

RESEARCH ARTICLE

Short-time exposure to light at night affects incubation patterns and correlates with subsequent body weight in great tits (*Parus major*)

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Funding information

Adaptive Life programme of the University of Groningen, The Netherlands

Abstract

Artificial light at night (ALAN) widely affects wildlife by blurring light-dark differences, including transitions such as sunrise and sunset, thereby affecting regulation of diel rhythms. As a result, activity onsets in many wild diurnal songbirds advance under ALAN. From chronobiological studies, it is known that the direction and strength of the response to light depends on when during the night exposure takes place. However, these experiments are mostly done under continuous light conditions, when animals have free-running rhythms. It remains unclear whether phase-dependence also holds in entrained, wild songbirds; i.e., does the effect of ALAN on activity patterns differ between exposure in the morning compared to the evening? This information is essential to assess the effects of mitigation measures by limiting ALAN to selected times of the night. We exposed incubating great tits (*Parus major*) inside the nest-box to 4 h of dim light, of which 1 h overlapped with dawn before sunrise or dusk after sunset. We found a small advancing effect of morning-light on activity onset and of evening-light on offset compared to dark controls but not *vice versa*. Breeding success and chick condition were unaffected by the light treatments. However, light-treated females had lower weights 9–18 days after the end of the treatment compared to the controls, independent of whether ALAN occurred in the morning or the evening, indicating possible costs of ALAN. Despite the weak behavioral response, ALAN might have affected the females' circadian clock or physiology resulting in lower body condition.

KEYWORDS

artificial light, circadian, female, part-night lighting, phase-dependent effects, rhythm, wildlife

1 | INTRODUCTION

Negative effects of artificial light at night (ALAN) on wildlife have received increasing attention in the scientific and popular press, highlighting the urge to take mitigation measures against light

pollution (Davies & Smyth, 2018). ALAN has several effects on wildlife, including attraction to the light source or aversion from light polluted areas. Beyond that, ALAN interferes with the natural light schedule, i.e. darkness at night, which is an important cue for organisms to regulate diel activity rhythms (i.e., with period length of

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1 day = diel) (Russart & Nelson, 2018). Thus, alteration of activity patterns is widespread as both nocturnal and diurnal animals may change their activity patterns in response to ALAN (Gaston et al., 2017; Sanders et al., 2021).

Most organisms exhibit rhythmic activity patterns that repeat every 24 h independently of their temporal niche i.e. nocturnal, diurnal or crepuscular (Refinetti, 2008; Yerushalmi & Green, 2009). These diel rhythms are driven by the internal circadian clock that adjusts to external cues such as daylight. In particular, light-dark transitions such as sunrise and sunset are very predictable due to the constant rotation of the earth around its own axis. Thus, light-dark transitions are usually major time-givers for entrainment of the internal circadian clock (Cassone, 2014). In addition to the long-term entrainment of the clock, diel rhythms can also be temporarily modified in response to the short-term changes in the environment. Those plastic changes are often referred to as "masking" the entrainment of the clock (Helm et al., 2017).

How the internal clock perceives, interprets and responds to information from light cues depends on the time of exposure. The internal circadian clock keeps (free-)running under constant conditions in the laboratory with a period length of ca 24 h ("circadian"), in which individuals exhibit subjective day-night patterns (Aschoff, 1989; Helm & Visser, 2010). As soon as a light pulse is provided the clock can respond accordingly by a phase shift. This response differs in strength and direction of the shift (e.g., advance or delay of the next active phase) depending on the intensity and timing of the light cue relative to the subjective night, which can be mapped using phase response curves. In general, activity is delayed after a light pulse at the end of the active phase and advanced after a light pulse before the start of the active phase (Binkley & Mosher, 1986; DeCoursey, 1986). Therefore, effects of ALAN on the diel rhythms of activity could potentially be dependent on the time of night animals are exposed to ALAN.

Light pollution often spans across the whole night so that previous research mainly investigated the effect of continuous ALAN, which abolishes darkness and dilutes light-dark transitions. Diurnal songbirds have been shown to advance their activity onset and exhibit more nocturnal activity, especially before sunrise, decreasing their time available for resting (e.g., Raap et al., 2015; Sanders et al., 2021). One of the mitigation measures proposed to limit the negative consequences is to restrict ALAN to a part of the night (Commonwealth of Australia, 2020; Donatello et al., 2019). But it is unclear whether ALAN in the morning versus evening causes different effects on the activity patterns under natural conditions. Here, we use a diurnal songbird, the great tit (*Parus major*) that has previously been shown to advance activity onset in response to ALAN under laboratory conditions as well as in the wild (e.g., de Jong et al., 2016a; Raap et al., 2015).

