is linked to migration route in the common cuckoo. *Nat. Commun.* 7:12296. doi: 10.1038/ncomms12296

Hiscock, H. G., Worster, S., Kattnig, D. R., Steers, C., Jin, Y., Manolopoulos, D. E., et al. (2016). The quantum needle of the avian magnetic compass. *Proc. Natl. Acad. Sci. U.S.A.* 113, 4634–4639. doi: 10.1073/pnas.1600341113

Jain, N., and Kumar, V. (1995). Changes in food intake, body weight, gonads and plasma concentrations of thyroxine, luteinizing hormone and testosterone in captive male buntings exposed to natural daylengths at 29^o N. *J. Biosci*. 20, 417–426. doi: 10.1007/bf02703845

Kelly, J. F., Horton, K., Stepanian, P., De Beurs, K., Fagin, T., Bridge, E., et al. (2016). Novel measures of continental-scale avian migration phenology related to proximate environmental cues. *Ecosphere* 7:e01434.

Kempenaers, B., Borgstrom, P., Loes, P., Schlicht, E., and Valcu, M. (2010). Artificial night lighting affects dawn song, extra-pairing success and lay-date in songbirds. *Curr. Biol.* 20, 1735–1739. doi: 10.1016/j.cub.2010.08.028

Kishkinev, D., Chernetsov, N., Pakhomov, A., Heyers, D., and Mouritsen, H. (2015). Eurasian reed warblers compensate for virtual magnetic displacement. *Curr. Biol.* 25, R822–R824.

Kumar, J., Malik, S., Bhardwaj, S. K., and Rani, S. (2018). Bright light at night alters the perception of daylength in Indian weaver bird (*Ploceus philippinus*). *J. Exp. Zool*. 329, 488–496. doi: 10.1002/jez.2201

Kumar, V., Kumar, B. S., and Singh, B. P. (1992). Photostimulation of blackheaded bunting: subjective interpretation of day and night depends upon both photophase contrast and light intensity. *Physiol. Behav*. 51, 1213–1217. doi: 10.1016/0031-9384(92)90311-o

Kumar, V., Rani, S., Malik, S., Trivedi, A. K., Schwabl, I., Helm, B., et al. (2007). Daytime light intensity affects seasonal timing via changes in the nocturnal melatonin levels. *Naturwissenschaften* 94, 693–696. doi: 10.1007/s00114-007-0243-1

Kumar, V., Singh, S., Misra, M., and Malik, S. (2001). Effects of duration and time of food availability on photoperiodic responses in the migratory male blackheaded bunting (*Emberiza melanocephala*). *J. Exp. Biol*. 204, 2843–2848. doi: 10.1242/jeb.204.16.2843

Kumar, V., Singh, S., Misra, M., Malik, S., and Rani, S. (2002). Role of melatonin in photoperiodic time measurement in the migratory redheaded bunting

(*Emberiza bruniceps*) and the nonmigratory Indian weaver bird (*Ploceus philippinus*). *J. Exp. Zool.* 292, 277–286. doi: 10.1002/jez.10079

Lahiri, D. K., Ge, Y. W., Sharman, E. H., and Bondy, S. C. (2004). Age-related changes in serum melatonin in mice: higher levels of combined melatonin and 6-hydroxymelatonin sulfate in the cerebral cortex than serum, heart, liver and kidney tissues. *J. Pineal Res.* 36, 217–223. doi: 10.1111/j.1600-079X.2004.00120.x

Larkin, R. P., and Frase, B. A. (1988). Circular paths of birds flying near a broadcasting tower in cloud. *J. Comp. Psychol.* 102, 90–93. doi: 10.1037/0735-7036.102.1.90

Liechti, F. (2006). Birds: blowin' by the wind? *J. Ornithol.* 147, 202–211. doi: 10.1007/s10336-006-0061-9

Longcore, T., and Rich, C. (2004). Ecological light pollution. *Front. Ecol. Environ.* 2, 191–198. doi: 10.1890/1540-92952004002[0191:ELP]2.0.CO;2

Malik, S., Rani, S., and Kumar, V. (2004). Wavelength dependency of light induced effects on photoperiodic clock in the migratory blackheaded bunting (*Emberiza melanocephala*). *Chronobiol. Int.* 21, 367–384. doi: 10.1081/CBI-120038742

Marra, P. P., Francis, C. M., Mulvihill, R. S., and Moore, F. R. (2005). The influence of climate on the timing and rate of spring bird migration. *Oecologia* 142, 307–315. doi: 10.1007/s00442-004-1725-x

McGuire, N. L., Kangas, K., and Bentley, G. E. (2011). Effects of melatonin on peripheral reproductive function: regulation of testicular GnIH and testosterone. *Endocrinology* 152, 3461–3470. doi: 10.1210/en.2011-1053

Miller, M. W. (2006). Apparent effects of light pollution on singing behavior of American robins. *Condor* 108, 130–139. doi: 10.1650/0010-5422.2006

Misra, M., Rani, S., Singh, S., and Kumar, V. (2004). Regulation of seasonality in the migratory male blackheaded bunting (*Emberiza melanocephala*). *Reprod. Nutr. Dev.* 44, 341–352. doi: 10.1051/rnd:2004039

Moore, M. V., Pierce, S. M., Walsh, H. M., Kvalvik, S. K., and Lim, J. D. (2000). Urban light pollution alters the diel vertical migration of Daphnia. *Verh. Int. Verein. Limnol.* 27, 779–782. doi: 10.1080/03680770.1998.11901341

Mouritsen, H., Heyers, D., and Güntürkün, O. (2016). The neural basis of long-distance navigation in birds. *Annu. Rev. Physiol.* 78, 133–154. doi: 10.1146/annurev-physiol-021115-105054

Navara, K. J., and Nelson, R. J. (2007). The dark side of light at night: physiological, epidemiological, and ecological consequences. *J. Pineal Res.* 43, 215–224. doi: 10.1111/j.1600-079X.2007.00473.x

Polak, T., Korine, C., Yair, S., and Holderied, M. W. (2011). Differential effects of artificial lighting on flight and foraging behaviour of two sympatric bat species in a desert. *J. Zool.* 285, 21–27. doi: 10.1111/j.1469-7998.2011.00808.x

Raap, T., Pinxten, R., and Eens, M. (2015). Light pollution disrupts sleep in free-living animals. *Sci. Rep.* 5:13557. doi: 10.1038/srep13557

Rani, S., Singh, S., and Kumar, V. (2005a). The pineal clock affects behavioral circadian rhythms but not photoperiodic induction in the Indian weaver bird (*Ploceus philippinus*). *J. Ornithol.* 146, 355–364. doi: 10.1007/s10336-007-0236-z

Rani, S., Singh, S., Misra, M., Malik, S., Singh, B. P., and Kumar, V. (2005b). Daily light regulates seasonal responses in the migratory male redheaded bunting (*Emberiza bruniceps*). *J. Exp. Zool.* 303A, 541–550. doi: 10.1002/jez.a.187

Rich, C., and Longcore, T. (eds) (2006). *Ecological Consequences of Artificial Night Lighting*. Washington, DC: Island Press.

Richardson, W. J. (1990). Wind and orientation of migrating birds: a review. *Experientia* 46, 416–425. doi: 10.1007/BF01952175

Roenneberg, T., Daan, S., and Merrow, M. (2003). The art of entrainment. *J. Biol. Rhythms* 18, 183–194. doi: 10.1177/0748730403018003001

Santos, C. D., Miranda, A. C., Granadeiro, J. P., Lourenco, P. M., Saraiva, S., and Palmeirim, J. M. (2010). Effects of artificial illumination on the nocturnal foraging of waders. *Acta Oecol.* 36, 166–172. doi: 10.1016/j.actao.2009.11.008

Singh, J., Rani, S., and Kumar, V. (2012). Functional similarity in relation to the external environment between circadian behavioral and melatonin rhythms in the subtropical Indian weaver bird. *Horm. Behav.* 61, 527–534. doi: 10.1016/j.yhbeh.2012.01.015

Singh, S., Misra, M., Rani, S., and Kumar, V. (2002). The photoperiodic entrainment and induction of the circadian clock regulating seasonal responses in the migratory blackheaded bunting. *Chronobiol. Int.* 19, 865–881. doi: 10.1081/CBI-120014570

Terzieva, D. D., Mateva, N. D., and Vladimirova-Kitova, L. G. (2009). Melatonin reference limits at 3:00 AM and 8:00 AM in healthy adults. *Clin. Lab.* 55, 359–361.

Titulaer, M., Spoelstra, K., Lange, C., and Visser, M. E. (2012). Activity patterns during food provisioning are affected by artificial light in free living great tits (*Parus major*). *PLoS One* 7:e37377. doi: 10.1371/journal.pone.0037377

Ubuka, T., Bentley, G. E., Ukena, K., Wingfield, J. C., and Tsutsui, K. (2005). Melatonin induces the expression of gonadotropin-inhibitory hormone in the avian brain. *Proc. Natl. Acad. Sci. U.S.A.* 102, 3052–3057. doi: 10.1073/pnas.0403840102

Vincze, O., Vágási, C. I., Pap, P. L., Osváth, G., and Møller, A. P. (2015). Brain regions associated with visual cues are important for bird migration. *Biol. Lett.* 11:20150678. doi: 10.1098/rsbl.2015.0678

Wiltschko, W., Munro, U., Ford, H., and Wiltschko, R. (1993). Red light disrupts magnetic orientation of migratory birds. *Nature* 364, 525–527. doi: 10.1038/364525a0

Yadav, G., Malik, S., Rani, S., and Kumar, V. (2015). Role of light wavelengths in synchronization of circadian physiology in songbirds. *Physiol. Behav.* 140, 164–171. doi: 10.1016/j.physbeh.2014.12.032

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

REVIEW
published: 08 December 2021
doi: 10.3389/fevo.2021.767177

11 Pressing Research Questions on How Light Pollution Affects Biodiversity

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Specialty section:
This article was submitted to
Urban Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: 30 August 2021
Accepted: 17 November 2021
Published: 08 December 2021

Citation:
Hölker F, Bolliger J, Davies TW, Giavi S, Jechow A, Kalinkat G, Longcore T, Spoelstra K, Tidau S, Visser ME and Knop E (2021) 11 Pressing Research Questions on How Light Pollution Affects Biodiversity. Front. Ecol. Evol. 9:767177. doi: 10.3389/fevo.2021.767177

Artificial light at night (ALAN) is closely associated with modern societies and is rapidly increasing worldwide. A dynamically growing body of literature shows that ALAN poses a serious threat to all levels of biodiversity—from genes to ecosystems. Many "unknowns" remain to be addressed however, before we fully understand the impact of ALAN on biodiversity and can design effective mitigation measures. Here, we distilled the findings of a workshop on the effects of ALAN on biodiversity at the first World Biodiversity Forum in Davos attended by several major research groups in the field from across the globe. We argue that 11 pressing research questions have to be answered to find ways to reduce the impact of ALAN on biodiversity. The questions address fundamental knowledge gaps, ranging from basic challenges on how to standardize light measurements, through the multi-level impacts on biodiversity, to opportunities and challenges for more sustainable use.

Keywords: ecological light pollution, biodiversity loss, thresholds, traits, populations, ecosystems, interdisciplinary, mitigation

INTRODUCTION

Our planet faces numerous challenges, many of which have direct and indirect connections to biodiversity (Díaz et al., 2020). One such challenge is artificial light at night (ALAN) leading to a fundamental change in the light environment over half of the Earth's surface—the Earth at night. ALAN has been growing exponentially since the nineteenth century and currently increases by 2–6% per year worldwide (Hölker et al., 2010a; Kyba et al., 2017). ALAN has been introduced in places, at times, spectra and intensities that do not occur naturally (Gaston et al., 2015). Ecosystems are largely organized by natural light-dark cycles, i.e., diurnal, yearly and lunar cycles, which have been stable over geological and hence evolutionary time scales. ALAN-induced disruptions of those cycles affect the structure and function of multiple levels of biodiversity that are again strongly interconnected (Longcore and Rich, 2004; Hölker et al., 2010b; Gaston et al., 2013). Given the growing global pervasiveness of ALAN (Kyba et al., 2017; Gaston et al., 2021), it is important to understand how multiple levels of biodiversity respond to it directly and indirectly (**Figure 1**).

Research into the ecological impacts of ALAN has exploded in recent years, mostly focusing on changes in species behavior and physiology (Gaston et al., 2015; Grubisic et al., 2019; Sanders et al., 2021). Yet, many "unknowns" remain that need to be addressed before we can understand and predict the impact of ALAN on multiple levels of biodiversity (genes and cells, individuals, populations, communities, ecosystems and landscapes, **Figure 1**), and develop effective mitigation measures. Here, we address these "unknowns" by synthesizing the results of a special session and follow-up discussions at the first World Biodiversity Forum in Davos, Switzerland, in 2020, where experts from across the world convened to discuss the impacts of ALAN on multiple levels of biodiversity over a broad spectrum of taxa in multiple biomes (aerial, aquatic, and terrestrial). We capture the complexity of the problem as broadly as possible by considering that different natural light cycles (**Figure 2A**) are affected by multiple forms of ALAN (e.g., streetlights, advertising lighting, skyglow, **Figure 2B**) with multiple effects (e.g., lethal attraction of organisms, disruption of circadian rhythms and erosion of ecosystem functions) at multiple levels of biodiversity in multiple realms (**Figure 2C**). Already at the World Biodiversity Forum it became clear that a transition toward the more sustainable use of ALAN is extremely challenging and requires answers to questions that can only be tackled by broadening the disciplinary perspective to strengthen transdisciplinary approaches.

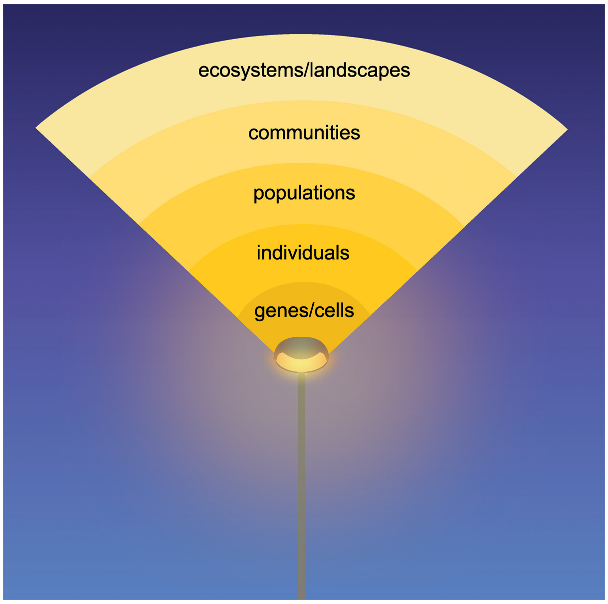

FIGURE 1 | Impact of artificial light at night (ALAN) on multiple levels of biodiversity. The multiple levels of biodiversity are interlinked, i.e., one level of biodiversity may respond to ALAN and modify processes at other biodiversity levels. For example, ALAN may impact the gene expression of certain clock genes, which results in a reduced fitness of individuals and a population decrease due to a phenological mismatch with other species and finally a changed community composition. This may impact ecosystem processes and nocturnal lightscapes (e.g., forests, coral reefs), which in turn influences all other levels.

11 PRESSING RESEARCH QUESTIONS

We identify 11 research questions that can be clustered into three main themes. We first outline questions linked to the diverse nature of natural and ALAN. Second, we outline questions related to the effects of ALAN on multiple levels of biodiversity. Finally, we formulate research needs on how to bend the curve of ALAN-induced biodiversity loss.

Interdisciplinary Barriers to Measuring Nocturnal Light

ALAN research is inherently interdisciplinary, with knowledge of the nighttime being fragmented across multiple subject areas including astronomy, physics, ecology, chronobiology, psychology, and engineering. Each field has different motivations for conducting ALAN research, and draws upon different instrumentation, measurement conventions, and experimental frameworks. Multiple units of measurement for light are encountered across the sciences, and many have little biological relevance. The absence of instruments capable of performing light at night measurements with the required level of detail, and insufficient training of biologists in radiometry and light propagation further compound this problem.

Q1 How to harmonize light measurement methods across disciplinary boundaries?

The interdisciplinary nature of ALAN research has resulted in different measurement approaches, procedures, and the use of various light units (Hänel et al., 2018), which complicates comparison of results (Kalinkat et al., 2021). Lighting engineers measure mainly in human-centric photometric SI units (mostly horizontal illuminance in lx, **Figure 3**), which some ALAN researchers have adapted for their studies to better facilitate the translation of results into lighting policy (see Q11). Astronomers mainly measure the radiance of the night sky (mostly at zenith) in units of magnitudes/arcsec^2 in different astronomical bands (see e.g., Patat, 2008). Some ALAN researchers have adapted the use of a simple radiometer, the Sky Quality Meter (SQM) that has its own spectral band (Hänel et al., 2018), which can be extended to multiple color channels (Kyba et al., 2012; Sánchez de Miguel et al., 2017). The meaningfulness of single point SQM measurements for biodiversity is, however, questionable and can be used in the wrong context (Longcore et al., 2020). Biologists tend to measure either irradiance or radiance in different spectral bands (e.g., photosynthetically active radiation—PAR) and sometimes report W (Watts) in micromole photons per seconds (μmol/s). Visual ecologists prefer wavelength resolved "hyperspectral" measurements, requiring high sensitivity spectrometers to resolve at low nighttime light levels (see Spitschan et al., 2016). Moreover, even within biodiversity research there are large disciplinary differences in the state of the art for measuring light. For example, while an array of measurement systems exists for terrestrial habitats, the attenuation of light in water makes hyperspectral light measurements even more challenging in aquatic ecosystems (Jechow and Hölker, 2019a; Tidau et al., 2021).

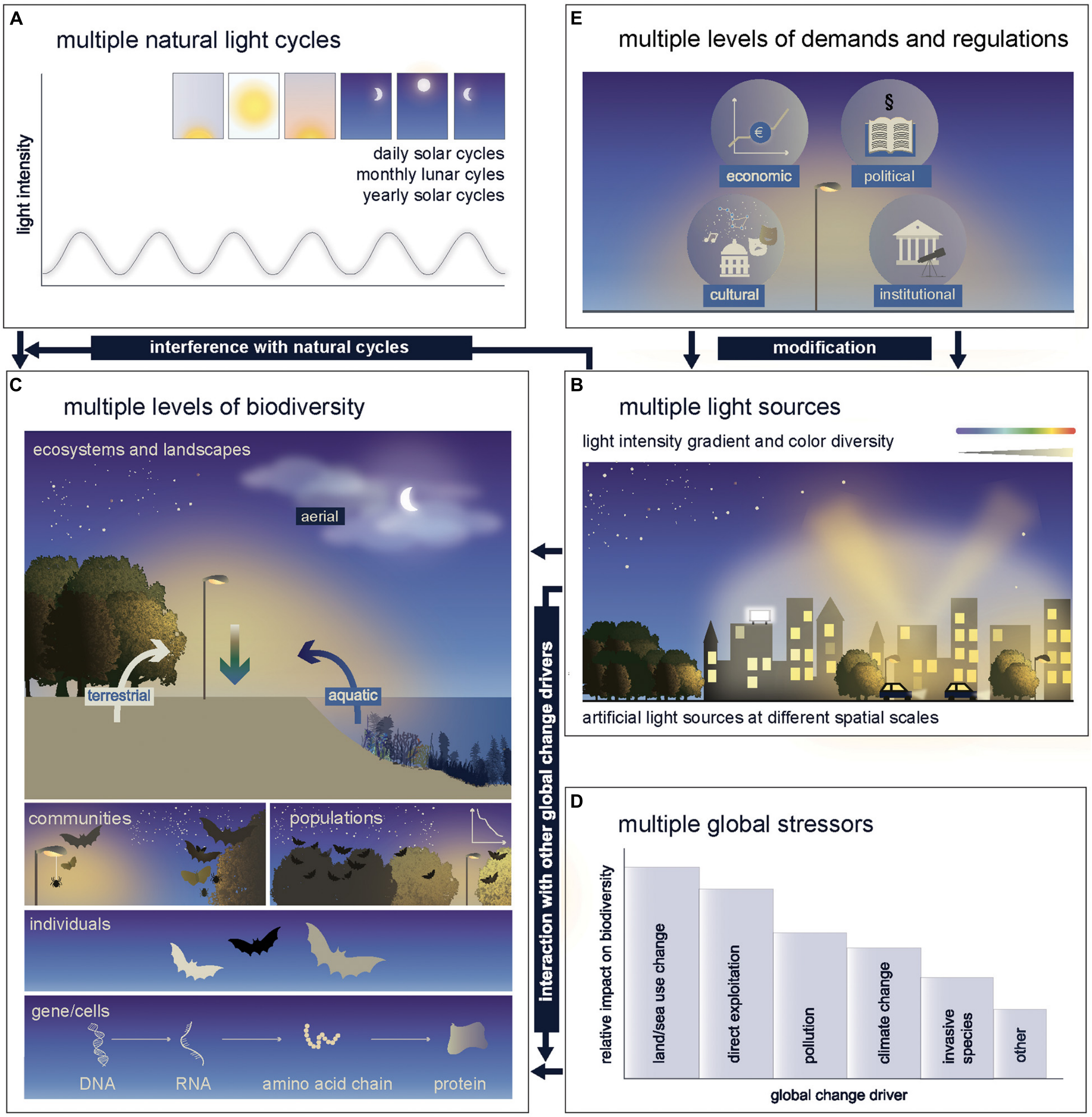

FIGURE 2 | Artificial light at night: Potential sources, biodiversity impacts and responses are complex. Different natural light cycles **(A)** are affected by multiple forms of ALAN **(B)** at multiple levels of biodiversity in multiple realms (e.g. from bottom up: gene expression; phenotypes; population dynamics, e.g. decline; community composition; species dispersal and/or organismic fluxes across ecosystem boundaries and bioms) **(C)**. ALAN can interact with multiple global change stressors **(D)**. Due to the potentially conflicting demands of ALAN a transition to a more sustainable use is extremely challenging and requires multiple levels of regulations **(E)**.

For biodiversity studies, nocturnal light ideally would be measured in biologically relevant ways, based on thresholds and spectral sensitivities of the species under question (see Q2 and Q5), because different light sources interfere differently with the large diversity of sensory systems in nature (Davies et al., 2013; see Q3). Furthermore, it is important to perform and provide ALAN-free natural light reference measurements for different habitats, seasons and weather conditions (Jechow and Hölker, 2019b). One challenge is to break disciplinary boundaries by, for example, connecting photometry of anthropogenic light sources (performed by lighting engineers) and night-sky or night-time radiometry (see e.g., Foster et al., 2021) to visual ecology and species responses (van Grunsven et al., 2014; Longcore et al., 2018; Seymoure et al., 2019). Thus, standardized light measurements that allow comparison across ALAN disciplines are desperately needed. Recent proposals favor spatially resolved multi-spectral night-time radiance measurements of the full light field (not just the upper hemisphere or at zenith) with digital cameras with fisheye lenses in the RGB bands (Jechow et al., 2019; Nilsson and Smolka, 2021), which is further supported by new calibration strategies (Fiorentin et al., 2020; Cardiel et al., 2021) and the proposal of a dark sky unit (Kolláth et al., 2020). This method has radiance and irradiance information in three spectral bands in one image. Additional hyperspectral measurements

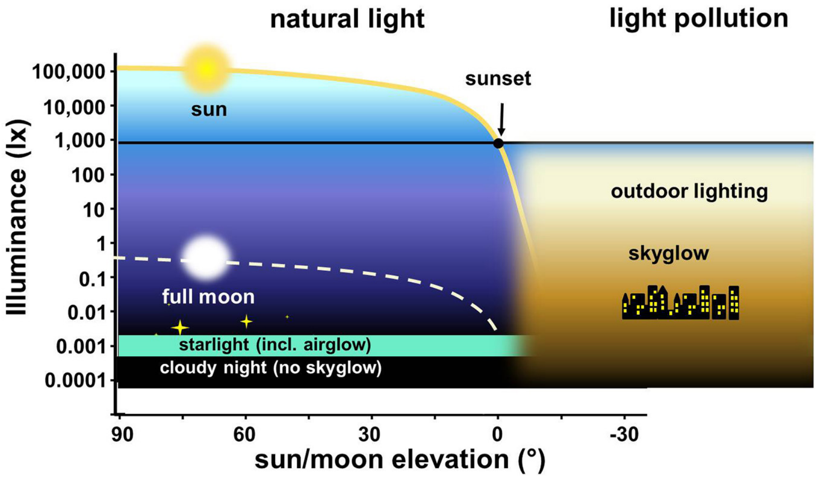

FIGURE 3 | Ranges of exposure that animals experience and respond to with natural variation in light and light intensities observed with ALAN (here using human-centric metric lux). Illuminance during day, twilight, and night as a function of elevation angle of sun and moon; yellow solid line—sun illuminance on clear day, gray dashed line—moonlight full moon.

or species-specific bands, however, are required for visual ecologists, and a translation to photometric units is essential for policy making and the connection to lighting professionals. Thus, a wider and more interdisciplinary harmonization of different needs for a broad application is required to help to establish standardized protocols that are currently lacking. These protocols should match the ecological and biological responses being investigated in terms of spectral band and resolution, directionality (radiance vs. irradiance; scalar vs. planar) and time scale (see Q2). Such consistent and cross-disciplinary standards for measurement are also necessary to formulate thresholds for mitigation and management (Jechow and Hölker, 2019b; Davies et al., 2020; see Q11).

Q2 What are biodiversity-relevant light-measurements and methods?

The multiple realms and levels of biodiversity all have specific photic properties and measurement requirements. At the same time, quantifying ALAN is rather a complex task that requires method development and training. Commercial, off-the-shelf measurement equipment is rarely appropriate because it lacks sensitivity and sufficient spatial and/or spectral resolution. This becomes particularly challenging in aquatic or aerial environments (Jechow and Hölker, 2019a). Furthermore, ecologists often lack sufficient understanding of radiometry in terms of units and measurement approaches, making it hard for ecologists to interpret measurements obtained outside of their field and translate them for their research (see Q1).

In ecological studies, information on ALAN is in most cases derived from single point ground-based measurements with limited spatial, temporal and spectral resolution, very often using human centric devices like lux meters. To be able to understand what an organism perceives, it is important to have the full spatial and spectral information of the light field. This could be acquired either with a spectroradiometer mounted on a rotational head that scans the radiance over the whole sphere (Kocifaj et al., 2018) or with a full-sphere hyperspectral camera that works at night-time, which is not available yet (but see Alamús et al., 2017 for night-time measurements and Shiwen et al., 2021 for full-sphere hyperspectral imaging in a forest during day). Both solutions are not technically mature, and the best current approximation is full-sphere imaging with a fisheye lens digital camera system with limited spectral resolution in the RGB bands (Jechow et al., 2019). Further technological development toward a hyperspectral solution are necessary and interim steps could be adding additional spectral bands to such imaging systems like in the ASTMON system (Aceituno et al., 2011) or by tailoring camera systems to achieve hypercolorimetric multispectral imaging (Colantonio et al., 2018). Ideally, the measurement strategy should also cover temporal variations in light on short time scales but also seasonal variations (**Figure 2A**). Remaining obstacles of such a holistic approach are the complexity of data and handling as well as potential high costs of a sophisticated device. A combination of multiple measurement devices (multispectral camera, hyperspectral single point, photometric single point) might be a more practical compromise.

Another pressing issue is the extrapolation of single point ground-based measurements to larger areas, which is relevant, for example, for migratory species. Here, challenges are posed by remote sensing approaches that are almost always only proxies for the ecological variable of interest. Night-time satellite data are limited in spatial, spectral and temporal resolution, but color imaging from the international space station ISS, airborne measurements, and particularly UAVs have the potential to fill the existing gaps in ALAN related biodiversity research (Bouroussis and Topalis, 2020; Levin et al., 2020; Sánchez de Miguel et al., 2021). A key component to improving the utility of these data would be research efforts that can translate what remotely sensed values could represent for conditions at ground level, where first steps have been taken but uncertainties remain high (Simons et al., 2020). Furthermore, there is an urgent need for stand-alone satellite missions focused on understanding light pollution and its effects on biodiversity (Barentine et al., 2021).

Focusing on skyglow is becoming a larger component of ALAN research on biodiversity (Kyba and Hölker, 2013). Skyglow occurs when ALAN radiates or reflects toward the sky and the light scatters at atmospheric particles and brightens the night sky (Aubé, 2015). In contrast to direct ALAN, skyglow can act as a pollutant far away from its origin and therefore has the potential to affect biodiversity over large spatial scales. Skyglow is dynamic as it depends on atmospheric constituents, seasonal effects such as leaf cover and ground albedo (Jechow and Hölker, 2019b) and can be dramatically amplified by clouds (Kyba et al., 2011) causing ground illuminance brighter than moonlight in extreme situations (Jechow et al., 2020; **Figure 3**). Skyglow is often insufficiently quantified with single channel spectral and spatial measurements at zenith. Again, multispectral (RGB) fisheye-lens digital camera systems are promising for skyglow measurements with more ecologically relevant information but such systems need wider application in ecology (Thums et al., 2016; Levin et al., 2020). Permanent installations can track skyglow dynamics across the full sky dome (Jechow et al., 2018) and if a similar measurement system is used for quantification of direct light pollution, results become comparable.

Linkage between skyglow and remotely sensed night-time light is possible via modeling. While a static world-wide model for skyglow exists and is being widely used in ecological studies (Falchi et al., 2016), a dynamic model that includes atmospheric changes, cloud cover, snow, vegetation cover and similar factors is still lacking.

Consequences for Biodiversity

Increasing evidence shows that the interference of ALAN with natural cycles of light and darkness, i.e., changes of photoperiod, intensity and spectra, influences a wide range of biological processes, from gene expression to ecosystem functioning, yet many questions remain about species and trait specific sensitivities to ALAN, and how these affect biodiversity at different scales (Gaston et al., 2015; Hopkins et al., 2018; Dominoni et al., 2020a; Falcon et al., 2020; Sanders et al., 2021; **Figure 2**). In this context, the following eight questions (Q3–Q10) need to be addressed.

Q3 What are the relevant photoreceptor systems and their key sensitivities?

Photosensory systems are near ubiquitous in nature and are found across the animal and plant kingdom down to single-celled organisms. Accordingly, photosensory systems and their response to light are hugely diverse, ranging from single photoreceptor cells to complex image forming camera-type eyes, which can capture (spatial) information and facilitate color guided behaviors as well as polarization patterns invisible to humans (Horváth et al., 2009; Land and Nilsson, 2012).

Photic stimuli, however, strongly vary between biological realms, across seasons and over the course of a day (**Figures 2A**, **3**). For example, the optical properties of freshwater and seawater strongly attenuate the light aquatic organisms are exposed to, shaping light environments that greatly differ in spectral composition, intensity and spatial information from those on land. Seawater in the open ocean attenuates blue light the least and hence many marine organisms are sensitive in this spectral region. In contrast, coastal waters and freshwater systems are transparent at different (typically longer) wavelengths and variable in their inherent optical properties, causing a less specific adaptation of organisms for specific wavelengths (Grubisic et al., 2019; Kühne et al., 2021).

Many nocturnal and crepuscular organisms are adapted to dim photic stimuli; some can use celestial bodies as a source of information (Dacke et al., 2003; Ugolini et al., 2003; Foster et al., 2018). Moonlight serves as a major environmental cue, for example entraining diel vertical migration on zooplankton down to 100 m (Last et al., 2016). On the other hand, the high sensitivity to low intensities of natural light makes those organisms (both terrestrial and aquatic) prone to disruptions even by low intensity ALAN, such as the globally widespread artificial skyglow (Moore et al., 2000; Kupprat et al., 2020; Torres et al., 2020; **Figure 3**).

ALAN research is inevitably inhibited by the lack of species for which photoreceptor systems and key spectral and light sensitivities of photobiological responses have been adequately described (e.g., overview for marine organisms in Tidau et al., 2021). For one of the best photobiologically studied classes, insects, a recent literature search revealed information on the spectral sensitivity of photoreceptors of only 221 insect species from 82 genera and 13 orders (van der Kooi et al., 2021). With almost 1 million species, half of which are nocturnal (Hölker et al., 2010b), this represents less than 0.03% of all insect species. Nonetheless, phylogenetically conserved patterns of sensitivity have been identified in some animal classes. Both visual and non-visual photoreceptor systems and their corresponding spectral sensitivities can be conserved within taxonomic groups (e.g., in mammals melanopsin and sensitivity to blue wavelengths). Most terrestrial insects are particularly attracted to ultraviolet and blue light (Donners et al., 2018; but see also Owens and Lewis, 2021). Similarly, most arthropods show a greater responsivity to blue light and most vertebrates show a lower responsivity to longer wavelengths (Davies et al., 2013; Longcore et al., 2018; Grubisic et al., 2019). Furthermore, within habitats, specific spectral sensitivities might be common to the organisms living there. For example, many species of aquatic turtles and fish are more sensitive to longer wavelengths in freshwaters and to shorter wavelengths in clear marine systems, i.e., their sensitivities relate to the optical water properties where they typically evolved (Grubisic et al., 2019; Wyneken and Salmon, 2020).

More fundamental research in visual biology is needed to describe both the inherent sensitivity of animal visual and non-visual systems to base ALAN research on. In addition, the spectral dependence of behavioral and physiological responses to light, and their relationship to intensity, ecological context, previous light exposure, and other factors demand attention.

Q4 Which species traits are most sensitive to ALAN?

Generalizations about which traits of species may be most sensitive to certain factors of global change are useful for predicting their ecological consequences. As with many manmade impacts on the natural environment, nighttime lighting can filter out species with functional response traits that cause species to be more sensitive to ALAN (Franzén et al., 2020;

Cox et al., 2021; Voigt et al., 2021). To give an example, high sensitivity to light in the eyes of North American bird species has been associated with a greater advancement of reproductive timing in response to light exposure, possibly leading to phenological mismatches (Senzaki et al., 2020). Elucidating biological traits that predicate sensitivity to ALAN is therefore critical for identifying ALAN-vulnerable species around the world (Secondi et al., 2020).

Light-dark cycles vary along latitudinal gradients (Hut et al., 2013), hence species traits that predicate sensitivity to ALAN are also expected to vary with latitude (Secondi et al., 2020). While birds in lower latitudes started to sing earlier when exposed to ALAN, in high latitudes the seasonal increase of natural light can mask ALAN effects on the onset of bird song (Da Silva and Kempenaers, 2017). Furthermore, 47 out of 140 bird species studied in North America are becoming more abundant with increasing light exposure during longer nights, probably because light extends the perceived photoperiod and birds take advantage of higher visibility (Wilson et al., 2021).

Meta-analyses of species demonstrably impacted by ALAN proved to be useful for identifying ALAN sensitive traits (e.g., Sanders et al., 2021), which may include eye/body size ratios indicating light sensitivity, aspects of eye morphology, mobility (e.g., sessile organisms vs. mobile), geographical range size (e.g., probability that migrating species have of encountering ALAN), life history traits, temporal niche (e.g., nocturnality or crepuscularity), habitat affiliation, and seasonal and lunar phenological events (e.g., timing of reproduction). We are just beginning to understand which biological traits predict sensitivity to the disruption of natural light intensity, cycles, and spectra due to ALAN (Grubisic et al., 2019; Sanders et al., 2021). Future studies should also examine in more animal and plant species how functional traits and contexts (e.g., latitude, habitat affiliation) relate to sensitivity to ALAN.

Q5 Above which thresholds does ALAN exposure become critical?

The majority of documented ALAN effects on species are in response to single exposure levels (Davies et al., 2017; Manfrin et al., 2017; van Grunsven et al., 2020). These studies have proved valuable for drawing attention to the sheer scale of ALAN impacts on individual species. In reality, irradiance of ALAN perceived by organisms varies spatially. An important prerequisite for upscaling to different ecosystems and landscapes (see Q9) is an understanding of changes in the measured biological responses as a function of multiple exposure levels (Brüning et al., 2015; Sanders et al., 2015; de Jong et al., 2016). Although challenging to deliver, and sometimes giving idiosyncratic results, dose-response experiments have proved powerful at identifying critical exposure thresholds in toxicology (Vandenberg et al., 2012), and should be a focal point for current and future ecological light pollution experiments (Brüning et al., 2015; de Jong et al., 2016; Kupprat et al., 2020). In particular, we need more studies testing for the effects of ALAN of lower intensities (e.g., from skyglow), which many organisms may experience throughout large areas worldwide (Kyba and Hölker, 2013; Grubisic et al., 2019) (see Q2).

Similarly, we lack an understanding of the sensitivity of species to the spectra of ALAN. Quantifying wavelength-dependent responses to ALAN is critical to predicting the impact of different lighting technologies and identifying spectra that can minimize deleterious impacts (Spoelstra et al., 2015; Brüning et al., 2016; Donners et al., 2018; Longcore et al., 2018). The utility of this approach is empirically well demonstrated; however, its application across a broader range of species is confined to those whose spectral response curves have been quantified. Consistencies in the number of photoreceptors and maximal wavelengths of sensitivity can and are used to form generalizations across broad taxonomic groupings (e.g., class, order; Davies et al., 2013; van Grunsven et al., 2014; Kühne et al., 2021). High precision insights into the responses of individual taxa however remain constrained by published spectral sensitivity information. Advances in our understanding of ALAN impacts are, in this way (and many others), partly limited by advances in fundamental photobiology and visual ecology (see Q3).

Q6 How and at what rates can populations adapt to ALAN?

The spatial light distribution, spectral composition, and the intensity of ALAN are unprecedented on evolutionary time scales (Hopkins et al., 2018). As when studying many anthropogenic impacts on the natural world, initial experiments have quantified behavioral and/or physiological responses to ALAN (Tuomainen and Candolin, 2011; Gaston et al., 2015). These effects on the individual will, however, compromise organism fitness (Sih et al., 2011) such as survival and reproductive success, ultimately manifesting impacts on population demography, and/or lead to microevolution (**Figure 2C**). To give one example, insects that are drawn to light will either die (e.g., by predation or exhaustion) or have reduced fitness compared to individuals of the same population that are less attracted by light. If this variation in light attraction has a heritable basis, this may lead to a response to the selection of genotypes in the population that are less attracted to light (Altermatt and Ebert, 2016). In those cases, ALAN will lead to micro-evolution. To date, much of the available knowledge on ALAN impacts is based on short-term experiments that are not able to observe evolutionary compensation mechanisms over long periods of time (Gaston et al., 2015; Kalinkat et al., 2021).

