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Restless roosts: Light pollution affects behavior, sleep, and physiology in a free-living songbird

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Abstract

The natural nighttime environment is increasingly polluted by artificial light. Several studies have linked artificial light at night to negative impacts on human health. In free-living animals, light pollution is associated with changes in circadian, reproductive, and social behavior, but whether these animals also suffer from physiologic costs remains unknown. To fill this gap, we made use of a unique network of field sites which are either completely unlit (control), or are artificially illuminated with white, green, or red light. We monitored nighttime activity of adult great tits, *Parus major*, and related this activity to within-individual changes in physiologic indices. Because altered nighttime activity as a result of light pollution may affect health and well-being, we measured oxalic acid concentrations as a biomarker for sleep restriction, acute phase protein concentrations and malaria infection as indices of immune function, and telomere lengths as an overall measure of metabolic costs. Compared to other treatments, individuals roosting in the white light were much more active at night. In these individuals, oxalic acid decreased over the course of the study. We also found that individuals roosting in the white light treatment had a higher probability of malaria infection. Our results indicate that white light at night increases nighttime activity levels and sleep debt and affects disease dynamics in a free-living songbird. Our study offers the first evidence of detrimental effects of light pollution on the health of free-ranging wild animals.

KEYWORDS
activity, artificial light, great tit, haptoglobin, malaria, oxalic acid, telomeres

1 | INTRODUCTION

Artificial lighting has transformed the global nighttime environment, which may have widespread consequences to humans and wildlife, including impacts on health and well-being (Dominoni, Borniger, & Nelson, 2016; Navara & Nelson, 2007; Zinsstag, Schelling, Waltner-Toews, & Tanner, 2011). An emerging, key issue is how to maximize the benefits of artificial light, for example, increased visibility, while minimizing the negative consequences. With the development of LED lighting that increases our flexibility to apply different light spectra, it might be possible to decrease the negative impacts of artificial lighting. However, this advance is hampered by the lack of information on the direct effects of light color on the behavior and physiology of free-living organisms. Thus, there is an urgent need to integrate research across multiple disciplines to fill in these gaps in our understanding and generate data that can be translated into policy and practice.

One reason that light pollution may have such a profound impact on organismal function is because light at night disrupts circadian and circannual timing (Dominoni, Helm, Lehmann, Dowse, & Partecke, 2013). In birds, artificial light affects the perceived photoperiod (Farner, 1964), changing their behavior (Dawson, 1998), and

ultimately their fitness (de Jong et al., 2015; Spoelstra & Visser, 2013). Compared to conspecifics in darker areas, free-living blackbirds, *Turdus merula*, in lit areas perceive a longer subjective day and extend activity further into the night (Dominoni & Partecke, 2015; Russ, Rüger, & Klenke, 2014). Studies in a laboratory setting show that birds exhibit restless behavior when exposed to light at night (De Jong, Jeninga et al., 2016; Yorzinski et al., 2015), but individuals in the wild may be able to avoid light exposure (de Jong, Ouyang, van Grunsven, Visser, & Spoelstra, 2016). Recently, a study showed that female great tits, *Parus major*, exposed to a light inside their nest box, spent a greater portion of the night awake than controls (Raap, Pinxten, & Eens, 2015). Lastly, these restless behaviors may translate into other behaviors, that is, dawn and dusk singing times are also altered by artificial light in the field (Da Silva, Valcu, & Kempenaers, 2015; Kempenaers, Borgstrom, Loes, Schlicht, & Valcu, 2010; Miller, 2006).

While artificial light affects circadian and circannual biology, the consequences on an individual's health and fitness under actual conditions remain unknown. We found that great tits roosting in white light have higher stress hormone concentrations (Ouyang et al., 2015), but it is uncertain if the cause is due to changes in behavior or the result of a direct physiological effect. Glucocorticoids, like those found to be elevated in the great tits, can be immunosuppressive (Nelson, Demas, Klein, & Kriegsfeld, 2002). Such a hormonal mechanism may help to explain why chronic exposure to dim light at night in laboratory hamsters suppresses the immune system (Bedrosian, Fonken, Walton, & Nelson, 2011). Another study on nestling great tits showed that two nights of artificial light increased haptoglobin and decreased nitric oxide levels, suggesting that artificial light could be increasing oxidative stress (Raap, Casasole, Pinxten, & Eens, 2016). To move forward, potential physiologic costs due to light pollution must be better quantified. Only then can mitigation measures, such as using alternative spectra, be properly implemented.