We aim to test whether effects of ALAN differ for birds' exposure to ALAN before sunrise or after sunset, and whether related fitness consequences, if any, differ depending on time of exposure. For this, we assessed activity patterns of female great tits during incubation from nest temperature data. Incubating females

adjust activity timing depending on incubation stage and environmental factors leading to differences between days (McGlade et al., 2023; Schlicht & Kempenaers, 2020). In particular, they delay their activity onset and advance their offset throughout incubation, while responses to environmental factors, such as temperature and rain fall, additionally increase variation between days. This large plasticity in females is also reflected in their repeatability of onsets ranging from 0.13 to 0.46 and offsets from 0.00 to 0.37 (Meijdam et al., 2022; Schlicht & Kempenaers, 2020; Stuber et al., 2015). It is important to experimentally disentangle daily effects from those of ALAN by looking at between- as well as within-individual responses.

We exposed two groups of incubating females for 5 days to 4 continuous hours of light inside their nest-box, each with a parallel dark control group. One treatment hour overlapped with twilight, either before sunrise or after sunset. In wild blue tits, 1 h of intense light exposure before sunrise was sufficient to advance their activity onset (Santema & Kempenaers, 2023; Schlicht et al., 2014). Thus, we expected to see a deviation from the natural activity patterns, visible in the dark controls, when individuals are exposed to light during their sensitive phase. Assuming a phase-dependent response of the internal clock, changes in the activity pattern could differ in their direction and magnitude between morning and evening exposure. In particular, the exposure before sunrise could advance the onset of activity compared to the control, while we would expect a delay for individuals exposed to light after sunset. For activity offset we expected a similar change if rest duration remains the same, otherwise the females would modify the length of their active day. These effects could carry over for some days, indicating a slow shift of the internal clock to the earlier onset of light and the reverse shift back after the treatment.

Manipulations of the natural conditions can cause fitness costs due to potential mismatches in timing with the environment (Greives et al., 2015; Verhulst & Nilsson, 2008). Therefore, we also assessed potential fitness consequences for both treatment groups compared to unmanipulated controls by investigating a number of fitness components: Hatch success could be altered as a direct result from a change in activity patterns during incubation. Also, the females' condition could be directly affected as light treatments are known to alter physiological processes due to potential changes in the circadian system (Dominoni, 2015; Nelson & Chbeir, 2018) or sleep disruption (Raap et al., 2015, 2016b). If any carry-over effects are present, females' biological rhythms might be still altered during early chick provisioning and thereby could influence females' performance affecting fledge success and chick condition.

2 | MATERIALS AND METHODS

2.1 | Study population and data collection

We manipulated light regimes inside the nest-box of incubating great tits during the breeding season of 2021 (i.e., activity records from 28th April to 10th June) to investigate their time-dependent

effects on activity patterns. The nest-boxes used were part of a long-term monitoring population on a Dutch island in the Wadden Sea (Vlieland: 53.28 N, 5.07 E). That population consists of about 400 nest-boxes spread across one large and four small forest patches, where different tit species have been individually marked with aluminium and color rings, and monitored once or twice a week during the breeding season (Kluyver, 1970). After monitoring nest building, the date of the first egg laid was recorded, back-calculated from the first observed clutch size assuming that females lay one egg per day. Around the expected hatch date (on incubation day 14), the clutch was visited more often where possible (up to intervals of 1–2 days) to record the observed hatch date (= chick day 0). Between chick days 8 and 11, we ringed chicks with an aluminium ring and caught parents using spring-loaded traps inside the nest-box. Captured females were individually marked, and her biometrics (3rd primary feather, tarsus length and body weight) were measured. On chick days 14–16, biometrics for chicks were taken. We accounted for differences in chick age statistically. Number of fledglings was recorded once the nest fledged (expected at chick day 21). Effects on fitness were investigated using parameters from brood monitoring data as well as biometric data of females and chicks (Strauß et al., submitted).

When two or more eggs were present, a temperature logger (iButton: Thermochron DS1922L-F5 by Maxim Integrated) was placed inside the nest-cup to estimate start of incubation and record incubation activity from nest temperature measurements. The iButtons were programmed to record in 3-min intervals and with an accuracy of 0.0625°C. Wrapped in a silk sock, they were secured among the eggs with a metal wire to avoid removal by the female. As reference, ambient temperature was recorded at four locations across the different forest patches using HOBO loggers (1-min interval, u12-012, ONSET).

All brood monitoring and experimental procedures were according to the Dutch Experiments on Animals Act and approved by the national Animal Ethics Committee (CCD Permit AVD80100 2017 831).