The challenge of analyzing ALAN-related trait changes (e.g., body size, relative eye size, and wing length, see Q4) over many generations could be addressed by examining museum vouchers collected for long periods of time (e.g., Keinath et al., 2021). Furthermore, ALAN research could adopt approaches and tools from quantitative genetics to understand and predict how species evolutionarily adapt to changes in their light environment. For this, both selection on ALAN-related traits needs to be measured and the heritability of these traits estimated. The rate of genetic change may, however, be small, as has been shown for wild species (Charmantier and Gienapp, 2014), and is likely to be too slow to adapt. This is because anthropogenic disturbances often introduce more rapid rates of environmental change compared to what organisms have experienced in their evolutionary past (Palumbi, 2001). The rate of genetic change is likely to be higher for species with a short generation time and standing genetic variation, such as microorganisms or insects. Indeed, some examples have been documented of the micro-evolution of insect species in response to climate change (Bradshaw and Holzapfel, 2001; Van Asch et al., 2007). In this context, evolutionary trap theory may offer a framework for understanding and mitigating the effects of ALAN (Haynes and Robertson, 2021).

Q7 How does ALAN alter biodiversity by redistributing species?

ALAN is known to affect the behavior of species, including altered orientation, navigation, foraging, and predator avoidance behaviors of a wide range of organisms (McLaren et al., 2018; Manríquez et al., 2019). One frequently observed phenomenon is the aggregation of individuals in artificially lit patches, reducing their presence in the darker surroundings. Conversely, the density of species repelled by light is likely to increase in dark locations neighboring illuminated areas (Manfrin et al., 2017; Giavi et al., 2020). Such heterogeneous responses to ALAN among and within taxonomic groups change species distribution patterns and create novel communities (Hölker et al., 2015; Sanders and Gaston, 2018; Voigt et al., 2021; **Figure 2C**) with potential cascading effects on ecosystem functions such as mineralization, pollination, or seed dispersal (Lewanzik and Voigt, 2014; Knop et al., 2017; van Grunsven et al., 2018). To date, our knowledge on how the effects of ALAN on community composition might be scaling up to affect ecosystem processes remains limited (Knop et al., 2017; Grubisic et al., 2018; Giavi et al., 2020).

Long term monitoring studies replicated at large spatial scales represent one option for quantifying changes in species distributions in response to ALAN, but they are still very rare (van Grunsven et al., 2020; Kalinkat et al., 2021). One reason is that such experiments present significant logistical and financial challenges, principally due to the large levels of replication required to control for multiple confounding environmental factors that are likely collinear with ALAN. A further challenge is to have data on how long and with which light properties ALAN has been applied (see Q2). Since there can be marked between-year variation in the influences of ALAN, it is critical to run such experiments linked to environmental context and seasonal timing over several generations of key species (ideally more than 10 years, van Grunsven et al., 2020; Kalinkat et al., 2021).

Q8 How does ALAN affect biodiversity through indirectly altering species interactions?

Global environmental pressures threaten biodiversity directly through changes in species' physiology and behavior, and indirectly through interactions between impacted species and other species within ecological communities (Tylianakis et al., 2008). To date only a few studies have quantified indirect effects of ALAN caused by altered species interactions within (Knop et al., 2017; Giavi et al., 2020, 2021), or across trophic levels (Bennie et al., 2018; Manfrin et al., 2018; Sanders et al., 2018; Maggi et al., 2020), and we are far from being able to predict where indirect effects occur and their likely importance for the wider ecosystem.

Further mesocosm experiments that manipulate a more diverse array of interacting communities are needed (Sanders et al., 2018). Also, further field studies are necessary such as studies on changes in the structure of entire species interaction networks and linking these to ecosystem functions (e.g., Knop et al., 2017). Furthermore, indirect approaches that document altered species interactions due to ALAN, such as stable isotope analyses (Manfrin et al., 2018), molecular analyses of gut contents or fecal samples (Cravens et al., 2018), GPS data analyses of predator–prey dynamics (Ditmer et al., 2021), or the analyses of pollen transport networks (Macgregor et al., 2017) are other promising approaches. Finally, more emphasis should also be placed on spatial (Giavi et al., 2020) and on temporal indirect effects of ALAN on species interactions.

Q9 What are the effects of ALAN on biodiversity at the ecosystem and at the landscape level?

Effects of global change drivers on biodiversity and ecosystem functioning might vary between ecosystems (Sage, 2020). In this vein, we can expect that the effect of ALAN on biodiversity will not be consistent across ecosystems and landscapes with some systems and areas being more susceptible to light pollution than others. On the one hand, the spread of light within ecosystems might vary depending on ecosystem type, lighting technology and medium (e.g., air, water, see Q3). The structure of a forest, for example, leads to a stronger attenuation of light (vertically and horizontally) compared to grasslands, which may make species adapted to closed habitats more vulnerable to ALAN compared to species adapted to open habitats (Voigt et al., 2021; Wilson et al., 2021). On the other hand, different latitudes harbor different ecosystems and hence species inhabiting them. Yet, the extent to which photoperiod and climate modulate exposure to ALAN at a given latitude is unknown (Secondi et al., 2020). Also, ecosystems interact and thus changes in one ecosystem due to ALAN will likely also impact linked ecosystems (e.g., Manfrin et al., 2017; **Figure 2C**). Finally, there is mounting evidence of ALAN impacts on ecosystem engineers such as corals and intertidal crabs, which can again modify the environmental context they are embedded in and hence affect biodiversity at the ecosystem level (Ayalon et al., 2021; Nuñez et al., 2021).

Light corridors and networks (e.g., illuminated roads), as well as light patches (e.g., an illuminated gas station in a dark environment) can impact landscapes by acting as barriers to movement and dispersal, and as population sinks (Degen et al., 2016; Laforge et al., 2019; van Grunsven et al., 2020). Changing the ability of species to move through landscapes may alter foraging and reproductive opportunities for individuals, modifying habitat connectivity and gene flow between populations, disrupting recolonization of habitat patches, and altering metapopulation dynamics (Caplat et al., 2016; Grubisic et al., 2018; Camacho et al., 2021; Gaston et al., 2021).

Even though the effects of ALAN on biodiversity might vary across ecosystems and landscapes, most research has focused on a very limited range of ecosystems, namely on natural terrestrial systems of temperate and developed regions. Also, nearly all documented effects are on individuals and ecological communities, while our understanding of effects on ecosystems and at the landscape level remains limited (Secondi et al., 2020). Empirical upscaling of individual level responses (see Q5) to changes in species biogeography would provide compelling evidence of ALAN's potential to reshape nature at the landscape scale. Thus, despite challenges in accessing certain ecosystems (marine offshore, high altitudes, lakes) remain, more work in a wider array of ecosystems and landscapes is warranted, both within and between biogeographic realms.

Q10 How does ALAN interact with multiple global change stressors?

ALAN is considered to be a major driver of global change with negative consequences for biodiversity (Hölker et al., 2010b; Davies and Smyth, 2018). It typically co-occurs with other global change drivers, such as climate change, anthropogenic noise, or land-use change, especially in urban areas (Perkin et al., 2011; Halfwerk and Slabbekoorn, 2015; Swaddle et al., 2015; Dominoni et al., 2020a; **Figure 2D**). Interactive effects of ALAN with other global change drivers are therefore likely (Rillig et al., 2019), which could be additive, antagonistic or synergistic (Jackson et al., 2016; Birk et al., 2020). To give one example, Miller et al. (2017) demonstrate that night-time warming combined with light pollution had non-additive impacts on predator–prey interactions. These stressors, however, often vary in parallel making it challenging to disentangle their effects on biodiversity.

A number of approaches exist to quantify the impact of ALAN on biodiversity in combination with other global change factors. While none of these in isolation will be enough to resolve the complexity of multiple interacting stressors, they can provide discrete novel insights that collectively provide a weight of evidence to direct future research. Firstly, under controlled conditions fully crossed factorial experiments can empirically quantify the existence of interactions between global change stressors (McMahon et al., 2017; Dominoni et al., 2020b). Secondly, when ALAN is experimentally controlled in natural situations for years, and a second factor varies over time, like temperature or precipitation, the interaction between ALAN and these stressors can be assessed. For instance, there is only an effect of ALAN on seasonal timing in great tits (*Parus major*) in cold springs, when the birds lay on average late (Dominoni et al., 2020c). A similar observation has been made for the impact of ALAN on the timing of bud-burst, with a pronounced effect of light on late-budding tree species (Ffrench-Constant et al., 2016). Thirdly, when the intensity or spectral characteristics of ALAN are changed and another stressor remains constant, a BACI (before-after, control-impact) approach could be used to quantify any emergent responses over time. Fourthly, in a recent study, Wilson et al. (2021) introduced another promising approach to disentangle the combined effects of different anthropogenic stressors. They used a large data set generated by community and citizen scientists[1] that allowed them to analyze effects of ALAN and noise pollution on bird occurrences. Although this approach is prone to various spatial and temporal biases (e.g., Geldmann et al., 2016), the large scale and high number of observations in such projects may enable insightful analyses given carefully tailored statistical models (e.g., Bird et al., 2014). In a similar vein, so-called distributed experiments by multiple research teams across countries and continents (e.g., the NutNet experiment; Borer et al., 2014) are another option to capture a much larger range of co-exposure to ALAN and other relevant stressors. To date we are not aware of any distributed experiments that explicitly address ALAN in combination with other stressors. Finally, the additive or interactive effects of stressors can be estimated from meta-analysis, provided that there are a sufficient number of studies (Birk et al., 2020).

Bending the Curve of Biodiversity Loss

Light pollution is only recently coming to the attention of those beyond the interested scientific communities such as lighting professionals (Schulte-Römer et al., 2019; Pérez Vega et al., 2021). The effect of light pollution in environmental and social considerations remains largely under-acknowledged. The consequence is that "sustainable lighting" currently aims mainly toward energy efficient technology to reduce the carbon footprint of lighting while ignoring the adverse effects of light pollution on biodiversity. To bend the curve of biodiversity loss (i.e., to reverse the decline) solid transdisciplinary solutions that have emerged from a collaboration of practice, research, production, decision-making and planning are crucial.

Q11 What are opportunities and challenges for an effective management of ALAN?

Although there is already evidence of readily available, and inexpensive, mitigation strategies that work (e.g., light orientation, proper shielding, intensity scaled to intended use, and spectral tuning (Hölker et al., 2010a; Gaston et al., 2012; Schroer and Hölker, 2017), uncertainty remains regarding which approaches are best for reducing the ecological effects of ALAN. The pros and cons of different approaches continue to be debated at length as a result of conflicting cultural, political, economic, and institutional demands (**Figure 2E**). At the same time, novel lighting technologies and concepts are constantly emerging. Thus, biodiversity-friendly ALAN solutions need to be solicited with a broad range of actors originating from different backgrounds, which makes successful negotiations for sustainable lighting challenging (see also Q1).

Many attempts to reduce light pollution run up against positive connotations of lighting (e.g., aesthetics, modernity, and security), which are deeply ingrained in modern societies (Jakle and Thompson, 2001; Hölker et al., 2010a). While there is a general perception that urban lighting improves safety and security regarding traffic accidents and crime, the empirical evidence is not very solid (Marchant et al., 2020). Awareness raising campaigns are needed to garner public support for implementing biodiversity friendly ALAN management strategies (Zielińska-Dabkowska et al., 2020), however such campaigns should draw on the benefits of darkness as a source of quietness and recovery, as much as its importance for biodiversity conservation.

Despite numerous attempts to control light pollution and to reduce its impact through policies regulating the use of outdoor ALAN, minimal success has been achieved in a limited number of geographic areas (Barentine, 2020). One reason for this is that the regulatory management of ALAN depends heavily on the political and administrative actors involved, who must take into account various aspects such as safety and security, energy efficiency, design, and health and environmental concerns. Future transdisciplinary policy initiatives to address light pollution must therefore consider

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[1] www.feederwatch.org

the many benefits of ALAN while addressing its negative impacts (Hölker et al., 2010a; Challéat et al., 2021). A legislative shortcoming in environmental protection is that often only species with special protection status are protected if they show, for example, avoidance behavior toward ALAN. Adverse effects on species and landscapes without special protection status are rarely considered by existing regulations (Schroer et al., 2020). In addition, outdoor lighting policies should consider the entire makeup of urban lighting (for example advertising, architectural lighting, and sports lighting), rather than focus solely on road lights (Kyba et al., 2021). A promising example is the German "insect protection" law recently implemented in the Federal Nature Conservation Act (Thomas, 2021), which aims to achieve a balance between emission regulation and immission control. The respective ordinance is still pending, where several issues on the measurement and assessment have to be specified (especially Q1–Q5). The conservation concept of implementing dark ecological networks consisting of core areas, corridors, and buffer zones to limit the impacts of light pollution on biodiversity at the landscape level is another interesting example (e.g., Challéat et al., 2021).

Although lighting professionals (e.g., design and industry) increasingly acknowledge ALAN as a threat to biodiversity, there are diverging views regarding potential obstacles to light pollution mitigation (Schulte-Römer et al., 2019; Pérez Vega et al., 2021). Current technological advancements in outdoor lighting, particularly LEDs, in principle allow developing lighting mitigation strategies that balance conflicting interests between humans and biodiversity, but this potential remains largely untapped (Longcore, 2018; Bolliger et al., 2020; Deichmann et al., 2021; Jägerbrand and Bouroussis, 2021). Furthermore, LEDs are an energy efficient technology that promises net savings in energy consumption. Past experience has shown that lighting is often subject to a strong rebound effect, where an increase in luminous efficacy resulted in higher light consumption rather than the targeted energy savings. Unfortunately, such a rebound effect is most likely also currently observed for LED technology, which can ultimately lead to further loss of natural nightscapes (Hölker et al., 2010a; Kyba et al., 2014).

A systematic consideration of ALAN issues that facilitates successful translation to a future sustainable lighting policy that harmonizes the needs of diverse stakeholder groups is still lacking (Pérez Vega et al., 2021). Achieving this demands inter- and transdisciplinary research involving collaboration between lighting engineers, ecologists, and other relevant stakeholder groups. Interdisciplinary institutions that work on the topic of light pollution and biodiversity conservation are currently lacking, in part because consideration of nighttime ecology is significantly underrepresented in ecological research. Gaston (2019) argues for a synthetic research program in this area of science. Interdisciplinary institutions such as research institutes or university departments for nighttime and light pollution research could be of great help to develop multi-level and cross-scale concepts, assessments, and evaluations of developments toward sustainable lighting (Kyba et al., 2020).

CONCLUSION

Due to anthropogenic activities, biodiversity has declined around the globe (IPBES, 2019) and global biodiversity is facing a sixth mass extinction (Barnosky et al., 2011). ALAN is one of the global change drivers (Davies and Smyth, 2018) contributing to the worldwide decline of biodiversity. Our summary of 11 key pressing questions shows that future research needs to address a mix of complex and interrelated questions to better assess the consequences of ALAN for biodiversity and to have a basis for designing efficient measures to minimize its ecological impacts. The goal for future interdisciplinary research should be to guide the diverse field of research, so information on biodiversity-relevant nocturnal light will be accessible, rigorous, and comparable across studies and disciplines. This requires a broader thinking about how to best characterize and measure ALAN from the perspective of the species or habitat of concern. Furthermore, we have to move from focusing on the physiological and behavioral effects on single species to how ALAN affects all levels of biodiversity including genotypes, communities, ecosystems, and landscapes, including direct and indirect interactions within and among those levels. The different natural light cycles, their role in shaping biodiversity, and their interaction with ALAN impacts needs more attention. Furthermore, ALAN should no longer be considered in isolation from other global change drivers but rather be addressed in a multiple stressor framework where sufficient knowledge of singular impacts allows. The same is true for considering multiple realms (e.g., aerial, aquatic, and terrestrial) and their interactions. This requires that we advance our techniques for quantifying spectral, spatial, and temporal ALAN patterns at multiple scales. Finally, it is not enough to only report that ALAN negatively impacts airborne, aquatic, and terrestrial organisms and ecosystems. More socio-ecological research needs to be directed toward understanding the cultural, political, economic, and institutional barriers that prevent implementation of mitigation measures and toward testing whether and how ALAN can be regulated and light pollution abated effectively.

We conclude that to further develop effective conservation measures aimed at reducing ALAN-induced biodiversity loss, a variety of challenges need to be addressed, ranging from broadening disciplinary perspectives (e.g., from individual species to communities) to strengthening transdisciplinary approaches and ultimately protecting species, ecosystems, and landscapes through effective conservation measures. For now, the information available to inform mitigation strategies remains modest, and as such the precautionary principle should be adopted as the basis for management recommendations while we answer the open questions identified in this study.

AUTHOR CONTRIBUTIONS

FH and EK contributed to the conception, design of the article, and wrote the first draft of the manuscript. FH, AJ, and EK produced the figures. All authors contributed manuscript text, manuscript revisions, and approved the final version.

FUNDING

The contributions of GK, AJ, and FH were supported by the projects “species protection through environmental friendly lighting” by the German Federal Agency for Nature Conservation (BfN) within the framework of the Federal Programme for Biological Diversity with funds from the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU; FKZ: 3518685A08) and “Beleuchtungsplanung: Verfahren und Methoden für eine naturschutzfreundliche Beleuchtungsgestaltung” by the BfN with funds from the BMU (FKZ: 3521 84 1000). The contributions of ST and TD were supported by the Natural Environment Research Council (grant no. NE/S003533/2 awarded to TD. The publication of this article was funded by the Open Access Publishing funds of the Leibniz Association and Agroscope.

ACKNOWLEDGMENTS

We would like to thank Catherine Pérez Vega who edited **Figures 1**, **2**.

REFERENCES

Aceituno, J., Sánchez, S. F., Aceituno, F. J., Galadí-Enríquez, D., Negro, J. J., Soriguer, R. C., et al. (2011). An all-sky transmission monitor: ASTMON. *Publ. Astron. Soc. Pac.* 123, 1076–1086.

Alamús, R., Bará, S., Corbera, J., Escofet, J., Palà, V., Pipia, L., et al. (2017). Ground-based hyperspectral analysis of the urban nightscape. *ISPRS J. Photogr. Rem. Sens.* 124, 16–26. doi: 10.1016/j.isprsjprs.2016.12.004

Altermatt, F., and Ebert, D. (2016). Reduced flight-to-light behaviour of moth populations exposed to long-term urban light pollution. *Biol. Lett.* 12:20160111. doi: 10.1098/rsbl.2016.0111

Aubé, M. (2015). Physical behaviour of anthropogenic light propagation into the nocturnal environment. *Philos. Trans. R. Soc. B* 370:20140117. doi: 10.1098/rstb.2014.0117

Ayalon, I., Rosenberg, Y., Benichou, J. I., Campos, C. L. D., Sayco, S. L. G., Nada, M. A. L., et al. (2021). Coral gametogenesis collapse under artificial light pollution. *Curr. Biol.* 31, 413–419. doi: 10.1016/j.cub.2020.10.039

Barentine, J. C. (2020). Who speaks for the night? The regulation of light pollution in the ‘Rights of Nature’ legal framework. *Int. J. Sust. Light.* 22, 28–36. doi: 10.26607/ijsl.v22i2.104

Barentine, J. C., Walczak, K., Gyuk, G., Tarr, C., and Longcore, T. (2021). A case for a new satellite mission for remote sensing of night lights. *Rem. Sens.* 13:2294. doi: 10.3390/rs13122294

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O., Swartz, B., Quental, T. B., et al. (2011). Has the Earth’s sixth mass extinction already arrived? *Nature* 471, 51–57. doi: 10.1038/nature09678

Bennie, J., Davies, T. W., Cruse, D., Bell, F., and Gaston, K. J. (2018). Artificial light at night alters grassland vegetation species composition and phenology. *J. Appl. Ecol.* 55, 442–450. doi: 10.1111/1365-2664.12927

Bird, T. J., Bates, A. E., Lefcheck, J. S., Hill, N. A., Thomson, R. J., Edgar, G. J., et al. (2014). Statistical solutions for error and bias in global citizen science datasets. *Biol. Cons.* 173, 144–154. doi: 10.1016/j.biocon.2013.07.037

Birk, S., Chapman, D., Carvalho, L., Spears, B. M., Andersen, H. E., Argillier, C., et al. (2020). Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nat. Ecol. Evol.* 4, 1060–1068. doi: 10.1038/s41559-020-1216-4

Bolliger, J., Hennet, T., Wermelinger, B., Bösch, R., Pazur, R., Blum, S., et al. (2020). Effects of traffic-regulated street lighting on nocturnal insect abundance and bat activity. *Basic Appl. Ecol.* 47, 44–56. doi: 10.1016/j.envpol.2021.117765

Borer, E. T., Harpole, W. S., Adler, P. B., Lind, E. M., Orrock, J. L., Seabloom, E. W., et al. (2014). Finding generality in ecology: a model for globally distributed experiments. *Methods Ecol. Evol.* 5, 65–73.

Bouroussis, C. A., and Topalis, F. V. (2020). Assessment of outdoor lighting installations and their impact on light pollution using unmanned aircraft systems–the concept of the drone-gonio-photometer. *J. Quant. Spectrosc. Radiat. Transf.* 253:107155.

Bradshaw, W. E., and Holzapfel, C. M. (2001). Genetic shift in photoperiodic response correlated with global warming. *Proc. Natl. Acad. Sci. U.S.A.* 98, 14509–14511.

Brüning, A., Hölker, F., Franke, S., Kleiner, W., and Kloas, W. (2016). Impact of different colours of artificial light at night on melatonin rhythm and gene expression of gonadotropins in European perch. *Sci. Total Environ.* 543, 214–222. doi: 10.1016/j.scitotenv.2015.11.023

Brüning, A., Hölker, F., Franke, S., Preuer, T., and Kloas, W. (2015). Spotlight on fish: light pollution affects circadian rhythms of European perch but does not cause stress. *Sci. Total Environ.* 511, 516–522. doi: 10.1016/j.scitotenv.2014.12.094

Camacho, L. F., Barragán, G., and Espinosa, S. (2021). Local ecological knowledge reveals combined landscape effects of light pollution, habitat loss, and fragmentation on insect populations. *Biol. Conserv.* 262:109311.

Caplat, P., Edelaar, P., Dudaniec, R. Y., Green, A. J., Okamura, B., Cote, J., et al. (2016). Looking beyond the mountain: dispersal barriers in a changing world. *Front. Ecol. Environ.* 14:261–268. doi: 10.1002/fee.1280

Cardiel, N., Zamorano, J., Bará, S., Sánchez de Miguel, A., Cabello, C., Gallego, J., et al. (2021). Synthetic RGB photometry of bright stars: definition of the standard photometric system and UCM library of spectrophotometric spectra. *Mon. Not. R. Astron. Soc.* 504, 3730–3748.

Challéat, S., Barré, K., Laforge, A., Lapostolle, D., Franchomme, M., Sirami, C., et al. (2021). Grasping darkness: the dark ecological network as a social-ecological framework to limit the impacts of light pollution on biodiversity. *Ecol. Soc.* 26:15. doi: 10.5751/ES-12156-260115

Charmantier, A., and Gienapp, P. (2014). Climate change and timing of avian breeding and migration: evolutionary versus plastic changes. *Evol. Appl.* 7, 15–28. doi: 10.1111/eva.12126

Colantonio, C., Pelosi, C., D’Alessandro, L., Sottile, S., Calabrò, G., and Melis, M. (2018). Hypercolorimetric multispectral imaging system for cultural heritage diagnostics: an innovative study for copper painting examination. *Eur. Phys. J. Plus* 133:526.

Cox, D. T. C., Gardner, A. S., and Gaston, K. J. (2021). Diel niche variation in mammals associated with expanded trait space. *Nat. Commun.* 12:1753. doi: 10.1038/s41467-021-22023-4

Cravens, Z. M., Brown, V. A., Divoll, T. J., and Boyles, J. G. (2018). Illuminating prey selection in an insectivorous bat community exposed to artificial light at night. *J. Appl. Ecol.* 55, 705–713.

Da Silva, A., and Kempenaers, B. (2017). Singing from North to South: latitudinal variation in timing of dawn singing under natural and artificial light conditions. *J. Anim. Ecol.* 86, 1286–1297.

Dacke, M., Nilsson, D. E., Scholtz, C. H., Byrne, M., and Warrant, E. J. (2003). Animal behaviour: insect orientation to polarized moonlight. *Nature* 424:33.

Davies, T. W., Bennie, J., Cruse, D., Blumgart, D., Inger, R., and Gaston, K. J. (2017). Multiple night-time light-emitting diode lighting strategies impact grassland invertebrate assemblages. *Glob. Change Biol.* 23, 2641–2648. doi: 10.1111/gcb.13615

Davies, T. W., Bennie, J., Inger, R., de Ibarra, N. H., and Gaston, K. J. (2013). Artificial light pollution: are shifting spectral signatures changing the balance of species interactions? *Glob. Change Biol.* 19, 1417–1423. doi: 10.1111/gcb.12166

Davies, T. W., McKee, D., Fishwick, J., Tidau, S., and Smyth, T. (2020). Biologically important artificial light at night on the seafloor. *Sci. Rep.* 10:12545. doi: 10.1038/s41598-020-69461-6

Davies, T. W., and Smyth, T. (2018). Why artificial light at night should be a focus for global change research in the 21st century. *Glob. Change Biol.* 24, 872–882. doi: 10.1111/gcb.13927

Degen, T., Mitesser, O., Perkin, E. K., Weiß, N. S., Oehlert, M., Mattig, E., et al. (2016). Street lighting: sex-independent impacts on moth movement. *J. Anim. Ecol.* 85, 1352–1360.

de Jong, M., Jeninga, L., Ouyang, J. Q., van Oers, K., Spoelstra, K., and Visser, M. E. (2016). Dose-dependent responses of avian daily rhythms to artificial light at night. *Physiol. Behav.* 155, 172–179. doi: 10.1016/j.physbeh.2015.12.012

Deichmann, J. L., Ampudia Gatty, C., Andía Navarro, J. M., Alonso, A., Linares-Palomino, R., and Longcore, T. (2021). Reducing the blue spectrum of artificial light at night minimises insect attraction in a tropical lowland forest. *Insect Conserv. Divers* 14, 247–259. doi: 10.1111/icad.12479

Díaz, S., Zafra-Calvo, N., Purvis, A., Verburg, P. H., Obura, D., Leadley, P., et al. (2020). Set ambitious goals for biodiversity and sustainability. *Science* 370, 411–413.

Ditmer, M. A., Stoner, D. C., Francis, C. D., Barber, J. R., Forester, J. D., Choate, D. M., et al. (2021). Artificial nightlight alters the predator–prey dynamics of an apex carnivore. *Ecography* 44, 149–161.

Dominoni, D. M., Halfwerk, W., Baird, E., Buxton, R. T., Fernández-Juricic, E., Fristrup, K. M., et al. (2020a). Why conservation biology can benefit from sensory ecology. *Nat. Ecol. Evol.* 4, 502–511. doi: 10.1038/s41559-020-1135-4

Dominoni, D. M., Jensen, J. K., de Jong, M., Visser, M. E., and Spoelstra, K. (2020c). Artificial light at night, in interaction with spring temperature, modulates timing of reproduction in a passerine bird. *Ecol. Appl.* 30, e02062. doi: 10.1002/eap.2062

Dominoni, D. M., Smit, J. A. H., Visser, M. E., and Halfwerk, W. (2020b). Multisensory pollution: artificial light at night and noise have interactive effects on activity patterns of a songbird. *Environ. Pollut.* 256:113314. doi: 10.1016/j.envpol.2019.113314

Donners, M., van Grunsven, R. H., Groenendijk, D., van Langevelde, F., Bikker, J. W., Longcore, T., et al. (2018). Colors of attraction: modeling insect flight to light behavior. *J. Exp. Zool. A* 329, 434–440.

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C., Elvidge, C. D., Baugh, K., et al. (2016). The new world atlas of artificial night sky brightness. *Sci. Adv.* 2:e1600377.

Falcon, J., Torriglia, A., Attia, D., Viénot, F., Gronfier, C., Behar-Cohen, F., et al. (2020). Exposure to artificial light at night and the consequences for flora, fauna, and ecosystems. *Front. Neurosci.* 14:1183. doi: 10.3389/fnins.2020.602796

Ffrench-Constant, R. H., Somers-Yeates, R., Bennie, J., Economou, T., Hodgson, D., Spalding, A., et al. (2016). Light pollution is associated with earlier tree budburst across the United Kingdom. *Proc. R. Soc. B* 283:20160813. doi: 10.1098/rspb.2016.0813

Fiorentin, P., Bertolo, A., Cavazzani, S., and Ortolani, S. (2020). Calibration of digital compact cameras for sky quality measures. *J. Quant. Spectrosc. Radiat. Transf.* 255:107235. doi: 10.1016/j.jqsrt.2020.107235

Foster, J. J., Smolka, J., Nilsson, D. E., and Dacke, M. (2018). How animals follow the stars. *Proc. R. Soc. B* 285:20172322.

Foster, J. J., Tocco, C., Smolka, J., Khaldy, L., Baird, E., Byrne, M. J., et al. (2021). Light pollution forces a change in dung beetle orientation behavior. *Curr. Biol.* 31, 3935–3942.

Franzén, M., Betzholtz, P. E., Pettersson, L. B., and Forsman, A. (2020). Urban moth communities suggest that life in the city favours thermophilic multi-dimensional generalists. *Proc. R. Soc. B* 287:20193014. doi: 10.1098/rspb.2019.3014

Gaston, K. J. (2019). Nighttime ecology: the "nocturnal problem" revisited. *Am. Nat.* 193, 481–502. doi: 10.1086/702250

Gaston, K. J., Davies, T. W., Bennie, J., and Hopkins, J. (2012). Reducing the ecological consequences of night-time light pollution: options and developments. *J. Appl. Ecol.* 49, 1256–1266.

Gaston, K. J., Ackermann, S., Bennie, J., Cox, D. T., Phillips, B. B., de Miguel, S., et al. (2021). Pervasiveness of biological impacts of artificial light at night. *Int. Comp. Biol.* 61, 1098–1110. doi: 10.1093/icb/icab145

Gaston, K. J., Bennie, J., Davies, T. W., and Hopkins, J. (2013). The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev. Camb. Philos. Soc.* 88, 912–927. doi: 10.1111/brv.12036

Gaston, K. J., Visser, M. E., and Hölker, F. (2015). The biological impacts of artificial light at night: the research challenge. *Philos. Trans. R. Soc. B* 370:20140133.

Geldmann, J., Heilmann-Clausen, J., Holm, T. E., Levinsky, I., Markussen, B., Olsen, K., et al. (2016). What determines spatial bias in citizen science? Exploring four recording schemes with different proficiency requirements. *Divers. Distrib.* 22, 1139–1149.

Giavi, S., Blösch, S., Schuster, G., and Knop, E. (2020). Artificial light at night can modify ecosystem functioning beyond the lit area. *Sci. Rep.* 10:11870. doi: 10.1038/s41598-020-68667-y

Giavi, S., Fontaine, C., and Knop, E. (2021). Impact of artificial light at night on diurnal plant-pollinator interactions. *Nat. Commun.* 12:1690. doi: 10.1038/s41467-021-22011-8

Grubisic, M., Haim, A., Bhusal, P., Dominoni, D. M., Gabriel, K., Jechow, A., et al. (2019). Light pollution, circadian photoreception, and melatonin in vertebrates. *Sustainability* 11:6400. doi: 10.3390/su11226400

Grubisic, M., van Grunsven, R. H., Kyba, C. C. M., Manfrin, A., and Hölker, F. (2018). Insect declines and agroecosystems: does light pollution matter? *Ann. Appl. Biol.* 173, 180–189.

Halfwerk, W., and Slabbekoorn, H. (2015). Pollution going multimodal: the complex impact of the human-altered sensory environment on animal perception and performance. *Biol. Lett.* 11:20141051. doi: 10.1098/rsbl.2014.1051

Hänel, A., Posch, T., Ribas, S. J., Aubé, M., Duriscoe, D., Jechow, A., et al. (2018). Measuring night sky brightness: methods and challenges. *J. Quant. Spectrosc. Radiat. Transf.* 205, 278–290.

Haynes, K. J., and Robertson, B. A. (2021). A transdisciplinary research agenda for understanding insect responses to ecological light pollution informed by evolutionary trap theory. *Curr. Opin. Insect Sci.* 45, 91–96. doi: 10.1016/j.cois.2021.02.004

Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C. C., Henckel, D., et al. (2010a). The dark side of light: a transdisciplinary research agenda for light pollution policy. *Ecol. Soc.* 15:13.

Hölker, F., Wolter, C., Perkin, E. K., and Tockner, K. (2010b). Light pollution as a biodiversity threat. *Trends Ecol. Evol.* 25, 681–682. doi: 10.1016/j.tree.2010.09.007

Hölker, F., Wurzbacher, C., Weißenborn, C., Monaghan, M. T., Holzhauer, S. I., and Premke, K. (2015). Microbial diversity and community respiration in freshwater sediments influenced by artificial light at night. *Philos. Trans. R. Soc. B* 370:20140130. doi: 10.1098/rstb.2014.0130

Hopkins, G. R., Gaston, K. J., Visser, M. E., Elgar, M. A., and Jones, T. M. (2018). Artificial light at night as a driver of evolution across urban rural landscapes. *Front. Ecol. Environ.* 16:472–479. doi: 10.1002/fee.1828

Horváth, G., Kriska, G., Malik, P., and Robertson, B. (2009). Polarized light pollution: a new kind of ecological photopollution. *Front. Ecol. Environ.* 7:317–325. doi: 10.1890/080129

Hut, R. A., Paolucci, S., Dor, R., Kyriacou, C. P., and Daan, S. (2013). Latitudinal clines: an evolutionary view on biological rhythms. *Proc. R. Soc. B* 280:20130433. doi: 10.1098/rspb.2013.0433

IPBES (2019). "Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the intergovernmental science-policy platform on biodiversity and ecosystem services," in *Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services*, ed. J. Díaz (Bonn: IPBES Secretariat).

Jackson, M. C., Loewen, C. J. G., Vinebrooke, R. D., and Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. *Glob. Change Biol.* 22, 180–189.

Jägerbrand, A. K., and Bouroussis, C. A. (2021). Ecological impact of artificial light at night: effective strategies and measures to deal with protected species and habitats. *Sustainability* 13:5991. doi: 10.3390/su13115991

Jakle, J. A., and Thompson, G. F. (2001). *City Lights: Illuminating the American Night*. Baltimore, MD: Johns Hopkins University Press.

Jechow, A., and Hölker, F. (2019a). How dark is a river? Artificial light at night in aquatic systems and the need for comprehensive night-time light measurements. *WIREs Water* 6:e1388. doi: 10.1002/wat2.1388

Jechow, A., and Hölker, F. (2019b). Snowglow–the amplification of skyglow by snow and clouds can exceed full moon illuminance in suburban areas. *J. Imaging* 5:69. doi: 10.3390/jimaging5080069

Jechow, A., Kyba, C. C. M., and Hölker, F. (2019). Beyond all-sky: assessing ecological light pollution using multi-spectral full-sphere fisheye lens imaging. *J. Imaging* 5:46. doi: 10.3390/jimaging5040046

Jechow, A., Kyba, C. C. M., and Hölker, F. (2020). Mapping the brightness and color of urban to rural skyglow with all-sky photometry. *J. Quant. Spectrosc. Radiat. Transf.* 250:106988. doi: 10.1016/j.jqsrt.2020.106988

Jechow, A., Ribas, S. J., Domingo, R. C., Hölker, F., Kolláth, Z., and Kyba, C. C. M. (2018). Tracking the dynamics of skyglow with differential photometry using a digital camera with fisheye lens. *J. Quant. Spectrosc. Radiat. Transf.* 209, 212–223.

Kalinkat, G., Grubisic, M., Jechow, A., van Grunsven, R. H., Schroer, S., and Hölker, F. (2021). Assessing long-term effects of artificial light at night on insects: what is missing and how to get there. *Insect Conserv. Divers.* 14, 260–270. doi: 10.1111/icad.12482

Keinath, S., Hölker, F., Müller, J., and Rödel, M. O. (2021). Impact of light pollution on moth morphology–a 137-year study in Germany. *Basic Appl. Ecol.* 56, 1–10. doi: 10.1016/j.baae.2021.05.004

Knop, E., Zoller, L., Ryser, R., Gerpe, C., Hörler, M., and Fontaine, C. (2017). Artificial light at night as a new threat to pollination. *Nature* 548, 206–209. doi: 10.1038/nature23288

Kocifaj, M., Kómar, L., and Kundracik, F. (2018). PePSS-A portable sky scanner for measuring extremely low night-sky brightness. *J. Quant. Spectr. Rad. Transf.* 210, 74–81.

Kolláth, Z., Cool, A., Jechow, A., Kolláth, K., Száz, D., and Tong, K. P. (2020). Introducing the Dark Sky Unit for multi-spectral measurement of the night sky quality with commercial digital cameras. *J. Quant. Spectrosc. Radiat. Transf.* 253:107162. doi: 10.1016/j.jqsrt.2020.107162

Kühne, J. L., van Grunsven, R. H. A., Jechow, A., and Hölker, F. (2021). Impact of different wavelengths of artificial light at night on phototaxis in aquatic insects. *Int. Comp. Biol.* 61, 1182–1190. doi: 10.1093/icb/icab149

Kupprat, F., Hölker, F., and Kloas, W. (2020). Can skyglow reduce nocturnal melatonin concentrations in Eurasian perch? *Environ. Pollut.* 262:114324. doi: 10.1016/j.envpol.2020.114324

Kyba, C., Pritchard, S. B., Ekirch, A. R., Eldridge, A., Jechow, A., Preiser, C., et al. (2020). Night matters—why the interdisciplinary field of "Night Studies" is needed. *J.* 3, 1–6. doi: 10.3390/j3010001

Kyba, C. C., Kuester, T., De Miguel, A. S., Baugh, K., Jechow, A., Hölker, F., et al. (2017). Artificially lit surface of Earth at night increasing in radiance and extent. *Sci. Adv.* 3:e1701528. doi: 10.1126/sciadv.1701528

Kyba, C. C. M., Hänel, A., and Hölker, F. (2014). Redefining efficiency for outdoor lighting. *Energ. Environm. Sci.* 7, 1806–1809.

