



Streetlights Disrupt Night-Time Sleep in Urban Black Swans

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Artificial light at night could have widespread and detrimental impacts on sleep. To reduce disruptive effects of artificial light on sleep in humans, most smartphones and computers now have software that reduces blue light emissions at night. Little is known about whether reducing blue light emissions from city lights could also benefit urban wildlife. We investigated the effects of blue-rich (white) and blue-reduced (amber) LED streetlights on accelerometry-defined rest, electrophysiologically-identified sleep, and plasma melatonin in a diurnal bird, the black swan (*Cygnus atratus*). Urban swans were exposed to 20 full nights of each lighting type in an outdoor, naturalistic environment. Contrary to our predictions, we found that night-time rest was similar during exposure to amber and white lights but decreased under amber lights compared with dark conditions. By recording brain activity in a subset of swans, we also demonstrated that resting birds were almost always asleep, so amber light also reduced sleep at night. We found no effect of light treatment on total (24 h) daily rest or plasma melatonin. Our study provides the first electrophysiologically-verified evidence for effects of streetlights on sleep in an urban animal, and furthermore suggests that reducing blue wavelengths of light might not mitigate these effects.

Keywords: accelerometry, artificial light at night, blue light, EEG, electrophysiology, light pollution, light spectra

INTRODUCTION

Over the past 150 years, the proliferation of artificial light at night has become a growing global issue (Hölker et al., 2010; Bennie et al., 2015; Falchi et al., 2016). By disrupting natural light cycles, artificial light can disrupt daily rhythms of behavior and physiology in humans and wildlife (Stevens and Zhu, 2015; Dominoni et al., 2016). Effects on behavior are particularly well-documented for diurnal birds, which often show earlier onset of daily activity (Kempenaers et al., 2010; Dominoni et al., 2013b; Da Silva et al., 2014; de Jong et al., 2017; Spoelstra et al., 2018; Ulgezen et al., 2019) and increased night-time activity (Dominoni et al., 2013a; de Jong et al., 2016, 2017; Ouyang et al., 2017; Alaasam et al., 2018; Ulgezen et al., 2019) when exposed to light at night. These behavioral changes may arise as a direct result of artificial light enhancing the visual environment, leading diurnal animals to forage more at night (Russ et al., 2014) or become more vigilant (Yorzinski et al., 2015). Alternatively, increased night-time activity could be driven by physiological impacts of light at night, including the suppression of melatonin – a hormone that drives endogenous day-night (circadian) rhythms (Dominoni et al., 2013a,b) and promotes sleep in diurnal animals

(Reiter et al., 2010; Gandhi et al., 2015). While increased nocturnal activity under artificial light has been linked with indicators of poorer health (Ouyang et al., 2017; Alaasam et al., 2018), the extent of associated costs remains largely unknown. However, if increased night-time activity reflects decreased sleep (Raap et al., 2015; Yorzinski et al., 2015; Ouyang et al., 2017), then there could be negative consequences for development, memory, learning, health, and fitness (Aulsebrook et al., 2016; Aulsebrook et al., 2018).

One approach to mitigate any negative effects of artificial light is to alter the color of lighting. Amber lights with lower emissions of short, blue wavelengths are broadly predicted to have less impact on wildlife than blue-rich, white lights (Longcore et al., 2018). A putative mechanism underpinning this rationale is that blue wavelengths of light (around 460 to 480 nm) have the most suppressive effect on the production of melatonin (Reiter et al., 2010; Dominoni, 2015). This effect is highly conserved across taxa (Pandi-Perumal et al., 2006; Jones et al., 2015) and may explain many of the disruptive impacts of artificial light on sleep (Aulsebrook et al., 2018). Accordingly, there is evidence that reducing exposure to blue wavelengths in the evening can improve sleep duration and quality in humans (Santhi et al., 2012; Ayaki et al., 2016). By implication, this strategy could also reduce impacts on night-time rest and sleep in diurnal wildlife.

To test this, we compared the effects of two types of streetlights on the behavior and physiology of an urban bird, the black swan (*Cygnus atratus*). The urban population of swans at Albert Park Lake (37°50'S, 144°58'E; Melbourne, VIC, Australia) has been well-studied for the past decade (Guay and Mulder, 2009). Swans in this population are exposed to light at night from streetlights, vehicles, buildings, sporting events, and sky glow. Swans are large and are re-captured relatively easily, making them ideal subjects for research involving repeated deployment of data loggers to record behavior, as well as repeated blood sampling. Although swans are mostly diurnal, they exhibit some flexibility in the timing of their behavior. For example, swans will fly long distances between water bodies at night (Frith, 1982). During breeding, males and females also show marked role division, with males typically incubating eggs during the day (while females presumably forage) and switching roles at night (unpublished data). Such flexibility in the timing of activity could allow swans to shift the timing of their sleep in response to light at night, rather than losing sleep overall. Furthermore, if artificial light suppresses sleep in birds with such flexible activity patterns, we would predict that other, less flexible, urban birds are similarly, if not more, affected.

We experimentally tested whether unfiltered white (blue-rich) and filtered amber (blue-reduced) light emitting diode (LED) streetlights would reduce rest, sleep, and circulating melatonin concentrations in black swans. The effects of ecologically-realistic artificial lighting on sleep are seldom tested; most studies have been on captive or laboratory animals, with relatively few exceptions (Aulsebrook et al., 2018). Although animals exposed to lights inside a cage or nest box show reduced sleep behavior and night-time rest (Raap et al., 2015; Yorzinski et al., 2015; de Jong et al., 2016; Raap et al., 2016; de Jong et al., 2017; Alaasam et al., 2018), it is unclear whether, in real-world,

heterogeneously-lit environments, animals would inhabit such intensely-lit spaces in the first place (Yorzinski et al., 2015; Aulsebrook et al., 2018; Raap et al., 2018; Caorsi et al., 2019). To address these gaps in the literature, we conducted our study in a large, naturalistic, heterogeneously-lit outdoor environment, where birds could limit their exposure to artificial lights by moving to less-lit areas at night.

An additional challenge for previous studies has been measuring sleep in naturalistic contexts (Aulsebrook et al., 2016). Sleep is best quantified by measuring brain activity with the electroencephalogram (EEG). Analysis of the EEG reveals two sleep states in birds and mammals called non-rapid eye movement (non-REM) sleep and REM sleep (Lesku and Rattenborg, 2014). Although advances in technology have facilitated EEG recordings in the wild (Aulsebrook et al., 2016; Rattenborg et al., 2017), studies outside the laboratory remain challenging. We therefore examined whether resting birds were indeed sleeping, or simply sitting quietly awake, by recording the EEG in a subset of swans (see also Lesku et al., 2012).

MATERIALS AND METHODS

Animals and Housing

We captured wild adult black swans (six males, five females) and transported them to Serendip Sanctuary, Lara, Australia (38°0'S, 144°24'E; **Figure 1A**). Swan sex was determined from standard genetic techniques and/or known morphological differences (see **Supplementary Methods**). Nine swans (two pairs, three single males, and two single females) were caught at Albert Park Lake (37°50'S, 144°58'E), and two swans (one pair) were from Altona Beach, Victoria (37°52'S, 144°59'E). Both sites are exposed to light pollution from streetlights, buildings, vehicles, and skyglow. All swans were individually identifiable from metal leg bands (Australian Bird and Bat Banding Scheme) and plastic neck collars as part of an ongoing research project (Guay and Mulder, 2009; Mulder et al., 2010). Breeding and pairing data for most swans were available from monthly censuses conducted at Albert Park Lake. At capture, no swan had a nest, cygnets, or dependent offspring.

At Serendip Sanctuary, all swans were housed together in a large (4,600 m²) naturalistic outdoor enclosure containing native terrestrial vegetation and a 1,470 m² pond, surrounded by a fox-proof fence (**Figure 1B**). Swans were fed poultry crumble (Barastoc Pullet Grower and Chick Starter, Ridley Corporation, Melbourne, Australia) and fresh lettuce daily to supplement foods (grasses) naturally available within the enclosure. To prevent escape, we trimmed the first seven primary feathers of each swan's wings; this allowed for take-off and gliding, but only at low heights. We captured swans fortnightly to ensure their weight was within a healthy range, check for any regrowth of feathers, and trim feathers when necessary. For all experimental procedures, swans were recaptured by herding them into a run that led to a small pen (~6 × 5 m), where they could be caught by hand.

All data were collected between November 2017 and March 2018 (spring and summer). Swans were given at least six

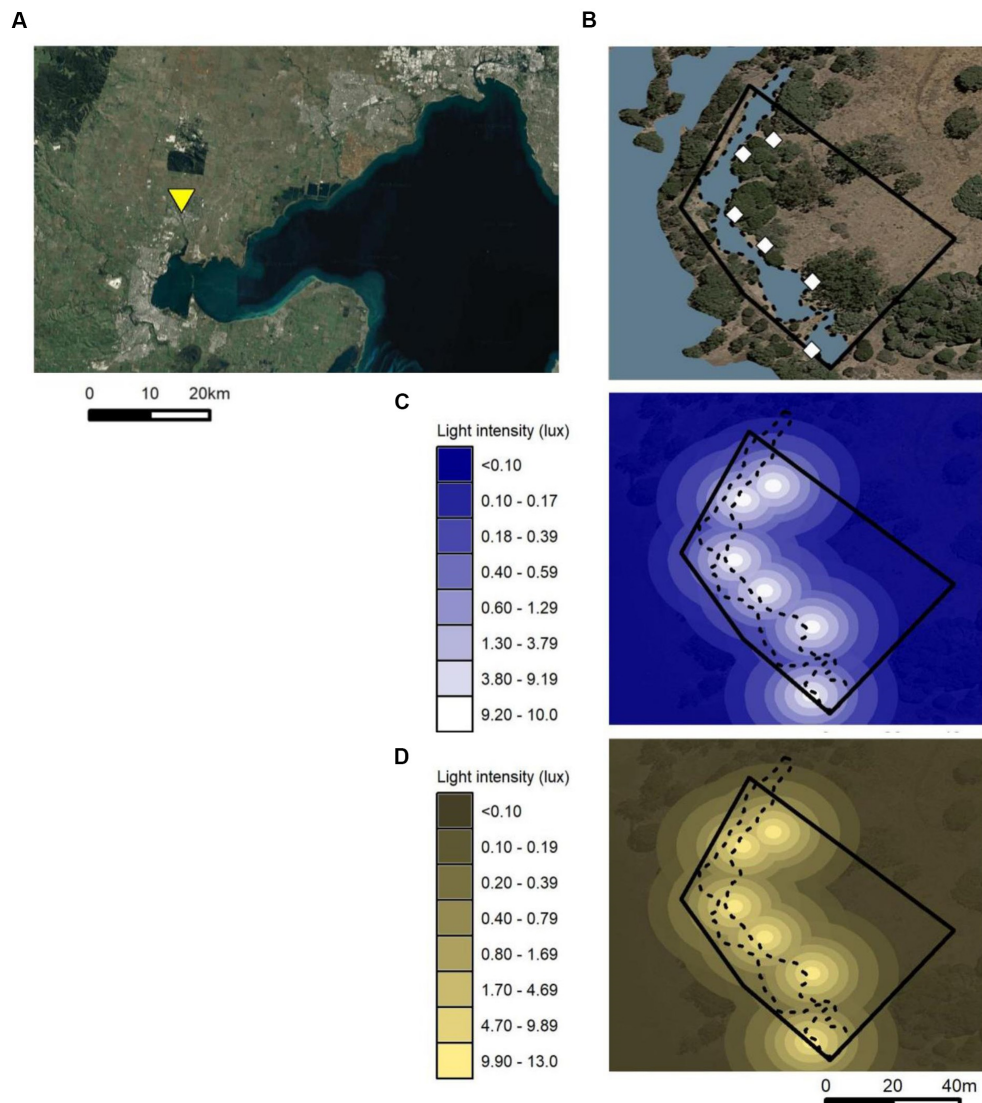


FIGURE 1 | Maps of swan enclosure. **(A)** Map showing location of swan enclosure at Serendip Sanctuary, Lara (38°0'S, 144°24'E; yellow triangle) to the south-west of Melbourne (at the northern end of Port Phillip Bay); **(B)** satellite image showing vegetation, ponds (blue), outer fence (solid black line), and positions of streetlights (white diamonds); **(C)** light intensity with distance from light poles during the white light treatment, and **(D)** during the amber light treatment. Light intensity values do not account for shielding provided by vegetation. Light intensity was measured at ground level facing the light pole using an SKL 30046473 lux meter with sensor SKL 310L 46472 (Skye Instruments, Llandrindod Wells, United Kingdom) on a single night. When facing away from the light, light intensity was similar to moonlight (<0.05 lux) 11 m away from the light pole. Maps were generated using Google Earth and *ggmap* (Kahle and Wickham, 2013).

weeks to acclimate to the enclosure before collecting data or implanting electrodes (see *sleep recordings*). During the acclimation period, swans experienced only minimal light at night (<0.01 lux during new moon; see “Environmental measurements”). After the study concluded, the swans were returned to Albert Park Lake.