2.2 | Experimental design

To manipulate the female's activity during incubation, we exposed incubating females to additional light inside the nest-box at twilight following the experimental design in Schlicht et al. (2014). We used two separate groups (morning and evening group) each containing 11 paired sets of light-treated and untreated females ($n = 22$; each pair constituted a Light Set). The light-treated individuals received 4 h of light either overlapping with sunrise (termed morning-treated group) or sunset (termed evening-treated group) for 5 days during incubation, compared to their paired untreated controls (termed morning-control and evening-control, respectively; Figure 1).

A light was placed into the nest-box by substituting its lid by an identical lid in which a small light was embedded. The experimental lid had a centrally positioned LED light that illuminated the nest-cup from close distance (ca 20 cm). We used a white LED with diffusive cap and a beam angle of 80° (3 mm diffuse, white LED, Opencircuit, Tallinn, Estonia) to illuminate the whole nest-cup evenly. The light spectrum was typical for a white LED (Figure S1) and its intensity was 9–30 lux at a distance of 20 cm. Therefore, we dimmed lights to on average 1.5 lux (range: 0.7–3.5 lux) using a shield made from partly transparent baking paper. In two occasions, the baking paper was removed or had fallen off, and females were exposed to brighter light conditions (12.8 in nest-box 225 and 20.5 lux in nest-box 664, respectively). However, these two individuals showed no noticeable abnormalities in their incubation patterns compared to the other

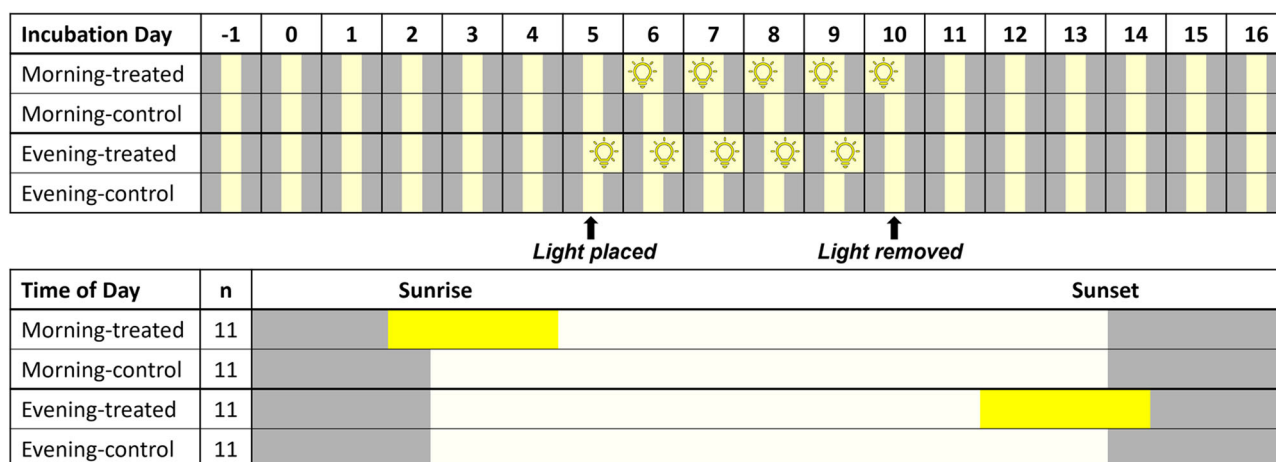


FIGURE 1 The experimental setup: Light treatment relative to incubation (top) and time of day (bottom). There were two treatment groups with either light in the morning (morning-treated) or the evening (evening-treated), and their paired dark controls (morning-control and evening-control, respectively). The light was placed at incubation day 5 and illuminated the next five nights for 4 h each. The morning-treated group received light from 1 h before until 3 h after sunrise, whereas for the evening-treated group, the light was on 3 h before until 1 h after sunset. Dark controls had no light treatment at any time.

individuals. Lights were programmed to automatically turn on and off depending on date and the treatment group. We programmed a small computer (NodeMCU Lolin V3 and DS3231 Real Time Clock Module I2C, AZDelivery) turning on the LED for a total of 4 h, either from 1 h before sunrise or from 3 h before sunset (Figure 1 bottom). In this way, both groups experienced 1 h of light treatment overlapping with civil twilight (i.e., with a sun angle below 0), and 3 h of light treatment during daytime (i.e., while the sun was elevated above the horizon). The whole setup was powered using a small battery (6 V 4.5 Ah, Ultracell; lasting up to 2 weeks in our setup) placed together with the custom-made timer in a box on the ground next to the tree trunk.