Kyba, C. C. M., and Hölker, F. (2013). Do artificially illuminated skies affect biodiversity in nocturnal landscapes? *Landsc. Ecol.* 28, 1637–1640.

Kyba, C. C. M., Ruby, A., Kuechly, H. U., Kinzey, B., Miller, N., Sanders, J., et al. (2021). Direct measurement of the contribution of street lighting to satellite observations of nighttime light emissions from urban areas. *Light Res. Technol.* 53, 189–211.

Kyba, C. C. M., Ruhtz, T., Fischer, J., and Hölker, F. (2011). Cloud coverage acts as an amplifier for ecological light pollution in urban ecosystems. *PLoS One* 6:e17307. doi: 10.1371/journal.pone.0017307

Kyba, C. C. M., Ruhtz, T., Fischer, J., and Hölker, F. (2012). Red is the new black: how the colour of urban skyglow varies with cloud cover. *Mon. Not. R. Astron. Soc.* 425, 701–708.

Laforge, A., Pauwels, J., Faure, B., Bas, Y., Kerbiriou, C., Fonderflick, J., et al. (2019). Reducing light pollution improves connectivity for bats in urban landscapes. *Landsc. Ecol.* 34, 793–809. doi: 10.1007/s10980-019-00803-0

Land, M. F., and Nilsson, D. E. (2012). *Animal Eyes*. Oxford: Oxford University Press.

Last, K. S., Hobbs, L., Berge, J., Brierley, A. S., and Cottier, F. (2016). Moonlight drives ocean-scale mass vertical migration of zooplankton during the Arctic winter. *Curr. Biol.* 26, 244–251. doi: 10.1016/j.cub.2015.11.038

Levin, N., Kyba, C. C. M., Zhang, Q., Román, M. O., Li, X., Portnov, B. A., et al. (2020). Remote sensing of night lights: a review and an outlook for the future. *Rem. Sens. Environ.* 237:111443. doi: 10.1016/j.rse.2019.111443

Lewanzik, D., and Voigt, C. C. (2014). Artificial light puts ecosystem services of frugivorous bats at risk. *J. Appl. Ecol.* 51, 388–394. doi: 10.1111/1365-2664.12206

Longcore, T. (2018). Hazard or hope? LEDs and wildlife. *LED Prof. Rev.* 70, 52–57.

Longcore, T., Duriscoe, D., Aubé, M., Jechow, A., Kyba, C., and Pendoley, K. L. (2020). Commentary: brightness of the night sky affects loggerhead (Caretta caretta) sea turtle hatchling misorientation but not nest site selection. *Front. Mar. Sci.* 7:706. doi: 10.3389/fmars.2020.00706

Longcore, T., and Rich, C. (2004). Ecological light pollution. *Front. Ecol. Env.* 2:191–198.

Longcore, T., Rodríguez, A., Witherington, B., Penniman, J. F., Herf, L., and Herf, M. (2018). Rapid assessment of lamp spectrum to quantify ecological effects of light at night. *J. Exp. Zool. A* 329, 511–521. doi: 10.1002/jez.2184

Macgregor, C. J., Evans, D. M., Fox, R., and Pocock, M. J. (2017). The dark side of street lighting: impacts on moths and evidence for the disruption of nocturnal pollen transport. *Glob. Change Biol.* 23, 697–707. doi: 10.1111/gcb.13371

Maggi, E., Bongiorni, L., Fontanini, D., Capocchi, A., Dal Bello, M., and Giacomelli, A. (2020). Artificial light at night erases positive interactions across trophic levels. *Funct. Ecol.* 34, 694–706. doi: 10.1111/1365-2435.13485

Manfrin, A., Lehmann, D., van Grunsven, R. H., Larsen, S., Syväranta, J., Wharton, G., et al. (2018). Dietary changes in predators and scavengers in a nocturnally illuminated riparian ecosystem. *Oikos* 127, 960–969.

Manfrin, A., Singer, G., Larsen, S., Weiß, N., van Grunsven, R. H., Weiß, N. S., et al. (2017). Artificial light at night affects organism flux across ecosystem boundaries and drives community structure in the recipient ecosystem. *Front. Env. Sci.* 5:61. doi: 10.3389/fenvs.2017.00061

Manríquez, P. H., Jara, M. E., Diaz, M. I., Quijón, P. A., Widdicombe, S., Pulgar, J., et al. (2019). Artificial light pollution influences behavioral and physiological traits in a keystone predator species, *Concholepas concholepas*. *Sci. Total Env.* 661, 543–552. doi: 10.1016/j.scitotenv.2019.01.157

Marchant, P., Hale, J. D., and Sadler, J. P. (2020). Does changing to brighter road lighting improve road safety? Multilevel longitudinal analysis of road traffic collision frequency during the relighting of a UK city. *J. Epidemiol. Community Health* 74, 467–472. doi: 10.1136/jech-2019-212208

McLaren, J. D., Buler, J. J., Schreckengost, T., Smolinsky, J. A., Boone, M., van Loon, E., et al. (2018). Artificial light at night confounds broad-scale habitat use by migrating birds. *Ecol. Lett.* 21, 356–364. doi: 10.1111/ele.12902

McMahon, T. A., Rohr, J. R., and Bernal, X. E. (2017). Light and noise pollution interact to disrupt interspecific interactions. *Ecology* 98, 1290–1299. doi: 10.1002/ecy.1770

Miller, C. R., Barton, B. T., Zhu, L., Radeloff, V. C., Oliver, K. M., Harmon, J. P., et al. (2017). Combined effects of night warming and light pollution on predator–prey interactions. *Proc. R. Soc. B* 284:20171195. doi: 10.1098/rspb.2017.1195

Moore, M. V., Pierce, S. M., Walsh, H. M., Kvalvik, S. K., and Lim, J. D. (2000). Urban light pollution alters the diel vertical migration of Daphnia. *Verh. lnternat. Verein. Limnol.* 27, 779–782.

Nilsson, D. E., and Smolka, J. (2021). Quantifying biologically essential aspects of environmental light. *J. R. Soc. Interface* 18:20210184.

Nuñez, J. D., Sbragaglia, V., Spivak, E. D., Chiaradia, N. M., and Luppi, T. A. (2021). The magnitude of behavioural responses to artificial light at night depends on the ecological context in a coastal marine ecosystem engineer. *Mar. Env. Res.* 165:105238. doi: 10.1016/j.marenvres.2020.105238

Owens, A. C. S., and Lewis, S. M. (2021). Narrow-spectrum artificial light silences female fireflies (Coleoptera: Lampyridae). *Insect. Conserv. Divers.* 199, 199–210. doi: 10.1111/icad.12487

Palumbi, S. R. (2001). Humans as the world's greatest evolutionary force. *Science* 293, 1786–1790.

Patat, F. (2008). The dancing sky: 6 years of night-sky observations at Cerro Paranal. *Astron. Astrophys.* 481, 575–591.

Pérez Vega, C., Zielinska-Dabkowska, K. M., and Hölker, F. (2021). Urban lighting research transdisciplinary framework—a collaborative process with lighting professionals. *Int. J. Environ. Res. Public Health* 18:624. doi: 10.3390/ijerph18020624

Perkin, E. K., Hölker, F., Richardson, J. S., Sadler, J. P., Wolter, C., and Tockner, K. (2011). The influence of artificial light on stream and riparian ecosystems: questions, challenges, and perspectives. *Ecosphere* 2, 1–16. doi: 10.1890/ES11-00241.1

Rillig, M. C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C. A., Buchert, S., Wulf, A., et al. (2019). The role of multiple global change factors in driving soil functions and microbial biodiversity. *Science* 366, 886–890. doi: 10.1126/science.aay2832

Sage, R. F. (2020). Global change biology: a primer. *Glob. Change Biol.* 26, 3–30. doi: 10.1111/gcb.14893

Sánchez de Miguel, A., Aubé, M., Zamorano, J., Kocifaj, M., Roby, J., and Tapia, C. (2017). Sky Quality Meter measurements in a colour-changing world. *Mon. Not. R. Astron. Soc.* 467, 2966–2979.

Sánchez de Miguel, A., Zamorano, J., Aubé, M., Bennie, J., Gallego, J., Ocaña, F., et al. (2021). Colour remote sensing of the impact of artificial light at night (II): calibration of DSLR-based images from the International Space Station. *Rem. Sens. Environ.* 264:112611. doi: 10.1016/j.rse.2021.112611

Sanders, D., Frago, E., Kehoe, R., Patterson, C., and Gaston, K. J. (2021). A meta-analysis of biological impacts of artificial light at night. *Nat. Ecol. Evol.* 5, 74–81. doi: 10.1038/s41559-020-01322-x

Sanders, D., and Gaston, K. J. (2018). How ecological communities respond to artificial light at night. *J. Exp. Zool. A* 329, 394–400. doi: 10.1002/jez.2157

Sanders, D., Kehoe, R., Cruse, D., van Veen, F. F., and Gaston, K. J. (2018). Low levels of artificial light at night strengthen top-down control in insect food web. *Curr. Biol.* 28, 2474–2478. doi: 10.1016/j.cub.2018.05.078

Sanders, D., Kehoe, R., Tiley, K., Bennie, J., Cruse, D., Davies, T. W., et al. (2015). Artificial nighttime light changes aphid-parasitoid population dynamics. *Sci. Rep.* 5, 15232–15232. doi: 10.1038/srep15232

Schroer, S., and Hölker, F. (2017). "Light pollution reduction," in *Handbook of Advanced Lighting Technology,* eds R. Karlicek, C. C. Sun, G. Zissis, and R. Ma (Cham: Springer International Publishing), 991–1010.

Schroer, S., Huggins, B. J., Azam, C., and Hölker, F. (2020). Working with inadequate tools: legislative shortcomings in protection against ecological effects of artificial light at night. *Sustainability* 12:2551. doi: 10.3390/su12062551

Schulte-Römer, N., Meier, J., Dannemann, E., and Söding, M. (2019). Lighting professionals versus light pollution experts? Investigating views on an emerging environmental concern. *Sustainability* 11:1696. doi: 10.3390/su11061696

Secondi, J., Davranche, A., Théry, M., Mondy, N., and Lengagne, T. (2020). Assessing the effects of artificial light at night on biodiversity across latitude–current knowledge gaps. *Glob. Ecol. Biogeogr.* 29, 404–419. doi: 10.1111/geb.13037

Senzaki, M., Barber, J. R., Phillips, J. N., Carter, N. H., Cooper, C. B., Ditmer, M. A., et al. (2020). Sensory pollutants alter bird phenology and fitness across a continent. *Nature* 587, 605–609. doi: 10.1038/s41586-020-2903-7

Seymoure, B. M., Linares, C., and White, J. (2019). Connecting spectral radiometry of anthropogenic light sources to the visual ecology of organisms. *J. Zool.* 308, 93–110.

Shiwen, L., Steel, L., Dahlsjö, C. A., Peirson, S. N., Shenkin, A., Morimoto, T., et al. (2021). Hyperspectral characterisation of natural illumination in woodland and forest environments. *SPIE J.* 11815, 1181506–1181511. doi: 10.1111/gcb.14903

Sih, A., Ferrari, M. C., and Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change. *Evol. Appl.* 4, 367–387. doi: 10.1111/j.1752-4571.2010.00166.x

Simons, A. L., Yin, X., and Longcore, T. (2020). High correlation but high scale-dependent variance between satellite measured night lights and terrestrial exposure. *Environ. Res. Commun.* 2:021006.

Spitschan, M., Aguirre, G. K., Brainard, D. H., and Sweeney, A. M. (2016). Variation of outdoor illumination as a function of solar elevation and light pollution. *Sci. Rep.* 6:26756. doi: 10.1038/srep26756

Spoelstra, K., van Grunsven, R. H., Donners, M., Gienapp, P., Huigens, M. E., Slaterus, R., et al. (2015). Experimental illumination of natural habitat—an experimental set-up to assess the direct and indirect ecological consequences of artificial light of different spectral composition. *Philos. Trans. R. Soc A* 370:20140129. doi: 10.1098/rstb.2014.0129

Swaddle, J. P., Francis, C. D., Barber, J. R., Cooper, C. B., Kyba, C. C., Dominoni, D. M., et al. (2015). A framework to assess evolutionary responses to anthropogenic light and sound. *Trends Ecol. Evol.* 30, 550–560. doi: 10.1016/j.tree.2015.06.009

Thomas, L. (2021). Lichtverschmutzung und die 3 Novelle zum BNatSchG. *Recht Nat.* 228, 42–46.

Thums, M., Whiting, S. D., Reisser, J., Pendoley, K. L., Pattiaratchi, C. B., Proietti, M., et al. (2016). Artificial light on water attracts turtle hatchlings during their near shore transit. *R. Soc. Open Sci.* 35:160142. doi: 10.1098/rsos.160142

Tidau, S., Smyth, T., McKee, D., Wiedenmann, J., D'Angelo, C., Wilcockson, D., et al. (2021). Marine artificial light at night: an empirical and technical guide. *Methods Ecol. Evol.* 12, 1588–1601. doi: 10.1111/2041-210X.13653

Torres, D., Tidau, S., Jenkins, S., and Davies, T. (2020). Artificial skyglow disrupts celestial migration at night. *Curr. Biol.* 30, R696–R697.

Tuomainen, U., and Candolin, U. (2011). Behavioural responses to human-induced environmental change. *Biol Rev.* 86, 640–657. doi: 10.1111/j.1469-185x.2010.00164.x

Tylianakis, J. M., Didham, R. K., Bascompte, J., and Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* 11, 1351–1363. doi: 10.1111/j.1461-0248.2008.01250.x

Ugolini, A., Fantini, T., and Innocenti, R. (2003). Orientation at night: an innate moon compass in sandhoppers (Amphipoda: Talitridae). *Proc. Biol. Sci.* 270, 279–281. doi: 10.1098/rspb.2002.2221

Van Asch, M., Van Tienderen, P. H., Holleman, L. J. M., and Visser, M. E. (2007). Predicting adaptation of phenology in response to climate change, an insect herbivore example. *Glob. Change Biol.* 13, 1596–1604. doi: 10.1111/j.1365-2486.2007.01400.x

van der Kooi, C. J., Stavenga, D. G., Arikawa, K., Belušič, G., and Kelber, A. (2021). Evolution of insect color vision: from spectral sensitivity to visual ecology. *Annu. Rev. Entomol.* 66, 435–461. doi: 10.1146/annurev-ento-061720-071644

van Grunsven, R. H., Donners, M., Boekee, K., Tichelaar, I., Van Geffen, K. G., Groenendijk, D., et al. (2014). Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. *J. Insect Conserv.* 18, 225–231. doi: 10.1007/s10841-014-9633-9

van Grunsven, R. H., Jähnichen, D., Grubisic, M., and Hölker, F. (2018). Slugs (Arionidae) benefit from nocturnal artificial illumination. *J. Exp. Zool. A* 329, 429–433. doi: 10.1002/jez.2170

van Grunsven, R. H., van Deijk, J. R., Donners, M., Berendse, F., Visser, M. E., Veenendaal, E., et al. (2020). Experimental light at night has a negative long-term impact on macro-moth populations. *Curr. Biol.* 30, 694–695. doi: 10.1016/j.cub.2020.04.083

Vandenberg, L. N., Colborn, T., Hayes, T. B., Heindel, J. J., Jacobs, D. R. Jr., Lee, D. H., et al. (2012). Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr. Rev.* 33, 378–455. doi: 10.1210/er.2011-1050

Voigt, C. C., Dekker, J., Fritze, M., Gazaryan, S., Hölker, F., Jones, G., et al. (2021). The impact of light pollution on bats varies according to foraging guild and habitat context. *BioScience* 71, 1103–1109.

Wilson, A. A., Ditmer, M. A., Barber, J. R., Carter, N. H., Miller, E. T., Tyrrell, L. P., et al. (2021). Artificial night light and anthropogenic noise interact to influence bird abundance over a continental scale. *Glob. Change Biol.* 27, 3987–4004. doi: 10.1111/gcb.15663

Wyneken, J., and Salmon, M. (2020). Linking ecology, morphology, and behavior to conservation: lessons learned from studies of sea turtles. *Int. Comp. Biol.* 60, 440–455. doi: 10.1093/icb/icaa044

Zielińska-Dabkowska, K. M., Xavia, K., and Bobkowska, K. (2020). Assessment of citizens' actions against light pollution with guidelines for future initiatives. *Sustainability* 12:4997. doi: 10.3390/su12124997

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
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ORIGINAL RESEARCH
published: 04 January 2022
doi: 10.3389/fevo.2021.779825

Welcome to the Dark Side: Partial Nighttime Illumination Affects Night- and Daytime Foraging Behavior of a Small Mammal

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Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *19 September 2021*
Accepted: *29 November 2021*
Published: *04 January 2022*

Citation:
Hoffmann J, Hölker F and Eccard JA (2022) Welcome to the Dark Side: Partial Nighttime Illumination Affects Night- and Daytime Foraging Behavior of a Small Mammal.
Front. Ecol. Evol. 9:779825.
doi: 10.3389/fevo.2021.779825

Differences in natural light conditions caused by changes in moonlight are known to affect perceived predation risk in many nocturnal prey species. As artificial light at night (ALAN) is steadily increasing in space and intensity, it has the potential to change movement and foraging behavior of many species as it might increase perceived predation risk and mask natural light cycles. We investigated if partial nighttime illumination leads to changes in foraging behavior during the night and the subsequent day in a small mammal and whether these changes are related to animal personalities. We subjected bank voles to partial nighttime illumination in a foraging landscape under laboratory conditions and in large grassland enclosures under near natural conditions. We measured giving-up density of food in illuminated and dark artificial seed patches and video recorded the movement of animals. While animals reduced number of visits to illuminated seed patches at night, they increased visits to these patches at the following day compared to dark seed patches. Overall, bold individuals had lower giving-up densities than shy individuals but this difference increased at day in formerly illuminated seed patches. Small mammals thus showed carry-over effects on daytime foraging behavior due to ALAN, i.e., nocturnal illumination has the potential to affect intra- and interspecific interactions during both night and day with possible changes in personality structure within populations and altered predator-prey dynamics.

Keywords: light pollution, inter-individual differences, animal personality, *Myodes glareolus*, ALAN

INTRODUCTION

When animals show foraging behavior, they constantly have to face the trade-off between acquiring food and avoiding predation (Sih, 1980; Lima and Dill, 1990). As predation risk varies across space and time, prey reduce foraging at certain times and/or in certain microhabitats to reduce the risk of being preyed upon (Lima and Dill, 1990; Jacob and Brown, 2000). A well-known environmental factor that influences foraging activity in many nocturnally active species is moonlight (Kronfeld-Schor et al., 2013). Under bright moonlight, animals often reduce foraging activity itself (Daly et al., 1992; Kotler et al., 2010) or shift their activity toward safer habitats with a higher amount of cover (Bowers, 1988; Perea et al., 2011). This shows that higher levels of nighttime illumination lead to an increase in the perceived predation risk in many species, especially in small mammals.

However, the use of artificial light at night (ALAN) has increased dramatically over the last decades, eliminating very dark conditions in large areas in and surrounding urban centers (Hölker et al., 2010a; Falchi et al., 2016). The spatial spread and intensity of nighttime illumination is predicted to steadily increase (Kyba et al., 2017) especially through the development and widespread adoption of new energy-efficient lighting systems such as the light-emitting diode (LED; Kyba et al., 2017; Donatello et al., 2019). Several studies show that ALAN influences several aspects of an animals' behavior such as timing of activity (de Jong et al., 2017; Eccard et al., 2018), movement (Hoffmann et al., 2018; Laforge et al., 2019), reproduction (Baker and Richardson, 2006; Russ et al., 2017) and foraging (Bird et al., 2004), which in turn can alter social interactions and group dynamics (Kurvers and Hölker, 2015). Under natural conditions, many animals experience dark nights as periods where the perceived predation risk is low and foraging can be extended or expanded to open habitats. In areas that are constantly polluted by ALAN, these foraging opportunities disappear. LED lamps are predicted to have particularly strong negative effects on the environment as they typically have a broad emission spectrum that can affect a large range of species (Gaston et al., 2013; Davies et al., 2017) and are used at higher light intensities due to their higher energy efficiency (rebound effect; Herring and Roy, 2007; Kyba et al., 2014, 2017). Light pollution often affects large areas and illuminates them homogenously through sky glow. However, LED street lamps and other strong light sources may create illumination of high intensity on a local scale. The attraction (e.g., insects, insect predators) or avoidance (e.g., prey animals) of these illuminated areas in an area of heterogenous light pollution is not well understood.

Most research focusses on the influence of ALAN on nocturnal species as they are expected to suffer severely from the loss of the nocturnal niche (Hölker et al., 2010b). Nevertheless, there are several mammal species such as shrews, voles and lemurs that show a polyphasic activity pattern or cathemerality with activity bouts during both day and night (Curtis and Rasmussen, 2006; Halle, 2006) that might be strongly affected by ALAN as well. However, there are few studies, which focus on this group of species and/or chronotypes in the context of changing nighttime conditions (Hoffmann et al., 2018, 2019; van Grunsven et al., 2018). These species might not only show changes in nocturnal behavior when subjected to ALAN but there might be carry-over effects to their diurnal behavior. For example, when animals face increased perceived predation risk during the night through elevated illumination levels and reduce their foraging accordingly, they might compensate for this by increasing foraging during the day exposing them to additional stressors or predation risks. Similarly, under heterogenous nighttime illumination animals might avoid brighter areas at night and seek them out during the day to compensate for lost foraging opportunities. Studies on the influence of moonlight on activity and foraging behavior of rodents show a reduced activity at night under full moon while activity at twilight and day, respectively, was increased (Daly et al., 1992; Gutman et al., 2011). However, studies on the influence of ALAN on behavior during the day using realistic and/or heterogenous light pollution scenarios are scarce (but see Kurvers et al., 2018).

Individuals within a species might respond differently to human-induced changes driven by personality traits (Gosling, 2001). This includes their risk-taking behavior (Wilson et al., 1994). While relatively shy individuals favor reducing predation risk over the opportunity to gain access to food resources or mates, bold individuals maximize their fitness through gaining access to more or higher-quality resources while as a consequence having a higher predation risk (Réale et al., 2000; Smith and Blumstein, 2008; Ballew et al., 2017; Kashon and Carlson, 2018). As ALAN is potentially altering the perceived predation risk of animals, individuals that show inter-individual differences in their risk-taking behavior might respond differently to this anthropogenic change (Merrick and Koprowski, 2017). Shy individuals might be at a disadvantage compared to bold individuals as they miss out on foraging opportunities by avoiding illuminated areas. Bold insectivorous animals might actively seek out the areas surrounding street lamps to take advantage of the higher abundance of insects that were attracted by light. On the other hand, bold individuals might be exposed to an increased predation risk if the respective predators are not deterred by nighttime illumination and are able to use the better visual conditions to increase predation success themselves. While some predatory animal species such as spiders (Willmott et al., 2019), bats (Stone et al., 2015) and owls (Canário et al., 2012) experience increased foraging opportunities around light sources and can themselves be increasingly preyed upon (Negro et al., 2000), it remains unclear how this is influenced by inter-individual differences.

We conducted a laboratory experiment and an experiment using large grassland enclosures to investigate the effects of partial illumination created by common LED lamps on the movement and foraging behavior of the bank vole (*Myodes glareolus*). The bank vole is a common polyphasic rodent species (Halle, 2006) that is widely distributed throughout Eurasia. It feeds on plant and animal matter (Hansson and Larsson, 1978) and is important prey for a wide range of avian and mammalian predators (Norrdahl and Korpimäki, 1995). As the species shows consistent inter-individual differences in behavior (Korpela et al., 2011; Šíchová et al., 2014; Mazza et al., 2018; Schirmer et al., 2019), it is a suitable model organism to investigate the impact of partial nighttime illumination on the foraging behavior of individuals differing in their personality. Under laboratory conditions we expect voles to reduce activity and foraging in illuminated areas due to a perceived increase in predation risk. As a consequence, higher nighttime giving-up densities (GUDs) should be measurable in illuminated compared to dark foraging patches. We further predict that voles compensate for the restricted foraging opportunities at night by foraging more in the formerly illuminated patches at day compared to the patches that are dark at night. Shy individuals should avoid illuminated areas while bold individuals may increase foraging due to higher risk-taking behavior.

In naturally vegetated outdoor enclosures we expect similar behavioral responses albeit potentially reduced compared to the laboratory experiment due to lower light intensity and dense vegetation cover. In a previous study we found that bank voles reduced their home ranges in outdoor enclosures under relatively homogeneous nighttime illumination (Hoffmann et al., 2018) but could not determine if this was due to voles opportunistically exploiting food resources from insect attraction to lighting or if it was due to reduced activity through perceived predation risk. This study measures foraging and movement behavior in both illuminated and dark patches to better address predation risk behavior under ALAN.

MATERIALS AND METHODS

Laboratory Experiment

Study Subjects and Housing

The experiment was conducted under laboratory conditions from April to June 2018 using 21 adult male bank voles. Voles were wild-captured from July to September 2017 at four sites in and surrounding Potsdam, Germany with low levels of light pollution (for GPS coordinates see **Supplementary Material 1**). Animals were housed individually in standard makrolon cages (Ehret GmbH Germany, type III; 42 cm × 27 cm × 16 cm) and were provided with *ad libitum* water, hay and food pellets (ssniff® NM, ssniff® R/M-H Ered II). The room was shielded from natural light while ceiling light tubes (Osram Lumilux T8 L 58W/830 3,000 K and L 58W/840 4,000 K, Osram, Germany; for spectral distributions see links in **Supplementary Material 2**) simulated daylight from 8 am to 8 pm with an average light intensity of 254 ± 44 lx at cage level. Millet was offered 1 week prior to testing so the animals could get accustomed to its taste and scent. Additionally, a wooden nest box (20 cm × 14 cm × 10 cm) was introduced to the cages so that animals could be moved with the nest box to the foraging arena without handling by humans. Animals were weighed directly before entering the arena.

Experimental Design

Four foraging arenas (240 cm × 175 cm × 75 cm) were set up in a room secluded from outdoor light. Each of the arenas was divided by a wall into two compartments of equal size. The separating wall stopped 10 cm above the ground so that the animals were able to move freely around the whole arena (**Figure 1**).

During daytime (8 am – 8 pm) the room was illuminated by the same lamps as the housing room with an average light intensity of 246 ± 22 lx at ground level. During nighttime (8 pm – 8 am), the room was either completely dark or one side of each arena was illuminated by a LED lamp (Paulmann Licht GmbH LED AGL: 6.5 W, 470 lm, 2,700 K, Germany) with an average light intensity of 8.5 ± 0.9 lx at ground level while the other side was shielded from that light by the separating wall. To create a light intensity similar to nighttime street light, the lamps were covered with two filter foils [Rosco e-color No. 209 (51% transmission) and No. 210 (24% transmission), United Kingdom]. Light intensities were measured using a lux meter (Extech HD450, United States, measuring range: 0.1 – 400,000 lx).

At the start of the experiment, each animal was placed individually with the nest box in the center of an arena. As the nest box had two exits on opposite sides, voles were able to enter either side of the arena directly. Water was provided *ad libitum* next to the nest box. Above each arena a camera (ABUS analog HD 720p outdoor dome camera, Germany) was mounted that video-recorded the movement of the animals outside of the nest box during the experiment.

Animals were placed in the arenas for four nights. They were introduced at 7 pm on the first day and left to habituate to the arena for the first two nights. During this period the animals were provided with eight seed trays (13 cm × 13 cm × 4.5 cm) containing 450 ml of sand and 0.6 g of millet each, which were placed pairwise in the corners of the arena 40 cm from the wall. These seed trays were replaced with new trays every 24 h and no nighttime illumination was present. In the following night, one side of the arena was illuminated. During the treatment night and the following day, seed trays were reduced to four per arena and were replaced at 8:30 am and 7:30 pm, respectively. The millet remaining in each seed tray was weighted to determine the giving-up density (GUD) for each tray and the variation among

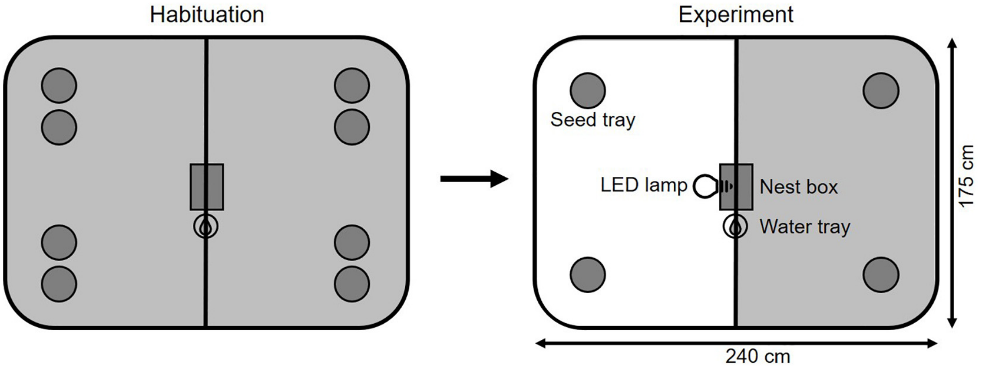

FIGURE 1 | Experimental arena with seed trays containing 450 ml sand and 0.6 g millet. Each of the arenas was divided by a wall into two compartments of equal size. The separating wall stopped 10 cm above the ground so that the animals were able to move freely around the whole arena. Number of seed trays was halved from the habituation phase to the experimental phase as seed trays were exchanged once a day in the habituation phase and twice in the experimental phase. The habituation phase was characterized by dark nights, in the experimental phase one side of the arena was artificially illuminated at night.

GUDs between the two seed trays within one side. This was done for the treatment night and the following day. Using video recordings, we analyzed the movement and foraging behavior of voles. To quantify time spent on either side of the arena (dark or illuminated) at night and day the position of the animal was recorded every 5 s. Additionally, we measured the cumulative time animals spent in the seed trays and the number of visits to the seed trays per side of the arena at night and day. As time spent on either side of the arena and time spent in seed trays are highly correlated (Spearman correlation; $\rho = 0.94$, $P < 0.001$, $N = 84$), we will only present and discuss the results of the behavioral variable time spent in trays.

To quantify the personality type of individuals along a shyness-boldness axis, we measured latency to emerge from the familiar nest box with the full body in the very first night (latency body). Emergence tests (Dark-Light tests) are a common measure of boldness in other studies of wild, small rodents and are related to other measures of risk taking (Herde and Eccard, 2013; Schirmer et al., 2019). A short latency body is regarded to characterize bolder individuals and a longer to characterize shyer individuals. We confirmed that first emergence from a nest box in the arena was repeatable in a pilot study ($R = 0.429$, $P = 0.014$, $N_{\text{Individuals}} = 26$; Hoffmann et al., 2020).

Data Analysis and Statistics

We analyzed the effects of light treatment, latency body, daytime and initial body mass on the response variables time in trays, number of tray visits, GUD and variation in GUD by using linear mixed models (LMMs). Full models included a three-way interaction of light treatment, latency body and daytime and the single factor initial body mass as fixed effects. The models further contained a random factor consisting of the individual nested in the experimental group to account for repeated measures. We log transformed the variables time in trays, number of tray visits and variation in GUD to be able to use LMMs. Latency body was scaled by dividing the centered data points by their standard deviations.

We used the Akaike Information Criterion (AIC) to reduce the models via stepwise backwards selection to find the most parsimonious model (**Table 1**). Non-significant interactions were excluded from the model to allow for interpretation of lower order effects. The random factor experimental group was excluded from the LMM analyzing GUD since it explained no variance. The proportion of explained variance in the most parsimonious models by the fixed factors alone (marginal R^2) and fixed and random factors combined (conditional R^2) was assessed according to Nakagawa and Schielzeth (2013). The Wald test (χ^2) and calculated confidence intervals were used to subsequently determine the significance of fixed factors from the minimal models. If the confidence intervals of fixed factors excluded zero *post hoc* tests were conducted. The most parsimonious model of variation in GUD included a three-way interaction of treatment, latency body and daytime while initial body mass had no effect (**Tables 1**, **2**). Since this interaction had a confidence interval including zero, we were not able to conduct a *post hoc* analysis.

Interactions of two categorical fixed factors were analyzed via a pairwise interaction comparison of factor levels of one variable while the other was held constant and vice versa. Interaction of a categorical and a continuous factor were analyzed via pairwise comparison of factor levels as a function of the continuous covariate. Furthermore, steepness of the slope was analyzed within all factor levels.

We calculated a Pearson correlation to test for an association between boldness (latency body) and change in body mass during the experiment.

For data analyses we used the software R Version 3.5.1 (R Core Team, 2018). Models were built with the function *lmer* from the R package "lme4" (Version 1.1 – 17, Bates, 2010) and *post hoc* tests were conducted using the R package "phia" (Version 0.2 – 1; Martínez, 2015). The mean and standard deviation for each response variable analyzed are presented.

Ethical Note

Experiments were conducted under the permission of the Landesamt für Arbeitsschutz, Verbraucherschutz und Gesundheit, Brandenburg (LAVG 2347-27-2017) and the Stadtverwaltung der Landeshauptstadt Potsdam, Bereich Veterinärwesen und Lebensmittelüberwachung (AZ 386-1-). All

TABLE 1 | Full and minimal linear mixed models testing the effects of independent variables and their interactions on response variables measured during the arena experiment in bank voles ($N_{\text{Individuals}} = 21$).

Dependent variable	Transformation	N	Model complexity	Fixed factors	Random effects	AIC
Σ Time in trays	log	84	Full	Treatment × Latency body × Daytime + Body mass	Group/ID	388
			Minimal	Treatment × Latency body + Treatment × Daytime + Latency body × Daytime	Group/ID	386
N Visits in trays	log	84	Full	Treatment × Latency body × Daytime + Body mass	Group/ID	194
			Minimal	Treatment × Daytime + Latency body × Daytime	Group/ID	189
Giving-up density		168	Full	Treatment × Latency body × Daytime + Body mass	Group/ID	−102
			Minimal	Treatment × Latency body × Daytime	ID	−106
Variation in GUD	log	84	Full	Treatment × Latency body × Daytime + Body mass	Group/ID	320
			Minimal	Treatment × Latency body × Daytime	Group/ID	319

Σ Time in trays – sum of time spent in trays per side of arena, N Visits in trays – number of visits in seed trays per side of arena, variation in GUD – difference in giving-up density between the two seed trays on either side of the arena, treatment – light treatment, latency body – latency to leave the nest box for the first time (measure of boldness), group – experimental group, ID – individual, tray – seed tray, AIC – Akaike Information Criterion.

TABLE 2 | Results of Wald chi-square (χ^2) tests for linear mixed models showing the effects of fixed factors on behavioral response variables of bank voles ($N_{Individuals} = 21$).

Dependent variable	Transformation	*N*	R_m	R_c	Fixed factor	Estimate	df	χ^2	*P*	CI [2.5%, 97.5%]
Σ Time in trays	log	84	0.434	0.616	Treatment	1.247	1	2.09	0.148	[0.093; 2.401]
					Latency body	−1.521	1	9.58	**0.002**	[−2.399; −0.587]
					Daytime	3.505	1	45.32	**<0.001**	[2.351; 4.659]
					Treatment × Latency body	−0.804	1	3.50	0.062	[−1.625; 0.017]
					Treatment × Daytime	−1.257	1	2.16	0.141	[−2.889; 0.375]
					Latency body × Daytime	1.672	1	15.14	**<0.001**	[0.852; 2.493]
N Visits in trays	log	84	0.415	0.785	Treatment	0.293	1	0.06	0.815	[−0.025; 0.612]
					Latency body	−0.630	1	6.32	**0.012**	[−0.969; −0.288]
					Daytime	1.427	1	89.50	**<0.001**	[1.108; 1.746]
					Treatment × Daytime	−0.641	1	7.51	**0.006**	[−1.092; −0.191]
					Latency body × Daytime	0.432	1	13.49	**<0.001**	[0.206; 0.659]
Giving-up density		168	0.204	0.521	Treatment	−0.053	1	0.31	0.578	[−0.117; 0.011]
					Latency body	0.059	1	6.79	**0.009**	[−0.010; 0.129]
					Daytime	−0.132	1	15.30	**<0.001**	[−0.196; −0.068]
					Treatment × Latency body	0.080	1	1.48	0.225	[0.015; 0.144]
					Treatment × Daytime	0.080	1	2.92	0.087	[−0.011; 0.171]
					Latency body × Daytime	0.006	1	3.57	0.059	[−0.058; 0.071]
					Treatment × Latency body × Daytime	−0.102	1	4.67	**0.031**	[−0.193; −0.011]
Variation in GUD	log	84	0.159	0.284	Treatment	0.210	1	0.28	0.594	[−0.622; 1.041]
					Latency body	−2.444	1	0.50	0.480	[−0.843; 0.356]
					Daytime	0.862	1	6.94	**0.008**	[0.031; 1.694]
					Treatment × Latency body	−0.616	1	0.12	0.731	[−1.450; 0.218]
					Treatment × Daytime	−0.089	1	0.02	0.886	[−1.265; 1.087]
					Latency body × Daytime	0.361	1	7.80	**0.005**	[−0.474; 1.195]
					Treatment × Latency body × Daytime	1.018	1	2.67	0.102	[−0.162; 2.197]

Σ Time in trays – sum of time spent in trays per side of arena, N Visits in trays – number of visits in seed trays per side of arena, variation in GUD – difference in giving-up density between the two seed trays on either side of the arena, treatment – light treatment, latency body – latency to leave the nest box for the first time (measure of boldness), R_m *– marginal* R^2 *value based on fixed factors,* R_c *– conditional* R^2 *value based on fixed and random factors, df – degrees of freedom.* $P < 0.05$ *is indicated in bold.*

applicable institutional and national guidelines for the care and use of animals were followed.