We made use of a unique network of field sites that are either completely unlit (control), or are artificially illuminated with white, green, or red light (Spoelstra et al., 2015). We measured nighttime activity in free-living great tits roosting in these areas and related their nighttime behavior to within-individual changes in physiology from March to May. Because restlessness by increased nighttime activity is expected to interfere with normal sleep patterns, we measured plasma concentrations of oxalic acid, a recently established cross-species marker of sleep restriction (Weljie et al., 2015). We also quantified acute phase protein concentration (Matson, Horrocks, Versteegh, & Tieleman, 2012) and malaria infection status (De Jong, Fokkema, Ubels, Van Der Velde, & Tinbergen, 2014; Piersma & van der Velde, 2012) as indices of immune function, and telomere length as an overall measure of metabolic costs and aging (Haussmann & Marchetto, 2010). In line with previous findings that link nighttime exposure to white light led to increased stress hormone concentrations and decreased immune function (Bedrosian et al., 2011; Ouyang et al., 2015), we predicted that great tits roosting closer to lampposts emitting white light would have higher activity at night, decreased haptoglobin concentration, and a higher probability of malaria infection, all leading to decreased immune function and accelerated biologic aging. We expect that birds roosting in nest boxes shielding them from ambient light show attenuated responses, and hypothesize that red and green light treatments can potentially reduce these costs (Ouyang et al., 2015). We hope that our data on physiologic change within individuals and their roosting behavior under different light spectra can be used to inform management decisions to limit the impact of artificial light at night.

2 | MATERIALS AND METHODS

2.1 | Study sites and standard protocols

Since early 2012, we illuminated eight, previously dark natural sites with street lamps (8.2 ± 0.3 SEM lux at ground level beneath the light posts) every night from sunset until sunrise. Each site contained four, 100 m long transects with five 4 m tall lampposts; along each transect lamps emanated either red, white or green light or no light (i.e., dark control treatment; see Spoelstra et al., 2015 for detailed field design). The order of the transects was randomly assigned per site. Green lamps emit less red and more blue light; red lamps emit less blue and more red light. As these lights are eventually intended for civil use, light intensity is normalized to lux for all three colors. Nine nest boxes were placed in each transect (see de Jong et al., 2015; 39% box occupancy). Adult birds ($n = 44$) were caught in March 2014 with mist nets at our sites. Following a blood sample, these birds were banded with a uniquely numbered aluminum ring and equipped with a radio transmitter (Biotrack Ltd, Dorset, UK) weighing 0.42 g. We glued this transmitter to the back of each bird using vetbond and eyelash glue (Ardell LashGrip transparant). In May, adults were recaptured in the nest box (81% recapture rate, Table 1). Recapture occurred on day 10, 11, or 12 of chick rearing using a spring trap. Blood samples were always collected within 3 min (mean ± *SE*: 1.1 ± 0.2 min) and placed on ice. All blood samples were taken from 8:00 to 12:00. Samples were separated into two aliquots: whole blood in telomere buffer and remaining samples were spun for 10 min at 16,000 *g* within 5 hr of collection; samples were frozen at −80°C until analysis.

2.2 | Activity measurements

We conducted pilot studies to validate the radio transmitter system for assessment of nighttime activity on four captive individuals in

TABLE 1 Sample size for the great tits caught separated by treatment and sex. Numbers in parentheses indicate number of recaptured individuals during breeding

	Treatment			
	Dark	Green	Red	White
Female	4 (3)	4 (3)	6 (6)	5 (4)
Male	5 (4)	5 (3)	7 (7)	6 (4)
Total	9 (7)	9 (6)	13 (13)	11 (8)