The females for both treatment groups (morning- and evening-treated group) were selected pair-wisely based on estimated start of their incubation at the same day (see below) and on breeding in spatial proximity. In the light-treated nest-boxes, the lid was replaced on incubation day 5 during daytime (10:00–19:00 CET) for both treatment groups (Figure 1 top). Lights were removed at incubation day 10 to allow us to investigate potential carry-over effects of light on females' activity patterns after the end of the treatment (see Results in Schlicht et al., 2014), and to avoid direct effects on possibly early-hatching chicks (Raap et al., 2016a; Ziegler et al., 2021). One nest of the morning-treated group hatched on the day of light removal and, therefore, the chicks might have been exposed to the light in the morning. Before the start of the treatment, we measured unmanipulated incubation activity of the females as baseline measurement. By placing the lights during daytime, the evening-treated individuals received the first treatment in the evening of incubation day 5, whereas the morning-treated individuals experienced the first treatment in the morning of day 6. The experimental phase was, therefore, calculated differently (i.e., –1 day) for offset of the evening group (i.e., evening-treated and evening-control). Clutches hatched 0–7 days after the end of the treatment resulting in a postexperiment phase where activity could be recorded before the chicks develop their own thermoregulation (Mertens, 1977). One female deserted after placing the lid and the treatment phases of two more females were missing due to malfunctional equipment (i.e., samples sizes are given in figures and tables).

For the comparison with the light-treated females, we additionally picked 11 dark controls for each treatment group ($n = 22$) that were within the temporal (± 2 days differences in start of incubation) and spatial distribution of their paired light-treated female. These subgroups were made to account for any variation from season, weather, and LED-setups used, and are further referred to as Light Set. Control nest-boxes were not additionally disturbed, due to the normal monitoring schedule, and were, therefore, visited once or twice less often than light-treated nest-boxes.

For assignment to experimental groups, we estimated the start of incubation (first day with 60% presence on the nest during the day) during field work. In the field, we used the *incR* package (Capilla-Lasheras, 2018), adjusting parameters by visual fit for each nest-box (fixed parameters for night limits: *lower.limit* = 20:00 to *upper.limit* = 5:00 h). In the finalized data processing of the incubation temperature data (see pipeline below), we also re-assessed the start of incubation

using the assigned presence (i.e., on-bouts) from the final pipeline. We found that the incubation start had been assessed inaccurately in the field for four light-treated broods (assessed to start 1–5 days later than the actual start of incubation). In most cases, nest attentiveness dropped below 60% between the actual and the previously assessed start of incubation in the field. In general, incubation delay and pauses were present in many broods leading to high variation in the incubation consistency, probably caused by the harsh and unpredictable weather conditions in 2021. Between April and June, ambient temperature was on average 10.89°C on Vlieland and, therefore, about 1.5°C colder compared to 2020 and 2022. This was more pronounced earlier in the season (–2.34°C in April) and diminished towards the end (–0.09°C in June) resulting in a colder and later spring in 2021. Additionally, May, i.e. the experimental phase, had 173.00 h less sun than the Mays of 2020 and 2022 but 125.45 h and 120.65 mm more rain (dataset from Vlieland, De Kooy and Hoorn (Terschelling) in 2020–2022: Royal Netherlands Meteorological Institute (Royal Netherlands Meteorological Institute KNMI, 2023).

2.3 | Onset and offset of activity from incubation activity

Following Capilla-Lasheras (2018), we assessed a female's presence and absence on the nest by evaluating changes in nest temperature compared to ambient temperature and to reference phases at night and day (Strauß et al., submitted). At first, we averaged and binned ambient temperatures for every 30 min across all HOBO loggers used. We calculated the differences between nest and ambient temperature, and the pattern resulting from that (i.e., differences between consecutive timepoints). Similarly, we also extracted the differences between consecutive temperature recordings of environment and nest separately, representing patterns of warming and cooling. Cumulative changes of nest warming (with positive sign) and cooling (with negative sign) were calculated by adding up these differences until the sign changed (hereafter called: cumulative warming or cooling, respectively). Additionally, the maximum change of each bout was recorded. In the second step, nest-date specific thresholds were extracted from night and day to assign on- and off-bouts. All thresholds and criteria below were determined by evaluating if they had a reasonable fit to a subset of the data. This subset included data of different quality, and breeding stages as well as deserted broods. For the processing pipeline here, we define night-time for the phase when the sun is more than 12° below the horizon (i.e., from after nautical dusk to before nautical dawn) and daytime for the sun with a higher elevation, respectively. During this night-time, females are expected to stay inside the nest-box for roosting and partially incubating (Podlas & Richner, 2013), and because foraging seems unlikely due to the very low light levels (Kacelnik, 1979; Kishida, 1989). Thus, we excluded nights where the data indicated female's absence or low-quality data, i.e., when the minimum nest temperature was smaller than 15°C and when

the average nest temperature was extremely similar to the average ambient temperatures (i.e., a difference of less than two standard deviation from the dataset's average). If data for the previous and subsequent nights were available, we picked the smaller thresholds for the day in between so that sensitivity of the thresholds was higher for rises and lower for drops.