Grassland Enclosure Experiment

Study Subjects and Experimental Design

Bank voles were equipped with a passive integrated transponder tag (PIT, Trovan ID-100, 2.12 mm × 11.5 mm, 0.1 g) for individual identification and kept in standard makrolon cages on a standard rodent diet until the start of the experiment. The study was conducted from November 2013 to April 2014 in six large naturally vegetated grassland enclosures near Potsdam, Germany. Each enclosure was 0.25 ha (50 × 50 m) in size and was surrounded by a galvanized metal wall extending 1 m below and 0.5 m above ground. Voles were protected against terrestrial predators through an electrical veterinary fence surrounding the facility. Enclosures were open to avian predation.

Artificial light at night was created by using small solar powered garden lamps with single LEDs (Conrad Electronics, Hirschau, Germany, Model 572129, for spectral distribution see Eccard et al., 2018). Four enclosures were partially illuminated (half of each enclosure, **Figure 2**), while two enclosures served as controls where the whole enclosure was uniformly dark and illuminated, respectively. Lamps were 60 cm high and were above the grass layer in winter, but immersed in the grass layer later in spring. Lamps emitted a cold white light with a high proportion of blue light (color temperature = 7,250 K) through one diode and contained a diffuser to scatter the light. Diode and diffuser created a brighter zone surrounding the lamp (radius $r = 25$ cm, illuminance $i = 0.8$ lx) and a dimmer outer zone ($r = 2.5$ m, $i < 0.1$ lx; for details see Eccard et al., 2018).

Thirty-six bank voles were released into enclosures from November until December with three females and three males in each enclosure. Multicapture live traps (Ugglan special No 2, Grahnab, Sweden) were evenly distributed across each enclosure ($N = 36$, 6 × 6 grid). Traps were sheltered against wind and sun by metal boxes (30 × 20 × 20 cm) and a tile as cover. Animals were captured in January and February to obtain survival estimates.

Radio Telemetry

We conducted 24 h radio telemetry at the beginning of April during half moon and at the end of April during new moon. To

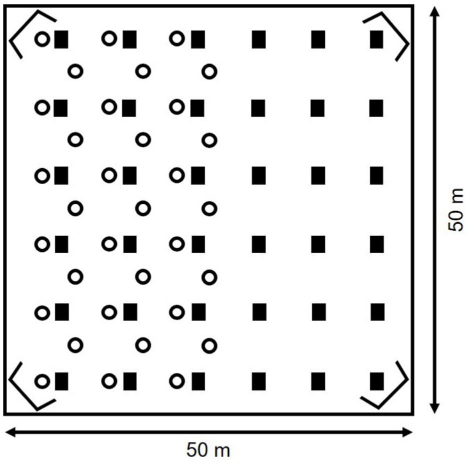

FIGURE 2 | Naturally vegetated outdoor enclosure with partial nighttime illumination. Artificial light at night was created using small LED garden lamps (circles). Multicapture live traps were evenly distributed across each enclosure (black boxes). A 24 h radio telemetry was conducted during half moon and new moon using Yagi antennae in the corners of the enclosure connected to an automatic receiving unit.

conduct the telemetry with sufficient sample size, we transferred additional animals (N = 24, two females and two males per enclosure) into the enclosure in mid-March. For detailed information on the methods see Hoffmann et al. (2018). In short, each enclosure was equipped with an automated radio telemetry system, consisting of eight Yagi antennae (Winkler-Spezialantennen, Germany) connected to an automatic receiving unit (Sparrow System, United States). After a calibration using stationary transmitters, locations of each transmitter could be calculated via trigonometry.

Voles were live-trapped and fitted with radio telemetry transmitters (Holohil BD-2C, ~1 g). In the half moon telemetry session 13 individuals were tracked (4 individuals in uniform enclosures, 9 individuals in partially illuminated enclosures) and in the new moon session 19 individuals were tracked (6 individuals in uniform enclosures, 13 individuals in partially illuminated enclosures). A location was calculated every 10 min resulting in 144 locations per animal in 24 h.

Statistical Analysis

All statistical analyses were performed with R version 4.0.3 (R Core Team, 2020) and for each analyzed variable we present the mean together with the standard deviation.

Side distribution of individuals within enclosures at day and night was analyzed using a Wilcoxon rank sum test. Separate tests were conducted for individuals tracked during half moon and new moon. Number of locations on the illuminated side divided by the total number of locations was compared to a theoretical value of 0.5 were no side preference or avoidance would be present. Additionally, side distribution of individuals within partially illuminated enclosures was compared to a random side distribution of individuals living in uniformly dark or illuminated enclosures at day and night using a Wilcoxon rank sum test. This was limited to new moon as under half moon only three control animals could be tracked.

Ethical Note

The experiment was conducted under the permission of the "Landesamt für Umwelt, Gesundheit und Verbraucherschutz" (LUGV; reference number V3-2347-44-2011) investigating effects of animal personality on risk taking (here: ALAN). Animals were housed under the permission and control of the LUGV (reference number 3854-1-132). All applicable institutional and national guidelines for the care and use of animals were followed.

RESULTS

Laboratory Experiment

Time Spent in Seed Trays

On average, voles spent 84.71 ± 125.74 min ($N_{\text{Individuals}}$ = 21) in all four seed trays combined during the treatment night and the following day. The interaction of latency body and daytime was significant (χ^2 = 15.14, $P < 0.001$, N = 84, **Table 2**). During the day, shy animals (longer latencies to emerge) spent less time in trays than bold animals, but during the night we detected no difference (**Figure 3** and **Table 3**). Initial body mass had no effect on time spent in trays (**Table 1**).

Number of Visits to Seed Trays

Animals conducted 50.3 ± 56.3 visits to all seed trays combined ($N_{\text{Individuals}}$ = 21). Animals visited seed trays more often during night than during day at the dark (night: 21.5 ± 25.0, N = 21; day: 6.5 ± 9.3, N = 21) and the illuminated side (night: 14.5 ± 16.0, N = 21; day: 7.9 ± 10.5, N = 21; **Tables 2**, **3** and **Figure 4A**).

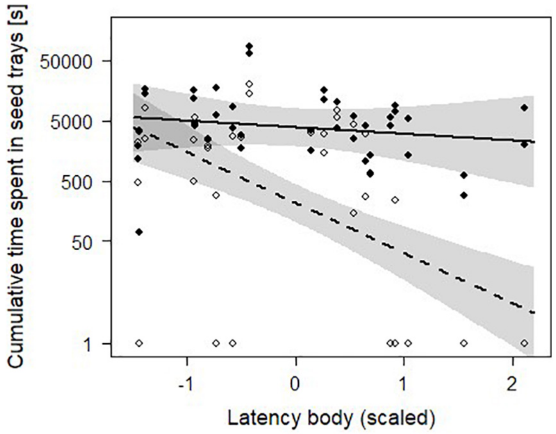

FIGURE 3 | Influence of daytime and latency body (scaled) on the cumulative time animals spent in seed trays on each side of the experimental arena of 21 bank voles. Black dots and the solid line show the raw nighttime data and the prediction line from the linear mixed effects model, respectively. White dots and the dashed line show the raw daytime data and the prediction line from the model. Gray areas represent 95%-confidence intervals. The y-axis is log scaled. The higher the latency body, the shyer is the animal.

TABLE 3 | Results of *post hoc* analyses of linear mixed models via Wald chi-square (χ^2) test.

Dependent variable	Interaction	Fixed	Across	Slope	Level	Estimate	χ^2	*P*
Σ Time in trays	Latency body × Daytime	Daytime		Latency body	Day	−1.923	21.82	**<0.001**
					Night	−0.251	0.37	0.543
N Visits in trays	Treatment × Daytime	Treatment	Daytime		Dark	−1.427	74.43	**<0.001**
					Light	−0.786	22.58	**<0.001**
		Daytime	Treatment		Day	−0.293	3.14	0.076
					Night	0.348	4.42	0.071
	Latency body × Daytime	Daytime		Latency body	Day	−0.630	12.98	**<0.001**
					Night	−0.198	1.28	0.258
Giving-up density	Treatment × Latency body × Daytime	Treatment	Daytime	Latency body	Dark	−0.006	0.04	0.848
					Light	0.095	8.20	**0.008**
		Daytime	Treatment	Latency body	Day	−0.080	5.70	**0.034**
					Night	0.022	0.45	0.503

Σ Time in trays – sum of time spent in trays per side of arena, N Visits in trays – number of visits in seed trays per side of arena, treatment – light treatment, latency body – latency to leave the nest box for the first time (measure of boldness). $P < 0.05$ is indicated in bold.

At night, voles tended to visit the seed trays at the dark side of the arena more often (21.5 ± 25.0, $N = 21$) than those on the illuminated side (14.5 ± 16.0, $N = 21$) while during the day voles tended to visit those trays more often on the side of the arena that had been illuminated at night (7.9 ± 10.5, $N = 21$) compared to those on the formerly dark side (6.5 ± 9.3, $N = 21$; **Table 3** and **Figure 4A**). While latency body did not explain the number of visits to the seed trays at night, bold animals (short latency) visited trays more often than shy animals (long latency) during the following day ($\chi^2 = 13.49$, $P < 0.001$, $N = 84$; **Table 3** and **Figure 4B**). Initial body mass had no effect on the number of visits to seed trays (**Table 1**).

Giving-Up Density

Averaged over all seed trays, giving-up density (GUD) was 0.33 ± 0.21 g of millet per 450 ml of sand ($N = 168$). GUD was influenced by an interaction of treatment, latency body and daytime but not by the initial body mass of the animal (**Tables 1**, **2**). The influence of latency body on GUD did not differ between day and night at the dark side of the arena while at the illuminated side the effect of latency body was stronger at day than at night (**Table 3** and **Figure 5**). Bold individuals (short latency) exploited trays to lower GUDs than shy individuals (long latency) but this difference was greater during the day at the formerly illuminated side of the arena compared to the formerly dark side (**Table 3** and **Figure 5**).

Exploitation Efficiency

To investigate differences in exploitation efficiency, we tried to fit population-level harvesting curves to total seeds taken out of the seed trays depending on the cumulative time animals spend in the tray for dark and illuminated trays at night and the subsequent day. However, we were not able to fit functions that explained enough variance (**Supplementary Material 3**).

Body Mass Change

Animals experienced a change in body mass of −1.62 ± 1.37 g over the course of the experiment. Body mass change did not differ depending on boldness (latency to emerge; $t = -1.36$, df = 19, $P = 0.191$).

Grassland Enclosure Experiment

Side Distribution

On average, 61.2 ± 36.6% ($N = 44$) of locations of voles living in partially illuminated enclosures were within the illuminated part of the enclosure. Rates did not differ from expected 50% during the day and during the night at half moon ($U_{day} = 29$, $P = 0.496$; $U_{night} = 20$, $P = 0.812$; $N = 9$) and new moon ($U_{day} = 61$, $P = 0.293$; $U_{night} = 68$, $P = 0.122$; $N = 13$). Additionally, rates did not differ at new moon between animals living in partially illuminated enclosures and animals living under uniformly dark or illumination conditions during day ($U_{day} = 27$, $P = 0.313$; $N_{Partial} = 13$, $N_{Uniform} = 6$) and night ($U_{day} = 35$, $P = 0.757$; $N_{Partial} = 13$, $N_{Uniform} = 6$).

DISCUSSION

We found that light treatment and individual boldness influenced the behavioral variables measured in the laboratory experiment. Animals tended to visit dark seed trays more often than illuminated trays during the night and tended to visit previously illuminated seed trays more often than previously dark trays during the day. Overall, boldness influenced foraging behavior more during day than during night with bold individuals spending more time in seed trays, visiting seed trays more often and having lower GUDs than shy individuals. While GUD was similar between dark and illuminated seed trays within individuals of varying boldness during the night, bold individuals had lower GUDs in previously illuminated trays than in dark trays during the day. In contrast, animals did not show avoidance or preference of the illuminated side in the naturally vegetated grassland enclosures.

While moonlight and skyglow (the reflection and scattering of ALAN by molecules or aerosols in the atmosphere) elicit a dim and spatially relatively homogeneous nighttime surface illumination (Kyba and Hölker, 2013), direct light sources often create a spatially heterogeneous "nightscape" with higher light intensities (Kuechly et al., 2012; Hale et al., 2013). We can show that small mammals adjust their foraging behavior to the artificial light conditions at the foraging side. Our findings of a

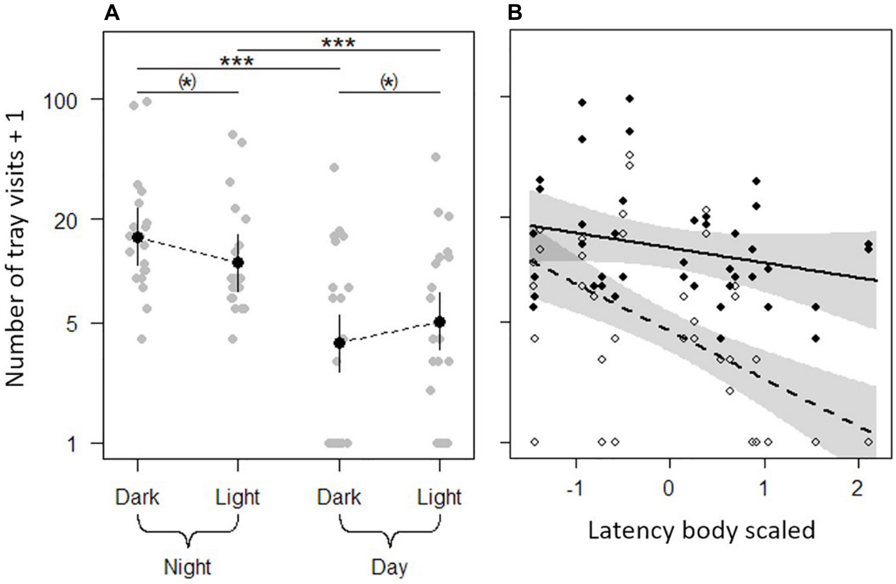

FIGURE 4 | Influence of **(A)** light treatment and daytime and **(B)** daytime and latency body (scaled) on the number of visits to seed trays on each side of the experimental arena of 21 bank voles. **(A)** Gray dots show the underlying raw data and black dots show the predicted means of the linear mixed effects model. Solid lines represent confidence intervals. $^*P < 0.1$, $^{***}P < 0.001$. **(B)** Black dots and the solid line show the raw nighttime data and the prediction line from the linear mixed effects model, respectively. White dots and the dashed line show the raw daytime data and the prediction line from the model. Gray areas represent 95%-confidence intervals. The higher the latency body, the shyer is the animal. The *y*-axes are log scaled.

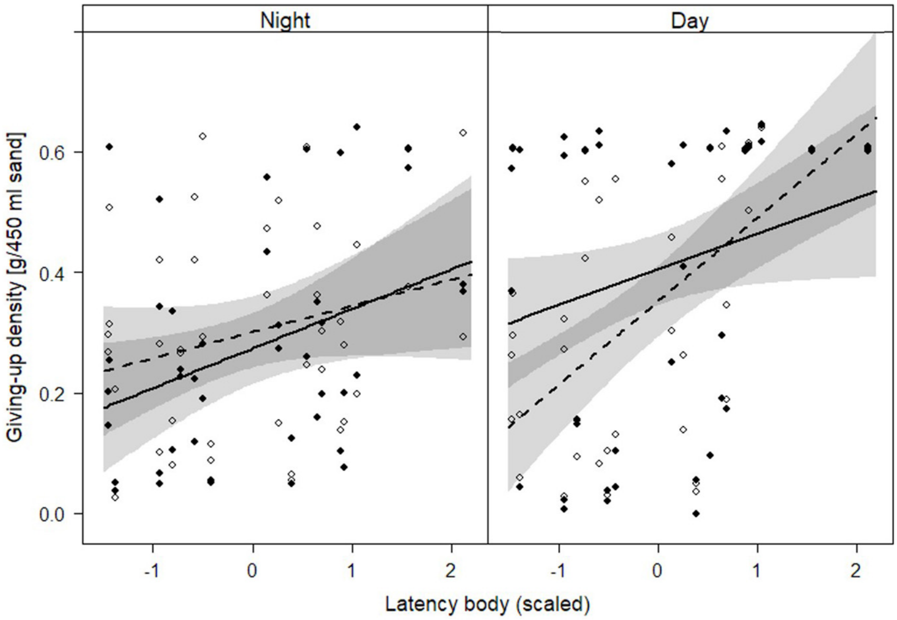

FIGURE 5 | Influence of light treatment, daytime and latency body (scaled) on giving-up density of seed trays. Both sides of the experimental arena contained two seed trays filled with millet and sand. Black dots and the solid line show raw data from the dark side of the arena and the prediction line from the linear mixed effects model, respectively. White dots and the dashed show raw data from the illuminated side of the arena and the prediction line from the model. Gray areas represent 95%-confidence intervals. The higher the latency body, the shyer is the animal.

reduced number of tray visits at illuminated trays at night are in accordance with Bird et al. (2004) who found that Beach mice decreased the number of patches they foraged in with increasing proximity to artificial light sources. On the other hand, time spent in trays and GUDs between dark and illuminated trays were similar at night. Animals might have reduced their movement between seed trays in the area subjected to ALAN to decrease visibility for predators. Nevertheless, they have to fulfill their energetic demands through foraging and might not be able to reduce the time they spent at the foraging site any further.

Shy individuals avoided foraging during the day more than bold individuals, leading to a reduced time spent in seed trays and number of tray visits. The arena provided no shelter except the nest box so that bank voles, which are common prey for a large variety of ground and aerial predators (Halle, 1993), might have experienced an increased perceived predation risk with increasing illumination. As the highest illumination level was present during the day, shy individuals who are more risk-averse might have reduced their diurnal foraging activity to a minimum while bold individuals did not reduce the time they spent in seed trays and the number of visits to trays. Thus, animals with varying personalities may differ in their perception of temporal risk distribution and in their risk allocation (Lima and Bednekoff, 1999). Alternatively, bold individuals might have to maximize foraging as they are shown to have a higher metabolism than shy individuals (Binder et al., 2016; von Merten et al., 2020). In that case, they are forced to show higher foraging activity even though perceived predation risk is high to fulfill their energetic needs.

Additionally, we can show a carry-over effect from the nighttime illumination to the foraging behavior shown at day as animals tended to visit seed trays more that were illuminated at night than those that previously were dark. This way, they could try to compensate for reduced nocturnal foraging. However, GUDs during the day differed depending on light treatment and boldness of the individual. With increasing boldness, the GUDs in the previously illuminated seed trays strongly decreased, suggesting that bold individuals try to maximize food consumption to compensate for missed foraging opportunities at night while shy individuals spent even more time on vigilance behavior. This might be a result of a so called "memory window" (Hughes et al., 1992). A foragers' behavior is influenced by its recent experiences in that environment. Perhaps shy individuals reduce their foraging at previously illuminated seed trays during the day as they remember the increased perceived predation risk during night and might attach more importance to that memory than bold individuals.

Depending on the behavioral changes caused by ALAN in predators of small mammals, the different responses of bold and shy individuals to spatially heterogeneous nighttime illumination can lead to a selection of a certain behavioral type and therefore to a loss of behavioral variation. If predators are avoiding illuminated sites, bold individuals should be at an advantage as they face no increased predation risk while having a higher access to food than shy individuals. On the other hand, studies show that aerial predators such as owls are able to increase hunting efficiency under increased nighttime illumination (Clarke, 1983). In that case, shy individuals might be at an advantage as their risk of being predated upon is lower than for bold individuals as they avoid illuminated habitat patches. Either way, ALAN has the potential to dramatically shift species interactions by a change in behavior of important prey species and thus can affect several trophic levels.

In contrast to the laboratory, we could not find an influence of partial nighttime illumination on the distribution of animals within grassland enclosures. In a previous study, we had found that animals increased their activity in illuminated enclosures compared to dark enclosures (Hoffmann et al., 2019), however, offering partial illumination in this study, we could not find a preference or avoidance for the illuminated part. A possible explanation for this could be that animals were able to avoid dim nighttime illumination by using vegetation cover and burrows. Furthermore, the light emitted by the solar garden lamps was more than ten times lower than the light used in the laboratory experiment. It resulted in surface illumination levels 2–3 times brighter than the maximum full moon illuminance on Earth (hypothetical value: 0.3 lx) down to a typical summer full moon in Berlin/Brandenburg (∼ 0.1 lx, Jechow et al., 2020) in only small parts of the enclosure while in most areas artificial illumination levels were below that level. The presence of high vegetation in April might have been sufficient to diminish the effect of increased predation risk sufficiently so that animals did not need to adjust their space use behavior. However, it may be interesting to investigate in future studies whether ALAN affects small-scale behavioral traits, such as time spent foraging above-ground, time spent hiding in burrows and burrowing activity or time spend foraging directly under lamps.

This study shows that partial nighttime illumination can affect night- and daytime foraging behavior of small mammals and provides further insights into how animal personality is related to behavioral changes caused by ALAN. While we can show that ALAN has carry-over effects regarding movement and foraging behavior into the daytime, it remains unclear how these affect interspecific interactions with diurnal species on the same and higher trophic levels. Thus, future studies on the effects of ALAN should extent their view from the night into the day to be able to better estimate the consequences of increasing nighttime illumination for ecosystems.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Landesamt für (Arbeitsschutz), Verbraucherschutz und Gesundheit, Brandenburg (reference numbers: LAVG 2347-27-2017 and LUGV V3-2347-44-2011). Keeping and breeding of animals was permitted by the Stadtverwaltung der Landeshauptstadt Potsdam, Bereich Veterinärwesen und Lebensmittelüberwachung (AZ 386-1).

AUTHOR CONTRIBUTIONS

JH designed the laboratory experiment. JE and JH designed the field experiment. JH secured the funding, supervised the collection of data, and analyzed the data with feedback from JE and FH. JH wrote the draft of the manuscript, which was further edited by FH and JE. All authors contributed to the article and approved the submitted version.

FUNDING

The Deutsche Bundesstiftung Umwelt (DBU) funded JHs studies on effects of light pollution (reference number 20015/374).

ACKNOWLEDGMENTS

We thank Andreas Rauch for his contribution during the laboratory experiment. We also thank Julia Köchling and Anne Gärtner for conducting field work and Elke Seydewitz for the analysis of telemetry data.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.779825/full#supplementary-material

REFERENCES

Baker, B. J., and Richardson, J. M. L. (2006). The effect of artificial light on male breeding-season behaviour in green frogs, *Rana clamitans* melanota. *Can. J. Zool.* 84, 1528–1532. doi: 10.1139/z06-142

Ballew, N. G., Mittelbach, G. G., Scribner, K. T., van Doorn, G. S., and Winn, A. A. (2017). Fitness consequences of boldness in juvenile and adult largemouth bass. *Am. Nat.* 189, 396–406. doi: 10.1086/690909

Bates, D. M. (2010). *lme4: Mixed-Effects Modeling with R.* Available online at: http://lme4. r-forge. r-project. org/book (accessed March 3, 2016).

Binder, T. R., Wilson, A. D. M., Wilson, S. M., Suski, C. D., Godin, J.-G. J., and Cooke, S. J. (2016). Is there a pace-of-life syndrome linking boldness and metabolic capacity for locomotion in bluegill sunfish? *Anim. Behav.* 121, 175–183. doi: 10.1016/j.anbehav.2016.09.006

Bird, B. L., Branch, L. C., and Miller, D. L. (2004). Effects of coastal lighting on foraging behavior of beach mice. *Conserv. Biol.* 18, 1435–1439. doi: 10.1111/j.1523-1739.2004.00349.x

Bowers, M. A. (1988). Seed removal experiments on desert rodents: the microhabitat by moonlight effect. *J. Mammal.* 69, 201–204. doi: 10.2307/1381778

Canário, F., Hespanhol Leitão, A., and Tomé, R. (2012). Predation attempts by short-eared and long-eared owls on migrating songbirds attracted to artificial lights. *J. Raptor Res.* 46, 232–234. doi: 10.3356/JRR-11-15.1

Clarke, J. A. (1983). Moonlight's influence on predator/prey interactions between short-eared owls (*Asio flammeus*) and deermice (*Peromyscus maniculatus*). *Behav. Ecol. Sociobiol.* 13, 205–209. doi: 10.1007/BF00299924

Curtis, D. J., and Rasmussen, M. A. (2006). The evolution of Cathemerality in primates and other mammals: a comparative and chronoecological approach. *Folia Primatol. (Basel)* 77, 178–193. doi: 10.1159/000089703

Daly, M., Behrends, P. R., Wilson, M. I., and Jacobs, L. F. (1992). Behavioural modulation of predation risk: moonlight avoidance and crepuscular compensation in a nocturnal desert rodent, *Dipodomys merriami*. *Anim. Behav.* 44, 1–9. doi: 10.1016/S0003-3472(05)80748-1

Davies, T. W., Bennie, J., Cruse, D., Blumgart, D., Inger, R., and Gaston, K. J. (2017). Multiple night-time light-emitting diode lighting strategies impact grassland invertebrate assemblages. *Global Change Biol.* 23, 2641–2648. doi: 10.1111/gcb.13615

de Jong, M., Caro, S. P., Gienapp, P., Spoelstra, K., and Visser, M. E. (2017). Early birds by light at night: effects of light color and intensity on daily activity patterns in blue tits. *J. Biol. Rhythms* 32, 323–333. doi: 10.1177/0748730417719168

Donatello, S., Rodríguez, R., Quintero, M. G. C., Jrc, O. W., Van Tichelen, P., Van, V., et al. (2019). *Revision of the EU Green Public Procurement Criteria for Street Lighting and Traffic Signals: Technical Report and Criteria Proposal.* Luxembourg: Publications Office of the European Union.

Eccard, J. A., Scheffler, I., Franke, S., and Hoffmann, J. (2018). Off-grid: solar powered LED illumination impacts epigeal arthropods. *Insect Conserv. Div.* 11, 600–607. doi: 10.1111/icad.12303

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C. M., Elvidge, C. D., Baugh, K., et al. (2016). The new world atlas of artificial night sky brightness. *Sci. Adv.* 2:e1600377. doi: 10.1126/sciadv.1600377

Gaston, K. J., Bennie, J., Davies, T. W., and Hopkins, J. (2013). The ecological impacts of nighttime light pollution: a mechanistic appraisal: nighttime light pollution. *Biol. Rev.* 88, 912–927. doi: 10.1111/brv.12036

Gosling, S. D. (2001). From mice to men: what can we learn about personality from animal research? *Psychol. Bull.* 127, 45–86. doi: 10.1037/0033-2909.127.1.45

Gutman, R., Dayan, T., Levy, O., Schubert, I., and Kronfeld-Schor, N. (2011). The effect of the lunar cycle on fecal cortisol metabolite levels and foraging ecology of nocturnally and diurnally active spiny mice. *PLoS One* 6:e23446. doi: 10.1371/journal.pone.0023446

Hale, J. D., Davies, G., Fairbrass, A. J., Matthews, T. J., Rogers, C. D. F., and Sadler, J. P. (2013). Mapping lightscapes: spatial patterning of artificial lighting in an urban landscape. *PLoS One* 8:e61460. doi: 10.1371/journal.pone.0061460

Halle, S. (1993). Diel pattern of predation risk in microtine rodents. *Oikos* 68:510. doi: 10.2307/3544919

Halle, S. (2006). Polyphasic activity patterns in small mammals. *Folia Primatol.* 77, 15–26. doi: 10.1159/000089693

Hansson, L., and Larsson, T.-B. (1978). Vole diet on experimentally managed reforestation areas in northern Sweden. *Ecography* 1, 16–26. doi: 10.1111/j.1600-0587.1978.tb00934.x

Herde, A., and Eccard, J. A. (2013). Consistency in boldness, activity and exploration at different stages of life. *BMC Ecol.* 13:49. doi: 10.1186/1472-6785-13-49

Herring, H., and Roy, R. (2007). Technological innovation, energy efficient design and the rebound effect. *Technovation* 27, 194–203. doi: 10.1016/j.technovation.2006.11.004

Hoffmann, J., Palme, R., and Eccard, J. A. (2018). Long-term dim light during nighttime changes activity patterns and space use in experimental small mammal populations. *Environ. Poll.* 238, 844–851. doi: 10.1016/j.envpol.2018.03.107

Hoffmann, J., Rüsen, L., Schirmer, A., and Eccard, J. A. (2020). *A Little is too Much: Already Low Levels of Artificial Light at Night Influence the Behavior of Small Mammals. Animal Ecology.* Potsdam: University of Potsdam.

Hoffmann, J., Schirmer, A., and Eccard, J. A. (2019). Light pollution affects space use and interaction of two small mammal species irrespective of personality. *BMC Ecol.* 19:26. doi: 10.1186/s12898-019-0241-0

Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C. C., Henckel, D., et al. (2010a). The dark side of light: a transdisciplinary research agenda for light pollution policy. *Ecol. Soc.* 15:13. doi: 10.5751/ES-03685-150413

Hölker, F., Wolter, C., Perkin, E. K., and Tockner, K. (2010b). Light pollution as a biodiversity threat. *Trends Ecol. Evol.* 25, 681–682.

Hughes, R. N., Kaiser, M. J., Mackney, P. A., and Warburton, K. (1992). Optimizing foraging behaviour through learning. *J. Fish Biol.* 41, 77–91. doi: 10.1111/j.1095-8649.1992.tb03870.x

Jacob, J., and Brown, J. S. (2000). Microhabitat use, giving-up densities and temporal activity as short- and long-term anti-predator behaviors in common voles. *Oikos* 91, 131–138. doi: 10.1034/j.1600-0706.2000.910112.x

Jechow, A., Kyba, C. C. M., and Hölker, F. (2020). Mapping the brightness and color of urban to rural skyglow with all-sky photometry. *J. Quant. Spectrosc. Radiat. Transfer* 250:106988. doi: 10.1016/j.jqsrt.2020.106988

Kashon, E. A. F., and Carlson, B. E. (2018). Consistently bolder turtles maintain higher body temperatures in the field but may experience greater predation risk. *Behav. Ecol. Sociobiol.* 72:9. doi: 10.1007/s00265-017-2428-8

Korpela, K., Sundell, J., and Ylönen, H. (2011). Does personality in small rodents vary depending on population density? *Oecologia* 165, 67–77. doi: 10.1007/s00442-010-1810-2

Kotler, B. P., Brown, J., Mukherjee, S., Berger-Tal, O., and Bouskila, A. (2010). Moonlight avoidance in gerbils reveals a sophisticated interplay among time allocation, vigilance and state-dependent foraging. *Proc. R. Soc. Lond. B Biol. Sci.* 277, 1469–1474. doi: 10.1098/rspb.2009.2036

Kronfeld-Schor, N., Dominoni, D., de la Iglesia, H., Levy, O., Herzog, E. D., Dayan, T., et al. (2013). Chronobiology by moonlight. *Proc. R. Soc. Lond. B Biol. Sci.* 280:20123088. doi: 10.1098/rspb.2012.3088

Kuechly, H. U., Kyba, C. C. M., Ruhtz, T., Lindemann, C., Wolter, C., Fischer, J., et al. (2012). Aerial survey and spatial analysis of sources of light pollution in Berlin, Germany. *Remote Sens. Environ.* 126, 39–50. doi: 10.1016/j.rse.2012.08.008

Kurvers, R. H. J. M., and Hölker, F. (2015). Bright nights and social interactions: a neglected issue. *Behav. Ecol.* 26, 334–339. doi: 10.1093/beheco/aru223

Kurvers, R. H. J. M., Drägestein, J., Hölker, F., Jechow, A., Krause, J., and Bierbach, D. (2018). Artificial Light at night affects emergence from a refuge and space use in guppies. *Sci. Rep.* 8:14131. doi: 10.1038/s41598-018-32466-3

Kyba, C. C. M., and Hölker, F. (2013). Do artificially illuminated skies affect biodiversity in nocturnal landscapes? *Landsc. Ecol.* 28, 1637–1640. doi: 10.1007/s10980-013-9936-3

Kyba, C. C., Hänel, A., and Hölker, F. (2014). Redefining efficiency for outdoor lighting. *Energy Environ. Sci.* 7, 1806–1809. doi: 10.1039/C4EE00566J

Kyba, C. C., Kuester, T., de Miguel, A. S., Baugh, K., Jechow, A., Hölker, F., et al. (2017). Artificially lit surface of Earth at night increasing in radiance and extent. *Sci. Adv.* 3:e1701528.

Laforge, A., Pauwels, J., Faure, B., Bas, Y., Kerbiriou, C., Fonderflick, J., et al. (2019). Reducing light pollution improves connectivity for bats in urban landscapes. *Landsc. Ecol.* 34, 793–809. doi: 10.1007/s10980-019-00803-0

Lima, S. L., and Bednekoff, P. A. (1999). Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *Am. Nat.* 153, 649–659. doi: 10.1086/303202

Lima, S. L., and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68, 619–640. doi: 10.1139/z90-092

Martínez, H. R. (2015). *Analysing Interactions of Fitted Models*. Available online at: https://cran.r-project.org/web/packages/phia/vignettes/phia.pdf (accessed November 7, 2015).

Mazza, V., Eccard, J. A., Zaccaroni, M., Jacob, J., and Dammhahn, M. (2018). The fast and the flexible: cognitive style drives individual variation in cognition in a small mammal. *Anim. Behav.* 137, 119–132. doi: 10.1016/j.anbehav.2018.01.011

Merrick, M. J., and Koprowski, J. L. (2017). Should we consider individual behavior differences in applied wildlife conservation studies? *Biol. Conserv.* 209, 34–44. doi: 10.1016/j.biocon.2017.01.021

Nakagawa, S., and Schielzeth, H. (2013). A general and simple method for obtaining R 2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* 4, 133–142. doi: 10.1111/j.2041-210x.2012.00261.x

Negro, J. J., Bustamante, J., Melguizo, C., Ruiz, J. L., and Grande, J. M. (2000). Nocturnal activity of Lesser Kestrels under artificial lighting conditions in Seville, Spain. *J. Raptor Res.* 34, 327–329.

Norrdahl, K., and Korpimäki, E. (1995). Mortality factors in a cyclic vole population. *Proc. R. Soc. Lond. B* 261, 49–53. doi: 10.1098/rspb.1995.0116

Perea, R., González, R., San Miguel, A., and Gil, L. (2011). Moonlight and shelter cause differential seed selection and removal by rodents. *Anim. Behav.* 82, 717–723. doi: 10.1016/j.anbehav.2011.07.001

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Réale, D., Gallant, B. Y., Leblanc, M., and Festa-Bianchet, M. (2000). Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Anim. Behav.* 60, 589–597. doi: 10.1006/anbe.2000.1530

Russ, A., Luèenièová, T., and Klenke, R. (2017). Altered breeding biology of the European blackbird under artificial light at night. *J. Avian Biol.* 48, 1114–1125. doi: 10.1111/jav.01210

Schirmer, A., Herde, A., Eccard, J. A., and Dammhahn, M. (2019). Individuals in space: personality-dependent space use, movement and microhabitat use facilitate individual spatial niche specialization. *Oecologia* 189, 647–660. doi: 10.1007/s00442-019-04365-5

Šíchová, K., Koskela, E., Mappes, T., Lantová, P., and Boratyński, Z. (2014). On personality, energy metabolism and mtDNA introgression in bank voles. *Anim. Behav.* 92, 229–237. doi: 10.1016/j.anbehav.2014.04.011

Sih, A. (1980). Optimal behavior: can foragers balance two conflicting demands? *Science* 210, 1041–1043. doi: 10.1126/science.210.4473.1041

Smith, B. R., and Blumstein, D. T. (2008). Fitness consequences of personality: a meta-analysis. *Behav. Ecol.* 19, 448–455. doi: 10.1093/beheco/arm144

Stone, E. L., Harris, S., and Jones, G. (2015). Impacts of artificial lighting on bats: a review of challenges and solutions. *Mammal. Biol.* 80, 213–219. doi: 10.1016/j.mambio.2015.02.004

van Grunsven, R. H. A., Jähnichen, D., Grubisic, M., and Hölker, F. (2018). Slugs (Arionidae) benefit from nocturnal artificial illumination. *J. Exp. Zool. A* 329, 429–433. doi: 10.1002/jez.2170

von Merten, S., Dingemanse, N. J., Mathias, M., da, L., and Rychlik, L. (2020). Individual behavior, behavioral stability, and pace of life within and among five shrew species. *Behav. Ecol. Sociobiol.* 74:15. doi: 10.1007/s00265-019-2793-6

Willmott, N. J., Henneken, J., Elgar, M. A., and Jones, T. M. (2019). Guiding lights: foraging responses of juvenile nocturnal orb-web spiders to the presence of artificial light at night. *Ethology* 125, 289–297. doi: 10.1111/eth.12852

Wilson, D. S., Clark, A. B., Coleman, K., and Dearstyne, T. (1994). Shyness and boldness in humans and other animals. *Trends Ecol. Evol. (Amst.)* 9, 442–446. doi: 10.1016/0169-5347(94)90134-1

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 18 January 2022
doi: 10.3389/fevo.2021.786557

Tracking Flights to Investigate Seabird Mortality Induced by Artificial Lights

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Light pollution causes attraction and/or disorientation of seabirds, leading to mortality events due to multiple threats. This is a poorly understood phenomenon, largely because of the challenge to track seabirds at night from their nests to the grounding light-polluted locations. New tracking technologies can inform about this phenomenon. Here, we used GPS transmitters with remote download to track the flights of Cory's shearwater *Calonectris borealis* fledglings from an inland experimental releasing site to the ocean. We released birds assigned to three experimental groups: GPS tagged, tape-labelled, and control birds. We assessed how both intrinsic (such as body mass, body condition, body size, and down abundance) and extrinsic (i.e., flight descriptors, such as distance, straightness, and flight duration, wind speed, or moon luminance) factors influenced light-induced groundings by using two datasets: one including the three groups and another including just the GPS tagged birds (as GPS devices provide unique information). We tested whether the probability of being grounded by artificial lights was related to intrinsic factors. With the use of the whole dataset, we found that birds with a higher down abundance had a higher probability of being grounded. GPS data revealed that the probability of being grounded was positively related to the tortuosity of flights and the overflown light pollution levels. Also, birds with slower flights were more likely to be grounded than birds with fast flights. Tortuosity increased with light pollution levels but decreased with the ambient light of the moon. GPSs with remote data download provided information on birds reaching the ocean, this being a substantial improvement to previous studies requiring recapture of the individuals to retrieve the data. GPS tracks of birds reaching the ocean allowed us to know that some birds overflew coastal urban areas so light-polluted as the landing sites of grounded birds. We provide novel scientific-based information to manage seabird mortality induced by artificial lights.