February 2014. These individuals were kept in a large (5 × 5 m) outdoor aviary, equipped with radio transmitters, and we used live behavioral observations and video recordings to determine a threshold for when a bird was active. The receiver (DataSika B, Biotrack Ltd, Dorset) logs the amplitude of predefined radio tag frequencies. In these preliminary tests, an amplitude difference of >10 dB between consecutive beeps was always a result of movement. This amplitude change can be induced by small movements, such as a change in angle of the radio tag antenna changes toward the receiver, and by larger movements like the bird flying away to another location. The 44 free-living individuals tagged in March were first manually tracked with Yagi antennas. We determined the precise roosting location and calculated the distance to the closest lamppost for each of the three consecutive nights. If birds roosted in nest boxes, they could easily be found by the clear signal increase when the receiver antenna was pointed to the nest box (from all angles). During these nights, birds were subsequently continuously monitored with two DataSika receivers, which scanned for 8 s per frequency (four birds simultaneously). Each receiver covered a circular area with a radius of 200 m, and which were installed such that the entire study area was covered. Treatment was assigned as the color of the closest light post during these nighttime roosts, and in all cases but one, birds also nested in the same treatment. We only included birds that roosted <160 m from the nearest light post (thereby excluding two birds). Activity of each individual was binned into 2-min intervals. A value of 1 (active) was assigned if there were two or more consecutive pulses in an interval with an amplitude difference of >10 dB, otherwise value 0 (inactive) was assigned. If no pulses were received, during these 2 min, the bin value was set to −1 (missing data). For analysis of activity at night, we took the sum of all active 2-min bins between civil dusk and dawn. Activity was plotted using CHRONOSHOP 1.1 (written by KS; Figure 1). We only included a bird 1) for which we received signals throughout the night, 2) when the bird also showed clear activity at the end of the previous evening and the beginning of the next morning, and 3) when we had determined the exact roost location using handheld telemetry receivers.

2.3 | Haptoglobin quantification

Haptoglobin is an acute phase protein that is produced in response to inflammation, infection, or trauma. We quantified haptoglobin concentrations (mg/ml) in 7 μl plasma samples using a commercially available kit (TP801; Tri-Delta Diagnostics, NJ, USA), which colorimetrically quantifies heme-binding capacity. We followed the "manual method" instructions from the manufacturer with minor modifications following Matson et al. (2012).

2.4 | Blood parasite screening

We extracted DNA from blood samples using an ammonium acetate protocol (Richardson, Jury, Blaakmeer, Komdeur, & Burke, 2001). With the primers HaemNFI and Haem NR3 (PCR product ±580 bp) and HaemF and Haem R2 (PCR product ±530 bp), we ran a nested PCR to amplify part of the cytochrome b gene of *Plasmodium* and *Haemoproteus* (Hellgren, Waldenström, & Bensch, 2004). Final PCR products (5 μl) of all samples were run with positive and negative controls on a 2% agarose gel. When a ± 530 bp band was visible in a gel lane, the corresponding individual was scored as infected; when this band was absent, the individual was scored as uninfected. Repeatability of this technique in this laboratory is high (ca. 94%–96%; Piersma & van der Velde, 2012; De Jong et al., 2014). As a quality control of our extracted DNA, samples were also analyzed using a PCR protocol designed to amplify the CDH gene on the sex chromosomes (Griffiths, Double, Orr, & Dawson, 1998). Samples that showed one (male) or two (female) bands were assumed to be of sufficient quality for use in the parasite screening protocol (Piersma & van der Velde, 2012). One sample for which no sexing bands were visible was assumed to be insufficient for parasite screening.

2.5 | Oxalic acid analyses

Plasma concentrations of oxalic acid were measured with a commercially available colorimetric assay kit following the instructions provided by the manufacturer (Biovision Inc., Milpitas, CA, USA). Briefly, 3 μl plasma samples were diluted with assay buffer to a total volume of 50 μl. Samples to generate a standard curve were included in the same assay. After adding reaction mix, experimental samples and standard samples were incubated at 37^{0}C for 1 hr. Then, absorbance (450 nm) was measured using a spectrophotometer. In addition, we also validated oxalic acid measurements as a measure of sleep debt in birds by analyzing samples from a previous experiment (De Jong, Jeninga et al., 2016). We show that laboratory birds exposed to 5 lux of light at night have a significant decrease in oxalic acid levels compared to exposure in less intense light at night (supplementary materials).