For each day, we assigned off-bouts for very low nest temperatures that follow the same pattern as ambient temperature, i.e., absolute temperature differences (nest – environment) of $<5^{\circ}\text{C}$ and absolute changes of these differences of $<0.5^{\circ}\text{C}$. Cumulative warming and cooling were assumed to be caused by the female absence or presence, respectively, when the maximal nest temperature changes were more than twice as the average observed at daytime and the cumulative changes were larger than the maximum drop or rise at night. Just as in Capilla-Lasheras (2018), strong rises and drops were assigned to on- and off-bouts when they exceeded the corresponding night-time maximal drop and rise, respectively. Additionally, presence of the female was assumed for nest temperature increases if cooling would be expected otherwise from large differences to ambient temperature, i.e., at nest temperatures 10°C warmer than ambient temperatures at daytime or nest temperature above 15°C during night-time (similar to the *temp.diff.threshold* setting in *IncR*; Capilla-Lasheras, 2018). In an automatic filtering step, standalone drops and rises were checked to be strong enough to be plausible for the 3-min interval (i.e., a reasonable off-bout if the drop was equal to or lower than the corresponding reference, and on-bout if the rise was equal to or above the corresponding reference and the temperature difference between nest and environment was smaller than 15°C). For details see Supporting Information Material S2 (Strauß et al., submitted).

The automatic assignment of on- and off-bouts was visually inspected, its quality scored (good, medium, and bad) by Aurelia F.T. Strauß, and onset and offset determined. Quality of the data was assessed by the visibility of differences in temperature variation between active and inactive phase, i.e., high variation versus low variation, respectively (Capilla-Lasheras, 2018; Diez-Méndez et al., 2021; Smith et al., 2015). Additionally, low absolute nocturnal temperatures lead to lower quality scores (medium: ca $15\text{--}20^{\circ}\text{C}$; bad: $<15^{\circ}\text{C}$) as the position of the iButton was assumed to be suboptimal in those cases. For further analyzes we used good and medium quality scores, resulting in the exclusion of single onsets (11%) or offsets (16%) of bad quality, and thus in a final dataset of 650 onsets and 615 offsets. The final dataset comprised multiple measurements per individual and experimental phase with only a few exceptions (i.e., two control females with only one onset after the light treatment phase, and only a single offset for four controls and one evening-treated female after the treatment, and one offset for one evening-control during the treatment phase). Onset and offset of activity corresponded to the first absence in the morning or end of last absence from the nest in the evening, respectively.

All data processing and statistical analyzes were performed in R (version 4.3.1; R Core Team, 2023) and Rstudio (version 2023.06.2).

2.4 | Statistics

Activity onset and offset of the light-treated individuals were tested for effects of the light treatment before, during and after the experiment compared to their paired controls. In particular, onset and offset were used relative to sunrise and sunset, respectively, as response variables for the morning and evening group separately. Thus, we had four main models with a Gaussian error distribution (i.e., for activity onset in the morning group, activity onset in the evening group, activity offset in the morning group and activity offset in the evening group; *lmer* function of *lme4* package version 1.1; Bates et al., 2015). As independent predictor we looked at the interaction of the light treatment (dark controls vs. light-treated) with the experimental phase (before, during and after the light treatment), which automatically included both covariates separately. Although birds change activity timing during incubation, we could not include time to hatching in the analyzes because it was confounded with experimental phase. To account for additional variation, we included three random effects: Female for the repeated measurements, April Day (i.e., date, where 31 March is April day 0) for variation due to weather and other variables that vary with date, and Light Set to account for seasonal, spatial and equipment-related differences between the light treated-dark control pairs.

For significant interactions, we ran five *post hoc* tests using subsets of the data to explore the details of the relationships. On one hand, we looked at differences across the treatment groups separately for the three treatment phases (three models: before, during and after). These models included light treatment as covariate, and Female, April Day and Light Set as random factors. On the other hand, we compared within individual changes in activity patterns across the treatment phase but separately for each treatment group (two models: dark controls and light-treated). Those models included experimental phase as covariate, and Female and April Day as random factors, while Light Set was no longer needed.