Environmental Measurements

Local times for sunrise, sunset, astronomical dawn, astronomical dusk, and lunar phase were obtained from Geoscience Australia¹.

¹<http://www.ga.gov.au/>

Wet bulb temperature data (recorded as an average per minute) were obtained from the Avalon Airport weather station (station reference: 087113; 38°1'S, 144°28'E; Bureau of Meteorology, Australia), ~8 km from the swan enclosure. Light intensity within the swan enclosure was recorded each minute throughout the study using custom-made light loggers (University of Konstanz, Konstanz, Germany; Dominoni et al., 2014; Table B1), each calibrated against a lux meter (SKL 30046473 with sensor SKL 310L 46472, Skye Instruments, Llandrindod Wells, United Kingdom) during evening twilight. We chose to measure light in lux, despite this measure being based on human vision, since human perspectives regarding illumination

are commonplace for lighting design and urban planning (Gaston et al., 2013). However, it is important to recognize that these units do not necessarily represent what would be perceived by the birds.

Light Treatments

Artificial light at night was manipulated by installing six LED streetlights (IP66 LEDway® SLM™ Streetlight 4,000 K, Ruud Lighting, Racine, WI, United States) mounted on steel poles ($3 \times 3 \times 196$ cm; see **Supplementary Methods**). We investigated the effects of two types of streetlighting: white (3,700 K) and amber (2,100 K). The amber lighting was similar in intensity to the white lighting (**Supplementary Table S1**) but was filtered to shift its spectral output toward longer, red wavelengths, almost eliminating emission of blue wavelengths (**Supplementary Figure S1**). Lights were positioned to mimic lighting conditions at Albert Park Lake and produced environmentally-realistic light intensities (0.1 – 10 lux around the pond, 10 lux directly underneath the light; **Figures 1C,D**). Light intensities within this range, and even lower, have previously been shown to have a detectable effect on bird behavior and physiology (e.g., Dominoni et al., 2013a; Alaasam et al., 2018; also see van Hasselt et al., 2020).

To investigate short-term effects of streetlights on sleep, we exposed swans to white light for one night in December 2017. To describe longer-term effects on time spent resting, circulating melatonin, and subsequent daytime behavior, we exposed the swans to at least 20 consecutive nights of white light and 20 consecutive nights of amber light. As a control, lights were switched off for 18 nights before (“first dark control”) and nine nights between (“second dark control”) the artificial light treatments. The durations of the dark controls were determined by lunar phase, so that blood samples for melatonin analysis could be collected at similar lunar illumination during light treatments (see melatonin analysis). Light intensity was higher during the first dark control than during the second owing to differences in lunar phase (**Supplementary Table S1**). Due to a technical malfunction, lights were switched off for part of Night 20 of the white light treatment. As the final blood samples for this light treatment had not yet been collected, the white light treatment was extended a further 13 nights (total duration: 33 nights). For consistency we only used data from the first 20 nights of each light treatment for our analysis of sleep and rest.

To observe directly how swans responded to the streetlights, one observer (A.E.A.) watched the swans from outside the enclosure when the lights first switched on. The birds were not visibly disturbed by either the white or amber lighting. The same observer also watched the swans from a bird hide during a night of white light exposure, after all experimental data had been collected. The birds did not appear to avoid the light and were mostly observed along the banks of the pond, <6 m from the nearest streetlight, which is where they were also frequently observed during the day. Some swans were even observed foraging, preening and resting almost directly beneath streetlights. We have seen a similar lack of overt responses to streetlights in wild swans. Many swans rested close to each other, although some did move during the night, and swans also rested

in close proximity (<1 m) to swans that were simultaneously active (e.g., preening).

Recording and Analyzing Resting Behavior

Activity from all swans was recorded near-continuously throughout the study using tri-axial accelerometer data loggers (Axivity AX3, Open Movement, Newcastle, United Kingdom). Using the raw accelerometry data, we calculated average overall dynamic body acceleration (ODBA; Wilson et al., 2006) for 4 s time bins. ODBA is a common measure of animal activity and allowed us to classify animals as resting or active (see **Supplementary Methods**). To define resting and active behaviors, we also observed swans and recorded time spent on various behaviors, including preening and foraging; these data were used to test whether light at night affected subsequent daytime behavior.

Recording and Analyzing Sleep

To record the electroencephalogram (EEG) and electromyogram (EMG), a subset of six swans (three females, three males) were surgically implanted with electrodes to measure brain waves and neck muscle tone, using standard stereotaxic techniques (Lesku et al., 2011a,b; see **Supplementary Methods**). Swans were given at least 2 weeks of post-operative recovery before commencing data collection. Data were recorded using a miniature data logger (Neurologger 2A, www.vyssotski.ch/neurologger2; Vyssotski et al., 2009; Anisimov et al., 2014), which had an inbuilt tri-axial accelerometer for capturing head movements.

Ultimately, due to logistic challenges, we obtained sleep data from two swans. Many recordings failed owing to water damage, or detachment of the logger or implant. Electrophysiological and accelerometry signals were imported into RemLogic v. 3.4.4 (Embla Systems, Pleasanton, CA, United States) for scoring and analysis. Data were manually scored by a single scorer (A.E.A.) from 600 h the day after the data logger was deployed (≥ 20 h post-handling, near sunrise) until 48 h later. Data were visually classified in 4 s epochs (time bins) as wakefulness, non-REM, or REM sleep following Martinez-Gonzalez et al. (2008; see **Supplementary Methods**).

Blood Collection and Melatonin Analysis

To assess daily variation in circulating melatonin under dark control conditions, we collected blood samples from each swan around sunset (–11 to 39 min relative to sunset), sunrise (15 to 56 min before sunrise), midday (5.2 to 6.1 h after sunrise), and night (6.2 to 7.3 h after sunset; 3.4 to 4.5 h before sunrise). These blood samples were collected after at least three nights without artificial light, and 4–8 nights from a new moon. We collected blood samples at least 24 h apart to minimize potential stress associated with repeated handling. All activity and sleep data collected <20 h after handling the birds were excluded from analyses.

To quantify any effects of light at night on plasma melatonin concentrations, we collected blood samples from all swans on six nights: two nights before each light treatment (during the first

and second dark controls), on Night 2 of each light treatment (white light and amber night), and on the final night of each light treatment (Night 33 of white light, Night 20 of amber light). Blood samples were collected around 6 h after sunset (mean \pm SE; first blood sample: 6.0 ± 0.1 h after sunset; final sample 7.0 ± 0.1 h after sunset), which was 4 h before sunrise (first sample: 4.4 ± 0.2 h before sunrise; final sample: 3.3 ± 0.3 h before sunrise), when circulating melatonin concentrations were expected to be highest (Zawilska et al., 2002, 2003). All blood samples were collected 4–9 nights from a new moon. Capture, handling, and bleeding of birds, as well as blood processing, was conducted under dim red light from head torches (see **Supplementary Methods**). Time between herding swans into the pen and collecting a blood sample from each swan ranged from 5 to 82 min.

Plasma concentrations of melatonin were determined by direct radioimmunoassay after extraction (Goymann et al., 2008). A four-parameter logistic curve was derived using Immunofit 3.0 (Beckman, Inc., Fullerton, CA, United States) to calculate sample concentration. Samples were run in two separate assays, and the lower detection limit was determined as the first value outside the 95% confidence intervals for the zero standard (B_{\max}): 0.80 and 0.86 pg/mL, respectively. The intra-assay co-efficients of variation (CV) of melatonin standards were 5.94 and 3.60%. The intra-extraction CVs of plasma pool samples ran in both assays were 5.07 and 0.44%. The inter-assay (standard melatonin) and inter-extraction CVs were 1.5 and 10.1%, respectively. Melatonin concentrations were adjusted for individual extraction recoveries (mean \pm SD, $91.7 \pm 5.02\%$).

Statistical Analysis

We performed statistical analyses using R version 3.5.1 (R Development Core Team, 2018). We used linear mixed effects models (LMM) to investigate effects of light at night on the duration of rest (based on accelerometry), circulating melatonin concentrations, and daytime behavior (based on behavioral observations) in swans. To determine the relationship between sleep and rest, we used a linear model that accounted for the temporal structure of the data. These analyses are described in further detail below.

LMM were fitted using the package *lme4* and *p*-values were calculated using the package *lmerTest* (Bates et al., 2015; Kuznetsova et al., 2017). We performed visual inspection of model residuals to confirm that linear model assumptions were met. The outcome of each model was also compared to a robust variant of the same model using the package *robustlmm* (Koller, 2016; Field and Wilcox, 2017). In all cases, outcomes from conventional and robust models were very similar; for ease of interpretation, we have chosen to report results from conventional models. Where a variable of interest had a significant effect ($p < 0.05$), *post hoc* tests were run using the function *emmeans* with a Tukey adjustment for multiple tests (Lenth, 2018). For analyses of rest, “night-time rest” was defined as the time spent resting between astronomical dusk and astronomical dawn (astronomical night). To account for changes in daylength (the duration of astronomical nights ranged from

5.3 to 8.1 h; **Supplementary Table S2**), the latest astronomical dusk and earliest astronomical dawn times were used for analyses involving multiple nights.

For LMM investigating effects on rest, dependent variables were individual data from swans on each night/date. Swan identity was included as a random factor to account for the repeated measures design. Date was also included as a random factor, since data collected from the same day cannot be treated as independent. A preliminary analysis indicated no difference in night-time rest duration between the first and second dark controls (mean \pm SE; first dark control: 3.35 ± 0.10 h, second dark control: 3.52 ± 0.09 h; LMM: $t_{18} = 0.48$, $p = 0.64$); we therefore pooled these data for analysis. We first tested for effects of lunar phase and mean night-time temperature on night-time rest during the dark control (**Table 1**). Lunar phase was defined as the absolute number of days from full moon (included in the model as a fixed numeric variable). We also included sex as a categorical variable in this model and subsequent models, to assess whether rest differed between males and females. We then investigated the effects of artificial light at night on night-time rest and total rest each 24 h day (with the 24 h day commencing at 1,900, around the time the lights were switched on; **Table 1**; also see **Supplementary Methods**). Initial models included an interaction term for sex and light treatment that was excluded if non-significant. Based on preliminary analyses that found an effect of light treatment on night-time, but not total daily rest, we made the *post hoc* decision to explore effects of light at night on dawn rest (rest during the hour preceding sunrise) and dusk rest (rest during the hour after sunset; **Table 1**). This decision was based on visual inspection of the data, which suggested that these were the only times of day when rest might have increased during light treatments. We also explored whether swans habituated to artificial light by examining whether night-time rest changed over time within light treatments, with mean night-time temperature (range: $9.6 - 21.5^{\circ}\text{C}$) included as a covariate (see **Supplementary Methods**).

For analysis of melatonin data, we tested whether circulating melatonin varied with time of day or light treatment in two separate models, which included time between capture (defined as when the swan was herded into the smaller pen) and bleeding as a fixed covariate. These models also included sex as a fixed categorical variable and swan identity as a random factor. We excluded two samples with much smaller volumes ($<45 \mu\text{L}$) from the light treatment model (one from the amber light treatment, another from dark control) and one from the time of day model (collected at midday).

To test for effects of light treatment on subsequent daytime behavior, dependent variables were the proportion of time spent resting, preening, and foraging, with observation duration (fixed covariate), sex (fixed categorical variable), and swan identity (random factor) included in each model (also see **Supplementary Methods**).

To investigate whether time spent resting is a reliable proxy for time spent sleeping, we modeled the relationship between these two variables with a linear regression model (LM) that accounted for the temporal structure of the data, using the

TABLE 1 | Results from linear mixed-effects models for impacts of light at night on time spent resting in black swans (b, unstandardized regression co-efficient; SE, standard error of regression co-efficient; df, degrees of freedom; t, t-ratio; p, p-value).