2.6 | Telomere analyses

DNA from whole blood was extracted (Gentra Purgene Blood Kit), digested with proteinase K, followed by restriction digestion with 15 U of HinfI, 75 U of HaeIII, and 40 U of RsaI at 37°C. Digested DNA samples (10 ug) were loaded into a 0.8% nondenaturing agarose gel. Early optimization gels showed that great tit telomere length ranges from 1 to 220 kb (Molecular markers: 1 kb Extend DNA Ladder (1–48.5 kB); MidRange I PFG marker (15–242.5kB); New England Biolabs, Ipswich, MA, USA). However, analysis of different molecular length windows across the entire range of telomere lengths showed that there is very little individual variation in telomere length above 40 kb. Therefore, we separated DNA using pulsed field gel electrophoresis to best resolve telomere lengths between 1 and 40 kb (3 V/cm, 0.5–7.0 s switch times, 14°C, 19 hr). Electrophoresis was followed by in-gel hybridization at 37°C overnight with a radioactive-labeled telomere-specific oligo $(CCCTAA)_4$. Hybridized gels were placed on a phosphorscreen (Amersham Biosciences, Buckinghamshire, UK), which was scanned on a Storm 540 Variable Mode

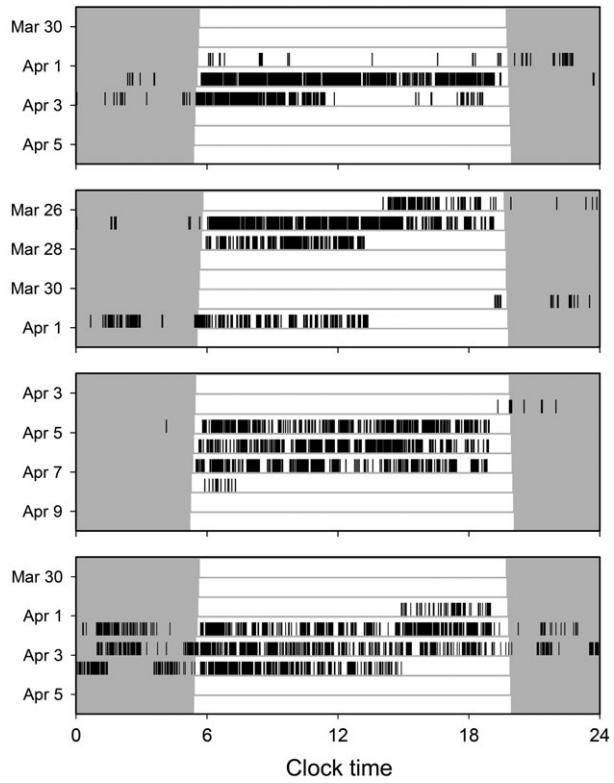

FIGURE 1 Example actograms. Four individuals were selected randomly and roosted at <25 m from the closest lamppost in (a) dark, (b) green, (c) red, and (d) white treatments

Imager (Amersham Biosciences). We used densitometry (ImageQuant 5.03v and ImageJ 1.42q) to determine the position and strength of the radioactive signal in each of the lanes compared to the molecular marker. The background was fixed as the nadir of the low MW region on the gel (<1 kb). Mean telomere length was calculated in the range of 1–40 kb (the limits of our molecular marker). Intra- and inter-gel coefficients of variation were, respectively: 2.6% and 3.4%.