Fitness parameters were analyzed for differences between treatment groups (morning-treated group, evening-treated group and the combined dark controls). Here, Treatment was used as independent predictor, Hatch Day as additional covariate to account for seasonal differences, and Light Set as random effect in all models with a Gaussian error unless otherwise specified. In the female weight model, we additionally accounted for body size and diel fluctuations by adding Tarsus Size, Time of Day and Squared Time as covariates. Fledge success was binomial and analyzed using a generalized mixed model with binomial error distribution (*glmer* function of *lme4* package). All models for the chick condition (i.e., weight, tarsus and P3 length) also included Chick Age (in chick days) as covariate and BroodID as random effect. Chick body weight had the Time of Day when measured and Squared Time included as covariates.

All estimates were extracted for the full models using *emmeans* (*emmeans* package version 1.8.4; Lenth, 2023) and are shown in the figures. Significance of the parameters was assessed using stepwise model reduction. In case of significant interactions, covariates were

not dropped separately because such procedure does not give meaningful insights beyond the significant interaction. *Post hoc* tests were conducted instead. To assess significance, we used likelihood ratio testing using *drop1* for fixed effects or *anova* for random effects and the binomial model.

3 | RESULTS

3.1 | Onset and offset of activity

In general, incubation patterns changed across the experimental phases (before, during and after the light treatment), which are inevitably correlated to the incubation stages (Figure 1 and Figure S2). In the morning- and evening-controls, onset and offset occur later over the course of incubation and thus also over the course of the experiment (Figure 2 and Figure S2). These temporal patterns differed between the light-treated birds and their respective dark controls.

For activity onset, we found a significant interaction of light treatment and experimental phase ($F_{2,322} = 3.54$, $p = 0.03$) in the morning group (Figure 2a and Table 1). Comparing the groups in all phases separately, we found that the morning-treated females had an 11-min earlier activity onset than morning-controls during the light treatment. However, this effect was marginally non-significant (*post hoc* treatment: $F_{1,100} = 4.33$, $p = 0.07$). There was no significant difference in onset between these subgroups before ($F_{1,130} = 0.88$, $p = 0.36$) and after the treatment ($F_{1,92} = 0.43$, $p = 0.53$). We then compared within-individual differences across experimental phases separately for the treatment groups. The morning-controls delayed their onset by 17 min during the treatment and thereafter advanced it again (*post hoc* phase: $F_{2,1157} = 5.40$, $p = 0.01$), while the morning-treated females did not change their onsets (−1 min difference; $F_{2,164} = 0.36$, $p = 0.70$; Figure 2a and Table S1). This implies that light-treated females in the morning group advanced their onsets by 18 min compared to their dark controls, by not showing a natural delay during mid-incubation. In contrast, for onset of the evening group, there was no difference between light-treated individuals and their dark controls ($F_{2,322} = 0.21$, $p = 0.81$; Figure 2b and Table 1).

For offset of activity we found a reversed pattern. While there was no interaction effect between light treatment and experimental phase on the offset in the morning group ($F_{2,304} = 1.69$, $p = 0.19$, Figure 2c), the offsets in the evening group significantly differed depending on treatment and experimental phase ($F_{2,307} = 4.13$, $p = 0.02$; Figure 2d and Table 1). Before the treatment started, evening-treated individuals had a marginally non-significant later offset of 35 min than evening-controls (*post hoc* treatment: $F_{2,110} = 4.82$, $p = 0.06$). This difference was not present thereafter, i.e., during ($F_{2,90} = 0.41$, $p = 0.52$) or after the treatment ($F_{2,103} = 0.44$, $p = 0.52$; Table S2). Likewise, within individuals, we found a significant 41-min shift in the evening-controls to a later offset during treatment (*post hoc* phase: $F_{2,145} = 11.38$, $p < 0.01$), whereas

the evening-treated individuals did not change (−1 min difference; $F_{2,160} = 1.17$, $p = 0.31$; Figure 2d and Table S2). This confirmed that the difference between the treatments came from the difference in the within-individual shifts. Birds that experienced light in the evening, therefore, advanced their activity offset by 42 min relative to their controls.

This asymmetric shift of activity onset versus offset led to changes in the duration of the active day in the light-treated females compared to their controls. While the duration of the nocturnal attendance generally decreased by 53 min within individuals throughout the experimental phases (range: 32–72 depending on the group), this change progressed quicker in the morning-treated females and more slowly in the evening-treated females during the treatment.

3.2 | Fitness

Light-treated females were 0.7 g lighter in body weight compared to the combined control females ($F_{2,34} = 7.06$, $p < 0.01$), with no difference between females from the morning- and evening-treated groups (*post hoc*: $F_{1,19} = 0.01$, $p = 0.94$; Figure 3 and Table 2). This was also true when controlling for body size (i.e., tarsus length: $F_{1,34} = 4.81$, $p = 0.04$). Other variables such as hatch day and time of day were non-significant (Table 2). Tarsus length, however, did not differ between the groups ($F_{2,34} = 0.64$, $p = 0.54$; Table S3).