Predictor	b	SE	df	t	p
Model 1: Effect of lunar phase on night-time rest during dark control conditions					
Intercept	5.23				
Lunar phase	<0.01	0.02	12.3	0.17	0.87
Sex	−0.03	0.20	9.5	0.15	0.89
Temperature	−0.12	0.05	11.6	2.29	0.04
Model 2: Effect of light treatment on night-time rest					
Intercept	3.44				
Dark control – white light	0.37	0.17	43.6	2.23	0.08
Dark control – amber light	0.61	0.18	47.3	3.47	<0.01
White light – amber light	0.24	0.17	45.1	1.42	0.34
Sex	−0.09	0.24	9.0	0.39	0.71
Model 3: Effect of light treatment on total daily rest					
Intercept	9.88				
Dark control – white light	0.51	0.37	36.0	1.36	0.37
Dark control – amber light	0.55	0.40	38.4	1.36	0.37
White light – amber light	0.04	0.36	37.1	0.11	0.99
Sex	0.51	0.61	9.0	0.84	0.42
Model 4: Effect of light treatment on dawn rest (hour preceding sunrise)					
Intercept	0.48				
Dark control – white light	−0.09	0.05	44.3	2.00	0.12
Dark control – amber light	−0.11	0.05	48.4	2.39	0.05
White light – amber light	−0.02	0.05	46.1	0.52	0.86
Sex	<0.01	0.04	9.0	0.04	0.97
Model 5: Effect of light treatment on dusk rest (hour after sunset)					
Intercept	0.28				
Dark control – white light	−0.04	0.04	49.8	1.02	0.57
Dark control – amber light	0.04	0.04	53.8	0.87	0.66
White light – amber light	0.08	0.04	51.7	1.78	0.19
Sex	−0.05	0.04	9.1	1.20	0.26

The dark control was the reference group and its estimated mean is therefore the intercept for each model. All models included swan identity and date as random factors. Statistically significant results are highlighted in bold.

function *gls* in the package *nlme*. This model incorporated a first-order autoregressive process, with time spent resting (per hour) as the dependent variable and time spent sleeping (per hour) as the independent variable. For ease of interpretation, we have also presented correlation co-efficients obtained using Pearson's product-moment correlation.

RESULTS

Rest and Behavior

Night-Time Rest

We found a strong effect of light treatment on night-time rest [$F_{(2,45)} = 6.14$, $p < 0.01$; **Figure 2** and **Table 1**]. Birds rested 40 min (19.5%) less during amber nights compared with dark control nights (mean night-time rest during amber light \pm SE: 2.76 ± 0.07 h; dark control: 3.43 ± 0.07 h; $t_{47} = 3.47$, $p < 0.01$)

and 25 min (12.0%) less during white nights than dark control nights (white light: 3.02 ± 0.06 h; $t_{43} = 2.23$, $p = 0.08$). Night-time rest did not differ between the two artificial light treatments ($t_{44} = 1.42$, $p = 0.34$). During dark control conditions, the amount of night-time rest was not related to lunar phase ($t_{12} = 0.17$, $p = 0.87$). Although warmer nights during dark control were associated with less night-time rest ($t_{12} = 2.29$, $p = 0.04$), this effect did not persist during white ($t_{172} = 0.20$, $p = 0.84$) or amber ($t_{99} = 0.78$, $p = 0.44$) light treatments. Night-time rest was comparable for males and females across all light treatments (**Table 1**). In our model comparing night-time rest across light treatments, we found substantial variance in night-time rest within individual swans, with 49.7% of total variance attributed to differences within individuals. There was less variance in night-time rest between swans, with 22.0% of total variance attributed to differences between individuals (with the remaining 28.4% variance attributed to differences between dates). We found no evidence to suggest that swans habituated to the presence of light at night during our study timeframe (**Supplementary Figure S2**).

Daily Rest

Despite disrupted night-time rest, we found no effect of light treatment on total rest across the 24 h day [$F_{(2,37)} = 1.18$, $p = 0.32$; **Figure 2** and **Table 1**]. The amount of daily rest was highly variable (range: 4.4 to 15.6 h) but averaged ~10 h per 24 h day (dark control: 10.20 ± 0.15 h; white light: 9.66 ± 0.13 h; amber light: 9.66 ± 0.17 h). Our model indicated that 32.9% of total variance could be attributed to differences between birds and 41.0% to differences within individuals (the remaining 26.1% variance was attributed to differences between dates). The only time of day when the amount of rest appeared greater during amber or white light, compared with the dark control, was in the hour preceding sunrise (dawn rest; **Figure 3**). This difference was small (<10 min), with swans tending to rest slightly more at dawn under amber light (0.60 ± 0.02 h; $t_{50} = 2.39$, $p = 0.05$), but not white light (0.57 ± 0.02 h; $t_{46} = 2.00$, $p = 0.12$) compared to the dark control (0.47 ± 0.02 h). There was no difference in dawn rest between the amber and white light treatments ($t_{47} = 0.52$, $p = 0.86$), and no effect of light treatment on rest during the hour after sunset [dusk rest: $F_{(2,52)} = 1.61$, $p = 0.21$].

Daytime Behavior

Light at night did not strongly influence the proportion of time spent resting, preening, or foraging during the subsequent day (**Supplementary Figure S3**).

Sleep

Sleep electrophysiology (EEG-based sleep) was successfully recorded from one female and one male swan, at separate times (**Figure 4**). These data are thus preliminary but are also the first recordings of sleep in this species. We analyzed 48 h of sleep data for each swan, commencing from sunrise ≥ 20 h after capture. The female swan experienced the dark control condition on both nights, whereas the male swan experienced dark control conditions on Night 1 and was exposed to white light throughout Night 2. The male swan's

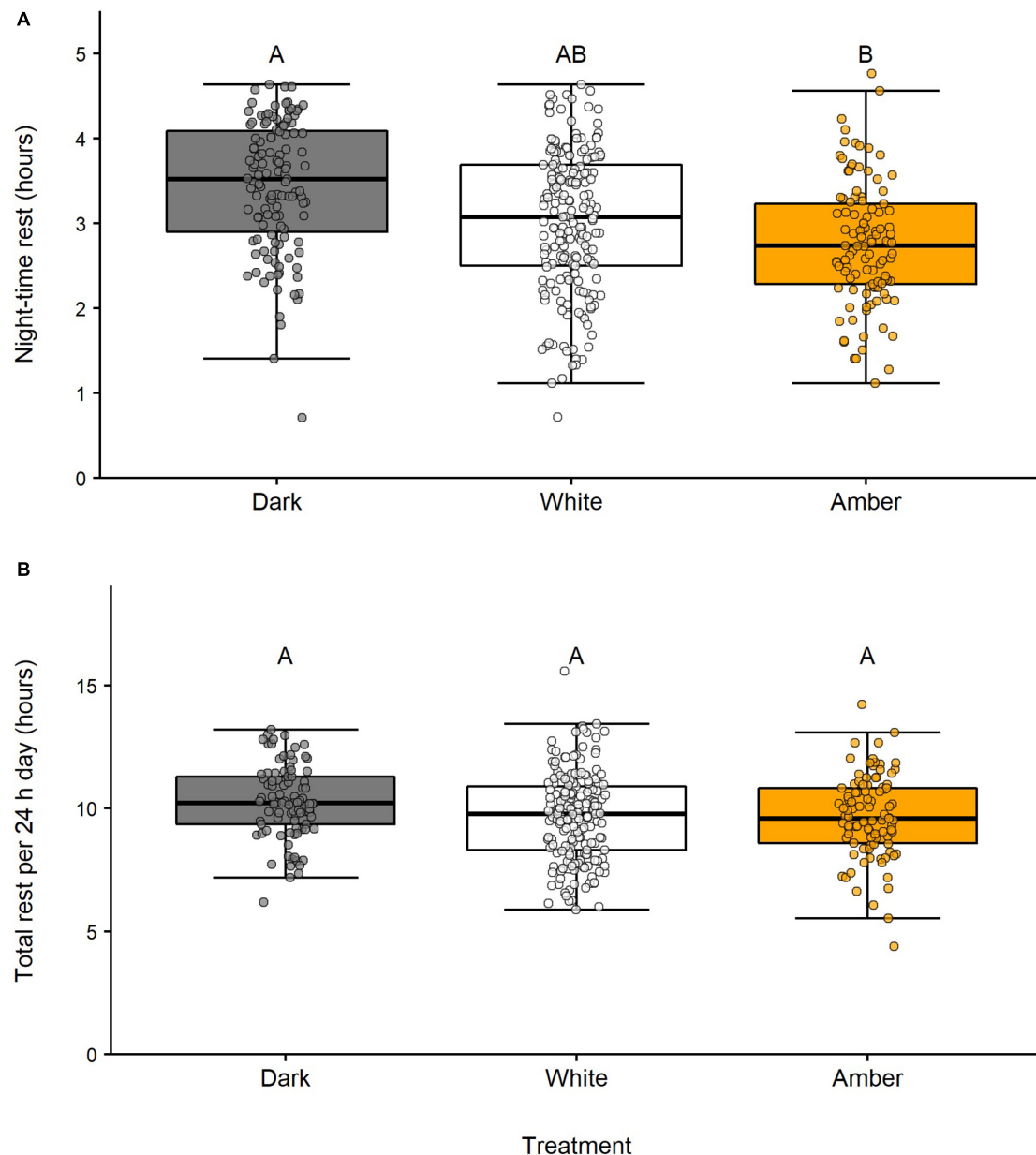


FIGURE 2 | Effects of streetlighting at night on **(A)** time swans spent resting during astronomical night and **(B)** total rest over 24 h days. Different letters denote significant differences between treatments; treatments with no letters in common were significantly different to each other ($p < 0.05$). Boxplots are Tukey boxplots showing median values (horizontal lines), interquartile ranges (IQR; boxes), and ranges excluding outliers (whiskers; outliers defined as values more than $1.5 \times$ IQR beyond box edges). Individual points represent data for each swan ($n = 11$) on each day/night, including outliers (total observations: dark $a = 123$, dark $b = 93$, white $a = 185$, white $b = 185$, amber $a = 109$, amber $b = 96$). Differences in the numbers of observations were due to exclusion of data after handling swans, loss of data loggers, and temporary removal of data loggers from individuals due to abrasion of feathers.

data logger was damaged the morning after Night 2, so that we were unable to investigate potential recovery of sleep after the light exposure.

Dark Control Conditions

The female swan slept a total of 5.40 h on day one and 7.72 h on day two; the male swan slept for 8.02 h. Both swans slept mostly between sunset and sunrise (66.8 – 74.3%

of total sleep time); 41.2 – 48.9% of their sleep occurred between astronomical dusk and dawn (**Figure 4**). Non-REM sleep constituted 81.3 – 85.6% of total sleep time and 15.2 – 21.0% of the 24 h day was spent in non-REM sleep. Daily trends in REM sleep appeared similar to non-REM sleep, with swans spending little of the day (2.3 – 3.6%) and more of the night (7.7 – 15.5 %) in REM sleep. The maximum recorded duration of REM sleep was 44 s; however, almost all other bouts of