Keywords: Canary Islands, coastal environment, conservation, management, mortality, light pollution, phototaxy

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Specialty section:
This article was submitted to
Urban Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *30 September 2021*
Accepted: *16 December 2021*
Published: *18 January 2022*

Citation:
Rodríguez A, Rodríguez B,
Acosta Y and Negro JJ (2022)
Tracking Flights to Investigate Seabird
Mortality Induced by Artificial Lights.
Front. Ecol. Evol. 9:786557.
doi: 10.3389/fevo.2021.786557

INTRODUCTION

Light pollution is an important threat to biodiversity conservation because it can cause cascading effects on ecosystem functioning (Longcore and Rich, 2004; Hölker et al., 2010). Despite the numerous studies and reviews of the field, the underlying factors are far from being understood (Gaston et al., 2014, 2021), especially for secretive species. From a conservation point of view, mass mortality events of organisms are one of the most severe ecological consequences of light pollution, involving a wide range of taxa such as moths, sea turtles, passerine birds, and seabirds (Rich and Longcore, 2006).

Seabirds are one of the most endangered groups of birds (Croxall et al., 2012; Dias et al., 2019). Within seabirds, petrels and shearwaters (Order Procellariiformes; hereafter petrels) are mainly threatened by introduced predators in their nesting grounds and commercial fisheries at sea (Rodríguez et al., 2019). Petrels also suffer from mass mortality episodes caused by artificial night lighting (Rodríguez et al., 2017). For a long time, it has been known that petrel fledglings are attracted and/or disorientated by artificial lights when they are leaving their nests for the first time and fly towards the sea (Imber, 1975). More than 56 burrow-nesting petrel species, some of them critically endangered, are affected by lights (Rodríguez et al., 2017; Silva et al., 2020). Every fledging season, on islands where humans and petrels coexist, thousands of fledglings of different species are grounded by light pollution. This phenomenon called "fallout" exposes grounded birds to injuries or death by collision with human structures or vehicles, as well as predation by introduced or domestic animals (e.g., rats, cats, and dogs), but also plumage soiling, inanition, or dehydration. To mitigate light-induced mortality, rescue campaigns are conducted by local governments and NGOs, releasing into the ocean a high proportion of the admitted birds. Without human intervention (i.e., the rescue actions) it is assumed that most grounded birds would die (Le Corre et al., 2002; Rodríguez and Rodríguez, 2009; Fontaine et al., 2011). Around 10% of birds collected in the campaigns die before release back to the wild, although light-induced mortality could be higher as laypeople do not usually report dead birds, thus 40% is possibly a more accurate estimate (Podolskyi et al., 1998; Ainley et al., 2001; Rodríguez et al., 2014). Why petrels become disorientated by lights is far from being fully understood (Atchoi et al., 2020). The majority of our knowledge about the fallout comes from observational data from rescue campaigns and mainly consists of the reporting of species identification, individual numbers, dates, and locations (Rodríguez et al., 2017).

Analyses of rescue campaign data have uncovered the main factors determining the number of grounded birds. Thus, more fledglings are grounded during moonless, windy, and around peak fledging period nights (Telfer et al., 1987; Rodríguez et al., 2014; Syposz et al., 2018). Rescue campaign data have also described the spatial distribution of the fallout, showing the most dangerous areas. However, the highest numbers of grounded birds have not been reached in the most light-polluted areas according to satellite imagery (Troy et al., 2011, 2013; Rodrigues et al., 2012). It is probably because of the interaction with other influential factors, such as distribution of the breeding colonies and distance to artificial lights. Unfortunately, rescue campaigns cannot identify the colony of origin of birds grounded by artificial lights. Therefore, questions regarding the distance at which birds are attracted to lights and light intensity thresholds leading to fallout events, remains unanswered (Troy et al., 2011; Rodríguez et al., 2015b). In this sense, in Hawaii, it has been suggested that fledglings could be attracted to lights from a long-distance and a substantial number of birds are attracted back to land after reaching the sea (Troy et al., 2013).

Current technological advances in remote tracking systems can help to understand why petrels are attracted to lights. One of our previous studies using GPS data-loggers to track the flights of Cory's shearwater fledglings from nest-burrows to light-polluted areas on Tenerife revealed that: (a) 50% of the birds were grounded within a 3 km radius from the nest-site; and (b) flight distance was positively related to light pollution levels (Rodríguez et al., 2015b). In this study, we conducted an experiment to assess the percentage of birds grounded by light pollution and to assess intrinsic and extrinsic factors related to the probability of grounding. We assessed intrinsic factors, such as body mass, body size, body condition, and down abundance, and extrinsic factors, such as moon luminance, wind speed, and overflown light pollution levels. We hypothesized that fledglings in a poor body condition have a higher chance of grounding due to lights and that light levels (from artificial light sources or the moon) play an important role in the probability of grounding. We expected that the higher moon illuminance and the lower overflown light pollution levels would result in a lower probability of groundings for shearwater fledglings. Given that shearwater fledgling flights are favoured by wind gusts; wind speed could help fledglings to keep on flight for longer. Consequently, we hypothesized that windy conditions reduce the probability of being grounded. This may seem contradictory to previous observational studies, which reported higher number of grounded birds during windy nights (e.g., Rodríguez et al., 2014; Syposz et al., 2018). However, the increase of groundings could be a consequence of a higher number of fledglings flying around and then being susceptible to artificial lights. To achieve these aims, we employed miniaturised GPS-GSM data loggers on Cory's Shearwater fledglings from rescue campaigns. This technology enables downloading positional data remotely, with no need for recapturing the individuals. Further, we assessed light pollution levels by using high-resolution nocturnal satellite imagery. As precise knowledge of factors affecting fallout could have important implications for conservation and management, we discuss our findings in relation to previous studies.

MATERIALS AND METHODS

Study Area and Species

The study was conducted on the south face of Tenerife Island, the largest and the highest of the Canary Islands (2,034 km^2 and 3,718 m a.s.l.). The coastline (342 km) is predominantly rocky with boulder shores, and cliffs up to 300 m high. The climate is subtropical and oceanic. Oceanographic conditions are influenced by northeast trade winds and marine upwelling that occur off the northwest African coast (Barton et al., 1998). In 2019, the local human population was around 949,471 inhabitants. In addition, as the island's economy is heavily dependent on tourism, several millions of visitors are received each year, and they mainly stay in touristic resorts in coastal areas (Martín-Ruiz, 2001; ISTAC, 2020).

Cory's shearwater (*Calonectris borealis*) is a medium-sized pelagic seabird (body mass, 600–800 g; wingspan, 112–126 cm), that breeds underground in Azores, Berlengas, Madeira, Selvagems, and the Canary Islands. Its diet is composed of pelagic fishes, cephalopods, and crustaceans, and it spends the non-breeding period in the southern coasts of America and Africa (Reyes-González and González-Solís, 2016). Shearwaters are present for breeding in Canarian waters from February to November. Single-egg clutches are laid in early June, and thus only up to one individual may fledge per nest and year during late October to early November (Martín and Lorenzo, 2001). Adults visit the colonies only at night, and juvenile birds usually leave the nest in the first 3 h after sunset (Rodríguez et al., 2015b). Cory's shearwater is the most abundant seabird species in the Canary Islands (Martín and Lorenzo, 2001). On Tenerife, its breeding population has been estimated at 8,200–16,600 pairs (Rodríguez et al., 2015b).

Cory's shearwater fledglings are attracted to artificial lights during their initial flights from their colonies to the Ocean. This attraction occurs in Azores, Madeira, and the Canary Islands causing the death of thousands of individuals every year (Rodríguez and Rodríguez, 2009; Fontaine et al., 2011; Rodrigues et al., 2012), but also in Mediterranean colonies of the related Scopoli's shearwater (*Calonectris diomedea*; formerly considered a single species) (Baccetti et al., 2005; Rodríguez et al., 2015a). In Tenerife, there is an island-wide rescue programme implemented by La Tahonilla Wildlife Rehabilitation Centre (thereafter TWRC) and funded by the local Government (Cabildo Insular de Tenerife). According to the TWRC database, the number of fledglings recovered by this programme varied annually according to the moon cycle, but overall it has increased since its inception in 1990 (Rodríguez et al., 2012b). During the last five fledging seasons (2016–2020), the annual average of birds rescued on Tenerife by the programme after being grounded by artificial lights was 2,124 ± 526.1 (mean ± *SD*; range: 1,441–2,610).

Experimental Design

Tracking shearwater fledglings from their nests to grounding locations in Tenerife Island is a daunting task. First, Cory's shearwater nests underground, in deep and inaccessible burrows (Rodríguez et al., 2022), located on high vertical cliffs or steep terrain. Therefore, extracting the nestlings for handling and marking is precluded for most nests. Second, being unable to monitor nestling growth, we cannot predict fledging dates with the accuracy needed. Batteries would get flat before birds fledge as energy consumption is higher when the GPSs are underground trying to connect with satellites. Third, sampling nestlings at their natural nests renders a smaller sample size as many devices would not work (see Rodríguez et al., 2015b for a complete discussion on the technical challenges). Given the above shortcomings, our sample size consisted of fledglings presumably affected by light pollution and later found by volunteers during the rescue campaign in 2017, 2018, and 2019. All birds were rescued the night before our handling took place and admitted to the programme conducted by TWRC. A visual inspection assessed the healthy status of birds. Birds underweight (<420 g), lethargic (with no perceptible reactions when handled), bleeding, with injuries, or with other clear evidence of violent collisions (e.g., damaged plumage or broken limbs) were excluded. Therefore, we only selected individuals among apparently healthy birds and thus able to fly.

We released the birds in a natural cave (simulating a natural burrow) situated 8 km inland and at 900 m a.s.l. on the south face of the island. In this part of the island, which concentrates the most largest and densest tourist urban areas of the island, more than 50% of the total number of stranded fledglings are reported annually (Rodríguez and Rodríguez, 2009). Before release, each bird was measured (see below) and randomly allocated to one of the three experimental treatments: GPS-tagged, tape-labelled, and control (see procedure details below). This procedure allowed us to quantify the percentage of birds attracted to artificial lights for each experimental treatment. Local authorities and environmental NGOs made a special effort on the media to involve the general public to rescue as many stranded birds as possible. The public was requested to collect and retain the birds, especially those wearing markings, and then call and inform TWRC. Rescued birds were examined by TWRC staff before their subsequent release into the sea.

Morphological Measurements

All birds were banded with metal rings and handled as described in Rodríguez et al. (2012a). For every fledgling, date, recovery location, body mass, wing length (W), tarsus length (T), skull length (S), and four bill morphometric measurements were recorded. Bill measurements were culmen (C, from the base of forehead feathers in centre of nasal tube to distant part of the curve of the hooked bill), bill length at nostril (BL, from centre of dorsomedial part of tube to distant part of the curve of the hooked bill), bill depth (BD, from the base of forehead feathers to ventral surface of lower mandible), and bill depth at nostril (BDN, from the base of nasal tube at nostrils to ventral surface of bill). The biometrics were taken by the same person (BR) using a spring balance (nearest 5 g), a rule (precise to 1 mm), and an electronic calliper (nearest 0.01 mm). The presence of down in the head and belly was assessed in an ordinal scale (0 = absence, 1 = presence of down, and 2 = entirely covered by down), and the sum of the two values (head and belly) was used as a down index (DI), ranging from 0 (down absence) to 4 (head and belly entirely covered by down).

Treatments

We randomly assigned birds to one of the three treatments: GPS-tagged, tape-labelled, and control birds. Birds of the first treatment (GPS-tagged) were tagged with customised GPS-GSM devices designed and provided by DigitAnimal[1] (Móstoles, Spain). Each device was put in a heat-shrink tube for waterproofing, and its final size was 28 × 55 × 18 mm. The device

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[1] www.digitanimal.com

weighed 23 g, which represented less than 4% of the body mass of Cory's shearwater fledglings (mean ± *SD* = 3.4% ± 0.3; *n* = 87). Devices were attached to the mid-dorsal feathers of birds with TESA tape (Puitz et al., 1997). GPSs were programmed to record a position every 30 s from approximately one hour after sunset on the day of deployment. Data were remotely transmitted and downloaded to an internal server in spreadsheet files. To the just tape-labelled birds (second treatment), we attached a 4–5 cm long stripe of TESA tape to the mid-dorsal feathers. Then, we wrote a code number with a black permanent ink pen. This second treatment was intended as a control given that birds without marks on their back could be overlooked by rescue workers and volunteers (see section "Discussion"). Control birds (third treatment) were not GPS nor tape-labelled, but they were handled as long as the other treatment birds, and they were also measured and banded to allow identification.

Data Processing

The positional data recorded by the GPS devices were imported in *Qgis* (version 3.14.16.; Open Source Geospatial Foundation Project[2]). We built two databases: one of the complete tracks (*n* = 43), i.e., those containing all locations from the release point to the ending sites, and another containing environmental information of each GPS position of complete tracks (*n* = 1,985 locations). For each complete track, we calculated fifteen descriptive variables (**Table 1**). For each point location, we calculated land altitude and irradiance. We extracted the irradiance values of nighttime lights from nocturnal satellite imagery as a proxy of light pollution. Three cloud-free composite of VIIRS nighttime lights corresponding to November 2017–2019 and produced by the Earth Observation Group, NOAA National Geophysical Data Center[3] were used. A Digital Elevation Model (DEM) with a cell size of 25 × 25 m and an accuracy of 1 m of horizontal and vertical resolution, respectively, was obtained from the Digital Atlas of Tenerife Cabildo de Tenerife[4] and used to calculate the elevation of every GPS location. Sunset times in minutes were obtained from the NOAA Global Monitoring Laboratory.[5] Moon luminance (continuous variable) was measured as the percentage of luminance at full moon at zenith at distance equal to the mean equatorial parallax (Austin et al., 1976). We calculated moon luminance for each 10-min period by using the moonlight Fortran software (Austin et al., 1976). Wind direction and wind speed were taken at 10-min periods from a meteorological station located at 10 km from the releasing site (Las Galletas, Agrocabildo, Cabildo de Tenerife[6]).

To identify the areas where birds concentrated, we created kernel density estimations for the GPS positions of all tracked birds by using the heatmap algorithm (Interpolation) at the processing toolbox of *Qgis*. We also created animations of movement trajectories (Schwalb-Willmann et al., 2020; **Supplementary Animations 1**).

TABLE 1 | Variables used to describe flights of GPS-tagged Cory's Shearwater (*Calonectris borealis*) fledglings in Tenerife, Canary Islands, during 2017–2019.

Variable	Description
Ending site	Three categories depending on where the departure flight ended: (a) ground (if the bird was stranded by light pollution), (b) coastal sea (if the bird rested on the sea surface closer than 1 km from the coastline), or (c) sea (if the bird rested on the sea surface farther than 1 km offshore).
Sea	Yes or no, if the bird overflew the sea surface and come back to land prior to reaching its ending site.
Straight distance	Minimum distance (km) from the release point to the ending site.
Flight distance	Distance (km) covered from the release point to the ending site.
Duration	Time (minutes) since the bird left the release point until it reached the ending site.
Flight speed	Mean speed (km/h) calculated by dividing covered distance by duration.
Bearing	Horizontal angle (degrees) between the direction from the release point to the ending site with respect to the true north.
Straightness	Index calculated as the ratio between straight distance and flight distance.
Moon luminance	Percentage of moon luminance at the onset of the flight.
Sunset time	Time elapsed from sunset to flight initiation (minutes).
Wind direction	Wind direction at the time of the flight starting (degrees).
Wind speed	Wind speed at the time of the flight starting (m/s).
Mean light pollution	Mean irradiance from all GPS locations of each flight.
SD light pollution	Standard deviation of irradiance from all GPS locations of each flight.
Maximum light pollution	Maximum value of irradiance overflew from all GPS locations of each flight.

Variables included in the univariate GLMs explaining the probability of being grounded are in bold (see main text).

Data Analysis

To estimate bird size and bird body condition, we first ran a principal component analysis (PCA) on the seven centred and scaled morphometric variables (W, T, S, C, BL, BD, and BDN; see above for details). The first principal component was used as a body size index (BSI). The first principal component retained 67.8% of the variation (**Supplementary Table 1**). The seven morphometric variables showed positive factor loadings (factor loadings: 0.28, 0.35, 0.43, 0.41, 0.41, 0.38, and 0.36 for W, T, S, C, BL, BD, and BDN, respectively) and highly significant correlations to the first principal component (**Supplementary Figure 1**). Then, we run a linear model of body mass on BSI (the first principal component). This regression showed a R^2 = 0.35 and it was statistically significant (F = 150.5, df = 1, 275, P < 0.001). Diagnostic plots indicated that model assumptions were not violated (see **Supplementary Figure 2**). Finally, we extracted the standardised residuals of this model and used them as a body condition index (BCI), where positive and negative values indicate that birds are heavier and lighter

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[2] http://qgis.osgeo.org

[3] https://eogdata.mines.edu/products/vnl/#monthly

[4] http://atlastenerife.es/portalweb

[5] https://www.esrl.noaa.gov/gmd/grad/solcalc

[6] www.agrocabildo.org

than the average in the population, respectively (Green, 2001; Rodríguez et al., 2012a).

We ran three linear models to test for potential differences in body mass, body size index (BSI), and body condition index (BCI) of birds allocated to the three treatments. We also tested for potential differences in body mass, BSI, and BCI among the study years (2017, 2018, and 2019). No significant differences were observed among treatments or years (see section "Results"). Chi-square tests were used to test for differences in the frequency of groundings among treatments and years.

We ran a generalised linear model (GLM) with binomial error and logit link function to model the probability of being grounded as a function of bird traits (body mass, BSI, BCI, and DI) and experimental treatments (categorical variable: GPS-tagged, tape-labelled, or control). As a response variable, we included the fate of each bird as 1, if the bird was grounded and reported in the rescue programme, or 0, if the bird was not reported by the rescue programme. For the latter case, we assumed the bird reached the ocean successfully. As explanatory variables, we included body mass, BSI, BCI, and DI, and the three-level factor treatment. To avoid multicollinearity, we calculated variance inflation factors (VIFs) for explanatory variables, and those with VIF values higher than 2 were excluded (Zuur et al., 2010). Body mass, which was positively related to BSI and BCI, reached the highest VIF value (>500), and, consequently, it was excluded from the analyses. After that, all VIF values were lower than 1.03.

We also assessed the probability of being grounded by using the dataset of complete tracks. We used GLMs with binomial errors, logit link functions, and the fate of birds (0 = non-reported in rescue programmes; 1 = grounded) as a response variable. Given our sample size (n = 43), we ran univariate GLMs to avoid overparameterization. Explanatory variables in univariate models were bird traits (i.e., body mass, BSI, BCI, and DI) and the variables describing flight traits in **Table 1** (excepting the factor ending site, straight distance, and the two circular variables: bearing and wind direction). To assess the variables involved in the flight straightness, we ran univariate linear models, i.e., with an explanatory variable per model. As explanatory variables in univariate models, we used bird traits (i.e., body mass, BSI, BCI, and DI) plus the variables wind speed, time since sunset, moon luminance, and mean, standard deviation, and maximum of light pollution.

We ranked the models according to the AICc value to assess the most explaining variables (the lower the AICc, the better the model). Because variables were taken on different scales, we standardised continuous explanatory variables (mean = 0; SD = 1) prior to modelling. Statistical analyses were conducted in *R* (version 4.0.0; R Foundation for Statistical Computing, Vienna, Austria). We used the packages *ggplot2*, *MASS*, *car*, *circular*, and *MuMIn*.

RESULTS

Recovery Rates and GPS Performance

We handled and released 277 fledglings, which were allocated to the three treatments: 87 GPS-tagged, 92 tape-labelled, and 98 control birds (**Table 2**). Body mass, body size index (BSI), and body condition index (BCI) did not differ among treatment groups (Body mass: F = 1.248, df = 2, 274, p-value = 0.289; BSI: F = 1.460, df = 2, 274, p-value = 0.234; BCI: F = 0.414, df = 2, 274, p-value = 0.662) nor among years (Body mass: F = 2.26, df = 2, 274, p-value: 0.106; BSI: F = 0.459, df = 2, 274, p-value = 0.632; BCI: F = 2.092, df = 2, 274, p-value = 0.125).

Thirty-seven out of 277 fledglings were later recovered when they got stranded inland after release (**Table 2**). The percentage of grounded birds was slightly higher for GPS-tagged birds (19.5%) than for tape-labelled and control birds (13% and 8.2%, respectively), but differences were statistically non-significant (Pearson's Chi-squared test = 5.166, df = 2, p-value = 0.076) (**Table 2**). However, we observed significant differences in grounding rate among the years, with higher rates in later years as follow: 2019 > 2018 > 2017 (Pearson's Chi-squared test = 8.084, df = 2, p-value = 0.018). A majority of birds (18; 49%) were rescued within 24 h after release, while thirteen (35%), four (11%), and two (5%) birds were rescued 24–48 h, 3 days, and 5 days after release, respectively. Body mass decreased for all the rescue fledglings and this variation ranged from −103 to −5 g. No obvious differences among treatments were observed in days elapsed and body mass loss from release to rescue (**Figure 1**).

The probability of being grounded for control birds decreased in relation to the two other treatments (estimate ± SE = −1.114 ± 0.470, 95% CI = −2.085, −0.220). No differences in the probability of being grounded by artificial lights were detected between GPS-tagged and tape-labelled birds (estimate ± SE = −0.640 ± 0.427, 95% CI = −1.500, 0.187 using GPS-tagged birds as the reference value). The probability of being grounded was positively related to the down index (estimate ± SE = 0.485 ± 0.189, 95% CI = 0.123, 0.866), but BSI and BCI were not significant (BSI 95% CI = −0.607, 0.134; BCI 95% CI = −0.465, 0.285). The GLM including BSI, BCI, DI, and treatment was significant in relation to the null model (Deviance = 14.924, df = 5, p-value = 0.011; AICc = 215.2; null model AICc = 219.8).

Flight Characteristics

Seventy-one of the 87 deployed GPS devices recorded positions, but we got complete tracks, i.e., with GPS locations from the release site to the grounding location or the ocean, from just 43 birds. Additionally, we got incomplete tracks from nine birds (not included in the statistical analyses). Most incomplete tracks lack the first few meters of the flight, probably due to the difficulties the devices experienced to connect with satellites from inside the releasing cave. Thus, loggers unable to connect with satellites before flight initiation started to log data once the birds were flying.

Bird flights covered distances of 18.7 ± 10.8 km and lasted for 47.3 ± 45.3 min on average (mean ± SD; **Table 3**). According to the binomial GLMs, the explanatory variables that reached significance to explain the probability of grounding (i.e., 95% confidence intervals did not include the 0) were straightness, those related to light pollution, and flight speed (**Table 4**). The probability of being grounded decreased with the straightness and the speed of the bird flight, but it increased with light pollution levels (**Table 4** and **Figure 2**). According to linear

TABLE 2 | Number of Cory's Shearwater (*Calonectris borealis*) fledglings experimentally released, and those recovered during rescue campaigns (R) in Tenerife in 2017–2019.

Treatment	2017		2018		2019		Total	
	N	***R* (%)**	***N***	***R* (%)**	***N***	***R* (%)**	***N***	***R* (%)**
GPS-tagged	24	2 (8.3)	21	2 (9.5)	42	13 (31.0)	87	17 (19.5)
Tape-labelled	33	2 (6.1)	24	6 (25.0)	35	4 (11.4)	92	12 (13.0)
Control	32	1 (6.3)	29	2 (6.9)	37	5 (13.5)	98	8 (8.2)
Total	89	5 (5.6)	74	10 (13.5)	114	22 (19.3)	277	37 (13.4)

N = number of handled birds; R = number of rescued birds.

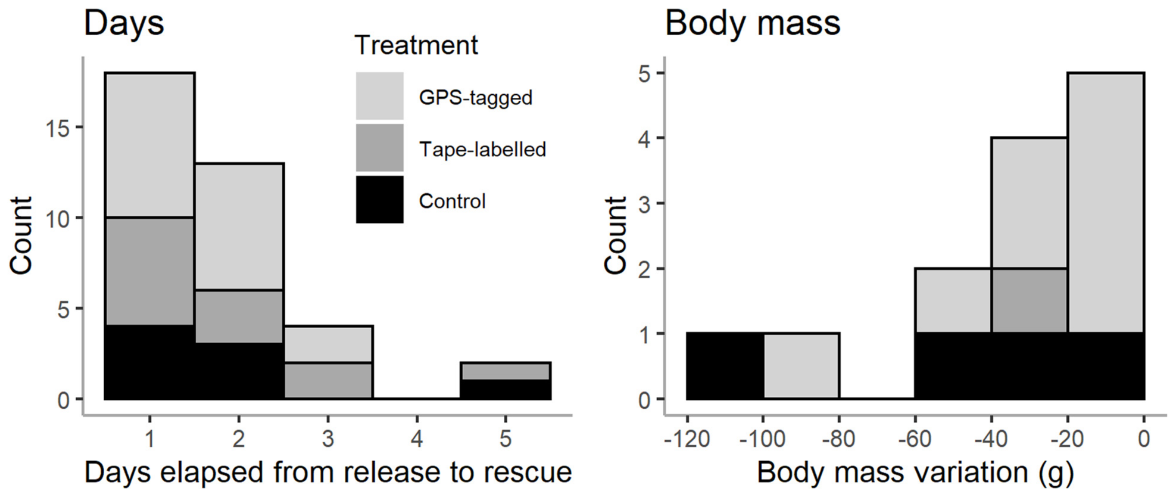

FIGURE 1 | Histograms of the days elapsed from release to rescue and body mass loss between experimental release date and rescue date.

models, flight straightness was related to environmental light levels. Flights were more tortuous with high light pollution levels, but straightness increased with moon luminance (**Table 5** and **Figure 3**).

TABLE 3 | Flight descriptors (mean ± *SD*) of GPS-tagged Cory's Shearwater (*Calonectris borealis*) fledglings in Tenerife, Canary Islands, during 2017–2019.

Variable	2017	2018	2019	Total
	(*n* = 14)	**(*n* = 6)**	**(*n* = 23)**	**(*n* = 43)**
Ending site				
Ground	1	0	10	11
Sea close	2	3	3	8
Sea far	11	3	10	24
Sea				
Yes	2	1	8	11
No	12	5	15	32
Straight distance (km)	12.1 ± 1.4	11.8 ± 1.1	10.7 ± 2.1	11.3 ± 1.9
Flight distance (km)	15.0 ± 2.5	14.9 ± 3.2	22.0 ± 13.9	18.7 ± 10.8
Duration (min)	24.6 ± 3.5	31.5 ± 9.3	58.5 ± 58.2	43.7 ± 45.3
Flight speed (km/h)	36.9 ± 5.9	29.7 ± 6.4	29.2 ± 10.3	31.8 ± 9.2
Bearing (°)	187.2 ± 26.8	200.8 ± 27.0	211.0 ± 27.9	201.8 ± 28.9
Straightness index	0.8 ± 0.1	0.8 ± 0.1	0.6 ± 0.3	0.7 ± 0.2
Moon luminance	20.6 ± 4.2	5.9 ± 9.1	3.3 ± 6.1	8.8 ± 9.8
Sunset time (min)	78 ± 65	259 ± 274	93 ± 65	110 ± 123

Hotspots

All birds left the release site towards low altitude areas following a southern heading, irrespective of wind direction at flight initiation (**Figure 4**). The directions birds followed corresponded to the most light-polluted areas in the south of our study area (**Figure 5**). The areas highlighted by the kernel density estimation (heatmap) overlapped with light-polluted and urban areas in coastal sectors (**Figure 5** and **Supplementary Animations 1**).

DISCUSSION

Several methodologies, i.e., radar, night-vision scopes, and GPS data-loggers, have been employed to study the attraction of seabirds to artificial lights (Day and Cooper, 1995; Day et al., 2015; Rodríguez et al., 2015b). Radar and night-vision scopes do not allow identifying the individual, so that intrinsic information is not provided. Formerly used GPS data-loggers involved recapturing tagged individuals to retrieve the information. As such, information was only available for grounded individuals (Rodríguez et al., 2015b). Our GPS-GSM devices with remote data download let us obtain information from all fledglings, even those not grounded and reaching the sea. They provide substantially more data. However, our approach used a non-random sample of birds, i.e., they had been previously exposed to light pollution and rescued in the programme. Uncertainty in predicting the fledging date and battery lifespan preclude the use

TABLE 4 | Univariate GLMs explaining the probability of being grounded ranked by AICc.

Explanatory variable	AICc	ΔAICc	Estimate	Lower limit	Upper limit
Straightness	**32.14**	**0**	**−1.55**	**−2.75**	**−0.67**
Mean light pollution	**32.60**	**0.46**	**1.45**	**0.61**	**2.56**
SD light pollution	**33.60**	**1.46**	**1.54**	**0.62**	**2.81**
Maximum light pollution	**34.66**	**2.52**	**1.65**	**0.59**	**3.36**
Flight speed	**41.57**	**9.43**	**−0.82**	**−1.78**	**−0.02**
Body mass	41.83	9.69	−0.83	−1.81	0.01
Down index	42.00	9.86	0.83	−0.02	1.91
Body condition index	42.19	10.05	−0.79	−1.80	0.04
Sea	42.83	10.69	1.39	−0.25	3.06
Null model	43.42	11.28	–	–	–
Flight distance	43.54	11.4	0.49	−0.19	1.22
Moon luminance	43.97	11.83	−0.56	−1.61	0.27
Duration	44.38	12.24	0.38	−0.33	1.15
Body size index	45.36	13.22	−0.20	−1.05	0.58
Wind speed	45.49	13.35	0.13	−0.71	0.84
Sunset time	45.59	13.45	−0.07	−1.26	0.64

Estimate and 95% confidence intervals are showed.
Significant explanatory variables are highlighted in bold, i.e., confidence intervals not including the 0.

of birds fledging from their natural nests (see a full discussion in Rodríguez et al., 2015b). In addition, the narrow and long entrances of the underground nests, as well as the steep nesting colonies in cliffs, make studying fledglings at their natural nests a challenging, even life-risky, task (**Supplementary Figure 3**). Despite this shortcoming, our experimental design has shed light on the grounding risk of young seabirds in relation to their exposure to light pollution, as well as their pathways to the sea, two pieces of information critical for the management of artificial lights around seabird colonies. We also identified the most dangerous light-polluted areas in the south of Tenerife, where more than 50% of birds rescued on the whole island are grounded (Rodríguez and Rodríguez, 2009).

All tracked birds oriented their flights towards low altitude, coastal areas, which is consistent with previous research (Rodríguez et al., 2015b; Syposz et al., 2021). The rate of grounding, including all birds, was similar to that reported in a previous tracking study (this study 13.4 *vs.* 14% in Rodríguez et al., 2015b). Interestingly, according to the GLM, the probability of being grounded differed among treatments. The significant differences detected in the frequencies of grounding for control birds and tape-labelled or GPS-tagged birds, but not between tape-labelled or GPS-tagged birds, suggest that control birds are overlooked during the rescue programme. Small metal rings are harder to spot than artificial marks on the back of the birds (whether labels or GPSs), particularly for shearwaters which hide the legs under their belly when they are on land. In fact, we, the authors, noticed two ringed birds, which had been overlooked by the rescue volunteers, among the birds we handled at the main rescue station (note we just checked and handled a minority of rescued birds). Another non-exclusive potential explanation is that the labels and GPSs could affect the flight performance of birds by disturbing aerodynamic. This is supported by our finding on the positive correlation between down abundance and the probability of being grounded. Fluffy down protruding from feathers may negatively affect the aerodynamics of the birds (Aldheeb et al., 2016). Therefore, the flight performance of younger or less developed fledglings may diminish. In this sense, the probability of releasing a rescued fledgling back to the wild decreases with the abundance of down, but not with body condition (Rodríguez et al., 2012a; Cuesta-García et al., 2022). Dedicated rehabilitation centres that are equipped with facilities to provide food, liquid and shelter in a safe place, could additionally consider allowing birds to stay up until they grow the plumage completely to increase their chances of survival.

Regarding the differences in grounding rates among years, they may be related to the ambiance light level (moon illuminance) according to the moon cycle. It is well known that seabird groundings are reduced during the moon-lit nights probably related to the less conspicuousness of focal artificial lights (Imber, 1975; Telfer et al., 1987; Ainley et al., 2001; Le Corre et al., 2002; Rodríguez and Rodríguez, 2009; Miles et al., 2010). The full moon of November 2017 coincided with the emancipation peak of Cory's shearwater fledglings on Tenerife (Rodríguez and Rodríguez, 2009). Thus, the recaptured birds were fewer in 2017 in comparison with the two following years (see **Table 2**), which is in line with the number of rescued birds during the whole campaigns during the three study years (2017 = 1,692; 2018 = 2,610; 2019 = 2,539 birds).

Most birds were rescued during the first 24 h after grounding, but some of them were rescued up to 5 days after their experimental release. All birds lost body mass, as expected given that no food or water was provided. Body mass at fledging is a crucial trait for the future survival and recruitment into the breeding population for seabirds (Becker and Bradley, 2007; Meathrel and Carey, 2007; Maness and Anderson, 2013; Perrins, 2014), and this is also true for our model species (Mougin et al., 2000). Thus, rescuing and delivering the birds to the sea as

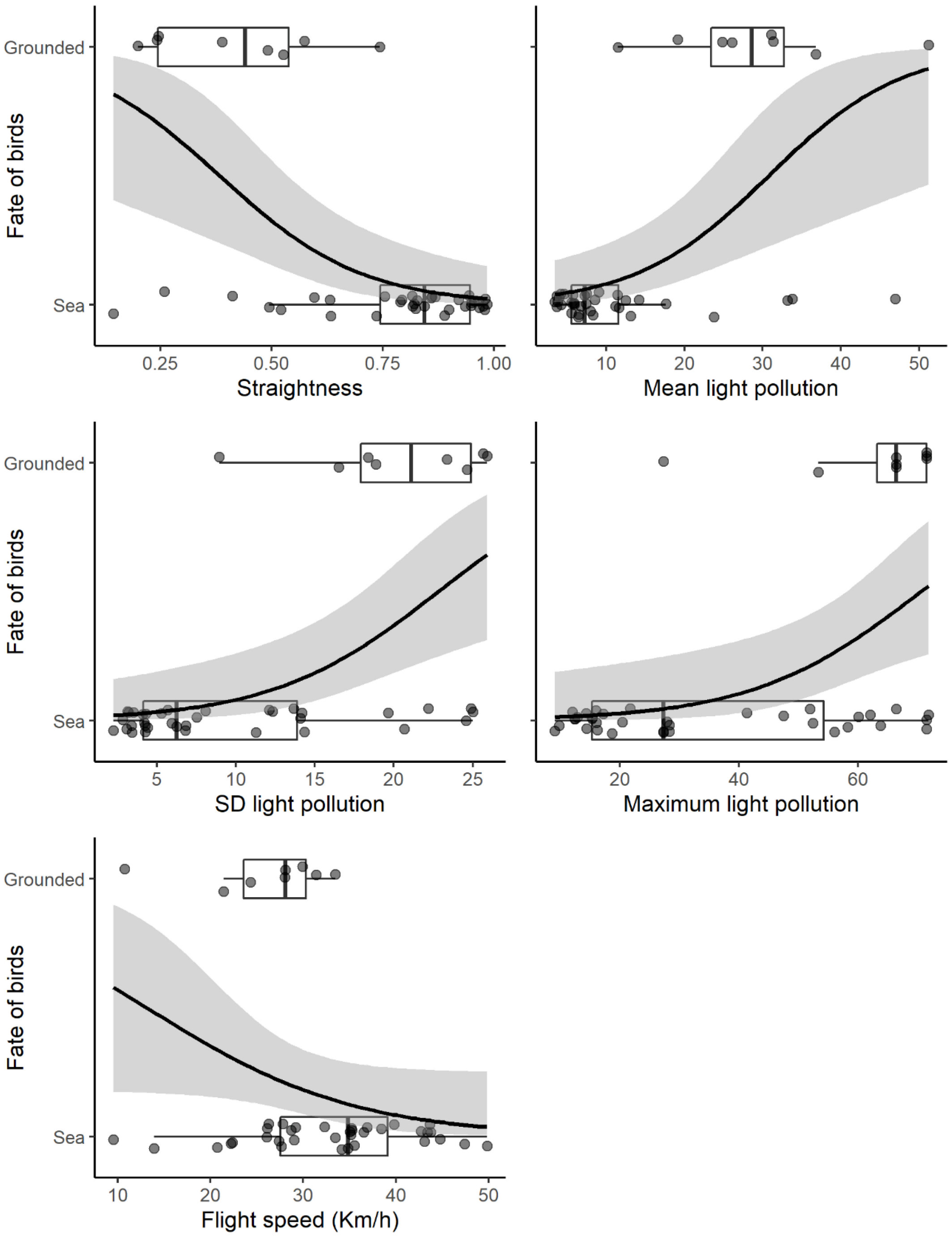

FIGURE 2 | Relation of the fate of birds (grounded birds *vs.* birds which reached the ocean successfully) with the significant explanatory variables (see **Table 4**). Black lines and grey areas indicate the predicted effects and the 95% confidence intervals according to the GLMs (**Table 4**). Dots have been jittered for better visualization. The line within boxes indicated the median, the right and the left edges of the boxes represent the first and third quartiles, and the whiskers extend 1.5 times the interquartile range. Straightness is the ratio between straight distance and flight distance.

soon as possible increases survival during the first weeks, and consequently, increases the probability of recruitment into the breeding population (Cuesta-García et al., 2022).