There was no effect of the light treatments on the breeding success. Most females hatched at least one egg. Only three dark controls failed completely, and one female deserted after placing the LED lid (excluded). The other breeding traits were also not significantly affected by the light treatment (number of hatchlings: $F_{2,41} = 1.81$, $p = 0.18$; number of fledglings: $F_{2,38} = 0.18$, $p = 0.83$; fledge success: $\chi^2_{2,n=40} = 0.77$, $p = 0.68$; Table S4). Fledging was more successful in later hatched broods (Table S4). Chick biometrics were only taken for 27 broods due to the low survival of broods until the measurement on chick days 14–16 during the wet weather conditions in May 2021. In these broods, the light treatments could not explain variation in chick weight ($F_{2,160} = 0.88$, $p = 0.43$) or size (tarsus: $F_{2,160} = 1.09$, $p = 0.38$; P3 feather: $F_{2,160} = 0.57$, $p = 0.57$; Table S5). Later hatching broods had larger P3 feathers, and all biometrics were highly dependent on chick age (in chick day) (Table S5).

4 | DISCUSSION

We found an effect of light at night on activity onset when provided in the morning, and of light in the evening on activity offset but not *vice versa*. Both effects came from differences in the within-individual changes of activity from before to during the experimental treatment between light-treated females and their dark controls. Breeding success was similar between