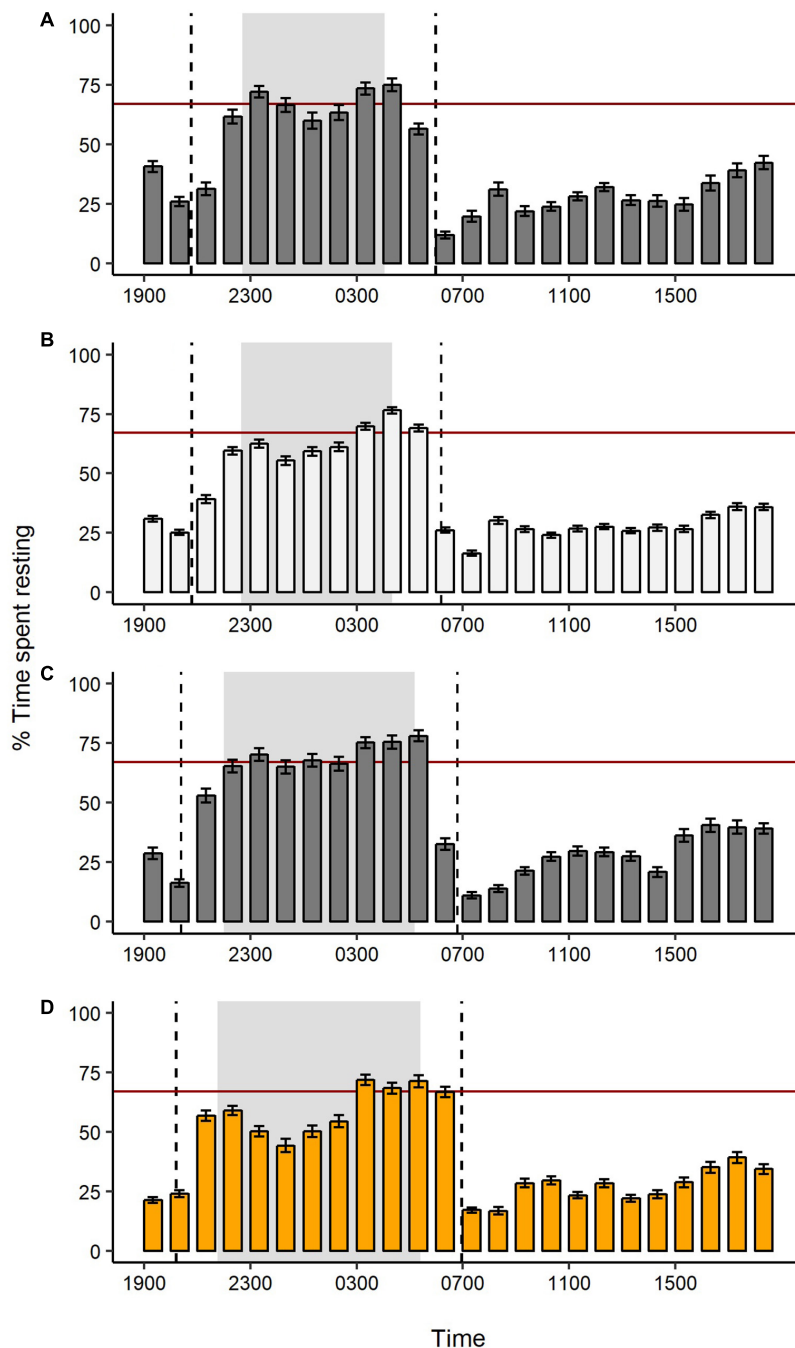


FIGURE 3 | Percentage of time swans spent resting each hour (mean ± SE) during four consecutive light treatments: **(A)** first dark control, **(B)** white light at night, **(C)** second dark control, and **(D)** amber light at night. Daylength decreased during the study, such that the earliest sunrise and latest sunset times for each treatment are marked by dashed vertical lines; astronomical night is shaded in gray. Mean night-time rest during the first dark control is marked for visual reference (red horizontal line at 67%).

REM sleep were <12 s long (median duration = 4 s, 98% of bouts ≤ 12 s).

White Light at Night

When the male swan was exposed to a single night of white light, the time spent in non-REM and REM sleep at night decreased

(Figure 4). Specifically, there was a 13% decrease in non-REM sleep and a 26% decrease in REM sleep during astronomical night of the white light treatment, relative to the previous (dark) night. This decline arose from a decrease in the number of bouts of non-REM and REM sleep, accompanied by an increase in the mean duration of wakefulness (Supplementary Figures S4–S6).

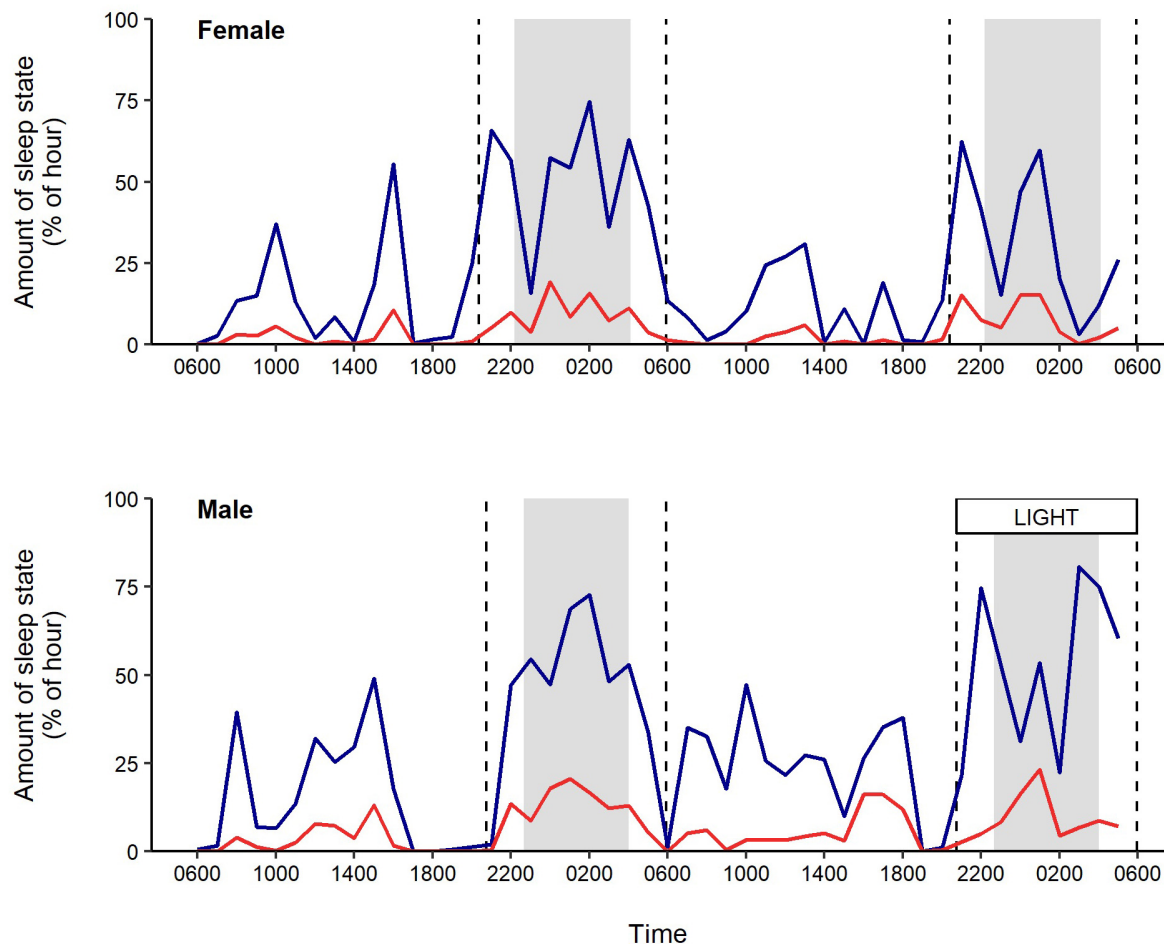


FIGURE 4 | Percentage of time spent in non-REM (blue) and REM sleep (red) per hour in an adult female and male black swan. The male swan was exposed to light from white streetlights throughout the second night. Sunrise and sunset times are marked by dashed vertical lines; astronomical night is shaded in gray.

Relationship Between Rest and Sleep

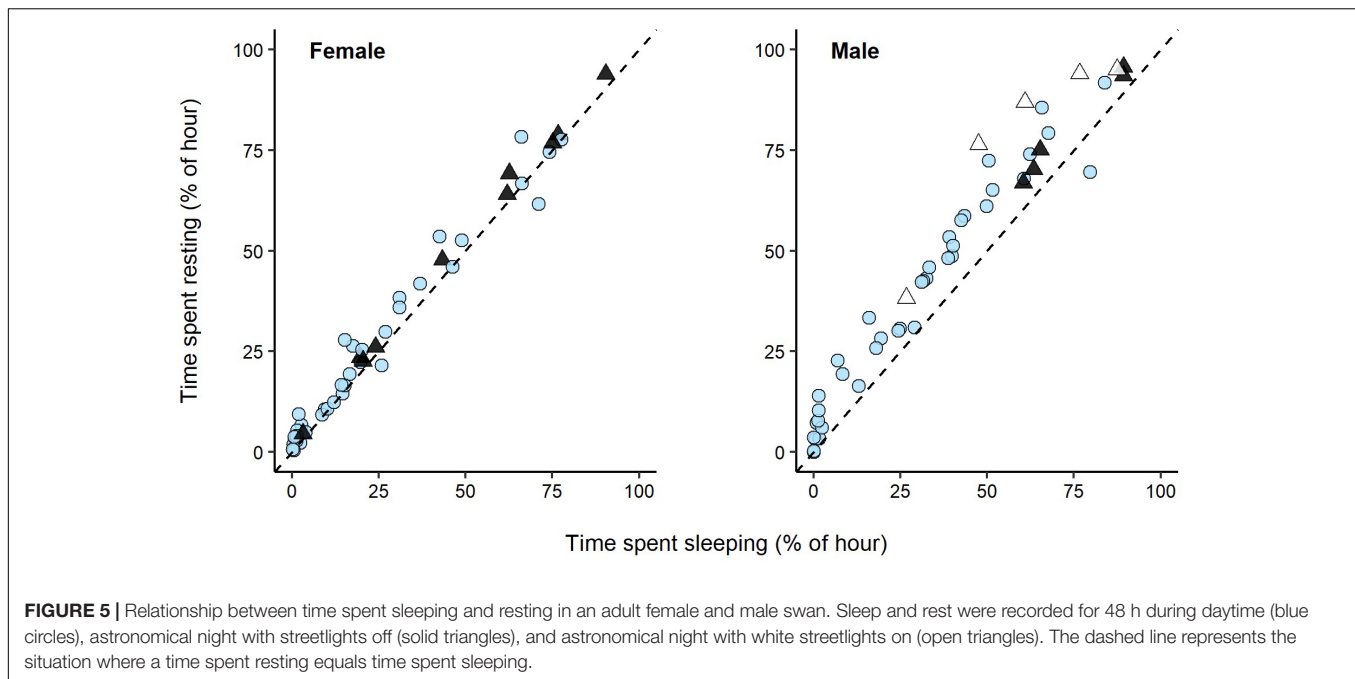
We found a strong linear relationship between rest and sleep for both swans (Pearson's correlation; male: $r = 0.98$; female: $r = 0.99$; **Figure 5**), which was highly significant (LM; male: $t_{46} = 27.61$, $p < 0.01$; female: $t_{46} = 50.11$, $p < 0.01$). This relationship applied to daytime (female: $t_{36} = 37.95$, $p < 0.01$; male: $t_{36} = 25.36$, $p < 0.01$), dark night-time (female: $t_8 = 54.25$, $p < 0.01$; male_{darkcontrol}: $t_3 = 30.93$, $p < 0.01$), and white light at night data (male_{whitelight}: $t_3 = 10.54$, $p < 0.01$). For the male swan, but not the female swan, the amount of time spent resting appeared to overestimate the amount of time spent sleeping by $\sim 10\%$ (mean \pm SE: $9.7 \pm 1.0\%$). Interestingly, the male swan also seemed to spend more time inactive but awake during exposure to white light, with inactivity overestimating sleep by $18.3 \pm 4.1\%$ during this light treatment. This might suggest that the male was more vigilant during exposure to white light. Nevertheless, this overestimation of sleep would cause us to underestimate the disruptive effects of light pollution on sleep. As such, inactivity is a reasonable and conservative proxy for sleep within our study context.

Melatonin

Plasma melatonin concentrations in swans tended to be very low, irrespective of time of day [$F_{(3,30)} = 1.33$, $p = 0.28$; **Supplementary Figure S7**]. We found no evidence for an effect of light treatment on circulating melatonin concentrations at night [$F_{(5,47)} = 1.23$, $p = 0.31$]. We also found no effect of time between herding swans into the pen and collecting blood samples on plasma melatonin [time of day model: $F_{(1,26)} = 2.64$, $p = 0.12$; light treatment model: $F_{(1,44)} = 0.11$, $p = 0.74$].

DISCUSSION

In this study, we provide evidence that streetlights disrupt night-time rest in urban birds in a naturalistic setting. Furthermore, we use electrophysiological data to verify that resting black swans are almost always sleeping (and vice versa), allowing us to infer impacts of streetlights on sleep in swans. We therefore refer to rest in swans as “sleep” from here onwards. We found that lighting of similar intensity, spectral composition, and spatial heterogeneity to real-world streetlighting reduced night-time



sleep in black swans. Furthermore, the amount of night-time sleep was similar during exposure to amber (blue-reduced) and white (blue-rich) lights. As circulating melatonin concentrations were low, irrespective of time of day or light at night, it is unclear whether reductions in sleep were related to the suppression of melatonin. Streetlighting also did not appear to influence the total amount of sleep each day, with swans showing substantial variation in total sleep time and potentially recovering a small amount of lost sleep toward sunrise. Overall, our findings indicate that having streetlights switched on all night can alter sleep in urban birds and that, contrary to expectation, filtering out blue wavelengths of light might not mitigate these effects.