2.7 | Statistical analyses

Statistical analyses were conducted using R (version 3.1.2). Body condition was estimated as a scaled mass index (Peig & Green, 2009), using each individual's body mass and tarsus. We initially included age of the individual (1-year old or >1-year old) in all models, but this factor was never significant we removed it from all analyses. We used generalized linear models with a Poisson distribution to test if total activity bouts (i.e., total number of active 2-min bins) per night differed by *treatment* and roosting *distance* to the nearest lamppost. We included *date* as a covariate. As some birds during some nights roosted in nest boxes, whereas on other nights roosted on tree branches (two-level variable *roost type*), we started by testing the interaction of *treatment*distance*roost type*. If this three-way interaction was significant, we separated the analysis for nights that an individual roosted on a tree and nights when they roosted inside a nest box. Individual identity and site were included as random effects. We tested if nighttime activity levels differed by treatment and the interaction term with a Tukey posthoc test. Repeatability in nighttime activity was calculated from the within- and between-variance components of a linear mixed effects model (LMM) using the restricted maximum-likelihood method (REML) and with bird identity as the random factor (Lessells & Boag, 1987; Nakagawa & Schielzeth, 2010). We used the package RPTR developed and explained in Nakagawa and Schielzeth (2010) to calculate repeatabilities. Briefly, confidence intervals and standard errors were calculated from parametric bootstraps that created distributions of likelihood ratios (1,000 times). We analyzed whether physiological change (May values–March values) for quantitative physiologic variables (i.e., malaria presence or absence was calculated for March and May separately) were related to activity levels using a LMM with individual and site as random effects. The presence or absence of malaria was analyzed in a GLMM with binomial errors, haptoglobin, oxalic acid, and telomere lengths were analyzed using normal errors. We included *sex* and *body condition* (measured as the residuals of a regression of mass and tarsus). Lastly, to analyze a component of fitness, we tested if number of fledglings was related to change in physiologic parameters and activity levels with a LMM with individual and site as random effects. All final models met assumptions and significance was taken at $\alpha = 0.05$.

3 | RESULTS

3.1 | Nighttime activity

Nighttime activity levels were highly repeatable ($R = 0.58$, CI (0.39, 0.72), $p < .0001$). There were significant effects on nighttime activity of *treatment*, *distance*, *roost type*, and their three-way interaction ($F_{8, 74.8} = 11.99$, $p < .0001$). Individuals that roosted on tree branches had more bouts of activity than individuals roosting in nest boxes (coef = 0.2, $SE = 0.06$, $z = 3.13$, $p = .001$). We note that all birds roosted on tree branches at least one of the nights of study, but the frequency was independent of light treatment ($p > .05$). Therefore, we analyzed the tree-roosting and the nest box roosting individuals separately. For the individuals roosting in nest boxes, there were no significant effects of treatment and distance to the closest lamppost (both $p > .4$). However, for the individuals roosting on the tree branches, there was a significant effect of light treatment and a significant interaction between light treatment and distance to the closest lamppost (Table 2, Figure 2a).

Posthoc tests of the main effect of treatment showed that birds in white light had more activity bouts during the night than birds in the red ($p = .001$), dark ($p = .01$), and green treatments ($p = .002$; Figure 2b). The effect of roosting distance to the closest lamppost on activity was significantly different between the white and red (estimate = −0.7, $SE = 0.26$, $p = .038$) and white and dark treatments (estimate = −0.5, $SE = 0.18$, $p = .018$). Individuals roosting in white light were more active closer to the lamppost than farther away, and this slope was steeper than the slope of the dark treatment (for which, as expected, there was no effect of distance on activity levels). Individuals roosting in red light also had a steeper negative

slope than in the white light, which means that the individuals roosting in red closer to the lamppost had higher activity than farther away. Individuals roosting in the green treatment did not differ in slope from red, white, and dark treatments. Day-time activity levels did not differ by treatment and distance to the closest lamppost (all $p > .05$). Sex, body condition, and date were insignificant in all models (including those on physiologic traits).

3.2 | Acute phase protein and blood parasites

Individuals in better body condition, that is, heavier mass corrected for tarsus length, also had higher haptoglobin concentrations (coef = 6.66, $SE = 1.71$, $t = 3.89$, $p < .0001$). The change in haptoglobin concentrations from March to May was unrelated to nighttime activity bouts or light treatment ($p = .9$).

The probability of malaria infection in March, but not in May, was higher for birds roosting in white light than in other treatments (coef = 21.6, $SE = 9.7$, $z = 2.22$, $p = .02$). Infection probability in both March and May was unrelated to nighttime activity levels and roosting distance from the closest lamppost (both $p > .1$).

3.3 | Oxalic acid

The change in oxalic acid levels between March and May was negatively related to March nighttime activity levels (coef = −0.001, $SE = 0.00$, $z = -2.07$, $p = .038$). In other words, birds that had the highest activity levels at night showed the strongest reduction in oxalic acid levels from March to May (Figure 3).