The flight trajectories provided by our GPS devices are particularly useful as they provided basic descriptive statistics for the birds grounded by light pollution, but also for those birds who reached the ocean successfully (**Figure 2**). Straightness and flight speed are two flight traits related to grounding. Birds with more tortuous and slower flights were more susceptible to grounding. The observed correlation between the chance to be grounded

TABLE 5 | Univariate linear models explaining flight straightness ranked by AICc.

Explanatory variable	AICc	ΔAICc	Estimate	Lower limit	Upper limit
Mean light pollution	**−24.83**	**0**	**−0.18**	**−0.23**	**−0.13**
Max light pollution	**−9.84**	**14.99**	**−0.14**	**−0.21**	**−0.08**
SD light pollution	**−8.34**	**16.49**	**−0.14**	**−0.20**	**−0.07**
Moon luminance	**1.54**	**26.37**	**0.09**	**0.02**	**0.16**
Body condition index	5.53	30.36	0.06	−0.02	0.13
Null model	5.53	30.36	–	–	–
Body size index	7.26	32.09	−0.03	−0.11	0.05
Body mass	7.31	32.14	0.03	−0.05	0.11
Time since sunset	7.36	32.19	0.03	−0.05	0.10
Down index	7.83	32.66	0.01	−0.07	0.08
Wind speed	7.84	32.67	0.00	−0.08	0.08

Estimate and 95% confidence intervals are shown.
Significant explanatory variables are highlighted in bold, i.e., confidence intervals not including the 0.

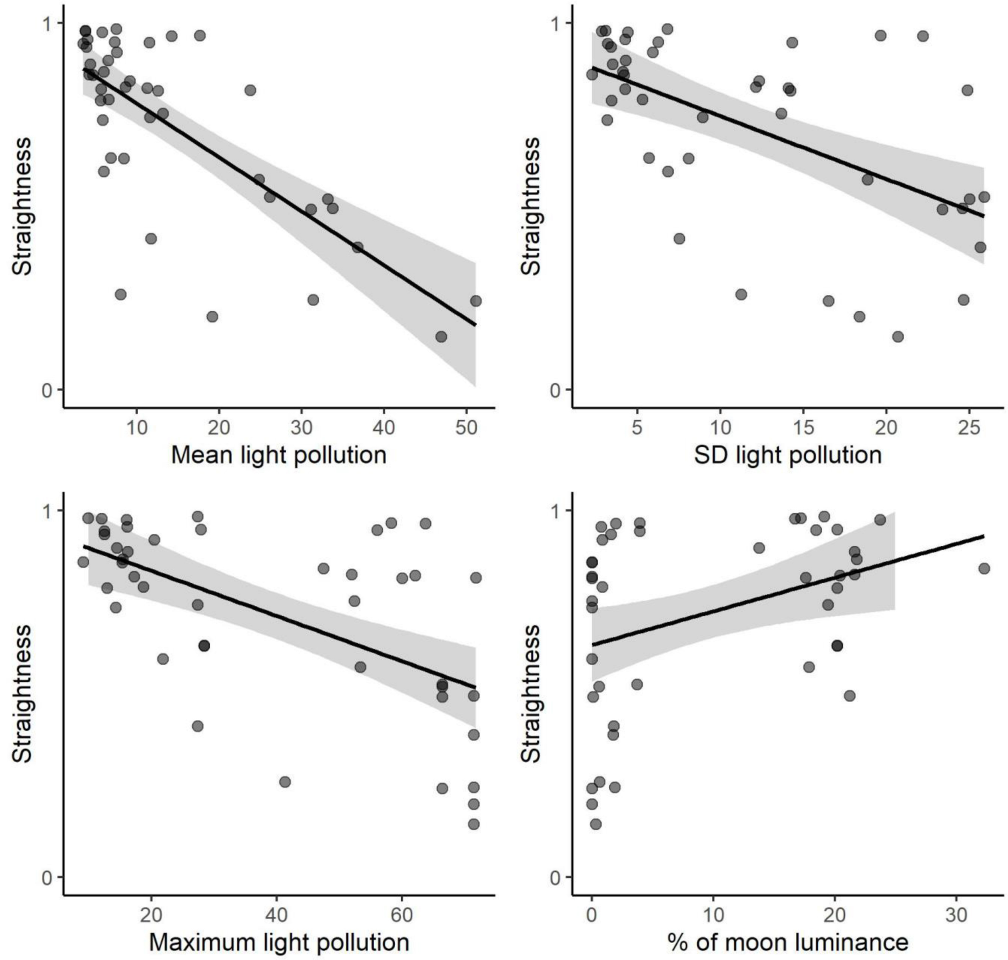

FIGURE 3 | Relationships between flight straightness (calculated as the ratio between straight distance and flight distance) and significantly correlated variables according to the linear models (see **Table 5**). Black lines and grey areas indicate the predicted effects and the 95% confidence intervals of univariate linear models (**Table 5**).

and the above-mentioned traits could be due to the necessity the birds had to land. In other words, landing birds might need to slow down their flight speed to avoid abrupt collisions. Similarly, because they were seemingly forced to do what we may call an "emergency landing" (because they were, for example, blinded by the light, or dazzled, they went exhausted, they perceived the urban matrix as an unsafe place, or whatever the reason made them land), they changed their flight directions multiple times.

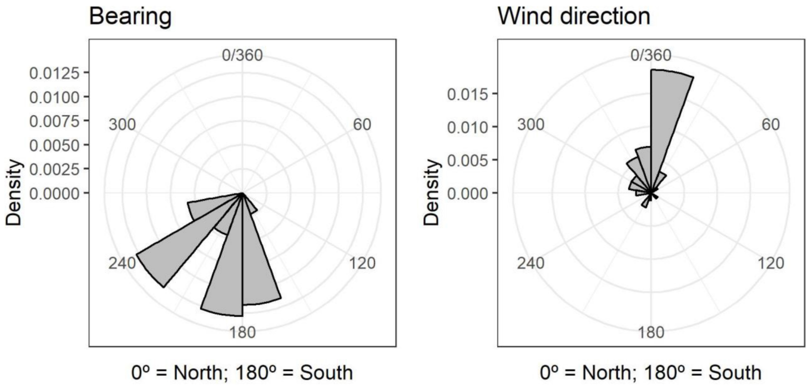

FIGURE 4 | Flight direction (bearing) and wind direction at the time of flight initiation.

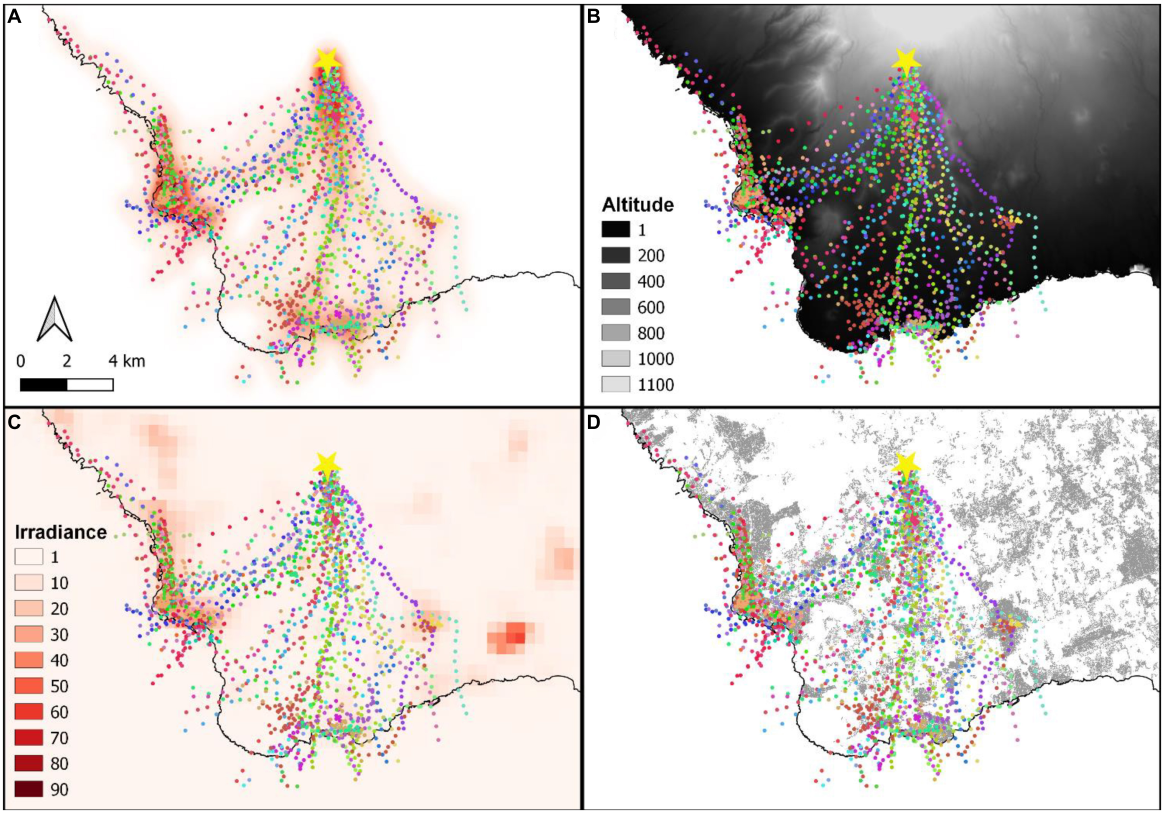

FIGURE 5 | GPS locations of Cory's shearwater *Calonectris borealis* fledglings tracked in the south of Tenerife, Canary Islands, during the fledging seasons of 2017–2019. **(A)** Heat map of all GPS locations. **(B)** Digital elevation model. **(C)** Irradiance levels taken as a proxy of light pollution from a monthly composite of November 2019 VIIRS. **(D)** Urban areas are depicted in grey. Yellow star indicates the release site.

Light pollution levels, measured as the mean, the standard deviation, and the maximum irradiance from satellite imagery, increased the probability of being grounded. These findings are in line with our predictions and previous research (Rodríguez et al., 2015b). However, some birds safely reached the ocean having flown over more light-polluted areas than those overflown by some grounded birds (**Figure 2** and **Supplementary Animations 1**). How these birds managed to reach the ocean is still an open question that deserves further research. It would be interesting to know, for instance, if there is a positive selection of birds able to negotiate a lighted nightscape, and whether we should expect less grounded birds in the future. Although certain variables, such as the increasing light pollution levels and the rescue awareness by the general public, could mask a decline, the number of admitted birds into the rescue programme increases annually (Rodríguez et al., 2012b).

Therefore, the information available does not seem to support the latter hypothesis.

CONCLUSION

The attraction and disorientation of seabirds by artificial lights is a poorly understood phenomenon causing mass mortality events of seabirds (Rodríguez et al., 2017). Contrary to other marine animals stranded by light pollution, e.g., sea turtles that leave tracks on beach sand (Hirama et al., 2021), tracking the fledgling flights from their nests to grounding locations is challenging. Our experimental study has revealed that intrinsic factors, such as down abundance in the plumage, are associated with the probability of being grounded by artificial lights. Our GPS devices with remote downloads have provided new insights into this poorly known conservation issue, as they rendered information on flight characteristics for both birds that successfully reached the ocean and those that got stranded inland. However, given that battery life was limited to a few hours, we had to resort to deploying GPS tags and tracking individual birds that had previously been grounded once after leaving their natal nests. Longer-lasting GPS devices that may be deployed on birds that have never experienced flight would be the next technological development needed to provide novel information to better understand the process of seabird fallout.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the article/**Supplementary Material** and the Digital.CSIC repository (Rodríguez et al., 2021). Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by insular and regional governments (Cabildo de Tenerife: AFF82/17, AFF94/18, and AFF142/19; Gobierno de Canarias: 2017/10464 and 2018/6842).

AUTHOR CONTRIBUTIONS

AR and BR conceived and designed the study, and conducted the fieldwork. AR analysed the data and wrote the first draft of the manuscript. YA and JN contributed materials and tools. YA managed and coordinated the research project. All authors improved the manuscript and approved the submitted version.

FUNDING

This study was funded by European Commission through the project INTERREG MAC/4.6d/157 LuMinAves.

ACKNOWLEDGMENTS

We are grateful to the people who kindly rescue grounded birds, contributing both to save birds and to collect data. We thank the special collaboration and help of Enrique Sacramento, M. Nazaret Carrasco, José Juan Hernández, Acaimo Martínez, María del Carmen Martín, Felipe Rodríguez, and the staff of La Tahonilla Wildlife Rehabilitation Center (Cabildo Insular de Tenerife). Special mention deserves the staff and the volunteers of Asociación para el Desarrollo de Actuaciones en Emergencias (ADAE), who allowed us to use its installations in Arona. The work was coordinated by the Canary Islands regional SEO/BirdLife's office, then our special thanks to its staff, Juan Antonio Lorenzo and Elena Ramos. We also thank Rubén Blanco of DigitAnimal for customizing the GPS loggers. Permits to conduct fieldwork were granted by insular and regional governments (Cabildo de Tenerife: AFF82/17, AFF94/18, and AFF142/19; Gobierno de Canarias: 2017/10464 and 2018/6842).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.786557/full#supplementary-material

REFERENCES

Ainley, D. G., Podolsky, R., Deforest, L., and Spencer, G. (2001). The status and population trends of the Newell's Shearwater on Kaua'i: insights from modeling. *Stud. Avian Biol.* 22, 108–123.

Aldheeb, M. A., Asrar, W., Sulaeman, E., and Omar, A. A. (2016). A review on aerodynamics of non-flapping bird wings. *J. Aerosp. Technol. Manag.* 8, 7–17.

Atchoi, E., Mitkus, M., and Rodríguez, A. (2020). Is seabird light-induced mortality explained by the visual system development? *Conserv. Sci. Pract.* 2:e195. doi: 10.1111/csp2.195

Austin, R. H., Phillips, B. F., and Webb, D. J. (1976). A method for calculating moonlight illuminance at the Earth's surface. *J. Appl. Ecol.* 13, 741–748.

Baccetti, N., Sposimo, P., and Giannini, F. (2005). Artificial lights and mortality of Cory's shearwater *Calonectris diomedea* on a Mediterranean island. *Avocetta* 29, 89–91.

Barton, E. D., Aristegui, J., Tett, P., Canton, M., García-Braun, J., Hernández-León, S., et al. (1998). The transition zone of the canary current upwelling region. *Prog. Oceanogr.* 41, 455–504. doi: 10.1016/S0079-6611(98)00023-8

Becker, P. H., and Bradley, S. J. (2007). The role of intrinsic factors for the recruitment process in long-lived birds. *J. Ornithol.* 148, 377–384. doi: 10.1007/s10336-007-0157-x

Croxall, J. P., Butchart, S. H. M., Lascelles, B., Stattersfield, A. J., Sullivan, B., Symes, A., et al. (2012). Seabird conservation status, threats and priority actions: a global assessment. *Bird Conserv. Int.* 22, 1–34. doi: 10.1017/S0959270912000020

Cuesta-García, M., Rodríguez, A., Martins, A. M., Neves, V., Magalhães, M., Atchoi, E., et al. (2022). Targeting efforts in rescue programmes mitigating light-induced seabird mortality: first the fat, then the skinny. *J. Nat. Conserv.* 65:126080. doi: 10.1016/j.jnc.2021.126080

Day, R. H., and Cooper, B. A. (1995). Patterns of movement of dark-rumped petrels and Newell's shearwaters on Kauai. *Condor* 97, 1011–1027. doi: 10.2307/1369540

Day, R. H., Rose, J. R., Prichard, A. K., and Streever, B. (2015). Effects of gas flaring on the behavior of night-migrating birds at an artificial oil-production island, Arctic Alaska. *Arctic* 68, 367–379. doi: 10.14430/arctic4507

Dias, M. P., Martin, R., Pearmain, E. J., Burfield, I. J., Small, C., Phillips, R. A., et al. (2019). Threats to seabirds: a global assessment. *Biol. Conserv.* 237, 525–537. doi: 10.1016/j.biocon.2019.06.033

Fontaine, R., Gimenez, O., and Bried, J. (2011). The impact of introduced predators, light-induced mortality of fledglings and poaching on the dynamics of the Cory's shearwater (*Calonectris diomedea*) population from the Azores, northeastern subtropical Atlantic. *Biol. Conserv.* 144, 1998–2011. doi: 10.1016/j.biocon.2011.04.022

Gaston, K. J., Ackermann, S., Bennie, J., Cox, D. T. C., Phillips, B. B., Sánchez de Miguel, A., et al. (2021). Pervasiveness of biological impacts of artificial light at night. *Integr. Comp. Biol.* 61, 1098–1110. doi: 10.1093/icb/icab145

Gaston, K. J., Duffy, J. P., Gaston, S., Bennie, J., and Davies, T. W. (2014). Human alteration of natural light cycles: causes and ecological consequences. *Oecologia* 176, 917–931. doi: 10.1007/s00442-014-3088-2

Green, A. J. (2001). Mass/length residuals: measures of body condition or generators of spurious results? *Ecology* 82, 1473–1483.

Hirama, S., Witherington, B., Kneifl, K., Sylvia, A., Wideroff, M., and Carthy, R. (2021). Environmental factors predicting the orientation of sea turtle hatchlings on a naturally lighted beach: a baseline for light-management goals. *J. Exp. Mar. Bio. Ecol.* 541:151568. doi: 10.1016/J.JEMBE.2021.151568

Hölker, F., Wolter, C., Perkin, E. K., and Tockner, K. (2010). Light pollution as a biodiversity threat. *Trends Ecol. Evol.* 25, 681–682. doi: 10.1016/j.tree.2010.09.007

Imber, M. J. (1975). Behaviour of petrels in relation to the moon and artificial lights. *Notornis* 22, 302–306.

ISTAC (2020). *ISTAC. Instituto Canario de Estadística. Gobierno de Canarias.* Available online at: www.gobiernodecanarias.org/istac/ (accessed September 15, 2020)

Le Corre, M., Ollivier, A., Ribes, S., and Jouventin, P. (2002). Light-induced mortality of petrels: a 4-year study from Réunion Island (Indian Ocean). *Biol. Conserv.* 105, 93–102. doi: 10.1016/S0006-3207(01)00207-5

Longcore, T., and Rich, C. (2004). Ecological light pollution. *Front. Ecol. Environ.* 2:191–198.

Maness, T. J., and Anderson, D. J. (2013). Predictors of juvenile survival in birds. *Ornithol. Monogr.* 78, 1–55. doi: 10.1525/om.2013.78.1.1.1

Martín, A., and Lorenzo, J. A. (2001). *Aves del Archipiélago Canario*. La Laguna: Francisco Lemus Editor.

Martín-Ruiz, J.-F. (2001). *Geografía de Canarias. Sociedad y Medio Natural*. Las Palmas de Gran Canaria: Ediciones Cabildo de Gran Canaria.

Meathrel, C. E., and Carey, M. J. (2007). How important are intrinsic factors to natal recruitment in short-tailed shearwaters *Puffinus tenuirostris*? *J. Ornithol.* 148, 385–393. doi: 10.1007/s10336-007-0204-7

Miles, W., Money, S., Luxmoore, R., and Furness, R. W. (2010). Effects of artificial lights and moonlight on petrels at St Kilda. *Bird Study* 57, 244–251. doi: 10.1080/00063651003605064

Mougin, J.-L., Jouanin, C., Roux, F., and Zino, F. (2000). Fledging weight and juvenile survival of Cory's shearwaters *Calonectris diomedea* on selvagem grande. *Ringing Migr.* 20, 107–110. doi: 10.1080/03078698.2000.9674231

Perrins, C. M. (2014). Factors affecting survival of fledgling manx shearwaters *Puffinus puffinus*. *Seabird* 27, 62–71.

Podolskyi, R., Ainley, D. G., Spencer, G., Leah, D., and Nur, N. (1998). Mortality of Newell's shearwaters caused by collisions with urban structures on Kauai. *Colon. Waterbirds* 21, 20–34.

Puitz, K., Wilson, R. P., Culik, B., Scolaro, J. A., and Charrassin, J. (1997). Long-term attachment of transmitting and recording devices to penguins and other seabirds. *J. Wildl. Manage.* 25, 101–106.

Reyes-González, J. M., and González-Solís, J. (2016). "Pardela cenicienta atlántica – *Calonectris borealis*," in *Enciclopedia Virtual de los Vertebrados Españoles*, eds A. Salvador and M. B. Morales (Madrid: Museo Nacional de Ciencias Naturales).

Rich, C., and Longcore, T. (2006). *Ecological Consequences of Artificial Night Lighting*. Washington, DC: Island Press.

Rodrigues, P., Aubrecht, C., Gil, A., Longcore, T., and Elvidge, C. (2012). Remote sensing to map influence of light pollution on Cory's shearwater in São Miguel Island, Azores Archipelago. *Eur. J. Wildl. Res.* 58, 147–155. doi: 10.1007/s10344-011-0555-5

Rodríguez, A., Arcos, J. M., Bretagnolle, V., Dias, M. P., Holmes, N. D., Louzao, M., et al. (2019). Future directions in conservation research on petrels and shearwaters. *Front. Mar. Sci.* 6:94. doi: 10.3389/fmars.2019.00094

Rodríguez, A., Burgan, G., Dann, P., Jessop, R., Negro, J. J., and Chiaradia, A. (2014). Fatal attraction of short-tailed shearwaters to artificial lights. *PLoS One* 9:e110114. doi: 10.1371/journal.pone.0110114

Rodríguez, A., Rodríguez, B., and Negro, J. J. (2015b). GPS tracking for mapping seabird mortality induced by light pollution. *Sci. Rep.* 5:10670. doi: 10.1038/srep10670

Rodríguez, A., García, D., Rodríguez, B., Cardona, E., Parpal, L., and Pons, P. (2015a). Artificial lights and seabirds: is light pollution a threat for the threatened Balearic petrels? *J. Ornithol.* 156, 893–902. doi: 10.1007/s10336-015-1232-3

Rodríguez, A., Holmes, N. D., Ryan, P. G., Wilson, K.-J., Faulquier, L., Murillo, Y., et al. (2017). Seabird mortality induced by land-based artificial lights. *Conserv. Biol.* 31, 986–1001. doi: 10.1111/cobi.12900

Rodríguez, A., and Rodríguez, B. (2009). Attraction of petrels to artificial lights in the Canary Islands: effects of the moon phase and age class. *Ibis* 151, 299–310. doi: 10.1111/j.1474-919X.2009.00925.x

Rodríguez, A., Rodríguez, B., Acosta, Y., and Negro, J. J. (2021). *GPS and Intrinsic Data of Cory's Shearwater Fledglings of Tenerife, Canary Islands [dataset]*. Digital.CSIC. doi: 10.20350/digitalCSIC/14132

Rodríguez, A., Rodríguez, B., and Lucas, M. P. (2012b). Trends in numbers of petrels attracted to artificial lights suggest population declines in Tenerife, Canary Islands. *Ibis* 154, 167–172. doi: 10.1111/j.1474-919X.2011.01175.x

Rodríguez, A., Rodríguez, B., Curbelo, ÁJ., Pérez, A., Marrero, S., and Negro, J. J. (2012a). Factors affecting mortality of shearwaters stranded by light pollution. *Anim. Conserv.* 15, 519–526. doi: 10.1111/j.1469-1795.2012.00544.x

Rodríguez, B., Siverio, F., Acosta, Y., and Rodríguez, A. (2022). Breeding success of Cory's shearwater in relation to nest characteristics and predation by alien mammals. *Ardeola* 69, 1–14. doi: 10.13157/arla.69.1.2022.sc1

Schwalb-Willmann, J., Remelgado, R., Safi, K., and Wegmann, M. (2020). moveVis: animating movement trajectories in synchronicity with static or temporally dynamic environmental data in R. *Methods Ecol. Evol.* 11, 664–669. doi: 10.1111/2041-210X.13374

Silva, R., Medrano, F., Tejeda, I., Terán, D., Peredo, R., Barros, R., et al. (2020). Evaluación del impacto de la contaminación lumínica sobre las aves marinas en chile: diagnóstico y propuestas. *Ornitol. Neotrop.* 31, 13–24.

Syposz, M., Gonçalves, F., Carty, M., Hoppitt, W., and Manco, F. (2018). Factors influencing manx shearwater grounding on the west coast of Scotland. *Ibis* 160, 846–854. doi: 10.1111/ibi.12594

Syposz, M., Padget, O., Wynn, J., Gillies, N., Fayet, A. L., and Guilford, T. (2021). An assay to investigate factors influencing initial orientation in nocturnally fledging seabirds. *J. Avian Biol.* 52:e02613. doi: 10.1111/JAV.02613

Telfer, T. C., Sincock, J. L., Byrd, G. V., and Reed, J. R. (1987). Attraction of hawaiian seabirds to lights: conservation efforts and effects of moon phase. *Wildl. Soc. Bull.* 15, 406–413.

Troy, J., Holmes, N., Veech, J., and Green, M. (2013). Using observed seabird fallout records to infer patterns of attraction to artificial light. *Endanger. Species Res.* 22, 225–234. doi: 10.3354/esr00547

Troy, J. R., Holmes, N. D., and Green, M. C. (2011). Modeling artificial light viewed by fledgling seabirds. *Ecosphere* 2:art109. doi: 10.1890/ES11-00094.1

Zuur, A. F., Ieno, E. N., and Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1, 3–14. doi: 10.1111/j.2041-210x.2009.00001.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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in Ecology and Evolution

MINI REVIEW
published: 25 January 2022
doi: 10.3389/fevo.2021.768090

Artificial Light as a Modulator of Mosquito-Borne Disease Risk

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Light is a fundamental cue regulating a host of biological responses. The artificial modification thereof demonstrably impacts a wide range of organisms. The use of artificial light is changing in type, extent and intensity. Insect vector-borne diseases remain a global scourge, but surprisingly few studies have directly investigated the interactions between artificial light and disease vectors, such as mosquitoes. Here we briefly overview the progress to date, which highlights that artificial light must be considered as a modulator of mosquito-borne disease risk. We discuss where the mechanisms may lie, and where future research could usefully be directed, particularly in advancing understanding of the biological effects of the light environment. Further understanding of how artificial light may modulate mosquito-borne disease risk may assist in employing and redesigning light regimes that do not increase, and may even mitigate, already significant disease burdens, especially in the developing world.

Keywords: ALAN, light emitting diode, vector control, sustainable development goals, disease vectors

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Specialty section:
This article was submitted to Behavioral and Evolutionary Ecology, a section of the journal Frontiers in Ecology and Evolution

Received: *31 August 2021*
Accepted: *24 November 2021*
Published: *25 January 2022*

Citation:
Coetzee BWT, Gaston KJ, Koekemoer LL, Kruger T, Riddin MA and Smit IPJ (2022) Artificial Light as a Modulator of Mosquito-Borne Disease Risk. Front. Ecol. Evol. 9:768090. doi: 10.3389/fevo.2021.768090

INTRODUCTION

Research into mosquito-borne disease risk covers a rich tapestry of approaches and fields. The work has tackled this pernicious societal problem in a variety of ways. Chiefly amongst these are advances in understanding of mosquito and parasite biology, ecology, biochemistry, genetics, control strategies and how these interact with human activities (White et al., 2011; Gatton et al., 2013; Caminade et al., 2014; Neafsey et al., 2015; Otto et al., 2018; Mordecai et al., 2019; Mozūraitis et al., 2020). Nonetheless, a new frontier of work is exploring the ways in which light, and the Artificial Light at Night (ALAN) from human forcing, may alter mosquito borne-disease risk. We here explore such advances, and highlight the complex ways in which artificially lit environments may change human-vector interactions and in consequence alter mosquito borne-disease risk.

Artificial Light at Night is produced from a range of anthropogenic sources, and is increasingly recognized as a global change driver (Davies and Smyth, 2018). Light regulates a range of physiological and behavioral responses, and so ultimately can also influence the fitness of species. ALAN therefore has impacts across the biological organizational hierarchy, from genes to communities, and across a diversity of taxonomic groups, from bacteria to higher vertebrates (Gaston et al., 2013; Sanders et al., 2021).

The interactions of artificial light with a host of aspects of insect biology are now well established (Wakefield et al., 2016; Knop et al., 2017; Owens et al., 2020; Sanders et al., 2021). If ALAN increases mosquito fitness and biting behavior, it may increase mosquito-borne disease risk (Barghini and de Medeiros, 2010; Martinez-Bakker and Helm, 2015; Kernbach et al., 2018). Conversely, if ALAN suitably changes fitness and feeding behavior it could be implemented and operationalized as an additional mosquito-control strategy to reduce mosquito-borne diseases (Barghini and de Medeiros, 2010; Martinez-Bakker and Helm, 2015; Kernbach et al., 2018). With mosquito-borne diseases, such as malaria, West Nile, chikungunya, dengue, lymphatic filariasis and zika, accounting for 17% of all global infectious diseases and cause an estimated 7,00,000 human deaths annually (WHO, 2021a), artificial drivers are integral aspects to understand. From a research and monitoring perspective, it is also critical to understand how mosquito trapping techniques using light for monitoring purposes may be optimized to ensure a broader suite of species are captured with greater reliability (Wilson et al., 2021). For example, *Culex pipiens* shows negative phototaxis, and so removal of light from regularly used CDC light traps may improve collection efficiency and understanding of species ecology (Boze et al., 2021).

Here, we consider artificial light as a modulator of mosquito-borne disease transmission. First, we touch on the burgeoning literature demonstrating that artificial light is affecting mosquito-borne disease risk. Then, we discuss the mechanisms that may underpin how ALAN is altering mosquito biology by highlighting how biological responses interact with the characteristics of the light environment and how these may influence responses of mosquitoes to light regimes. Throughout, we discuss where ongoing research in this critical field may be usefully focused, particularly advancing understanding of the biological effects of light and the characteristics of the light environment itself.

MOSQUITOES AND ARTIFICIAL LIGHT

Light at night as a modulator of circadian rhythms in anophelines was first experimentally demonstrated in 1966, where recording the flight activity of *Anopheles gambiae* under different light regimes showed that light can have an inhibitory effect on activity (Jones et al., 1966). Since then, a cohesive quantitative link between artificial light and mosquito biology has emerged, although the work is scattered across a range of different responses, from attraction and changes in biting rates, to impacts on different aspects of the biology of mosquitoes, and from genes to organismal physiology. We highlight some examples below, and note that unless otherwise stated, all studies used white Light Emitting Diode (LED) lights in cases where experimental treatments are used.

- There is extensive circadian and light regulation of the transcriptome (i.e., sum of all the messenger RNA) in *An. gambiae*, influencing genes from metabolic detoxification, immunity to nutrient sensing (Rund et al., 2013) and clock gene expression in *Culex pipiens f. molestus* (Honnen et al., 2019).
- Acute and sustained photic suppression of biting activity in *An. gambiae* (Sheppard et al., 2017), and their blood feeding is modulated by light (Das and Dimopoulos, 2008).
- *Aedes aegypti* may increase its biting rate under 50 lux incandescent lights (Rund et al., 2020), while ultraviolet (UV) light suppresses *An. gambiae* activity compared to no night time light (Baik et al., 2020).
- *Culex* mosquitoes in areas with even dim skyglow ALAN (∼4 lx) reproduce and bite later into the season, thus extending the period of disease risk for urban residents (Fyie et al., 2021).
- Artificial Light at Night reduces diapause incidence in *Aedes albopictus* by 40% (Westby and Medley, 2020; high-pressure sodium lights).
- Artificial Light at Night can alter West Nile virus exposure risk across Florida (Kernbach et al., 2021) and increases virus competence in its ubiquitous passerine reservoir species (Kernbach et al., 2019); both studies investigated ALAN at broad spatial scales.

Although such work takes a range of forms and has little consistency in the responses measured, there is now consensus that ALAN is altering mosquito biology (Longcore and Rich, 2004; Rund et al., 2016; Wilson et al., 2021).

BIOLOGICAL EFFECTS OF ARTIFICIAL LIGHT

Artificial Light at Night may modulate disease vector biology and hence disease risk (**Figure 1**). Organisms either use light as a resource (*e.g.,* partitioning, and thus activity) or use light as an information source [*e.g.,* for vision, circadian clocks and photoperiodism; following Gaston et al. (2013)]. In consequence, the characteristics of the light environment can modulate biological responses. We broadly focus on such patterns – but we note that intra- and interspecific differences also need to be accounted for. For instance, light responses in both *Anopheles* and *Aedes* are strongly affected by sex (Honnen et al., 2016; Baik et al., 2020). For this reason, we caution against generalizations between different species. At this stage, in our assessment of the literature, the understanding of the impacts of ALAN on mosquitoes is still incomplete. Only some aspects have been touched on thus far, which we highlight below, and also where work can usefully be expanded.

Much attention has been paid to the circadian rhythms of vectors since endogenous circadian clocks regulate many aspects of behavior, physiology and metabolism (Rund et al., 2013; Prior et al., 2019; Baik et al., 2020). There is extensive circadian and light regulation of the transcriptome in *An. gambiae* (Rund et al., 2013), and host circadian rhythms are disrupted during malaria infection (Prior et al., 2019). Circadian rhythms also alter detoxification and insecticide resistance in the malaria mosquito *An. gambiae* (Balmert et al., 2014). In humans, artificial lights have altered and extended human activity beyond strictly diurnal

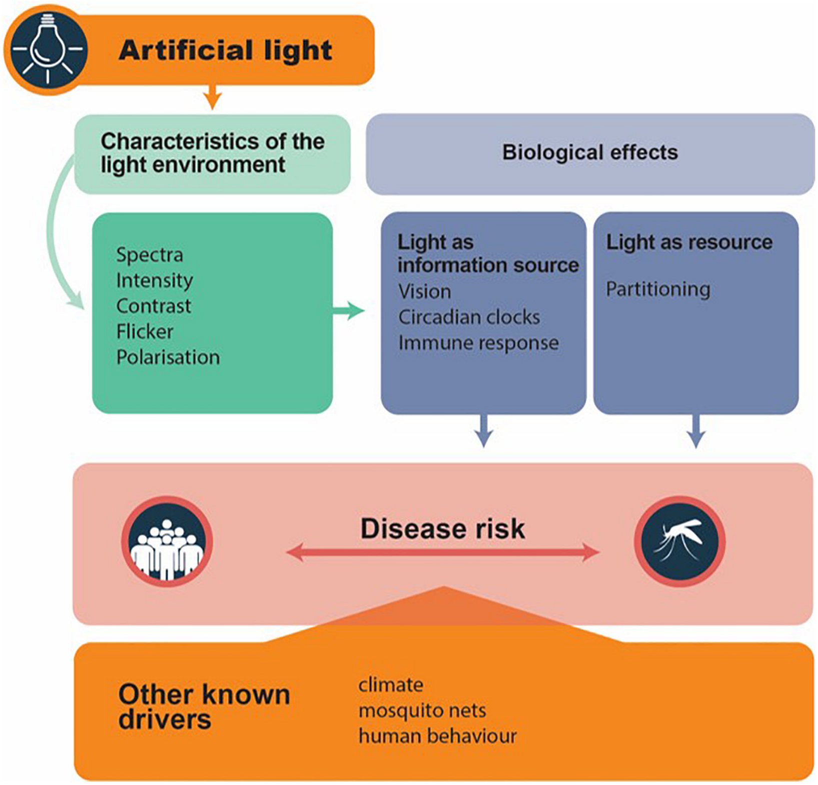

FIGURE 1 | Conceptual illustration to predict how artificial light may act as a modulator to mosquito-borne disease transmission. It captures the characteristics of the light environment which alter species biologically respond to light, which then may modulate disease transmission risk. Note that all the other known drivers can interact with that of artificial light to influence disease transmission risk. Simplified from Gaston et al. (2013).

into extensive night time hours (Chepesiuk, 2009). The causes and consequences of daily rhythms in the interactions between vectors, their hosts and the parasites they transmit is an emerging research area [most recently reviewed by Rund et al. (2016)].

Increases in human activity into the nighttime mean that nocturnal mosquitoes have a greater chance of obtaining a blood meal, if it extends human activity outside of other prevention methods, such as bed-net use (Barghini and de Medeiros, 2010). In the Bolivian Amazon, 48% of *An. darlingi* total night biting takes place from 19:00 to 21:00pm (Harris et al., 2006), and there is a similarly early biting time in other anophelines in Kenya (Cooke et al., 2015). Furthermore, *Anopheles* and *Culex* are predominantly crepuscular and nocturnal, while *Aedes* spp. are predominantly diurnal (Honnen et al., 2019; Baik et al., 2020). As such, the timing of artificial light outside of natural regimes is a key aspect that alters mosquito biology (Sheppard et al., 2017). As one might expect, in the nocturnal *An. gambiae* biting rates are suppressed under artificial light (Sheppard et al., 2017), particularly UV light (Baik et al., 2020). Conversely, under LED lights, *Ae. aegypti* may increase their biting rates on humans (Rund et al., 2020). This limited evidence suggests that ALAN reduces biting rates in nocturnal mosquitoes, but that it may increase those of diurnal ones. How increased biting rates translate into mosquito fitness and disease prevalence can be further explored, especially in both laboratory and field settings.

From a community ecology perspective, alterations to mosquito composition and behavior due to artificial lights may conceivably alter competitive interactions, community assembly and predation risk (for a recent review see Grubisic and van Grunsven, 2021). How artificial light may interact with various aspects of mosquito ecology, and how this alters their fitness and potentially disease risk, could be usefully advanced. At the moment, however, the theoretical framework on how ALAN impacts insect communities and populations is poorly developed (Grubisic and van Grunsven, 2021).