Disruption of sleep by light at night could have important implications (Aulsebrook et al., 2016). Sleep is important for early development (Kayser et al., 2014), learning (Derégnaucourt et al., 2005), memory (Vorster and Born, 2015), and immune function (Dinges et al., 1995), as well as for repairing DNA damage (Zada et al., 2019) and removing neurotoxic metabolites (Xie et al., 2013) in the brain. Although previous studies have found effects of light at night on night-time activity and behavior in birds (e.g., Dominoni et al., 2013a; Russ et al., 2014; Raap et al., 2015, 2016; Ouyang et al., 2017), this study provides the first evidence that increased night-time activity under artificial light at night (captured by accelerometry) reflects decreased sleep (based on the EEG) in a naturalistic environment. Interestingly, during exposure to white light at night, the male swan also spent more time inactive, yet awake. Our results therefore might even underestimate the suppressive effects of light at night on sleep. Furthermore, we found little evidence for habituation to light at night over multiple weeks of exposure. Together, these findings might suggest that long-term exposure to light from streetlights has negative consequences for waking performance and health in birds.

Still, decreased night-time sleep in swans might not necessarily have adverse implications. We found no evidence for effects of light at night on subsequent daytime behavior in swans, or their total amount of sleep each 24 h day. Swans also showed substantial inter-individual variation in total daily sleep, and even more substantial intra-individual variation, which surpassed any differences due to light at night. Such plasticity in sleep duration might mean that birds are well-adapted to varying their sleep in response to environmental change. For example, a recent study found striking variation in the daily amount of sleep in European starlings (*Sturnus vulgaris*) in relation to season and lunar phase (van Hasselt et al., 2020). Finally, swans may have also recovered sleep lost during the night by sleeping more intensely. Sleep intensity (or depth) is typically measured as the amount of EEG slow waves during non-REM sleep (slow wave activity; Tobler, 2011). Our EEG data did not allow us to fully explore this possibility, as we only obtained EEG data from one swan under light at night, who damaged his data logger the following morning. Future studies should test for sleep homeostasis following light-induced wakefulness.

Our study cannot determine the mechanisms by which light at night affected sleep, nor explain why amber and white light had such similar effects on sleep. Nevertheless, we can offer some possible explanations. Light at night affects physiology and visual capacity, both of which could influence sleep (Aulsebrook et al., 2018). Physiologically, light at night can disrupt sleep by suppressing the production of melatonin (Lewy et al., 1980; Zawilska, 1996; Cajochen et al., 2000), changing the phase relationship between activity and the circadian clock (Evans et al., 2005), and shifting the timing of the circadian clock (Boivin et al., 1996). These processes are most sensitive to short, blue wavelengths of light (Dominoni, 2015), which would suggest that blue-reduced amber lighting should affect sleep less

than blue-rich white lighting. However, sustained exposures to long-wavelength red light can still affect melatonin production and circadian rhythms (Dauchy et al., 2015). While we found no effect of white or amber light at night on melatonin in our study, these results should be interpreted cautiously; melatonin concentrations were very low even under naturally dark conditions, which might have limited our ability to detect decreases under light at night. These results suggest that either swans produce little melatonin or that there was an issue with our blood sampling protocol, potentially because swans were active during the period they were herded for blood sampling (Fusani et al., 2011). The effects of white and amber lighting on physiology might also depend on other lighting characteristics, such as light intensity (de Jong et al., 2017), which warrants further investigation.

Another obvious impact of light at night is that it enhances the visual environment, particularly for diurnal animals. These visual changes might reduce night-time sleep by facilitating other behaviors at night, such as foraging (Russ et al., 2014) or anti-predator vigilance (Yorzinski et al., 2015). The effects of different types of lighting on sleep might therefore depend on how the animal perceives that lighting. While there is no information on the visual sensitivity of black swans, photopic vision in domestic ducks is more sensitive to light in the yellow to red range (577 to 633 nm) than it is for humans (Barber et al., 2006). For our study, this could mean that swans actually perceived the amber lighting as more “intense” than the white lighting, even though both lighting types provided similar illuminance as measured by lux (which is weighted toward the sensitivity of human daytime vision; Lucas et al., 2014). In addition, how animals perceive light at night – and their subsequent behavioral response – could influence the physiological impacts of light at night. For example, if swans avoided exposure to white light at night, then white lighting might have had relatively little effect on their physiology. Anecdotally, the swans in our study did not appear to avoid light at night, which is consistent with evidence from some other birds (Ulgezen et al., 2019) but not all (Dominoni et al., 2014; Yorzinski et al., 2015).

Given that the swans in our study were from urban areas, they may already be relatively tolerant of light at night, and thus our findings could underestimate the possible impact of light at night. Swans also display considerable flexibility in the timing of their behavior – they fly long distances at night (Frith, 1982) and alternate the timing of egg incubation with their partner. Because female swans primarily incubate their eggs at night (and males during the day), disruption of sleep by light at night could disproportionately affect one sex. Studies of the sleep and activity patterns of swans during various life history stages (e.g., breeding and incubation, as well as offspring development) could therefore offer further interesting insights. Moreover, other species that are less flexible than swans in their daily timing of behavior might experience greater impacts of light at night on sleep.

Light at night can have complex effects on wildlife (e.g., Witherington and Bjørndal, 1991; Sella et al., 2006), and use of amber lighting may be beneficial in some contexts (Longcore et al., 2018). However, our findings raise the possibility that filtering streetlights to reduce emission of blue light may not help

reduce impacts of light at night on sleep in birds. This is in direct contrast to studies of humans (Santhi et al., 2012; Ayaki et al., 2016), highlighting the need to consider species-specific impacts of both the intensity and color of streetlights. Our research further demonstrates that streetlights can disrupt night-time sleep even in urban-tolerant birds. Although we found no further adverse effects on total sleep, behavior or circulating melatonin, our results and the findings of other studies nevertheless suggest that land managers should consider switching off lights when they are not in use, particularly around parks, reserves and other wildlife habitat.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

All methods were approved by The University of Melbourne Animal Ethics Committee (1513532.1), La Trobe University Animal Ethics Committee (AEC13-42.4), Department of Environment, Land, Water, and Planning (permit: 10008176), and the Australian Bird and Bat Banding Scheme (Authority 1405).

AUTHOR CONTRIBUTIONS

AA, TJ, RM, and JL conceived and designed the study. AA performed the data collection and data analysis with assistance from JL, TJ, and RM and those mentioned in the acknowledgments. JL conducted surgeries with assistance from AA and those mentioned in the acknowledgments. WG facilitated and interpreted the hormone analysis. AV developed and provided Neurologgers. AA wrote the manuscript. All authors commented on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2020.00131/full#supplementary-material>

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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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Supplementary Material

1 Supplementary methods

1.1 Determining swan sex

Swans were sexed genetically from blood samples using standard techniques (Bruford et al. 1992). Genetic sexing failed for two individuals and these were therefore sexed using either morphological attributes, pairing history, or a combination of both. In our study population, all genetically sexed swans ($n = 730$ individuals) with a head-bill length greater than 145 cm ($n = 45$) were male, while 95% of adults with a head-bill length shorter than 135 cm ($n = 169$) were female. One of the unsexed swans had a head-bill length of 145.5 cm and was previously paired with two different female swans. The other swan had a head-bill length of 134.3 cm and was currently paired with a male swan. We are therefore certain that all individuals in the study were correctly sexed.

1.2 Light treatments

Streetlights were fitted 2.5 m above ground and angled 45° above the horizontal plane. They were positioned 10-20 m apart on the northern bank of the pond (see Figure 1c, d). Lights were powered by electrical mains and controlled by a timer; they switched on 1.5-3 h before sunset and switched off 1-2 h after sunrise.

For white lighting (3700 K), we covered the LEDs with white diffusion filters (416 Three Quarter White Diffusion filters, Lee Filters, Andover, England), which dimmed the lights to an ecologically-realistic intensity (approx. 10 lux underneath the light). To produce amber lighting (2100 K), the same lights were filtered with both white diffusion filters and amber filters (770 Burnt Yellow filter, Lee Filters, Andover, United Kingdom), which almost entirely eliminated the emission of blue wavelengths.

1.3 Recording and analyzing time spent resting

Tri-axial accelerometer data loggers (Axivity AX3, Open Movement, Newcastle, United Kingdom) were programmed to continuously record total acceleration (sampling rate: 25 Hz) over 5 weeks. For waterproofing, each logger was wrapped in kinetic thread seal tape and duct tape. We attached the logger to the swan's identification collar with tesa® 4651 tape, so that each logger was positioned in a standardized orientation at the base of the neck. The combined weight of the collar, accelerometer, and tape was no more than 60 g ($<1.5\%$ of a swan's body weight). Data loggers were replaced during routine catching events every 2-5 weeks.

To calculate overall dynamic body activity (ODBA), we first removed static acceleration (acceleration due to gravity and logger orientation) from total acceleration for each axis, using a running mean of 2 s (Wilson et al. 2006). The remaining acceleration is due to body motion, or dynamic acceleration. Absolute values of dynamic acceleration for the three axes were summed together to calculate ODBA. To examine rest and activity over more biologically meaningful timeframes (rather than fractions of a second), we calculated mean ODBA for 4 s time 'bins'.

To determine a threshold for rest and activity, we compared mean ODBA to corresponding focal observations of swans (see below). When swans were resting (upright resting or relaxed resting) for a consolidated period, their mean ODBA was almost always less than or equal to 0.04 (96.9% of resting time bins; $n = 709$ occurrences of resting). Likewise, active swans (engaged in preening, foraging, agonistic, locomotory, drinking, and vigilance behaviors) almost always had a mean ODBA greater than 0.04 (96.7% of active time bins; $n = 1326$ occurrences of activity). Consequently, a 4 s mean ODBA less than or equal to 0.04 was deemed to be a reasonable proxy for resting behavior in swans.

1.4 Behavioral observations

Swan behavior was recorded by a single observer (A.A.) 0.50-5.45 h after sunrise during the first dark control, long-term white light, second dark control, and long-term amber light conditions. An instantaneous sampling method was used, in which the behavior of the focal swan was recorded every 15 s for 10 min (Guay et al. 2013). To permit synchronization with accelerometer data loggers, the precise timing of observations was recorded using Time.is (<http://time.is>). Changes in behavior between sampling intervals were also noted, allowing us to identify consolidated periods of rest and activity. Swans were observed from a bird hide to minimize influence of the observer on swan behavior. If the focal swan moved out of sight during the observation period, observation resumed when the swan was in view. If the focal swan remained out of view, the observation ceased, and the total duration of the observation was recorded (minimum observation duration: 6 min). Commonly observed behaviors included upright rest (when the swan was stationary on land or drifting slowly on the water with head upright), relaxed rest (stationary or drifting with bill tucked into scapular feathers), preening, foraging, locomotion (swimming or walking), drinking, and watchful behavior (alert and turning head). Swans rarely exhibited social behaviors, including agonistic interactions (hissing, lunging at or chasing another bird).

1.5 Implanting EEG and EMG electrodes

Seven adult swans (three males, four females) underwent surgery to implant EEG and EMG electrodes, using standard techniques (Lesku et al. 2011; Lesku et al. 2012; Rattenborg et al. 2016). All procedures were performed by an experienced surgeon (J.L.) and assisted by a team of three people, including a veterinarian and two people focused on monitoring responses to the anesthetic. Briefly, the swan was administered with a non-steroidal anti-inflammatory (meloxicam 0.1 mg/kg) and sedative (midazolam 1-2 mg/kg) via intramuscular injection before being anesthetized with isoflurane vaporized in 100% oxygen (induction at 4%, maintenance at 1 – 3.5%). After induction of anesthesia via facemask, endotracheal intubation was used to administer anesthetic and oxygen. A midline incision was made to expose the cranium. Four holes (0.5 mm diameter) were drilled through the exposed cranium to the level of the dura (membrane overlying the brain), allowing for the insertion of EEG electrodes. EEG electrodes consisted of gold-plated, round-tipped pins (0.5 mm diameter, 3 mm length) connected to electrode wire (AS633, Cooner Wire Co., Chatsworth, USA). Five such electrodes were placed on the dura, with two EEG electrodes over the left hyperpallia, two over the right hyperpallia, and an additional electrode over the left hemisphere as the ground. As the bone over the hyperpallium is relatively thin, the hyperpallium could be visually identified through the cranium. EEG electrodes were fixed in place using superglue and dental acrylic (Paladur, Kulzer, Hanau, Germany). In addition, two EMG electrodes consisting of the same electrode wire (approx. 47 and 57 mm length) were placed on the nuchal (neck) muscle. All seven electrodes connected to a small plug and were fixed to the

swan's head using dental acrylic. The incision was closed around the headplug using non-toxic skin adhesive (Histoacryl, B. Braun, Melsungen, Germany).