3.4 | Telomere dynamics and reproductive success

The change in telomere lengths from March to May did not relate to nighttime activity levels nor to treatment (all $p > .2$). Fledgling success for the 34 recaptured birds did not differ by light treatments or activity levels. Fledgling success was also not related to differences between March and May in telomere lengths or oxalic acid levels (both $p > .05$). However, individuals that increased their haptoglobin concentration from March to May fledged more young (coef = 9.56, $SE = 2.53$, $t = 3.77$, $p = .0002$) than those that decreased their haptoglobin concentrations from March to May, regardless of treatment. In addition, individuals that tested positive for malaria in May but not in March fledged more young (coef = 2.06, $SE = 0.8$, $t = 2.57$, $p = .01$), regardless of treatment.

4 | DISCUSSION

We measured the effect of different light spectra on nighttime behavior and physiologic change for the first time in a free-living songbird. We showed that with exposure to white light, nighttime activity was twice as high compared to other treatments. Moreover, the most active individuals showed the strongest reduction in oxalic acid levels from March to May. In the white light treatment, individuals were more likely to be infected with malaria. Lastly, fledgling success was unrelated to light treatment but correlated with changes in parental infection status.

TABLE 2 Model estimates for the effect of light treatment on nighttime activity bouts. All data are from individuals that roosted on tree branches, that is, outside of nest boxes. Individual estimates are given from summary statistics of the GLMM ($df = 108$, Log likelihood = −974.2). Random effects include individual ID ($n = 42$ over three nights) and site

Variable	*df*	*F*	Estimate	*SE*	*p* Value
Treatment					
Dark	7, 69.2	2.29	2.19	0.62	.030
Green			4.02	0.78	
Red			3.98	0.61	
White			4.00	0.61	
Treatment: distance to lamppost					
Dark: distance to lamppost	3, 59.4	4.18	0.004	0.002	.013
Green: distance to lamppost			−0.019	0.004	
Red: distance to lamppost			−0.013	0.003	
White: distance to lamppost			−0.002	0.001	
Distance to lamppost			0.001	0.003	.679
Date	1, 149	0.46			.496

We provide the first and only evidence of differences in nighttime activity levels in free-living songbirds as a result of artificial light spectra. When we located birds at night in March, we found individuals to be relatively consistent in their roost location over three consecutive nights, hence staying within the same treatment, in the proximity of their nest. Their consistent roosting choices indicate that birds tend to stay in the same area, affected by the light treatment throughout the breeding season. We found differences in the behavior of birds roosting in nest boxes versus on tree branches. The nest box offers shelter from external light sources: inside a nest box, light levels are nearly zero Lux at night. Activity of birds roosting on tree branches was highest in the white light treatment. The increased nightly activity due to light aligns with the observation that great tits exposed to a light source inside the nest box show disturbed sleep (Raap et al., 2015). As birds were located at the same spot during consecutive nights, and there was a high repeatability between these nights, this activity is most likely caused by increased vigilance and restlessness while perched, rather than by birds flying around. The increased activity rates, which suggest birds are active throughout the night, might relate to increased predator vigilance or direct disturbance (Russ et al., 2014). In addition, individuals that roosted closer to the lampposts in red light were more active than those that roosted farther away. This inverse relationship between activity and distance to red light is corroborated by a previous study in the same network of field sites. In that study, stress hormones of individuals in the red light treatment increased with