Light is also used as an information source *via* the insect eye which can affect their visual ecology in complex ways (Land and Nilsson, 2012). Detailed modeling of spectra, intensity, reflectance and the vision of hawk-moths (*Deilephila elpenor*), revealed that artificial lighting can disrupt the visual ecology links between flowers, pollinators and predators (Briolat et al., 2021). Such detailed work does not exist for mosquitoes, and so the role of mosquito vision in increasing vector disease transmission is currently not well known (Gibson, 1995; Land et al., 1997; Moon et al., 2015). Light is known to drive both rhodopsin maturation and recycling (Metoxen et al., 2016) and Op10 rhodopsin is co-expressed with Op8 rhodopsin in *Ae. aegypti* and *An. gambiae* R7 photoreceptor cells (Hu et al., 2014; Zhan et al., 2020). *Anopheles gambiae*, as well as other disease vectors, may be particularly sensitive to even small fluctuations in the night environment. There are fundamental structural differences in the eyes of diurnal and nocturnal mosquitoes, illustrating that it's a key morphological trait responding to the light environment (Land et al., 1999; Land and Nilsson, 2012). While most nocturnal insects have superposition eyes, curiously, *An. gambiae* has retained structures more akin to an apposition eye more typical for diurnal species, such as a wide conical lens and short thick rhabdoms (Land et al., 1999). Its eye is adapted for high sensitivity in dim environments, where selectivity to, and a high ability for, photon capture takes precedence over finer resolutions in vision. This means even small fluctuations in the light environment may therefore influence behavior and hence modulate risk for disease transmission (Land et al., 1999; Barghini and de Medeiros, 2010; Land and Nilsson, 2012; Martinez-Bakker and Helm, 2015; Kernbach et al., 2018).

Mosquitoes make use of an attraction cascade to find potential hosts, relying on a range of sensory cues, using vision to associate odor plumes with thermal targets (Van Breugel et al., 2015; Zhan et al., 2020). The range of attraction, the maximum distance at which light may attract insects, is variable but generally low in mosquitoes. In *Anopheles* mosquitoes, it is less than 5 m (Costantini et al., 1998), and more variable in *Culicoides* at 10–30 m (Rigot and Gilbert, 2012; Kirkeby et al., 2013; Wilson et al., 2021). However, of greater importance may be how mosquitoes perceive their lit environment, as light from a source (radiance) is not akin to light reflected off a surface (irradiance). For instance, red objects are attractive to mosquitoes but not red light (Wilson et al., 2021).

The use of ALAN may increase transmission risk if it is occurs at times and places that attract vectors, and so increases the potential for disease transfer. How typical objects in human environments reflect lights in different artificially lit environments, and how this may attract or repel mosquito behavior, may be another fruitful research avenue.

Artificial light may suppress the immune responses of a range of mammals, including humans (Navara and Nelson, 2007; Gaston et al., 2013; Fonken and Nelson, 2014). Exposure to artificial light alters hormone production, in particular by interfering with the regular expression of melatonin (Navara and Nelson, 2007; Gaston et al., 2013; Fonken and Nelson, 2014). Since it is critical to the sleep cycle and removal of free-radicals, changes in melatonin may alter an organism's ability to fight off diseases. Melatonin itself may also modulate the *Plasmodium* (malaria parasite) cell cycle (Lima et al., 2013). Melatonin expression in mosquitoes is less well known, but it seems likely that it will also have a sensitivity to blue wavelengths, and may similarly be involved in regulating immune responses to infection from parasites like *Plasmodium*. c.

CHARACTERISTICS OF THE LIGHT ENVIRONMENT

Expanding understanding of how light may modulate the above biological responses and alters mosquito-borne disease risk requires increased attention. This includes the impact of the characteristics of the light environment (spectra, intensity, contrast, flicker, and polarization) on mosquito species and their biology.

The spectrum of light is arguably its most fundamental characteristic and is often included as a key characteristic in ALAN research. Mosquitoes are known to respond to light of different spectra. For instance, electroretinograms show that both female *Aedes* (Muir et al., 1992) and female *Culex* spp. (Peach et al., 2019) have dual peaks of sensitivity of insect eye receptors to UV and blue/green light, respectively. *Anopheles* spp., especially females, show a strong aversion for light in the UV and bluer spectra (Baik et al., 2020). This likely indicates fewer photoreceptors in mosquitoes than other groups (Van Der Kooi et al., 2021), which may in turn indicate that lighting approaches can be devised that do not attract them by being outside of their visual spectrum, but that appear as regular lighting to humans. Conceptually, light in the red spectrum could be less attractive to these groups (Wilson et al., 2021). However, given the diversity of responses from earlier studies, no single spectral solution will act as a deterrent for different species of mosquitoes, but there may be opportunities for exploring more targeted species-specific approaches (Wilson et al., 2021).

Insights into how spectra and intensity interact to alter mosquito behavior can also be advanced. Different intensities do alter behavior in *Anophelines* (Sheppard et al., 2017). Two lights that appear equally bright to humans may look fundamentally different to insects. We posit that laboratory-reared mosquitoes are typically also kept in environments darker than even the faint celestial light and lunar cycles that would occur naturally, and so at very low light intensities. How this alters circadian rhythms over time, and inferences made from laboratory populations, could be usefully investigated.

Less well studied than light spectra and intensity is the influence of contrast, flicker and polarization. Objects in the environment reflect different wavelengths of lights to different extents, which means the spectral environment experienced by an individual mosquito may differ depending on the makeup of objects and their contrast with each other, and the composition of different artificial lights in the local environment (Land and Nilsson, 2012; Wilson et al., 2021). For *Ae. aegypti*, it has been shown that mosquito attraction to the thermal and odorant cues of host decoy traps is modulated by visual information, and changes with the color of the traps (Tang et al., 2021). In the context of mosquitoes, this may be particularly important in how they respond to isolated lights vs. more diversely lit environments.

Flicker frequency, aka critical fusion frequency (CFF), is the frequency at which flickering light appears as a continuous light source to the observer. For instance, human CFF is around 50–60 Hz, but in nocturnal insects it averages around 70 Hz (Barroso et al., 2017). In consequence, insects may observe flickering differently to other organisms and so it may affect light attractiveness and/or repulsion (Wilson et al., 2021). Indeed, manipulating flicker frequency of UV LED light can improve the capability of light traps to attract mosquitoes (Liu et al., 2017), but it nonetheless remains an understudied component of artificial light.

Navigating by polarized light is important to many aquatic insects. However, no evidence for positive polarotaxis was found in *Ae. aegypti* (Bernáth et al., 2008), and so other mainly olfactory cues are thought to be of greater importance for finding suitable egg-laying water in mosquitoes (Heinloth et al., 2018). Nevertheless, as with contrast and CFF, the role of polarized light in mosquito biology may be overlooked as it has rarely been explicitly considered in previous studies.

CONCLUSION

Research conducted to date on disease transmission and risk has mainly focused on the impacts of artificial light on vectors in laboratory settings rather than on how real-world environments are artificially lit, and how humans and vectors respond and interact in those environments. While the evidence-base for artificial light as a modulator of mosquito-borne diseases is now established, there are still many unanswered questions and future research can be informed by what has already been learned from laboratory experiments and responses in other taxonomic groups. Many questions may be answered by testing how different characteristics of the lit environment may be translated into the myriad of biological effects that it influences. New approaches can quantify the key features of lit environments and the generally overlooked components we mentioned above (Nilsson and Smolka, 2021). Of course, ALAN can interact with other, better known anthropogenic drivers of increased disease risk like land transformation (Meyer Steiger et al., 2016; Lee et al., 2020) and changing climatic conditions (Medlock and Leach, 2015). Teasing their individual contributions apart experimentally will advance understanding into disease risk. Popular disease frameworks such as R0 equations (Hartemink et al., 2015), Dynamic SEIR (Susceptible, Exposed, Infectious, Removed) compartmental models (Getz et al., 2019) and/or agent based models (Smith et al., 2018), could usefully incorporate the impacts of artificial light on vector biology. However, a range of new data from laboratory and field studies will be required to parametrize such models, and much work remains to be done to develop them.

At present, the World Health Organization (WHO, 2021b) does not recognize artificial light as a modulator of vector borne disease risk. The extent to which artificial light may be used as a vector control strategy remains unknown (Wilson et al., 2021). The burgeoning scientific study of the interactions of artificial light and mosquitoes now clearly demonstrates that it needs greater consideration as such. Only once the consequences of artificial lighting technologies are fully understood can we ensure that current developmental expansion does not unexpectedly compromise human health by increasing disease risk. Mosquito-borne diseases compromise the attainment of the Sustainable Development Goals (SDGs) and artificial lights of the correct kinds, used in optimal places and times, could help reduce vector-borne disease burdens.

AUTHOR CONTRIBUTIONS

BC, KG, and IS conceptualized the study. BC wrote the first draft. All authors then contributed and wrote all subsequent drafts.

FUNDING

The Jennifer Ward Oppenheimer Research Grant funds BC and LK is supported by a DST/NRF Research Chair Initiative grant award (UID: 64763).

REFERENCES

Baik, L. S., Nave, C., Au, D. D., Guda, T., Chevez, J. A., Ray, A., et al. (2020). Circadian regulation of light-evoked attraction and avoidance behaviors in daytime- versus nighttime-biting mosquitoes. *Curr. Biol.* 30, 3252–3259.e3. doi: 10.1016/j.cub.2020.06.010

Balmert, N. J., Rund, S. S. C., Ghazi, J. P., Zhou, P., and Duffield, G. E. (2014). Time-of-day specific changes in metabolic detoxification and insecticide resistance in the malaria mosquito *Anopheles gambiae*. *J. Insect Physiol.* 64, 30–39. doi: 10.1016/j.jinsphys.2014.02.013

Barghini, A., and de Medeiros, B. A. S. (2010). Artificial lighting as a vector attractant and cause of disease diffusion. *Environ. Health Perspect.* 118, 1503–1506. doi: 10.1289/ehp.1002115

Barroso, A., Haifig, I., Janei, V., Da Silva, I., Dietrich, C., and Costa-Leonardo, A. M. (2017). Effects of flickering light on the attraction of nocturnal insects. *Light. Res. Technol.* 49, 100–110. doi: 10.1177/1477153515602143

Bernáth, B., Horváth, G., Gál, J., Fekete, G., and Meyer-Rochow, V. B. (2008). Polarized light and oviposition site selection in the yellow fever mosquito: no evidence for positive polarotaxis in *Aedes aegypti*. *Vision Res.* 48, 1449–1455. doi: 10.1016/j.visres.2008.04.007

Boze, B. G. V., Renfro, K., Markowski, D., and Lozano-Fuentes, S. (2021). Effect of incandescent light on collection of West Nile Virus vectors using CDC miniature light traps in Northern Colorado. *J. Insect Sci.* 21, 21–24. doi: 10.1093/jisesa/ieab069

Briolat, E. S., Gaston, K. J., Bennie, J., Rosenfeld, E. J., and Troscianko, J. (2021). Artificial nighttime lighting impacts visual ecology links between flowers, pollinators and predators. *Nat. Commun.* 12:4163. doi: 10.1038/s41467-021-24394-0

Caminade, C., Kovats, S., Rocklov, J., Tompkins, A. M., Morse, A. P., Colón-González, F. J., et al. (2014). Impact of climate change on global malaria distribution. *Proc. Natl. Acad. Sci. U.S.A.* 111, 3286–3291. doi: 10.1073/pnas.1302089111

Chepesiuk, R. (2009). Missing the dark: health effects of light pollution. *Environ. Health Perspect.* 117, 20–27. doi: 10.1289/ehp.117-a20

Cooke, M. K., Kahindi, S. C., Oriango, R. M., Owaga, C., Ayoma, E., Mabuka, D., et al. (2015). "A bite before bed": exposure to malaria vectors outside the times of net use in the highlands of western Kenya. *Malar. J.* 14:259. doi: 10.1186/s12936-015-0766-4

Costantini, C., Sagnon, N., della Torre, A., Diallo, M., Brady, J., Gibson, G., et al. (1998). Odor-mediated host preferences of West African mosquitoes, with particular reference to malaria vectors. *Am. J. Trop. Med. Hyg.* 58, 56–63. doi: 10.4269/ajtmh.1998.58.56

Das, S., and Dimopoulos, G. (2008). Molecular analysis of photic inhibition of blood-feeding in *Anopheles gambiae*. *BMC Physiol.* 8:23. doi: 10.1186/1472-6793-8-23

Davies, T. W., and Smyth, T. (2018). Why artificial light at night should be a focus for global change research in the 21st century. *Glob. Chang. Biol.* 24, 872–882. doi: 10.1111/gcb.13927

Fonken, L. K., and Nelson, R. J. (2014). The effects of light at night on circadian clocks and metabolism. *Endocr. Rev.* 35, 648–670. doi: 10.1210/er.2013-1051

Fyie, L. R., Gardiner, M. M., and Meuti, M. E. (2021). Artificial light at night alters the seasonal responses of biting mosquitoes. *J. Insect Physiol.* 129:104194. doi: 10.1016/j.jinsphys.2021.104194

Gaston, K. J., Bennie, J., Davies, T. W., and Hopkins, J. (2013). The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev.* 88, 912–927. doi: 10.1111/brv.12036

Gatton, M. L., Chitnis, N., Churcher, T., Donnelly, M. J., Ghani, A. C., Godfray, H. C. J., et al. (2013). The importance of mosquito behavioural adaptations to malaria control in Africa. *Evolution (N. Y).* 67, 1218–1230. doi: 10.1111/evo.12063

Getz, W. M., Salter, R., and Mgbara, W. (2019). Adequacy of SEIR models when epidemics have spatial structure: ebola in Sierra Leone. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 374:20180282. doi: 10.1098/rstb.2018.0282

Gibson, G. (1995). A behavioural test of the sensitivity of a nocturnal mosquito, Anopheles gambiae, to dim white, red and infra-red light. *Physiol. Entomol.* 20, 224–228. doi: 10.1111/j.1365-3032.1995.tb00005.x

Grubisic, M., and van Grunsven, R. H. (2021). Artificial light at night disrupts species interactions and changes insect communities. *Curr. Opin. Insect Sci.* 47, 136–141. doi: 10.1016/j.cois.2021.06.007

Harris, A. F., Matias-Arnéz, A., and Hill, N. (2006). Biting time of *Anopheles darlingi* in the Bolivian Amazon and implications for control of malaria. *Trans. R. Soc. Trop. Med. Hyg.* 100, 45–47. doi: 10.1016/j.trstmh.2005.07.001

Hartemink, N., Cianci, D., and Reiter, P. (2015). R0 for vector-borne diseases: impact of the assumption for the duration of the extrinsic incubation period. *Vector Borne Zoonotic Dis.* 15, 215–217. doi: 10.1089/vbz.2014.1684

Heinloth, T., Uhlhorn, J., and Wernet, M. F. (2018). Insect responses to linearly polarized reflections: orphan behaviors without neural circuits. *Front. Cell. Neurosci.* 12:50. doi: 10.3389/fncel.2018.00050

Honnen, A. C., Kypke, J. L., Hölker, F., and Monaghan, M. T. (2019). Artificial light at night influences clock-gene expression, activity, and fecundity in the mosquito *Culex pipiens* f. *molestus*. *Sustainability* 11:6220. doi: 10.3390/su11226220

Honnen, A., Johnston, P. R., and Monaghan, M. T. (2016). Sex-specific gene expression in the mosquito *Culex pipiens* f. *molestus* in response to artificial light at night. *BMC Genomics* 17:22. doi: 10.1186/s12864-015-2336-0

Hu, X., Leming, M. T., Whaley, M. A., and O'Tousa, J. E. (2014). Rhodopsin coexpression in UV photoreceptors of *Aedes aegypti* and *Anopheles gambiae* mosquitoes. *J. Exp. Biol.* 217, 1003–1008. doi: 10.1242/jeb.096347

Jones, M. D. R., Ford, M., and Gillet, J. D. (1966). Light-on and light-off effects on the ciracadian flight activity in the mosquito *Anopheles gambia*. *Nature* 211, 871–872.

Kernbach, M. E., Hall, R. J., Burkett-Cadena, N. D., Unnasch, T. R., and Martin, L. B. (2018). Dim light at night: physiological effects and ecological consequences for infectious disease. *Integr. Comp. Biol.* 58, 995–1007. doi: 10.1093/icb/icy080

Kernbach, M. E., Martin, L. B., Unnasch, T. R., Hall, R. J., Jiang, R. H. Y., and Francis, C. D. (2021). Light pollution affects West Nile virus exposure risk across Florida. *Proc. R. Soc. Lond. B Biol. Sci.* 288:20210253. doi: 10.1098/rspb.2021.0253

Kernbach, M. E., Newhouse, D. J., Miller, J. M., Hall, R. J., Gibbons, J., Oberstaller, J., et al. (2019). Light pollution increases West Nile virus competence of a ubiquitous passerine reservoir species. *Proc. R. Soc. Lond. B Biol. Sci.* 286:20191051. doi: 10.1098/rspb.2019.1051

Kirkeby, C., Græsbøll, K., Stockmarr, A., Christiansen, L. E., and Bødker, R. (2013). The range of attraction for light traps catching *Culicoides biting* midges (Diptera: Ceratopogonidae). *Parasit. Vectors* 6:67. doi: 10.1186/1756-3305-6-67

Knop, E., Zoller, L., Ryser, R., Gerpe, C., Hörler, M., and Fontaine, C. (2017). Artificial light at night as a new threat to pollination. *Nature* 548, 206–209. doi: 10.1038/nature23288

Land, M. F., and Nilsson, D.-E. (2012). *Animal Eyes*, 2nd Edn. Oxford: Oxford University Press.

Land, M. F., Gibson, G., and Horwood, J. (1997). Mosquito eye design: conical rhabdoms are matched to wide aperture lenses. *Proc. R. Soc. Lond. B Biol. Sci.* 264, 1183–1187. doi: 10.1098/rspb.1997.0163

Land, M. F., Gibson, G., Horwood, J., and Zeil, J. (1999). Fundamental differences in the optical structure of the eyes of nocturnal and diurnal mosquitoes. *J. Comp. Physiol.* 185, 91–103. doi: 10.1007/s003590050369

Lee, J. M., Wasserman, R. J., Gan, J. Y., Wilson, R. F., Rahman, S., and Yek, S. H. (2020). Human activities attract harmful mosquitoes in a tropical urban landscape. *Ecohealth* 17, 52–63. doi: 10.1007/s10393-019-01457-9

Lima, W. R., Holder, A. A., and Garcia, C. R. S. (2013). Melatonin signaling and its modulation of PfNF-YB transcription factor expression in *Plasmodium falciparum*. *Int. J. Mol. Sci.* 14, 13704–13718. doi: 10.3390/ijms140713704

Liu, Y. N., Liu, Y. J., Chen, Y. C., Ma, H. Y., and Lee, H. Y. (2017). Enhancement of mosquito trapping efficiency by using pulse width modulated light emitting diodes. *Sci. Rep.* 7:40074. doi: 10.1038/srep40074

Longcore, T., and Rich, C. (2004). Ecological light pollution. *Front. Ecol. Environ.* 2, 191–198. doi: 10.1890/1540-9295(2004)002[0191:ELP]2.0.CO;2

Martinez-Bakker, M., and Helm, B. (2015). The influence of biological rhythms on host-parasite interactions. *Trends Ecol. Evol.* 30, 314–326. doi: 10.1016/j.tree.2015.03.012

Medlock, J. M., and Leach, S. A. (2015). Effect of climate change on vector-borne disease risk in the UK. *Lancet Infect. Dis.* 15, 721–730. doi: 10.1016/S1473-3099(15)70091-5

Metoxen, A. J., Leming, M. T., Hu, X., Whaley, M. A., and O'Tousa, J. E. (2016). Light-driven processes control both rhodopsin maturation and recycling in mosquito photoreceptors. *J. Neurosci.* 36, 11051–11058. doi: 10.1523/JNEUROSCI.1754-16.2016

Meyer Steiger, D. B., Ritchie, S. A., and Laurance, S. G. W. (2016). Mosquito communities and disease risk influenced by land use change and seasonality in the Australian tropics. *Parasit. Vectors* 9:387. doi: 10.1186/s13071-016-1675-2

Moon, Y. M., Metoxen, A. J., Leming, M. T., Whaley, M. A., and Tousa, J. E. O. (2015). Rhodopsin management during the light-dark cycle of *Anopheles gambiae* mosquitoes. *J. Insect Physiol.* 70, 88–93. doi: 10.1016/j.jinsphys.2014.09.006.Rhodopsin

Mordecai, E. A., Caldwell, J. M., Grossman, M. K., Lippi, C. A., Johnson, L. R., Neira, M., et al. (2019). Thermal biology of mosquito-borne disease. *Ecol. Lett.* 22, 1690–1708. doi: 10.1111/ele.13335

Mozūraitis, R., Hajkazemian, M., Zawada, J. W., Szymczak, J., Pålsson, K., Sekar, V., et al. (2020). Male swarming aggregation pheromones increase female attraction and mating success among multiple African malaria vector mosquito species. *Nat. Ecol. Evol.* 4, 1395–1401. doi: 10.1038/s41559-020-1264-9

Muir, L. E., Thorne, M. J., and Kay, B. H. (1992). *Aedes aegypti* (Diptera: Culicidae) vision: spectral sensitivity and other perceptual parameters of the female eye. *J. Med. Entomol.* 29, 278–281. doi: 10.1093/jmedent/29.2.278

Navara, K. J., and Nelson, R. J. (2007). The dark side of light at night: physiological, epidemiological, and ecological consequences. *J. Pineal Res.* 43, 215–224. doi: 10.1111/j.1600-079X.2007.00473.x

Neafsey, D. E., Waterhouse, R. M., Abai, M. R., Aganezov, S. S., Alekseyev, M. A., Allen, J. E., et al. (2015). Highly evolvable malaria vectors: the genomes of 16 *Anopheles* mosquitoes. *Science* 347:1258522. doi: 10.1126/science.1258522

Nilsson, D. E., and Smolka, J. (2021). Quantifying biologically essential aspects of environmental light. *J. R. Soc. Interface* 18:20210184. doi: 10.1098/rsif.2021.0184

Otto, T. D., Gilabert, A., Crellen, T., Böhme, U., Arnathau, C., Sanders, M., et al. (2018). Genomes of all known members of a Plasmodium subgenus reveal paths to virulent human malaria. *Nat. Microbiol.* 3, 687–697. doi: 10.1038/s41564-018-0162-2

Owens, A. C. S., Cochard, P., Durrant, J., Farnworth, B., Perkin, E. K., and Seymoure, B. (2020). Light pollution is a driver of insect declines. *Biol. Conserv.* 241:108259. doi: 10.1016/j.biocon.2019.108259

Peach, D. A. H., Ko, E., Blake, A. J., and Gries, G. (2019). Ultraviolet inflorescence cues enhance attractiveness of inflorescence odour to *Culex pipiens* mosquitoes. *PLoS One* 14:e0217484. doi: 10.1371/journal.pone.0217484

Prior, K. F., O'Donnell, A. J., Rund, S. S. C., Savill, N. J., van der Veen, D. R., and Reece, S. E. (2019). Host circadian rhythms are disrupted during malaria infection in parasite genotype-specific manners. *Sci. Rep.* 9:10905. doi: 10.1038/s41598-019-47191-8

Rigot, T., and Gilbert, M. (2012). Quantifying the spatial dependence of *Culicoides* midge samples collected by Onderstepoort-type blacklight traps: an experimental approach to infer the range of attraction of light traps. *Med. Vet. Entomol.* 26, 152–161. doi: 10.1111/j.1365-2915.2011.00988.x

Rund, S. S. C., Gentile, J. E., and Duffield, G. E. (2013). Extensive circadian and light regulation of the transcriptome in the malaria mosquito *Anopheles gambiae*. *BMC Genomics* 14:218. doi: 10.1186/1471-2164-14-218

Rund, S. S. C., Labb, L. F., Benefiel, O. M., and Duffield, G. E. (2020). Artificial light at night increases *Aedes aegypti* mosquito biting behavior with implications for arboviral disease transmission. *Am. J. Trop. Med. Hyg.* 103, 2450–2452. doi: 10.4269/ajtmh.20-0885

Rund, S. S. C., O'Donnell, A. J., Gentile, J. E., and Reece, S. E. (2016). Daily rhythms in mosquitoes and their consequences for malaria transmission. *Insects* 7:14. doi: 10.3390/insects7020014

Sanders, D., Frago, E., Kehoe, R., Patterson, C., and Gaston, K. J. (2021). A meta-analysis of biological impacts of artificial light at night. *Nat. Ecol. Evol.* 5, 74–81. doi: 10.1146/annurev-ecolsys-110316-022745

Sheppard, A. D., Rund, S. S. C., George, G. F., Clark, E., Acri, D. J., and Duffield, G. E. (2017). Light manipulation of mosquito behaviour: acute and sustained photic suppression of biting activity in the *Anopheles gambiae* malaria mosquito. *Parasit. Vectors* 10:255. doi: 10.1186/s13071-017-2196-3

Smith, N. R., Trauer, J. M., Gambhir, M., Richards, J. S., Maude, R. J., Keith, J. M., et al. (2018). Agent-based models of malaria transmission: a systematic review. *Malar. J.* 17:299. doi: 10.1186/s12936-018-2442-y

Tang, J. Y., Kosgei, J., Ochomo, E., Ndenga, B. A., Ghiaseddin, R., Lobo, N. F., et al. (2021). Impact of visual features on capture of *Aedes aegypti* with host decoy traps (HDT). *Med. Vet. Entomol.* 35, 169–176. doi: 10.1111/mve.12482

Van Breugel, F., Riffell, J., Fairhall, A., and Dickinson, M. H. (2015). Mosquitoes use vision to associate odor plumes with thermal targets. *Curr. Biol.* 25, 2123–2129. doi: 10.1016/j.cub.2015.06.046

Van Der Kooi, C. J., Stavenga, D. G., Arikawa, K., Belušič, G., and Kelber, A. (2021). Evolution of insect color vision: from spectral sensitivity to visual ecology. *Annu. Rev. Entomol.* 66, 435–461. doi: 10.1146/annurev-ento-061720-071644

Wakefield, A., Broyles, M., Stone, E. L., Jones, G., and Harris, S. (2016). Experimentally comparing the attractiveness of domestic lights to insects: do LEDs attract fewer insects than conventional light types? *Ecol. Evol.* 6, 8028–8036. doi: 10.1002/ece3.2527

Westby, K. M., and Medley, K. A. (2020). Cold nights, city lights: artificial light at night reduces photoperiodically induced diapause in urban and rural populations of *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* 57, 1694–1699. doi: 10.1093/jme/tjaa139

White, B. J., Collins, F. H., and Besansky, N. J. (2011). Evolution of *Anopheles gambiae* in relation to humans and malaria. *Annu. Rev. Ecol. Evol. Syst.* 42, 111–132. doi: 10.1146/annurev-ecolsys-102710-145028

WHO (2021a). *Vector-Borne Diseases Factsheet*. Available online at: https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases (accessed August 31, 2021).

WHO (2021b). *Handbook of Integrated Vector Management*. Available online at: https://www.euro.who.int/en/health-topics/communicable-diseases/vector-borne-and-parasitic-diseases/integrated-vector-management-ivm (accessed August 31, 2021).

Wilson, R., Wakefield, A., Roberts, N., and Jones, G. (2021). Artificial light and biting flies: the parallel development of attractive light traps and unattractive domestic lights. *Parasit. Vectors* 14:28. doi: 10.1186/s13071-020-04530-3

Zhan, Y., Alonso, D., Alberto, S., Rusch, C., Riffell, J. A., and Montell, C. (2020). *Aedes aegypti* vision-guided target recognition requires two redundant rhodopsins. *bioRxiv* [Preprint]. doi: 10.1101/2020.07.01.182899

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 16 February 2022
doi: 10.3389/fevo.2022.751288

Light Attraction in Carabid Beetles: Comparison Among Animals From the Inner City and a Dark Sky Reserve

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Artificial light at night (ALAN) is altering the behaviour of nocturnal animals in a manifold of ways. Nocturnal invertebrates are particularly affected, due to their fatal attraction to ALAN. This selective pressure has the potential to reduce the strength of the flight-to-light response in insects, as shown recently in a moth species. Here we investigated light attraction of ground beetles (*Coleoptera*: *Carabidae*). We compared among animals (three genera) from a highly light polluted (HLP) grassland in the centre of Berlin and animals collected at a low-polluted area in a Dark Sky Reserve (DSR), captured using odour bait. In an arena setting tested at night time, HLP beetles (n = 75 across all genera) showed a reduced attraction towards ALAN. Tested during daytime, HLP beetles were less active in an open field test (measured as latency to start moving), compared to DSR (n = 143). However, we did not observe a reduced attraction towards ALAN within the species most common at both sides, *Calathus fuscipes* (HLP = 37, DSR = 118 individuals) indicating that not all species may be equally affected by ALAN. Reduced attraction to ALAN in urban beetles may either be a result of phenotypic selection in each generation removing HLP individuals that are attracted to light, or an indication for ongoing evolutionary differentiation among city and rural populations in their light response. Reduced attraction to light sources may directly enhance survival and reproductive success of urban individuals. However, decrease in mobility may negatively influence dispersal, reproduction and foraging success, highlighting the selective pressure that light pollution may have on fitness, by shaping and modifying the behaviour of insects.

Keywords: light pollution, artificial light at night (ALAN), Carabidae beetles, environmental change, Illuminance, solar powered light-emitting diode

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Specialty section:
This article was submitted to
Behavioral and Evolutionary Ecology,
a section of the journal
Frontiers in Ecology and Evolution

Received: *31 July 2021*
Accepted: *26 January 2022*
Published: *16 February 2022*

Citation:
Kaunath V and Eccard JA (2022)
Light Attraction in Carabid Beetles:
Comparison Among Animals From
the Inner City and a Dark Sky Reserve.
Front. Ecol. Evol. 10:751288.
doi: 10.3389/fevo.2022.751288

INTRODUCTION

Light pollution is defined as the alteration of natural light levels at night by anthropogenic artificial light sources (Cinzano et al., 2001; Falchi et al., 2016). It is no longer confined to urban areas, with the effects also visible in remote and rural areas, sometimes many kilometres away from the light source, i.e., sky glow (Grubisic et al., 2018). Nocturnal invertebrates, including both flying and ground dwelling insects, are especially affected from light pollution due to their attraction towards outdoor illuminations, such as street lanterns, spotlights at sport events and festivals, tourist sightseeing highlights (Rich and Longcore, 2013; Owens and Lewis, 2018), and even ornamental LED garden tools (Eccard et al., 2018; Grubisic et al., 2018; Owens et al., 2020).

Various function of natural light insects may explain how artificial light at night (ALAN) can interfere in the life history of insects in a powerful manner (Sanders et al., 2021). Natural light sources play a crucial role in the orientation of many insect species (Grubisic et al., 2018; Owens et al., 2020). African Dung beetles (*Scarabaeus satyrus*) use the Milky Way (Dacke et al., 2013), moths (*Noctua pronuba*) are known to combine stars and moon light (Sotthibandhu and Baker, 1979), and sand hoppers (*Talitrus saltator*) maintain their nightly route by the moon (Ugolini et al., 1999). Further, visual communication can also be part of reproductive behaviour: for example, firefly beetles (Lampyridae) depend on light signals to find suitable mating partners (Ineichen and Rüttimann, 2012). Polarised light, reflected from incoming light by aquatic surfaces, is used by aquatic insects, such as mayflies, to detect suitable oviposition locations (Longcore and Rich, 2004; Szaz et al., 2015).

Previous studies have mainly investigated the attraction of invertebrates towards ALAN (flight-to-light response) which causes an increase in local mortality, either by higher risk of predation, exhaustion, and death by burning (Eisenbeis et al., 2006). One third of attracted insects will suffer death before the next sunrise due to one or more of the previously named causes (Frank et al., 2006; Owens et al., 2020). Epigaeal ground-dwelling organism such as ants, ground beetles, amphipods and harvestmen are also affected by ALAN and accumulate in higher abundances under artificial light sources (Davies et al., 2012; Eccard et al., 2018; Owens et al., 2020). This behaviour is the basis for the widespread use of light-traps as comprehensive method for assessing the diversity of insects in which the light-trap consist e.g., of a basic pit trap with an additional light source to increase the number of specimen captured. Also, light trapping is a very cost and time-efficient sampling method to produce qualitative and quantitative data on species occurrence and distribution in a short time period (Szentkirályi, 2002).

Artificial bright light sources can work as an ecological trap (Longcore and Rich, 2004; Frank et al., 2006), i.e., reduce the fitness of animals attracted to them. In some cases they work as evolutionary traps, (Hopkins et al., 2018) implying large-scale effects on insect populations by adding a light specific mortality. Given their crucial role in ecosystems as pollinators and as pest control, this can potentially lead to a cascade across species networks (Jankielsohn, 2018).

The massive decline of insect diversity and biomass across ecosystems in human dominated landscapes (Hallmann et al., 2017; Powney et al., 2019; Seibold et al., 2019) has often been attributed to habitat loss (e.g., land use change, agricultural intensification), chemical pollution (increased use of fertilizers), climate change, invasive species, or a combination of these factors. However, Owen et al. (2019) suggested that researchers possibly suffer a "diurnal bias," in that most attention has been paid to daytime phenomena, and that we have not yet studied the potential effects of light on night time phenomena (Owens et al., 2020). Light pollution is itself potentially a severe threat to biodiversity (Hölker et al., 2010b; Grubisic et al., 2018). Still, ALAN it is often not recognised as potential driver: for example Hallmann et al. (2017) did not consider the potential role of ALAN on insect biomass decline, even though all study sites were located in the most light polluted areas of Germany [(Grubisic et al., 2018), see Krefeld lightpollutionmap.info/]. Since many taxa show alteration of physiological and behavioural traits by, and fatal attraction to ALAN [review see Sanders et al. (2021)], thus, light pollution as a driving factor for decline cannot be excluded (Hölker et al., 2010b; Grubisic et al., 2018). More recently, several years after Hölker et al.,'s proposal in 2010 to include light pollution in insects research, studies are beginning to investigate the role of light pollution when analysing cascading effects of insect decline (Kehoe et al., 2021).

When looking at potential long-term consequences of light pollution as a threat to insect populations, nocturnal, light-attracted micromoth populations suffer a stronger decline than corresponding diurnal species not attracted by light (van Langevelde et al., 2018; Wilson et al., 2018), demonstrating that, possibly combined with other factors, artificial night-time lighting affects moth populations (Wilson et al., 2018). Altermatt and Ebert (2016) also observed a reduced flight-to-light behaviour in moth population exposed to intense light pollution when compared to populations from Dark Sky Habitats (Altermatt and Ebert, 2016).

Given that alteration of activity patterns (e.g., increase, decrease, or shift of activity) are one of the main impacts of ALAN (Sanders et al., 2021), a possible, yet understudied outcome of constant light exposure in insects may be the general mobility decline, as suggested by Altermatt and Ebert (2016). In urban open spaces, highly mobile individuals encounter artificial light sources at a higher likelihood, hence, they are more easily captivated by light than less mobile individuals. Once attracted, insects are not able to escape from the illuminated zone ("captivity effect") and face death (Eisenbeis et al., 2006), reducing fitness of more active individuals compared to less active ones in the same environment. It remains to be seen whether these disadvantages for highly mobile individuals have long-term consequences, including possible directional selection as suggested by Altermatt and Ebert (2016).

In this study we investigate the attraction to light sources, and the importance of long-time exposure of carabid beetle populations by comparing animals from two populations – either originating from a grassland habitat exposed to intense light pollution for many decades, or from location exposed to low light pollution levels. Carabid beetles are known to show a positive phototaxis response to ALAN [(Owens et al., 2020) and see Eccard et al. (2018) for some species turning immobile at illumination], and illumination can even result in a shift in species composition (Davies et al., 2012; Eccard et al., 2018). We hypothesised that (a) light polluted grassland harboured a different species community, than less polluted areas and (b) carabid beetles (from any genus) from highly light polluted (HLP) areas are less attracted to artificial light sources than their counterparts. Further, we investigated general activity pattern in an open field test and hypothesised that HLP beetles may be less mobile in their general activity when compared to beetles from low light pollution. We compared behaviour of several genera captured at both very light polluted urban or very dark rural sites, but also compared within populations of the same species captured at both sites.

MATERIALS AND METHODS

Location and Animals

We collected carabid beetles from an urban area which has been exposed to intense light pollution over several decades in the Tiergarten (Central Berlin, Germany), and in rural areas with very low light pollution levels at the Biological Station Gülpe (Brandenburg, Germany), 80 km east of Berlin during August and September 2020 (**Table 1** and **Figure 1**). The latter area was awarded as an "Sternenpark" [Dark Sky Reserve (DSR)] by the International Dark Sky Association (IDA) in 2014.

We trapped adult carabid beetles with ten wine-baited pitfall traps at both sites for 4 days and nights in August 2020 (93 animals at DSR, 66 at HLP) and for 2 days and four nights in September 2020 (74 animals in DSR and 88 at HLP). Traps were emptied every 12 h, i.e., after sunrise and during sunset to distinguish dark and light capture intervals. Beetles were kept groupwise in plastic boxes (1.3 l, 12.5 cm × 19 cm × 7.5 cm; item number RK1285A) and were fed with pinkie maggots (*Lucilia Caesar*) or seeds every day. Boxes included shelters (pinecones, moss, leaves from originated site) and were moistened every day. Boxes were kept in an open garden shed outside the sun, in which beetles were exposed to the natural light-day cycle to ensure no effect of changing light conditions. During this time in captivity, beetles were not exposed to any artificial light sources unless they were tested in the arena.

Animals were kept from 3–44 days until the start of the experiment. The attraction experiment and the open field test were conducted on two separate days for each animal. Beetles were released at their origin afterwards.

Characterisation of Artificial Light Source Solar Powered LED

The artificial light source used in the experiment consisted of commercial solar powered LED (SPLED) garden lamps (Conrad Electronics, Hirschau, Germany, Model 5721129) which contained a 4 cm × 6 cm solar panel in its black plastic head (diameter 105 mm) and one diode (LED). Mainly cold white light was emitted from the integrated diode, but with a high percentage of blue light (correlated colour temperature = 7250 K). The spectral irradiance pattern of these lamps was published in Eccard et al. (2018).[1] The position of the diode and the shape of the diffusor created a small highly illuminated zone close by and around the LED lamp (radius $r = 25$ cm, illuminance $E_v = 0.8$ lx) and a further, less illuminated outer zone ($r = 2.5$ m, $E_v < 0.1$ lx). Each day the solar panel was placed in the sun to recharge its battery (1.2 V, 600 mAh).