Swans often ceased autonomous breathing while anesthetized, so manual ventilation was used when needed. Swans also showed variation in body temperature (38.6 - 41.6 °C) and heart rate (72 - 180 bpm) while anesthetized. The administration of intravenous fluids (Hartmann's Solution, Compound Sodium Lactate, 10 mL/kg/hour) via the medial tarsal vein, adding/removing blankets or icepacks, applying ethanol to the feet and underneath the wings, use of a temperature-controlled electric blanket, and adjusting maintenance levels of isoflurane all appeared to help manage these fluctuations. During the first surgery, a female swan developed hyperthermia (body temperature climbed to 48.6 °C) and died from cardiac arrest while under anesthetic. Subsequently, swans were not anesthetized and did not undergo surgery if their body temperature was over 41 °C or they showed any visual signs of stress (panting, salivating). There were subsequently no complications during or following surgery (six swans: three males, three females).

Swans took approximately 5 min to wake post-anesthesia and were then transported back to the enclosure for recovery. We held the swan in the shade until it was fully alert and capable of walking (typically around 60 min after anesthesia owing to the pre-operative injection of midazolam), then released it at the pond. We visually monitored the swan for another 20-30 min to ensure that it was displaying normal behavior (e.g. drinking, foraging, preening, walking, swimming, calling with partner).

All swans except one lost their dental acrylic headplug before the conclusion of the study, most within two months. When this occurred, the swan was captured as soon as possible and the headplug site was cleaned, and sealed with non-toxic skin adhesive. In all cases, the plug site healed rapidly until it was impossible to tell that there had ever been an incision. During this recovery period, and even immediately after the headplug came loose, swans showed normal behavior. The remaining female swan had much of her headplug removed at the conclusion of the study, prior to her release.

Five of the six birds that underwent surgery were seen in the wild five months after their release, and four of those were still observed 10 months later. The sixth bird disappeared after his first month back in the wild; however, he may have relocated (two of the non-surgery birds were eventually sighted at different locations within a year of their release). One of the swans that underwent surgery was observed with a partner and four cygnets 19 months after his release, indicating no lasting effects of the surgery or relocation.

1.6 Recording and analyzing sleep

EEG, EMG, and head acceleration were recorded using a miniature data logger (Neurologger 2A, www.vyssotski.ch/neurologger2). All signals were sampled at 100 Hz. The data logger was powered by a lithium thionyl chloride battery (LS14250, Saft, Paris, France; total weight of the logger and battery = 15g). Data loggers were deployed for a maximum of five days. The data logger and battery were variably waterproofed using a combination of H2OFF Electric (NanoProtech Italia, Milan, Italy), kinetic thread seal (PTFE) tape, and silicon adhesive.

Data were visually classified in 4 s epochs as wakefulness, non-REM or REM sleep by a single scorer (AA). When an epoch contained more than one state, it was scored as whichever state formed the majority of that epoch. Wakefulness was characterized by an activated (low-

amplitude, high-frequency) EEG, often accompanied by high and variable EMG activity and head movements. Particularly large and rapid head movements during wakefulness can also produce high-amplitude waves in the EEG (e.g. Lesku et al. 2012); these patterns were also scored as wakefulness. Non-REM sleep was characterized by high-amplitude, low-frequency EEG activity, at least twice the wave amplitude of quiet wakefulness in the absence of substantial head movements. Rapid eye movement (REM) sleep was characterized by a wake-like EEG, but without an increase in EMG activity, and only slight, slow head movements, consistent with drooping of the head as a result of reduced muscle tone (Lesku et al. 2011).

1.7 Blood sampling and melatonin analysis

Swans were captured by hand after herding them into a fenced pen within the enclosure. We collected up to 200 μ L blood from the tarsal vein using a needle and syringe. The blood was then immediately transferred to a heparinized microtube and stored on ice inside a dark box. To separate plasma from red blood cells, samples were spun at 12,000 rpm for 5 min within 2.25 h of collection. Plasma samples were then frozen temporarily on dry ice, and ultimately stored at -80°C . All blood collection and processing procedures were conducted under dim red light from head torches. Light from red head torches was additionally filtered using the same filter as the amber streetlights to minimize blue wavelengths of light.

In October 2018, samples were transported on dry ice to the Max Planck Institute for Ornithology, Seewiesen (Germany) for analysis. Samples remained frozen throughout transit and were stored at -80°C upon their arrival.

1.8 Statistical analysis

To investigate the effects of light treatment on swan activity, we analyzed data from only the first 20 days of each light treatment, to ensure similar durations of exposure and to reduce unequal sample sizes between treatments. Mean night-time temperatures were slightly warmer during the white light treatment ($16.4 \pm 3.3^{\circ}\text{C}$) than during the dark control ($14.3 \pm 0.6^{\circ}\text{C}$), though there was no difference in temperature between the dark control and the amber light treatment ($14.0 \pm 0.4^{\circ}\text{C}$). Variation in night-time temperature was also greater during the white light treatment than the amber light treatment or dark control. However, we found no effect of temperature on night-time rest within the white light treatment (in models testing for changes in night-time rest over time; see below). Given the possible confound between temperature and light treatment, and given that we had no compelling evidence or *a priori* predictions that such small differences in temperature might influence rest, we therefore chose to exclude temperature from our final models.

We also investigated whether swans habituated to artificial light at night by testing whether night-time rest changed over time within light treatments. Treatment day was incorporated in these models as a fixed categorical variable. Other variables included in the model were mean night-time temperature (fixed covariate), sex (fixed categorical variable) and swan identity (random factor). Linear and quadratic effects of treatment night were tested in separate models; results reported for effects of temperature within artificial light treatments are from the former. We used the same approach to investigate whether effects of artificial light carried over into either of the dark control periods.

To determine whether light at night affected the time budgets of swans the following day (based on behavioral observations), we first pooled data for each swan in each light treatment

(first dark control, white light, second dark control, and amber light). This decreased zero-inflation of the data such that linear mixed effects models were appropriate. As the total duration of these pooled observations varied in length, we included observation duration as a fixed covariate in each model. Dependent variables were proportion of time spent resting, preening, and foraging. We chose these behaviors because they were among the most commonly observed behaviors, and might indicate effects of light treatment on stress, health, and food intake.

1.9 Swan release at Albert Park Lake

After the study concluded, we returned the swans to Albert Park Lake, where they were visually monitored post-release for 2 h. The released swans were then observed at least every week for the next month, during which time their behavior was ostensibly indistinguishable from that of other wild swans, indicating no lasting effects of captivity or surgery.

2 Supplementary tables

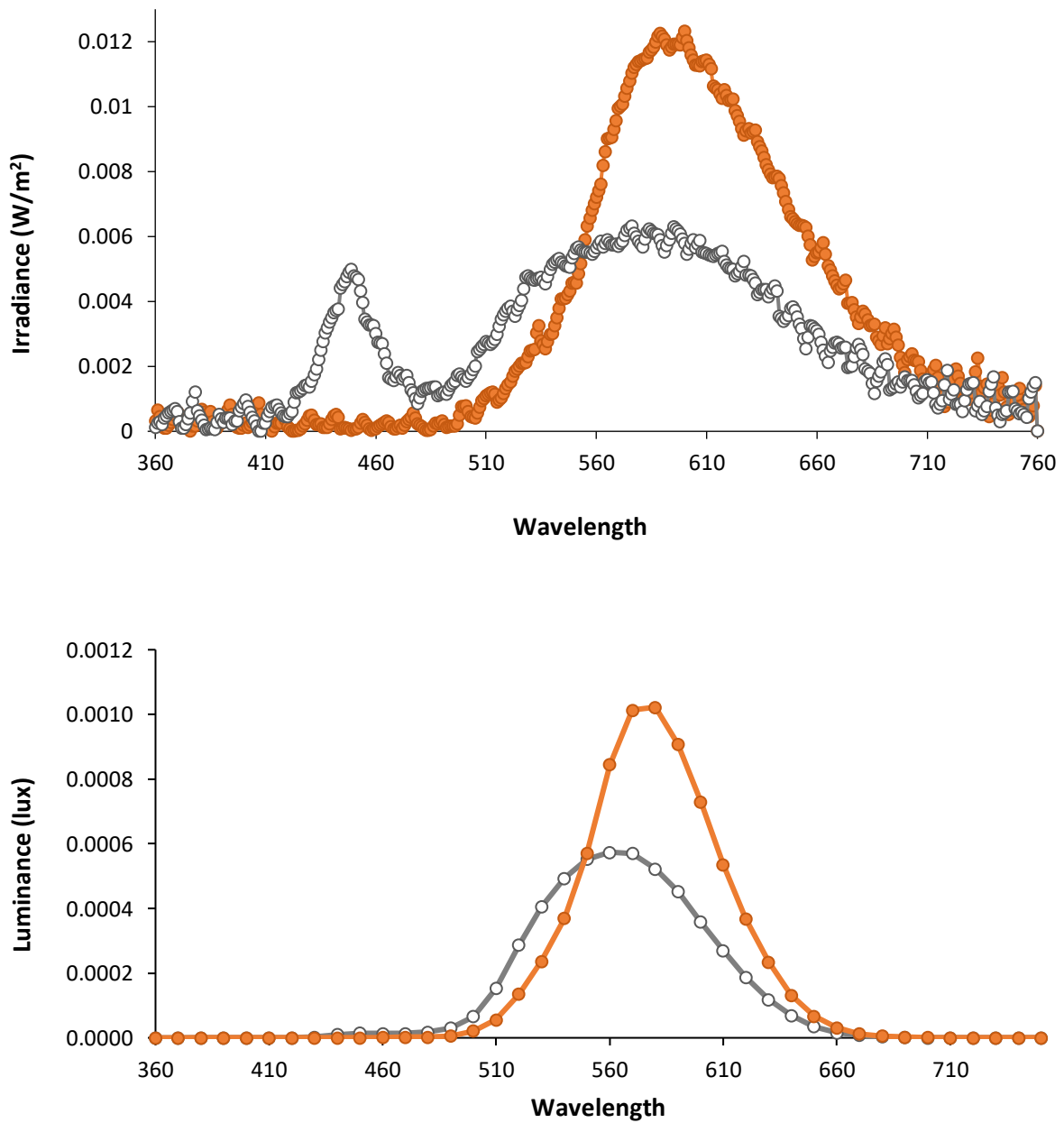
Supplementary Table 1: Mean night-time light intensity (mean lux \pm SE) for each light treatment, recorded from two locations each minute between astronomical dusk and astronomical dawn. Due to a technical malfunction, lights were switched off for part of Night 20 and Night 21 of the white light treatment; data from these nights is therefore excluded. Two loggers failed to record data during the first part of the amber light treatment; reliable data were therefore only obtained for the final 8 nights of amber light.

Light treatment	Light intensity (lux) \pm SE
First dark phase	0.03 \pm 0.07
White light: Nights 1-19	0.30 \pm 0.04
White light: Nights 22-33	0.26 \pm 0.02
Second dark phase	<0.01
Amber light: Nights 13-20	0.36 \pm 0.02

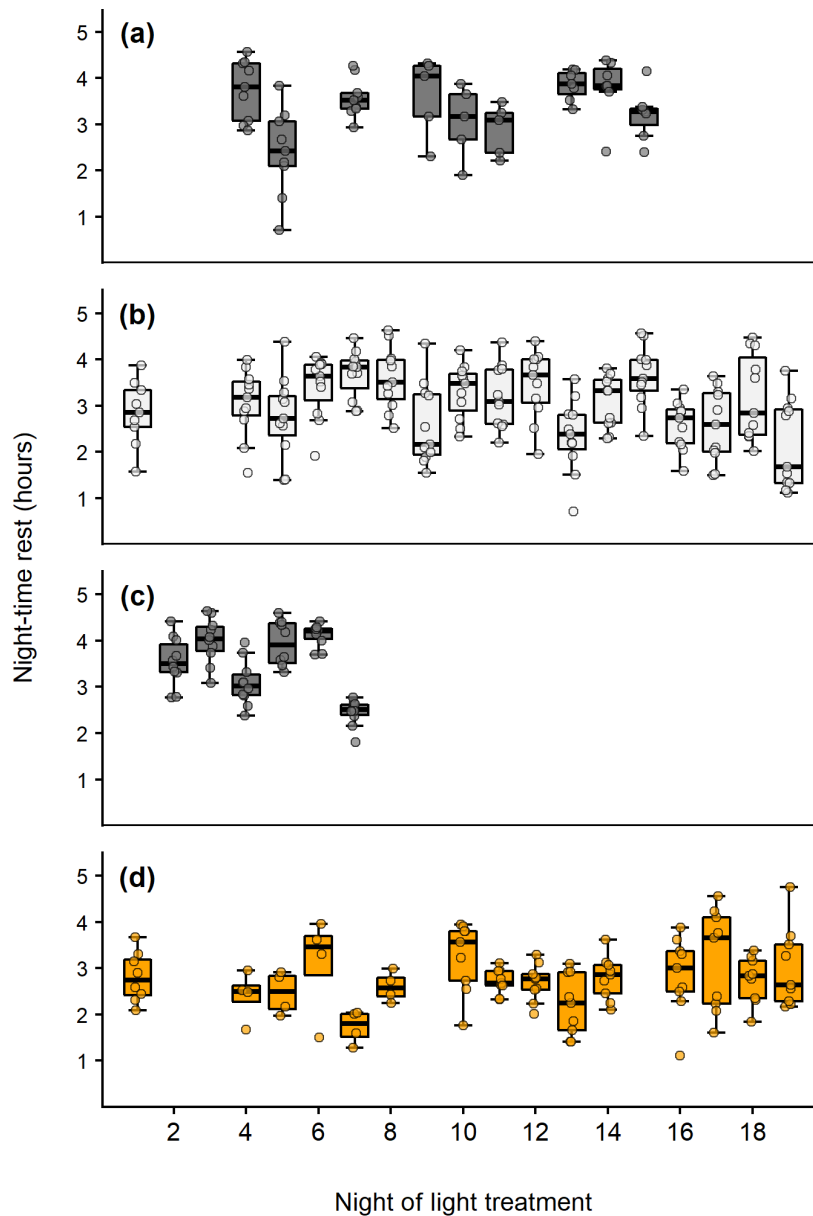
Supplementary Table 2: Mean values for environmental conditions between astronomical dusk and dawn during recordings of sleep and activity. Data were obtained from Avalon Airport weather station (station reference: 087113; 38°1'S, 144°28'E; Bureau of Meteorology, Australia), approximately 8 km from the swan enclosure.