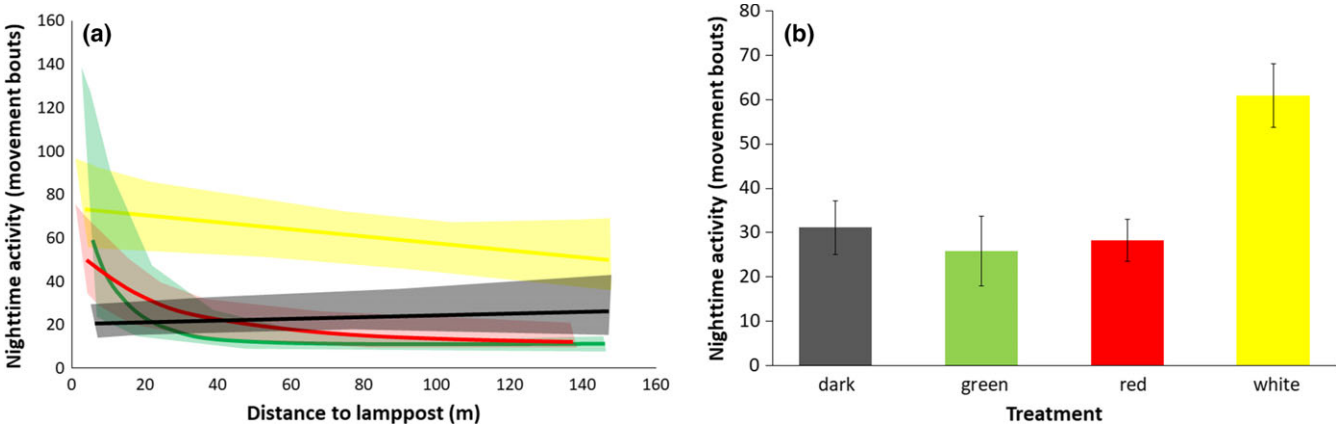

FIGURE 2 Nighttime activity of birds roosting in artificial light. (a) Predictions from a GLMM model of nighttime activity in great tits roosting in tree branches at difference distances from the closest lamppost under four different light colors. Shown are model prediction eans and 95% CI shaded. (b) Mean nighttime activity bouts for different light treatments. Mean ± 1 *SE*. To avoid the effect of distance, shown are birds that roosted within 25 m to the nearest lamppost. Note that each bird's activity was measured for three nights. Number of birds within 25 m of the lamppost: dark = 5, red = 8, white = 6, green = 5

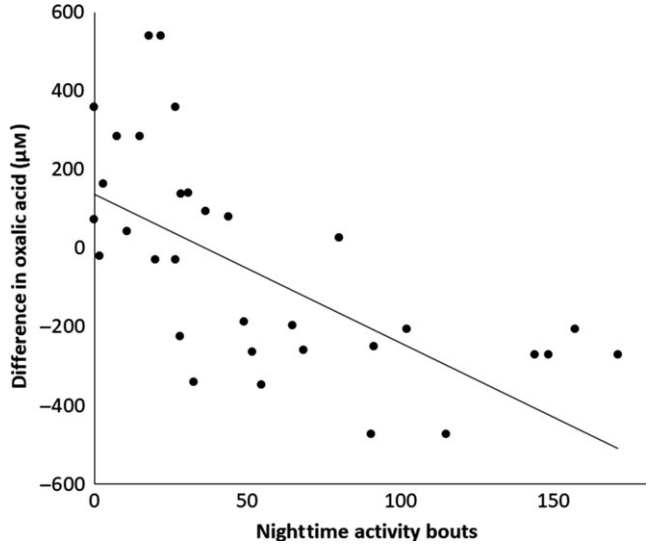

FIGURE 3 Change in oxalic acid levels (May–March) in relation to nighttime activity bouts. Individuals that had higher oxalic acid levels in May compared to their March levels had lower activity in March. $R^2 = 0.36$, $y = -3.8x + 136$

decreasing distance from the light source (Ouyang et al., 2015). We note that high nighttime activity did not result in birds being less active during the day. Therefore, light treatment only affected nighttime activity levels.

Decreases in oxalic acid concentrations have recently been used as biomarker for sleep debt (Weljie et al., 2015). In both rats and humans, sleep restriction was associated with a drop in plasma levels of oxalic acid. We measured oxalic acid concentrations for the first time in a free-living bird species and found that birds with the highest activity at night showed the strongest decrease in oxalic acid concentrations from March to May. These results support the view that birds with the highest nighttime activity have the strongest disruption of sleep. The finding that nighttime activity levels in March correlated with the change in oxalic acid levels from March to May can indicate that the degree of sleep disruption accumulated over a long period. This study occurred over lengthening days, and the sleep debt can accumulate in a more pronounced way during the breeding season than over winter. Natural patterns of nightly activity expressed by animals over time are currently unknown. For example, females may be accruing sleep debt over the breeding season (Steinmeyer, Schielzeth, Mueller, & Kempenaers, 2010), but we point out that oxalic acid decrease occurred for both sexes and only for birds with increased nighttime activity levels. Birds with high nighttime activity are likely to be lacking sleep, which may have continued to build up during April and May. This conclusion seems to be the most parsimonous explanation, and we corroborate this finding by showing that oxalic acid levels do decreased in controlled laboratory settings when birds were deprived of sleep (supplementary materials).