Behavioural Experiments

Attraction Towards Artificial Light at Night

We experimentally measured the attraction towards an artificial light source in elongated arenas (60 cm long × 8.5 cm wide tracks) where one SPLED was attached to one side of track (yellow field, Appendix). Approach to the lamp by a beetle was quantified by its presence in an area nearest to the lamp (8.5 × 8.5 cm, called illuminated zone). Six beetles were tested simultaneously (runs) in six adjacent tracks, and the position of the lamps was swapped between subsequent runs. The beetles were randomly selected from either HLP or DSR origins sites and species and placed gently into the middle of the track when the experiment started and observed for 15 min. Location within/outside the illuminated zone was recorded every 15 s (total: 60 observation intervals). Experiments were conducted at night.

Activity in the Open Field Test

Activity of the carabid beetles were observed for 3 min in an open field test (round plastic Petri dish, diameter 13.8 cm, rim height: 2.5 cm) under a desk light (110–240 V, E14, max. 40 W, mean lx 276. 62 ± 27.6). The open field was separated into eight 1/8 sectors. Activity was measured by counting the number of line crossings by an individual. The open field was further divided into an outer and inner zone with the same area size ($A = 74.78$ cm^2). The inner concentric zone was within a radius of ($r = 4.8$ cm), the outer a ring between inner and outer radius ($r = 6.9$ cm). Each beetle was released at one of the sectors in the outer zone and the following behaviour was recorded: time to start moving [*latency to move (sec)*] and number of line crossings (*number of crossings*). Open field were conducted

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[1] https://onlinelibrary.wiley.com/doi/full/10.1111/icad.12303

TABLE 1 | Sampling sites of carabid beetles in Tiergarten (High Light pollution, HLP) and in Gülpe (Dark Sky Reserve, DSR) in 2020.

Population	Latitude/ longitude	Light pollution radiance (10^{-9} Watt cm^2 × sr)	Sky brightness ratio	Sampling period	Numb. of captured beetles
Dark sky reserve (DSR)	52°44023″N, 12°15018″O	0.15	0.273	August September	93 74
High light pollution (HLP)	52°51426″N, 13°37576″O	75.57	35.3	August September	66 88

Light pollution levels (radiance and sky brightness) were taken from the lightpollutionmap.info (Stare, 2021).

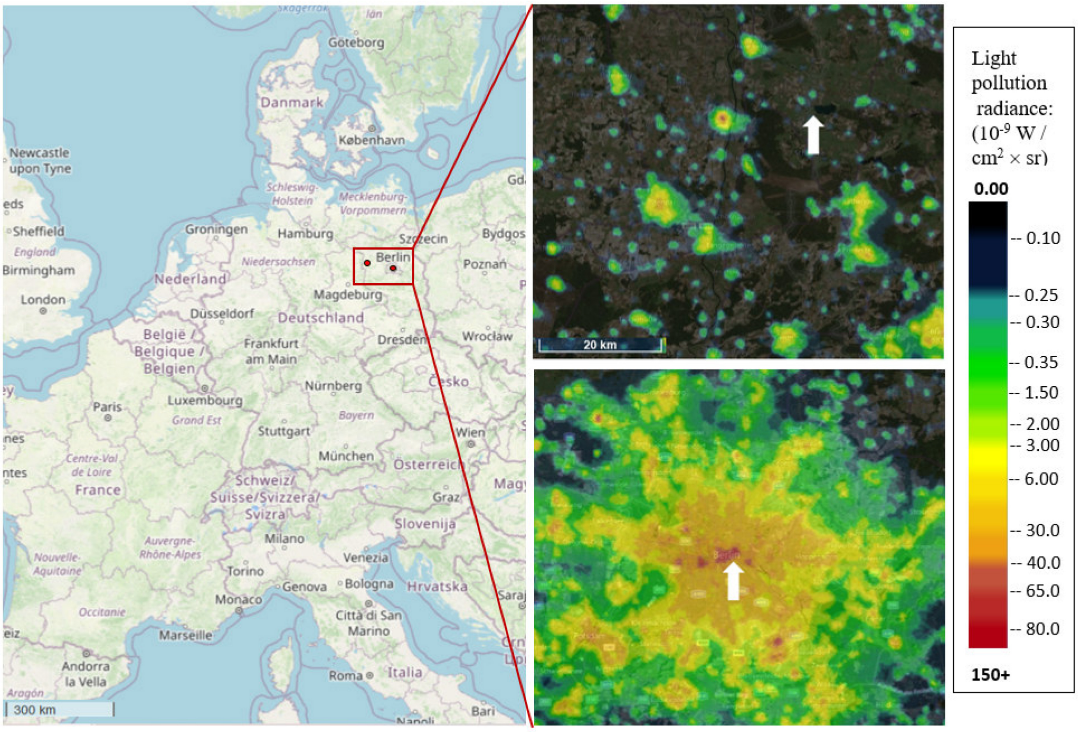

FIGURE 1 | Location of the sites in Germany (map data OpenStreetMap contributors, CC-BY-SA), whereas red rectangle shows close-up from Berlin and its surrounding federal state Brandenburg where the red points mark the exact location of the sites. Light pollution radiance maps (VIIRS 2020) are showing the capital Berlin (bottom right) and part of the federal state Brandenburg (top right). White arrows are highlighting the urban site (Tiergarten, HLP) and the rural site (Biological Station Gülpe, DSR). Colours represent the radiance values in which bright colours (green, yellow, orange, and red) show higher light pollution values (see legend/ lightpollutionmap.info).

during day times to also test for possible differences of main activity pattern of species.

Behavioural Analyses

Analyses of the individual behaviour were conducted for 218 beetles all species that occurred in both sites divided into 3 genera. Attraction towards the artificial light source was analysed in two sequences (hurdle model): firstly, a binary variable of attraction towards the illuminated zone (binomial value yes/no for the entire experiment) and secondly, if yes, the number of 15 s intervals spent in the illuminated zone.

In both sequences, general linear distribution models (GLMs) were used to analyse the effect of the origin (HLP vs. DSR) including the genus of the beetle species, the interaction term of light pollution*genus, the side of the attached LED, the capture interval of emptying the trap (day/night), the days in captivity and species-specific activity pattern (diurnal/nocturnal) as fixed factors. The species-specific activity pattern was defined by literature records, to be either nocturnal or diurnal (Thiele, 1977; Van Dijk and Den Boer, 1992; Kielhorn, 2005).

Activity in the open field test was analysed in two separate models [linear model (lm) for continuous variable (latency to start moving (seconds), generalized linear model (glm) for count data (number of sectors crossed)]. We included either the latency (seconds) to start moving, or the number of crossings of sector borders as a response variable, and, as fixed factors, we used genus, capture interval of emptying the traps (day/night), the days in captivity and species-specific activity pattern (diurnal/nocturnal).

Full models including all fixed factors were reduced *via* stepwise backwards model selection by comparing the Akaike Information Criterion (AIC). Factors that improved the model were included, even if the factor did not explain the variation. Factors genus and origin were always kept in the model. The interaction term (pollution*genus) was removed if it did not improve the model fit.

Across some variables, the addition of fixed factors as species-specific activity pattern (nocturnal/diurnal) and the capture interval of emptying the pit traps (morning/evening) did not improve the model (AIC selection) and did not explain variation in the variable impro. Thus, these factors were removed during the model reduction process.

The species *Calathus fuscipes* was captured with > 20 animals per origin, thus, we conducted a within-species comparison of behaviour among origins.

All analyses were done with R Version [4.0.3 (2020-10-10) using the lme4 package (version 1.1-25, (Bates et al., 2015)] and figures always represent raw data.

TABLE 2 | Species captured by odour trapping in grassland habitats in the city (HLP) and in a Dark Sky Reserve (DSR).

Species	HLP	DSR	Activity pattern	Perc. night captures (HLP/DSR)
C. fuscipes (GOETZE, 1777)	37	118	Nocturnal	86/87
C. melanocephalus (LINNÉ, 1758)	3	13	Nocturnal	100/38
H. anxius (DUFTSCHMIED, 1812)	12	3	Diurnal	58/33
A. bifrons (GYLLENHAL, 1810)	10	1	Nocturnal	100/100
C. ambiguus (PAYKULL, 1790)	5	1	Nocturnal	80/100
H. affinis (SCHRANK, 1781)	2	4	Diurnal	50/75
A. apricaria (PAYKULL, 1790)	4	1	Nocturnal	50/0
A. aenea (DEGEER, 1774)	2	2	Diurnal	100/50
only at HLP				
H. smaragdinus (DUFTSCHMIED, 1812)	76	–	Nocturnal	59
H. griseus (PANZER, 1796)	3	–	Nocturnal	100
only at DSR				
P. versicolor (STURM, 1824)	–	18	Diurnal	22
H. rufipes (DE GEER, 1774)	–	4	Nocturnal	100
H. pumilus (STURM, 1818)	–	1	Diurnal	100
C. erratus (SAHLBERG, 1827)	–	1	Nocturnal	0

Numbers refer to captured individuals, activity pattern refers to distribution of activity in response to natural light phases (Thiele, 1977; Van Dijk and Den Boer, 1992; Kielhorn, 2005) and the percentage of beetles captured during the night for HLP/DSR. Genera: Amara, Calathus, Harpalus, Poecilus.

Diversity Analysis

Species composition was compared among the plots, using the pit trapping rounds for animal collection as repeat. We analysed 11 sampling rounds (six rural and five urban with 11–60 individuals of 3–9 species captures) and removed three sampling rounds with < 3 species collected. Species composition was analysed using ordination to visualise the data, and multivariate, permutational analyses of variance (MPANOVA) to compare among the two plots (both with the R package vegan 2-5-5).

RESULTS

Carabid Communities and Diversity

A total of 14 carabid species (n = 321, Genus *Calathus, Harpalus, Amara* and, only at DSR, *Poecilus*) were captured (**Table 2**), of which eight species (218 individuals, 3 genera) were recorded at both sites.

The animal abundance [28.3 ± 14 (mean ± SD) animals per sampling round] and species richness (5 ± 1.9 species) did not differ among urban and rural sites (t-test abundance, t = 0.56, p = 0.59; Wilcoxon test species richness: W = 12.5, p = 0.711). However, their species composition was dissimilar between the rural and urban grassland plot [see **Figure 2**, MPANOVA (origin) = 9.9, p = 0.002, df = 1/10)], but without finding any difference in the Diversity Index (Shannon Wieder Index. urban: 1.2 ± 0.4, rural: 0.65 ± 0.2, student's t = -1,5, df = 9, pr = 0.158).

Behaviour

Behavioural experiments were performed with 218 beetles belonging to eight species present in both origins (**Table 2**) from three genera: *Amara* (20 individuals, 3 Species), *Calathus* (177 individuals, 3 Species), and *Harpalus* (21 individuals, 2 Species).

A lower proportion of beetles from HLP (57%) went into the illuminated sector compared to DSR beetles (72%, Chi^2 -Test, X^2 = 4.1, df = 1, p = 0.041, **Figure 3A**).

Among those beetles that entered the illuminated zone (n = 146), the number of time intervals in the illuminated zoner was lower for HLP [n = 43, 8.5 ± 1.29 time intervals (Mean ± SE)] than in DSR (n = 103, 10.4 ± 1.02 time intervals; **Table 3**), accordingly the DSR individuals stayed longer in the illuminated zone than their HLP counterparts (glm, pollution (HLP): Chi^2, df = 1, p = 0.04*, **Figure 3B**).

Activity in Open Field

Beetles from HLP origin began moving slower [46 ± 6.7 (Mean ± SE) seconds] than beetles from DSR origin which

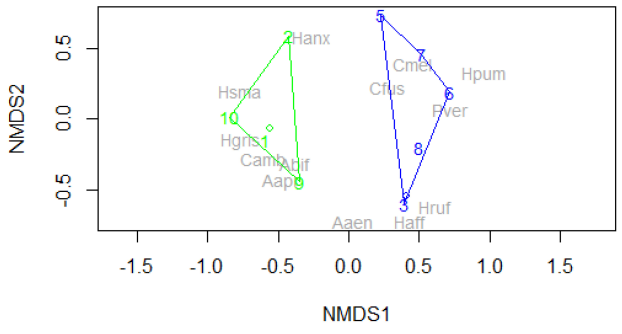

FIGURE 2 | Ordination plot of ground beetle species data (grey names, Family species = Ospe, Families include *Harpalus, Calathus, Poecilus*, and *Amara*), captured in 11 sampling rounds (numbers) on an urban (green) and a rural (blue) dry grassland. Model with two clusters, stress = 0.009. NMDS1 maybe interpreted as a urban–rural gradient with species left occurring only on urban, centre on both, and the right only rural plots. NMDS2 may be interpreted as an abundance axis.

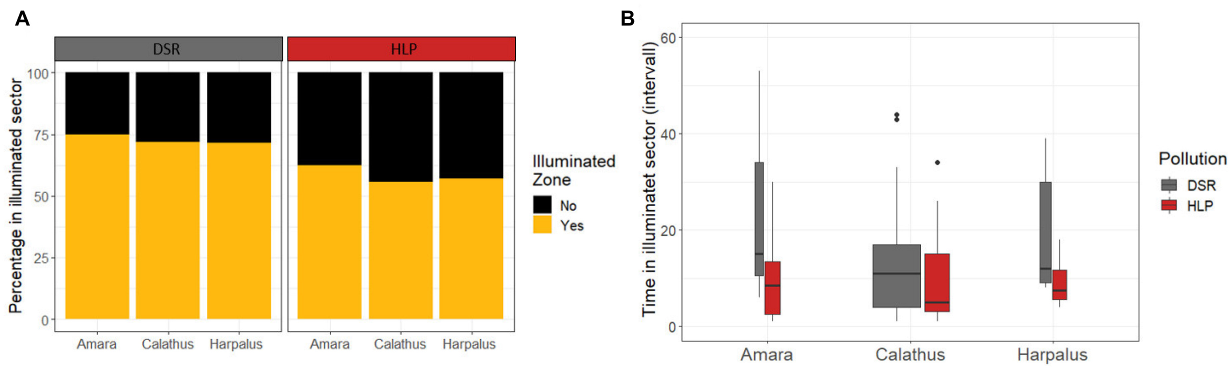

FIGURE 3 | Use of the illuminated sector in an experiment on light attraction of carabid beetles captured in a Dark Sky Reserve (DSR) and in an urban area with high light pollution (HLP) divided into three genera *Amara, Calathus, Harpalus*. **(A)** Percentage of animals approaching the LED (total *n* = 218), **(B)** time spend near the LED based on those animals from HLP (red color) and DSR (grey color) that approached LED (*n* = 146) divided into genera. The boxplot presents the median and the quartiles. Width of box relates to sample size.

TABLE 3 | General linear distribution model analysis of light attraction of ground beetles (step 1: *n* = 218, step 2: *n* = 146, three genera) investigated in an elongated arena comparing animals originating from two areas with different light pollution exposure.

	Step 1: enter the illuminated sector				**Step 2: time intervals in the illuminated sector**			
Fixed factor	**Estimate**	**SE**	***t*-value**	**Pr (>| t|)**	**Estimate**	**SE**	***t*-value**	**Pr (>| t|)**
Intercept	0.68	0.67	1.02	0.31	2.38	0.31	7.68	0
Pollution (HLP)	−0.71	0.33	−2.14	**0.0321***	−0.32	0.16	−1.99	**0.048***
Genus (Calathus)	−0.28	0.54	−0.52	0.61	−0.18	0.25	−0.72	0.47
Genus (Harpalus)	−0.13	0.68	−0.19	0.85	0.12	0.32	0.38	0.70
Days in captivity	0.03	0.02	1.51	0.13	0.01	0.01	1.92	0.06
Position LED	−0.21	0.30	−0.69	0.49	removed			
Captive interval (night)	0.20	0.34	0.58	0.56	−0.22	0.16	−1.41	0.16

*Effect sizes of HLP (highly light polluted) refer to Dark Sky Reserve (DSR). Effect sizes of genera refer to Amara and capture intervals to day capture. Variables that explained variation are highlighted in bold. Asterisks represent statistical significance (*p < 0.05).*

TABLE 4 | General linear distribution model for behavioural variables measured in an open field test with 218 ground beetles captured at two origins differing in light pollution intensity.

	Latency (seconds) to start moving				**Numbers of sectors crossed**			
Fixed factor	**Estimate**	**SE**	***t*-value**	**Pr (>| t|)**	**Estimate**	**SE**	***t*-value**	**Pr (>| t|)**
Intercept	1.25	0.22	5.69	0	2.8	0.6	4.69	0
Pollution (HLP)	0.25	0.12	2.20	0.029*	−2.05	0.6	−3.42	**0.00*****
Genus (Calathus)	−0.14	0.18	−0.78	0.43	−1.35	0.58	−2.33	**0.00****
Genus (Harpalus)	0.25	0.23	1.08	0.28	−0.21	0.69	−0.3	0.77
Days in captivity	−0.01	0.01	−2.11	**0.036***	0.01	0.01	1.42	0.16
Capture interval (night)	−0.07	0.12	−0.60	0.55	removed			
Activity pattern (Nocturnal)	removed				2.16	0.64	3.39	**0.00*****
Origin (HLP)* Genus (Calathus)	removed				1.82	0.62	2.94	**0.00****
Origin (HLP)* Genus (Harpalus)	removed				2.31	0.75	3.08	**0.00****

*Effect size reports behavioural difference for beetles from highly light polluted (HLP) area to a Dark Sky Reserve (DSR). Genera are compared to the behaviour of Amara, capture intervals to day capture, species-specific activity pattern to diurnal. Variables that explained variation are highlighted in bold. Asterisks represent statistical significance (*p < 0.05, **p < 0.01, ***p < 0.00).*

initiated their movement after (26 ± 3.8) seconds [glm, pollution (HLP): Chi^2, df = 1, $p = 0.029$].

Beetles originating from DSR crossed 42.7 ± 8.3 (mean ± SE) sectors and HLP beetles crossed 29.1 ± 5.4 sectors during the open-field test. Effect of pollution on the number of sectors crossed was only observed when looking at the effect size of the glm model (see **Table 4**), but were not supported in the final Anova

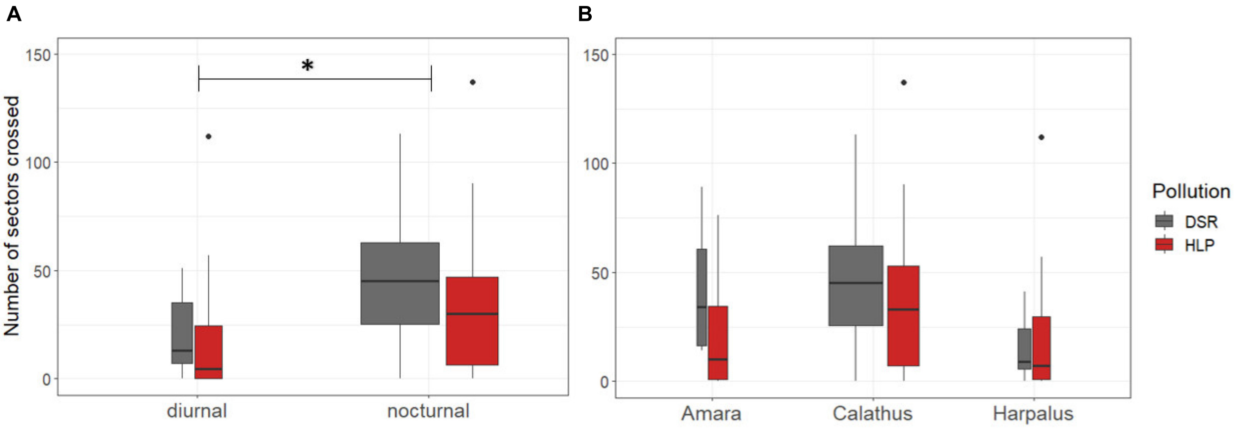

FIGURE 4 | Number of crossings in in nocturnal beetles originating from DSR beetles (*n* = 134, dark grey) and HLP areas (*n* = 59, red). **(A)** Division between nocturnal (*n* = 193) and diurnal beetles (*n* = 25) and **(B)** division into three genera of all beetle species independent their main activity pattern. Boxplots show median *and* the quartiles. Width of box relates to sample size.

TABLE 5 | General linear distribution model for behavioural variables measured in an open field test with 155 beetles *C. fuscipes* captured at two origins differing in light pollution intensity.

	Step 1: enter the illuminated sector				**Step 2: time intervals in the illuminated sector**			
Fixed factor	**Estimate**	**SE**	***t*-value**	**Pr (>| t|)**	**Estimate**	**SE**	***t*-value**	**Pr (>| t|)**
Intercept	0.99	0.44	2.26	0.024	2.31	0.22	10.42	0.00
Pollution (HLP)	−0.62	0.40	−1.55	0.122	−0.12	0.20	−0.61	0.54
Days in captivity	0.01	0.02	0.54	0.59	0.01	0.01	1.26	0.21
Position LED	−0.56	0.35	−1.58	0.12	removed			
Captive interval (night)	removed				−0.31	0.19	−1.61	0.11

Effect size reports behavioural difference for beetles from highly light polluted (HLP) area to a Dark Sky Reserve (DSR). Capture intervals are compared to day capture. Variables that explained variation are highlighted in bold.

TABLE 6 | General linear distribution model for behavioural variables {latency to start moving [log(seconds)], numbers of sectors crossed} measured in an open field test with 155 beetles *C. fuscipes* captured at two origins differing in light pollution intensity.

	Latency to start moving (seconds)				**Number of sectors crossed**			
Fixed factor	**Estimate**	**SE**	***t*-value**	**Pr (>| t|)**	**Estimate**	**SE**	***t*-value**	**Pr (>| t|)**
Intercept	2.32	0.39	5.98	0.00	2.31	0.22	10.42	0.00
Pollution (HLP)	0.59	0.33	1.79	0.076	−0.12	0.20	−0.61	0.54
Capture interval (night)	−0.04	0.34	−0.12	0.91	0.01	0.01	1.26	0.21
Days in captivity	−0.02	0.02	−1.50	0.14	−0.31	0.19	−1.61	0.11

Effect size reports behavioural difference for beetles from highly light polluted (HLP) area to a Dark Sky Reserve (DSR). Capture intervals are compared to day capture. Variables that explained variation are highlighted in bold.

output [negative glm, pollution (HLP): Chi^2, df = 1, $p = 0.106$].

Nocturnal beetles had a higher number of crossings 40.5 ± 3.9 than the diurnal species 18.8 ± 3.7, in which nocturnal ones are more active than diurnal species in the open field test [negative glm, species-specific activity pattern (nocturnal): Chi^2 -Test, df = 1, $p = 0.019$, **Figure 4A**].

The effect of origin was different among genera (Interaction pollution*genus: chi^2, df = 2, $p = 0.047^*$, **Figure 4B**) but in *post hoc* analyses no effects of origin within each genus were detected (all Turkey, $p > 0.1$).

Analyses Within *Calathus fuscipes*

In the most abundant species *C. fuscipes* with 37 and 118 individuals (HLP and DSR), differences in behaviour obtained from the beetle community were not confirmed. Effects of light pollution did not explain the percentage of animals which went into the illuminated zone, the times spent in it, or the number of crossings in the open field test [glm, pollution (HLP): Chi^2, $p > 0.5$, see **Tables 5**, **6**].

DISCUSSION

Our study confirmed that urban carabid beetles have a reduced attraction to artificial light sources (ALAN), relative to individuals with rural origins. Effects of origin did not differ among the 14 species of three genera investigated, which were all common at both sites of origin. However, different from Altermatt and Ebert (2016) that found different flight-to-light responses in urban and rural moths of the same species, we did not find different responses within the species most common at both origins *C. fuscipes*.

The species *C. fuscipes* was the most abundant species in both origins and was also collected both during day and night (approx. 86% during night, 24% during day, see **Table 2**). This suggests that this species may be very resilient to light pollution. Likewise, the lack of differentiation among species could be seen as evidence that a systematic change in behavioural patterns due to ALAN has already happened and that it has comprehensive implications beyond singular species levels, while resilient and ubiquitous species, such as *C. fuscipes,* are not strongly affected.

A reduced attraction to light at night of urban insect populations may affect results of light trapping, which is often used to assess the diversity of insects. The use (or addition) of a light-independent survey method should therefore be advised.

Species in human-altered environments (e.g., through the introduction of light, invasive species) have lower population sizes, demonstrating the potentially negative effect of anthropogenic activity on insects (Eisenbeis et al., 2006; Sih et al., 2011), including the fatal attraction to ALAN.

The findings in our study are coherent with the observation of reduction in flight-to-light behaviour in urban moth population seen by Altermatt and Ebert (2016). This suggests that the reduction in light attraction (or even possible avoidance of light sources) can lead to a higher survival rate in urban population by reducing mortality caused by ALAN. Reduction in attraction outweighs potential benefits of light attraction, instead favouring individuals that are not inclined to move towards ALAN (Frank et al., 2006; Altermatt and Ebert, 2016). We also found a reduction of the general activity of urban beetles, compared to rural. The potential underlying mechanism, general mobility reduction (number of crossings or latency to start movement), which was confirmed in one of the two measurements in the open field.

The parameters of the open field highlight the different responses between the genera, as well as a need for more caution in further studies when including both diurnal and nocturnal species in analyses. These are not equally affected by light pollution (Sanders et al., 2021). Here, nocturnal beetles were more active than diurnal ones (**Table 3**), alas runs were done during daytime and should have been either undertaken in accordance to species-specific activity pattern or both during day and night time. Higher activity in in nocturnal beetles can be caused by the urge to escape direct light exposure to hide under soil or leaves, as it is common for nocturnal carabid beetles over day time (Lindroth and Bangsholt, 1985). Nonetheless, treatment was equal to both cohorts and only partly revealed expected lower activity in beetles from HLP.

General activity decline can constrain migration and exchange between habitat patches on a regional scale (Bennie et al., 2015), thereby restricting gene flow and limiting the inflow of genetic diversity (Kotler, 1984; Eisenbeis et al., 2006; Sih et al., 2011; Hopkins et al., 2018). This can impact persistence of meta-populations in urban green spaces (Bennie et al., 2015) through negatively impacting reproduction success (Gaston and Bennie, 2014). Further, limited genetic exchange between rural and urban habitats can lead to evolutionary differentiation of urban and rural populations with the potential to create new species (Hopkins et al., 2018).

Much recent attention has been paid to light pollution and its manifold and complex impacts on individuals, populations and ecosystems. Our study demonstrates, firstly, that there is a behavioural change in urban population with high potential for adaptation, but with further need to investigate in long-term rearing experiments and in the physiological underlying mechanism; and secondly, that this behavioural change could negatively affect foraging capacity, dispersal and reproduction if lower activity levels are fully confirmed of urban settlers (Lenski, 1984; Hopkins et al., 2018). Previous research shows that rodents have reduced mobility or reduced foraging activity when exposed to increased illumination in open habitats (Kotler, 1984; Bird et al., 2004), but research regarding foraging success in insect under illumination is lacking.

In summary, light pollution can function as an agent of selection (Eisenbeis et al., 2006; Tuomainen and Candolin, 2011) and can shape the behavioural responses towards ALAN in beetle communities. Nonetheless, light pollution is still on the rise in both intensity and in its distribution around the globe (Kyba, 2018; Falchi et al., 2019), highlighting the need for action to develop lightning strategies that minimize the adverse ecological impacts. For example by preventing dark areas from being artificially lit, reduce trespassing of light sources, only illuminating surfaces intended to (by adjusting angle of light, baffles above lamps), changing the spectral composition of used light and further, to raise awareness about the conscious, sustainable use of light in our daily life [see review: Gaston et al. (2012), IDA (2021), Hölker et al. (2010a)]. It is time to turn off the lights.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

VK and JE designed the experimental set up and research question. VK performed the animal trapping, experiments and hence, data collection, and organizing of the database. Both authors performed the statistical analyses and contributed to discussion, manuscript revision, read, and approved the submitted version.

FUNDING

This study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 491466077. This study was part of the project "rural-urban coupling" [Ministry for Education and Science BMBF (Bridging in Biodiversity Science (BIBS), package "Rural-urban coupling," funding number 01LC1501B)].

ACKNOWLEDGMENTS

We would like to thank the Biological Station Gülpe of the University of Potsdam for housing us and the carabid beetles and of course, the colleges of the research group "Animal Ecology" at the University of Potsdam. Additionally, we like to express our gratitude to Jens Esser and Michael Schmitt for their Coleoptera expertise, also Sascha Buchholz and Christopher Kyba for support during study planning.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.751288/full#supplementary-material

REFERENCES

Altermatt, F., and Ebert, D. (2016). Reduced flight-to-light behaviour of moth populations exposed to long-term urban light pollution. *Biol. Lett.* 12:20160111. doi: 10.1098/rsbl.2016.0111

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.

Bennie, J., Duffy, J. P., Davies, T. W., Correa-Cano, M. E., and Gaston, K. J. (2015). Global trends in exposure to light pollution in natural terrestrial ecosystems. *Remote Sens.* 7, 2715–2730.

Bird, B. L., Branch, L. C., and Miller, D. L. (2004). Effects of coastal lighting on foraging behaviorof beach mice. *Conserv. Biol.* 18, 1435–1439. doi: 10.1111/j.1523-1739.2004.00349.x

Cinzano, P., Falchi, F., and Elvidge, C. D. (2001). The first world atlas of the artificial night sky brightness. *Mon. Not. R. Astron. Soc.* 328, 689–707. doi: 10.1046/j.1365-8711.2001.04882.x

Dacke, M., Baird, E., Byrne, M., Scholtz, C. H., and Warrant, E. J. (2013). Dung beetles use the Milky Way for orientation. *Curr. Biol.* 23, 298–300. doi: 10.1016/j.cub.2012.12.034

Davies, T. W., Bennie, J., and Gaston, K. J. (2012). Street lighting changes the composition of invertebrate communities. *Biol. Lett.* 8, 764–767. doi: 10.1098/rsbl.2012.0216

Eccard, J. A., Scheffler, I., Franke, S., and Hoffmann, J. (2018). Off-grid: solar powered LED illumination impacts epigeal arthropods. *Insect Conserv. Divers.* 11, 600–607. doi: 10.1111/icad.12303

Eisenbeis, G., Rich, C., and Longcore, T. (2006). "Artificial night lighting and insects: attraction of insects to streetlamps in a rural setting in Germany," in *Ecological Consequences of Artificial Night Lighting*, Vol. 2, eds G. C. Rich and T. Longcore (Washington, DC: Island Press), 191–198.

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C., Elvidge, C. D., Baugh, K., et al. (2016). The new world atlas of artificial night sky brightness. *Sci. Adv.* 2:e1600377.

Falchi, F., Furgoni, R., Gallaway, T. A., Rybnikova, N. A., Portnov, B. A., Baugh, K., et al. (2019). Light pollution in USA and Europe: the good, the bad and the ugly. *J. Environ. Manage.* 248:109227. doi: 10.1016/j.jenvman.2019.06.128

Frank, K. D., Rich, C., and Longcore, T. (2006). "Effects of artificial night lighting on moths," in *Ecological Consequences of Artificial Night Lighting*, eds C. Rich and T. Longcore (Washington, DC: Island Press), 305–344.

Gaston, K. J., and Bennie, J. (2014). Demographic effects of artificial nighttime lighting on animal populations. *Environ. Rev.* 22, 323–330.

Gaston, K. J., Davies, T. W., Bennie, J., and Hopkins, J. (2012). Reducing the ecological consequences of night-time light pollution: options and developments. *J. Appl. Ecol.* 49, 1256–1266. doi: 10.1111/j.1365-2664.2012.02212.x

Grubisic, M., van Grunsven, R. H., Kyba, C. C., Manfrin, A., and Hölker, F. (2018). Insect declines and agroecosystems: does light pollution matter? *Ann. Appl. Biol.* 173, 180–189.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., et al. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One* 12:e0185809. doi: 10.1371/journal.pone.0185809

Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C. C., Henckel, D., et al. (2010a). The dark side of light: a transdisciplinary research agenda for light pollution policy. *Ecol. Soc.* 15:13.

Hölker, F., Wolter, C., Perkin, E. K., and Tockner, K. (2010b). Light pollution as a biodiversity threat. *Trends Ecol. Evol.* 25, 681–682. doi: 10.1016/j.tree.2010.09.007

Hopkins, G. R., Gaston, K. J., Visser, M. E., Elgar, M. A., and Jones, T. M. (2018). Artificial light at night as a driver of evolution across urban–rural landscapes. *Front. Ecol. Environ.* 16:472–479. doi: 10.1002/fee.1828

IDA (2021). *International Dark-Sky Association: Light Pollution Sollution*. Available Online at: https://www.darksky.org/light-pollution/light-pollution-solutions/ [Accessed November 1, 2021]

Ineichen, S., and Rüttimann, B. (2012). Impact of artificial light on the distribution of the common European glow-worm, Lampyris noctiluca (*Coleoptera*: *Lampyridae*). *Lampyrid* 2, 31–36.

Jankielsohn, A. (2018). The importance of insects in agricultural ecosystems. *Adv. Entomol.* 6, 62–73.

Kehoe, R., Frago, E., and Sanders, D. (2021). Cascading extinctions as a hidden driver of insect decline. *Ecol. Entomol.* 46, 743–756. doi: 10.1111/een.12985

Kielhorn, K.-H. (2005). "Rote liste und gesamtartenliste der laufkäfer (*Coleoptera*: *Carabidae*) von Berlin," (Hrsg.) in *Der Landesbeauftragte für Naturschutz und Landschaftspflege/Senatsverwaltung für Stadtentwicklung* Berlin: Rote Listen der gefährdeten Pflanzen und Tiere von Berlin CD-ROM.

Kotler, B. P. (1984). Risk of predation and the structure of desert rodent communities. *Ecology* 65, 689–701. doi: 10.1007/s002449900150

Kyba, C. C. (2018). Is light pollution getting better or worse? *Nat. Astron.* 2, 267–269.

Lenski, R. E. (1984). Food limitation and competition: a field experiment with two Carabus species. *J. Anim. Ecol.* 53, 203–216. doi: 10.2307/4352

Lindroth, C. H., and Bangsholt, F. (1985). *The Carabidae-Coleoptera-Of Fennoscandia and Denmark*. Leiden: Brill Archive.

Longcore, T., and Rich, C. (2004). Ecological light pollution. *Front. Ecol. Environ.* 2:191–198.

Owens, A. C., and Lewis, S. M. (2018). The impact of artificial light at night on nocturnal insects: a review and synthesis. *Ecol. Evol.* 8, 11337–11358. doi: 10.1002/ece3.4557

Owens, A. C., Cochard, P., Durrant, J., Farnworth, B., Perkin, E. K., and Seymoure, B. (2020). Light pollution is a driver of insect declines. *Biol. Conserv.* 241:108259. doi: 10.1016/j.biocon.2019.108259

Powney, G. D., Carvell, C., Edwards, M., Morris, R. K., Roy, H. E., Woodcock, B. A., et al. (2019). Widespread losses of pollinating insects in Britain. *Nat. Commun.* 10:1018. doi: 10.1038/s41467-019-08974-9

Rich, C., and Longcore, T. (2013). *Ecological Consequences of Artificial Night Lighting*. Washington, DC: Island Press.

Sanders, D., Frago, E., Kehoe, R., Patterson, C., and Gaston, K. J. (2021). A meta-analysis of biological impacts of artificial light at night. *Nat. Ecol. Evol.* 5, 74–81. doi: 10.1038/s41559-020-01322-x

Seibold, S., Gossner, M. M., Simons, N. K., Blüthgen, N., Müller, J., Ambarlı, D., et al. (2019). Arthropod decline in grasslands and forests is associated with landscape-level drivers. *Nature* 574, 671–674. doi: 10.1038/s41586-019-1684-3

Sih, A., Ferrari, M. C., and Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change. *Evol. Appl.* 4, 367–387. doi: 10.1111/j.1752-4571.2010.00166.x

Sotthibandhu, S., and Baker, R. (1979). Celestial orientation by the large yellow underwing moth, *Noctua pronuba* L. *Anim. Behav.* 27, 786–800. doi: 10.1016/0003-3472(79)90015-0

Stare, J. (2021). *Light Pollution Map. Deneb Geoinformation Solutions*. Available Online at: https://www.lightpollutionmap.info/ [Accessed July, 30, 2021]

Szaz, D., Horvath, G., Barta, A., Robertson, B. A., Farkas, A., Egri, A., et al. (2015). Lamp-lit bridges as dual light-traps for the night-swarming mayfly, Ephoron virgo: interaction of polarized and unpolarized light pollution. *PLoS One* 10:e0121194. doi: 10.1371/journal.pone.0121194

Szentkirályi, F. (2002). Fifty-year-long insect survey in Hungary: T. Jermy's contributions to light-trapping. *Acta Zool. Academ. Sci. Hung.* 48(Suppl. 1), 85–105.

Thiele, H.-U. (1977). *Carbid Beetles in Their Environment: A Stud on Habitat Selection by Adaptations in Physiology and Behaviour*. Berlin: Springer-Verlag.

Tuomainen, U., and Candolin, U. (2011). Behavioural responses to human-induced environmental change. *Biol. Rev.* 86, 640–657. doi: 10.1111/j.1469-185x.2010.00164.x

Ugolini, A., Melis, C., and Innocenti, R. (1999). Moon orientation in adult and young sandhoppers. *J. Comp. Physiol. A* 184, 9–12. doi: 10.1098/rspb.2005.3199

Van Dijk, T. S., and Den Boer, P. (1992). The life histories and population dynamics of two carabid species on a Dutch heathland. *Oecologia* 90, 340–352. doi: 10.1007/BF00317690

van Langevelde, F., Braamburg-Annegarn, M., Huigens, M. E., Groendijk, R., Poitevin, O., van Deijk, J. R., et al. (2018). Declines in moth populations stress the need for conserving dark nights. *Glob. Change Biol.* 24, 925–932. doi: 10.1111/gcb.14008

Wilson, J. F., Baker, D., Cheney, J., Cook, M., Ellis, M., Freestone, R., et al. (2018). A role for artificial night-time lighting in long-term changes in populations of 100 widespread macro-moths in UK and Ireland: a citizen-science study. *J. Insect Conserv.* 22, 189–196. doi: 10.1007/s10841-018-0052-1

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