	Wet bulb temperature (°C)	Precipitation (mm)	Relative humidity (%)	Wind speed (km/h)	Night length (h)
<i>Sleep: Female swan</i>					
Night 1	17.8	<0.1	85.3	13.0	5.9
Night 2	19.1	0.4	73.7	19.1	5.9
<i>Sleep: Male swan</i>					
Night 1	15.1	<0.1	79.4	8.2	5.3
Night 2 (white light)	12.2	<0.1	89.0	9.4	5.3
<i>Activity: All swans</i>					
First dark phase	14.1	<0.1	78.5	12.2	5.5
White light	16.0	0.3	82.8	12.9	6.0
Second dark phase	14.2	<0.1	81.4	14.3	7.4
Amber light	14.4	<0.1	77.6	14.4	8.1

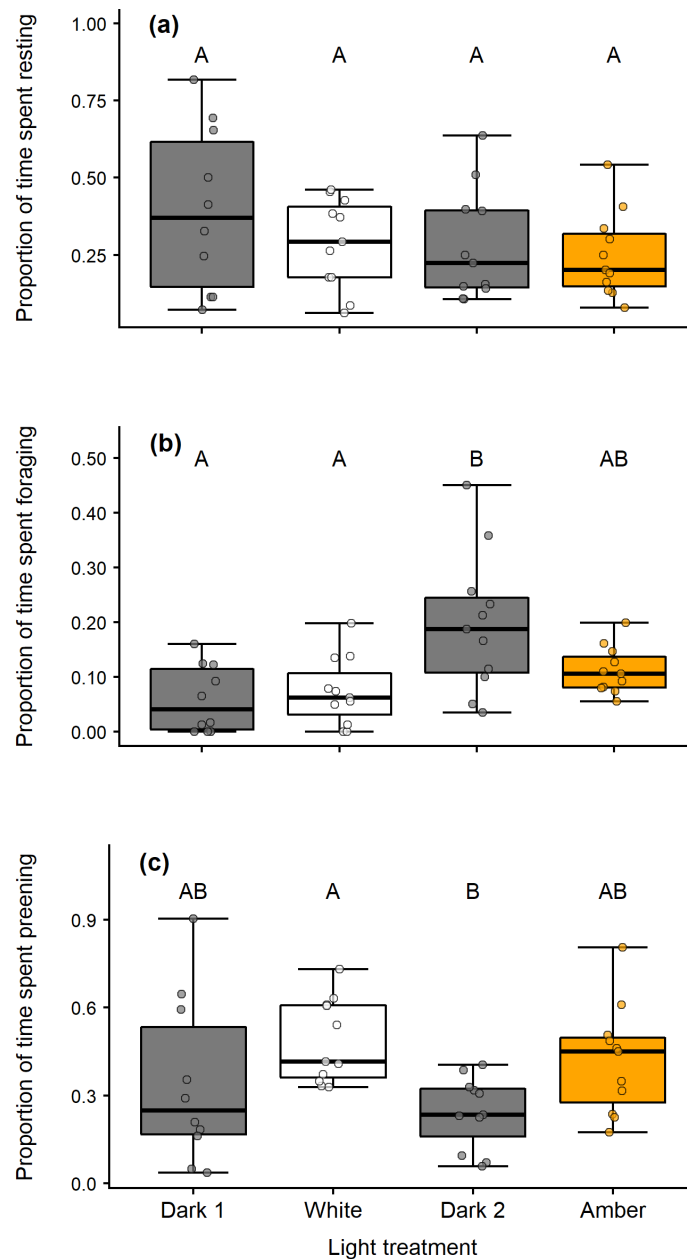
3 Supplementary figures



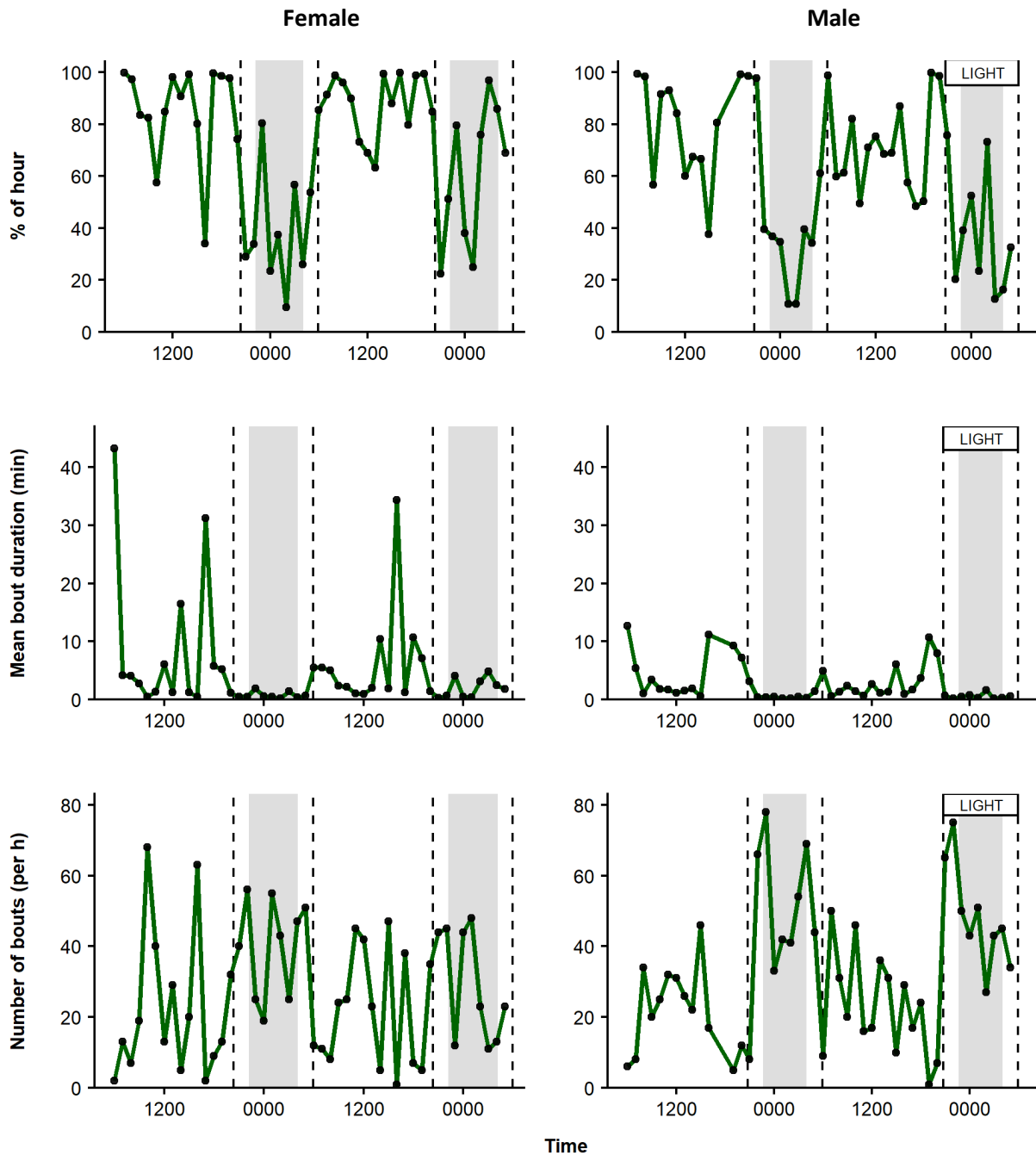
Supplementary Figure 1: Spectral irradiance (top) and luminance (bottom) output of white lighting (white) and amber lighting (orange). Light spectra were recorded at ground level using a MK350 LED Meter (UPRtek Zhunan, Taiwan) 5 m from the light pole. Conversions from irradiance to luminance (for wavelengths grouped into 10 nm bins) were performed according to Johnsen (2012).



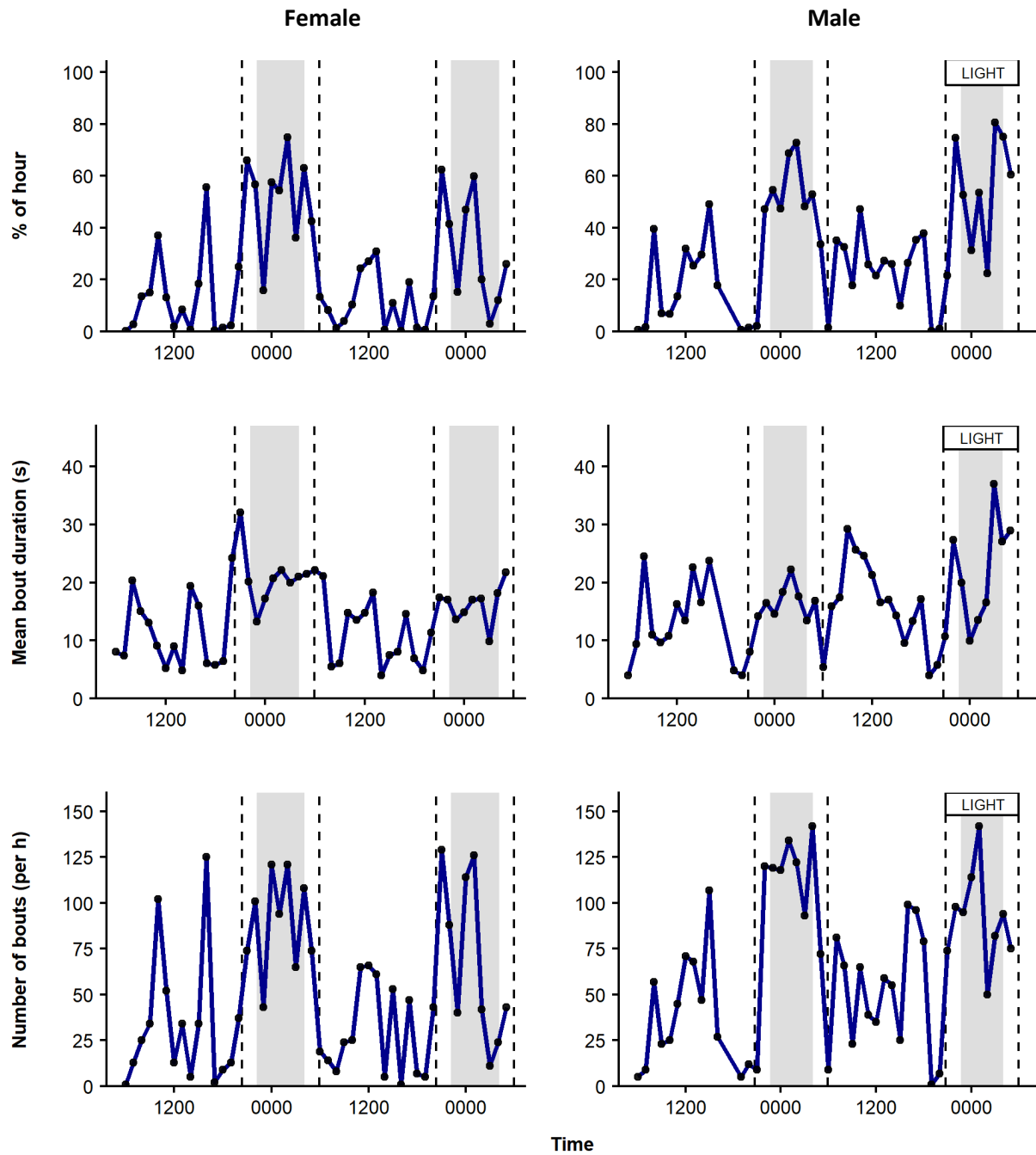
Supplementary Figure 2: Duration of time swans spent resting during each astronomical night of (a) first dark control (lights off; 18 nights), (b) exposure to white streetlights (20 nights), (c) second dark control (9 nights), and (d) exposure to amber streetlights (20 nights). During the white light treatment, there was a highly significant quadratic relationship between night-time rest and treatment night ($t_{171} = -3.54$, $p < 0.01$), whereby there was a modest increase in night-time rest over the first ten nights, followed by a sharper decrease over the subsequent nine nights. There were no trends associated with treatment night during the amber light treatment ($t_{98} = 1.14$, $p = 0.26$) or either of the dark control periods (first dark control: $t_{53} = 1.35$, $p = 0.18$; second dark control: $t_{47} = -1.12$, $p = 0.27$). These results suggest that swans did not habituate to the light treatments and effects of exposure to artificial light did not carry over into control conditions. Boxplots are Tukey boxplots showing medians as horizontal lines, interquartile ranges (IQR) as boxes, and ranges excluding outliers as whiskers (outliers being more than $1.5 \times \text{IQR}$ beyond box edges). Individual points represent data for each swan (including outliers). Missing data are due to the exclusion of data collected <20 h after handling swans to deploy data loggers or collect blood samples.