We assumed that this accumulation of sleep debt would also lead to decreases in telomere lengths as a sign of cellular aging; however, we did not find a difference in telomere loss among the treatment groups. In a recent study of great tits, cross-fostered nestlings from rural to urban areas, possibly exposed to more artificial light, had more telomere loss (Salmón, Nilsson, Nord, Bensch, & Isaksson, 2016). It could be that most telomere loss occurs early in life (Boonekamp, Mulder, Salomons, Dijkstra, & Verhulst, 2014) and the effect on adults, measured over our experimental period, is not as pervasive. Moreover, nestlings are stationary and adults are able to freely move as a possible counter to the negative effects of artificial light (de Jong, Ouyang et al., 2016). To our knowledge, no studies have looked at telomere loss in adults over a 3-month period. We note that we included age in our initial analyses, but age was categorized to 1-year old or older, and it could be that telomere differences due to age is only visible when the entire age distribution is known (Haussmann & Mauck, 2008). Alternatively, it could be that

higher activity results in other metabolic costs that do not directly translate to telomere shortening.

We acknowledge that individuals are free to choose their roost and nest location based on the light source or other factors. As possible evidence of differential settlement, in March, more individuals tested positive for malaria during March in the white light treatment than in the other groups. Perhaps lower quality individuals (infected) birds are forced to roost in light-polluted areas. Alternatively, the difference in malaria prevalence can be due to differences in host susceptibility or in infection expression due to stress from roosting in the white light treatment (Ouyang et al., 2015). For example, a spring relapse occurs during the breeding season when increased corticosterone concentrations in the host suppress the immune system (Lapointe, Atkinson, & Samuel, 2012). Lastly, this difference can be result of a difference in the distribution of mosquitoes amongst the treatment groups. We have known for a long time that many insects, such as Lepidoptera (Frank, 1988), but also insects of the orders Diptera, Trichoptera, Hemiptera, Coleoptera, and Hymenoptera are known to be attracted to light (van Grunsven et al., 2014; Longcore et al., 2015). Although mosquito attraction has not been well studied in relation to different light spectra, the species that act as malaria vector in western Europe seem to be attracted to light only in the fall (Silver, 2008). Therefore, increased prevalence of malaria is unlikely to be caused by phototaxis of the vector.

There were no differences in fledgling success between the treatment groups (de Jong et al., 2015). However, we show that birds in which haptoglobin concentrations increased from March to May and those that tested positive for malaria in May fledged more young. Such patterns can arise if birds with decreased haptoglobin concentration are suffering from lytic anemia, irrespective of light treatment. The stress of fledgling more young could lead to recurrence of latent infection or allow new infection (Lapointe et al., 2012). Due to malaria infection being higher in white light treatment, there can be indirect consequences on reproductive fitness as a result of anemia.

Our data clearly reveal that under white light birds are restless at night, and this restlessness coincides with decreased oxalic acid levels. In white light, birds also have higher malaria infection. Although the causal mechanisms behind these effects are unknown, we hypothesize that sleep deprivation due to nighttime activity in white light conditions resulted in increased stress levels (Ouyang et al., 2015) and decreased immune function, leading to infection. These behavioral and physiologic changes indicate that white light at night is affecting daily rhythms, resulting in individuals with an altered physiological state. Whether these negative effects also translate into reduced survival in fledglings and adults still needs to be addressed. In the green and red light treatments, the effects on physiology and behavior are dampened, which opens up the possibility of using alternative light spectra as a method to decrease physiologic costs. Whether these costs are the same for other taxa and species still needs to be tested. As governments and agencies become increasingly interested in replacing outdoor lighting with LED options to reduce economic costs, we suggest that managers look into using different LED light spectra to limit negative impacts for wildlife.

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