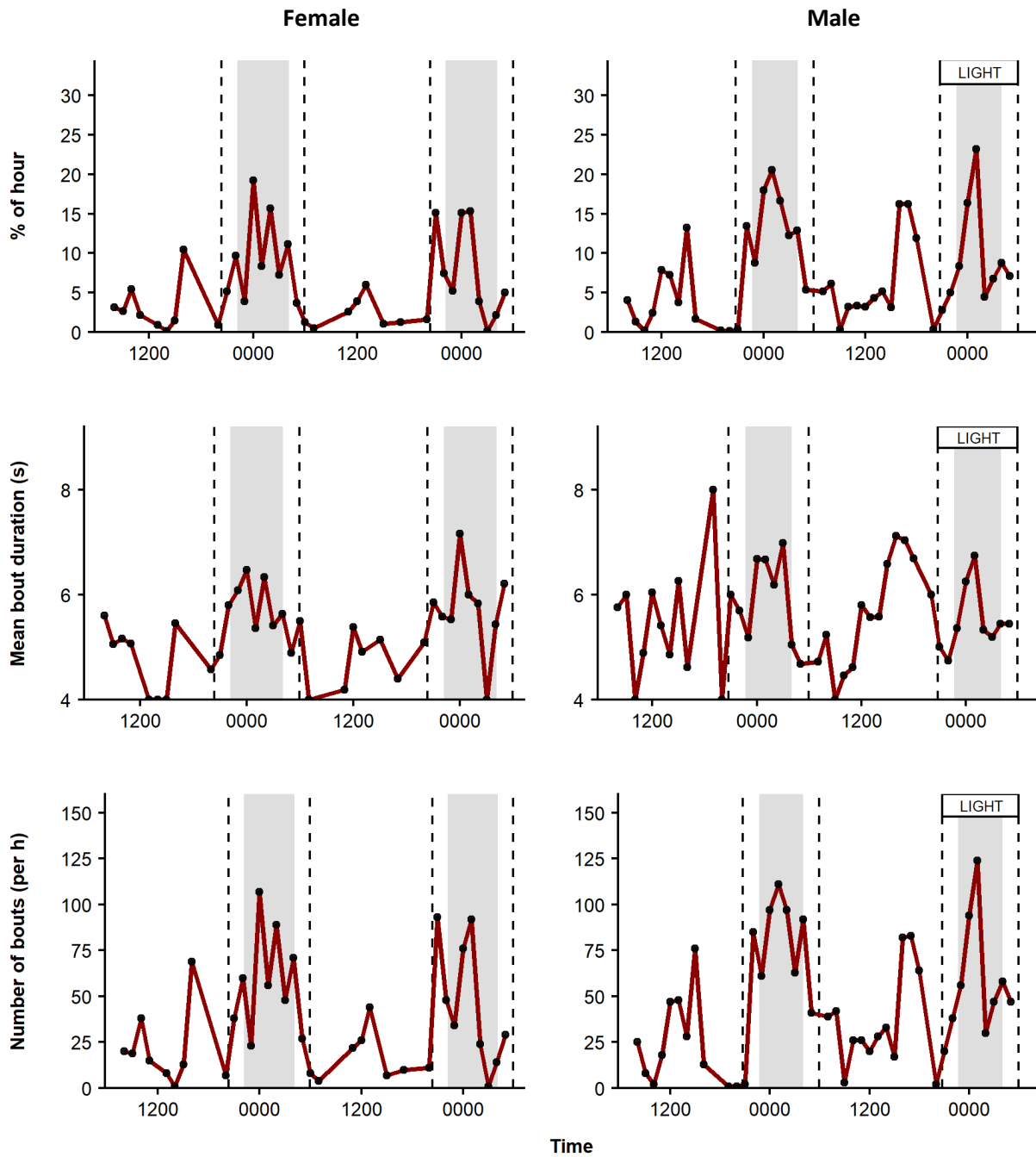
Supplementary Figure 3: Effects of exposure to light from streetlights at night on the proportion of time swans spent (a) resting (b) foraging, and (c) preening the following morning. There was no effect of light treatment on the proportion of time swans were observed resting ($F_{3,30} = 0.69$, $p = 0.56$). Swans tended to spend more time foraging during the second dark control than during the first dark control ($t_{32} = 2.85$, $p = 0.04$), white light treatment ($t_{28} = 4.03$, $p < 0.01$), and amber light treatment ($t_{28} = 2.70$, $p = 0.05$). Swans also spent more time preening after the white light treatment compared with the second dark control ($t_{29} = 3.19$, $p = 0.02$), but not the first dark control ($t_{34} = 1.88$, $p = 0.26$). Datapoints represent individual data from each swan per day. Different letters denote significant differences between treatments; treatments with no letters in common were significantly different to each other ($p < 0.05$). Boxplots are Tukey boxplots showing medians as horizontal lines, interquartile ranges (IQR) as boxes, and ranges excluding outliers as whiskers (outliers being more than $1.5 \times \text{IQR}$ beyond box edges). Individual points represent data for each swan (including outliers).



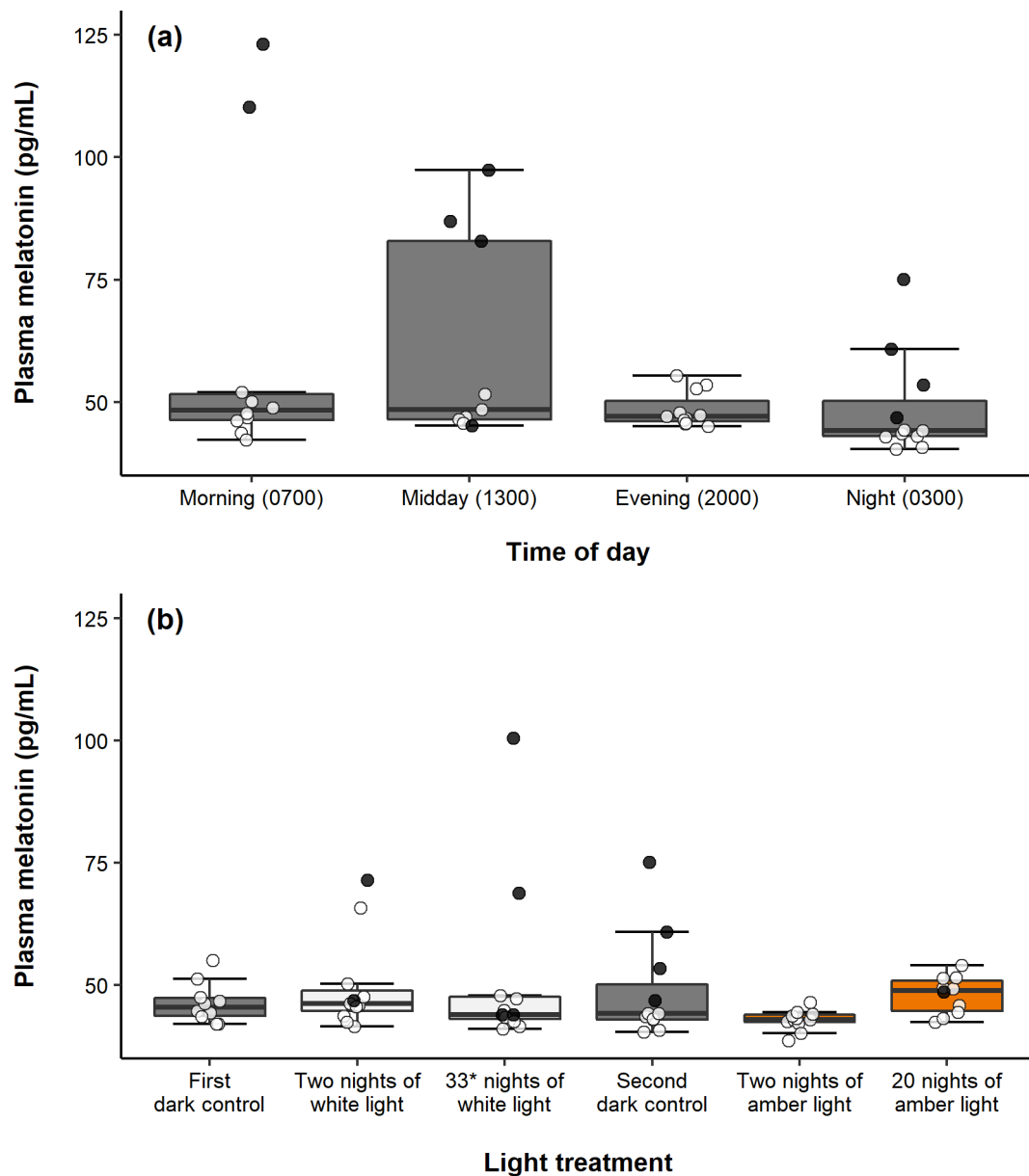
Supplementary Figure 4: Wakefulness in an adult female (left column) and male (right column) over two consecutive days (beginning at sunrise). (a) Total amount (% of hour), (b) mean bout duration and (c) number of bouts. Sunrise and sunset times are marked by dashed vertical lines; astronomical night is shaded in grey. The male swan was exposed to white light at night from streetlights throughout the second night. The increase in wakefulness during exposure to white light arose from an increase in the duration of wake bouts (from 0.28 min at 0100 to 1.61 min at 0200).



Supplementary Figure 5: Non-REM sleep in an adult female (left column) and male (right column) over two consecutive days (beginning at sunrise). (a) Total amount (% of hour), (b) mean bout duration and (c) number of bouts. Sunrise and sunset times are marked by dashed vertical lines; astronomical night is shaded in grey. The male swan was exposed to white light at night from streetlights throughout the second night. The 13% decrease in non-REM sleep during exposure to white light arose from a 19% decrease in the number of bouts of non-REM sleep.



Supplementary Figure 6: REM sleep in an adult female (left column) and male (right column) over two consecutive days (beginning at sunrise). (a) Total amount (% of hour), (b) mean bout duration and (c) number of bouts. Sunrise and sunset times are marked by dashed vertical lines; astronomical night is shaded in grey. The male swan was exposed to white light at night from streetlights throughout the second night. The 26% decrease in total REM sleep during exposure to white light arose largely from a 22% decrease in the total number of bouts of REM sleep.



Supplementary Figure 7: Plasma concentrations of melatonin (pg/mL) in swans (*a*) at four different times of day under no artificial light (dark control) and (*b*) under different exposures to white and amber light from streetlights. Plasma concentrations tended to be low, irrespective of time or light treatment (black datapoints represent samples above the detection limit of radioimmunoassay, white datapoints represent samples at or below the detection limit). Boxplots are Tukey boxplots showing medians as horizontal lines, interquartile ranges (IQR) as boxes, and ranges excluding outliers as whiskers (outliers being more than $1.5 \times$ IQR beyond box edges). Individual points represent data for each swan (including outliers). *Lights switched off during Night 20 of the white light treatment; the treatment was therefore extended for an additional fortnight.

4 References (Supplementary)

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