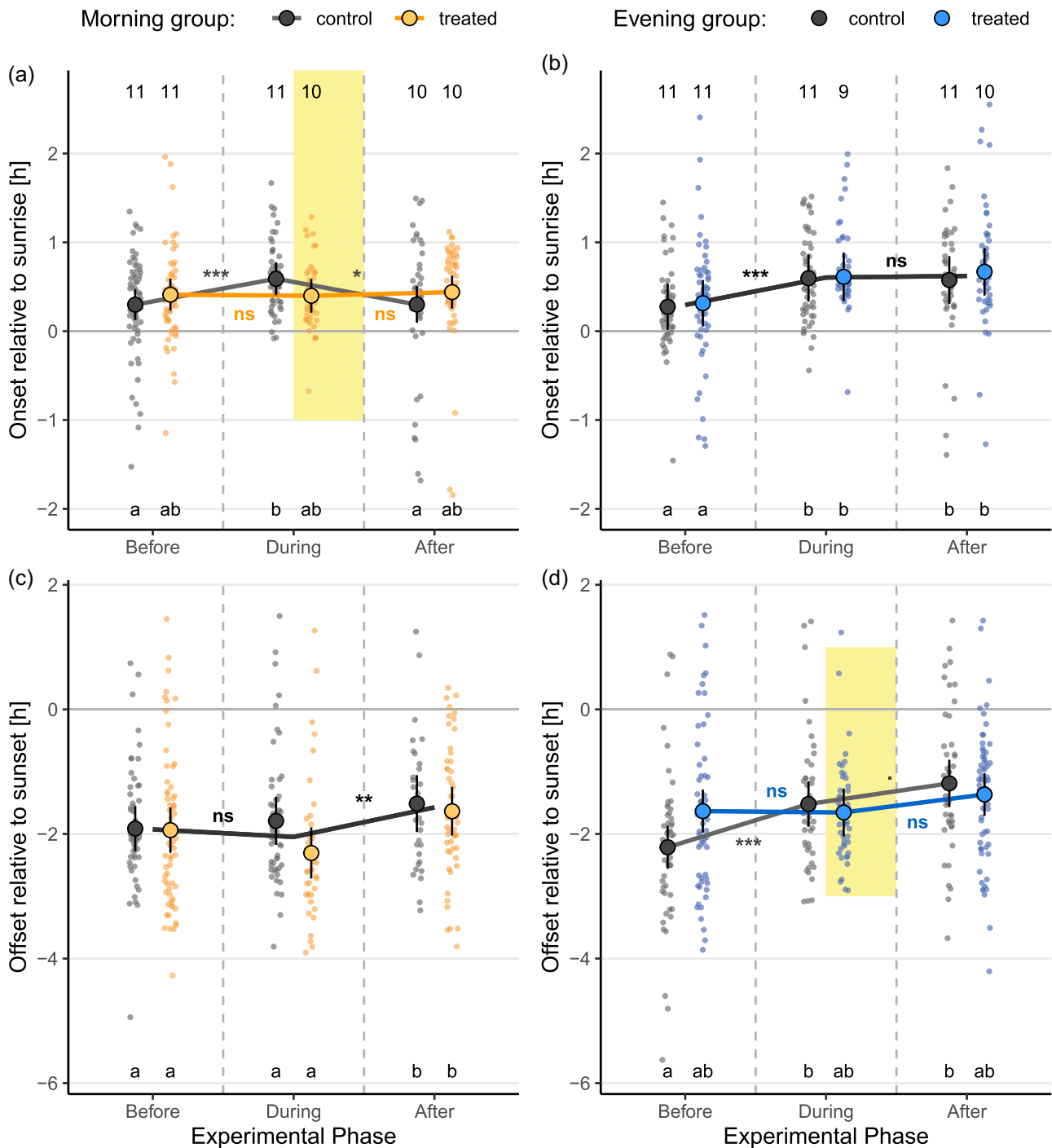


FIGURE 2 Activity onset (top) and offset (bottom) across experimental phases for morning (left) and evening group (right). Raw data are shown as well as model estimates (mean + CI). Treatment groups are in different colors. Temporal changes are indicated as lines in black for non-significant differences between treatments (b, c) and otherwise separately in their respective color (a, d). Sample sizes on top show the number of females measured, and the yellow field highlights the data collected from birds that were at this time exposed to ALAN. Significance levels from *post hoc* tests: letters were used for treatment differences within experimental phases, and stars for phase differences within treatments ns $p > 0.1$, $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

light-treated and control females. Nonetheless, females seemed to have lost energy reserves due to the light treatments as they were lighter than the control females when measured 9–18 days after the treatment.

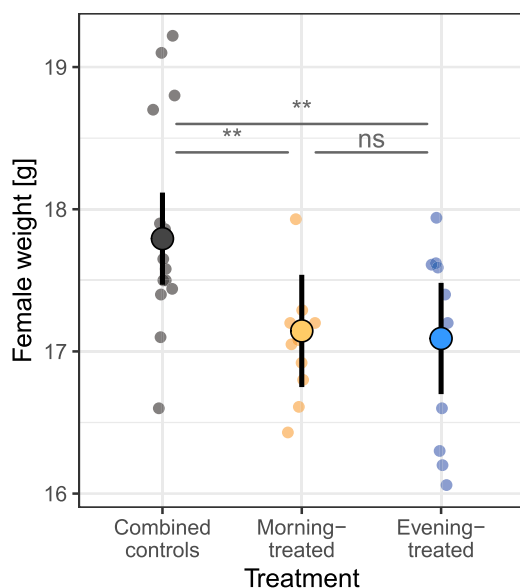
Untreated females naturally delayed their onset and offset of activity throughout incubation in our study. The same pattern has previously been observed for onset but the reverse for offset (McGlade et al., 2023; Schlicht & Kempnaers, 2020). Deviation

TABLE 1 Model statistics for activity onset relative to sunrise and offset relative to sunset using stepwise model reduction.

Onset Fixed	Morning group <i>n</i> = 22				Evening group <i>n</i> = 22			
	<i>F</i>	<i>df</i> ₁	<i>df</i> ₂	<i>p</i>	<i>F</i>	<i>df</i> ₁	<i>df</i> ₂	<i>p</i>
Phase					19.72	2	268.48	<<0.01***
Treatment					0.10	1	10.02	0.76
Treatment × phase	3.54	2	281.30	0.03*	0.21	2	276.66	0.81
Random	Chisq	<i>df</i>		<i>p</i>	Chisq	<i>df</i>		<i>p</i>
Female	11.90	1		<<0.01***	61.68	1		<<0.01***
April day	6.52	1		0.01*	1.75	1		0.19
Light set	0.00	1		1.00	0.15	1		0.70
Offset Fixed	Morning group <i>n</i> = 22				Evening group <i>n</i> = 22			
	<i>F</i>	<i>df</i> ₁	<i>df</i> ₂	<i>p</i>	<i>F</i>	<i>df</i> ₁	<i>df</i> ₂	<i>p</i>
Phase	4.79	2	291.17	0.01**				
Treatment	1.05	1	10.32	0.33				
Treatment × phase	1.69	2	288.15	0.19	4.13	2	273.20	0.02*
Random	Chisq	<i>df</i>		<i>p</i>	Chisq	<i>df</i>		<i>p</i>
Female	7.71	1		0.01**	2.43	1		0.12
April day	0.00	1		1.00	0.05	1		0.82
Light set	0.31	1		0.58	0.05	1		0.82

Note: For significant interactions, model statistics for the separate covariates are unavailable (i.e., phase and treatment, see *post hoc* testing instead). Sample sizes are included as the number of females.

p* < 0.05; *p* < 0.01; ****p* < 0.001.

**FIGURE 3** Female weight (measured 9–18 days after the end of the treatment) and model estimates (mean + CI) depending on treatment group. Significance levels from *post hoc* test: ns *p* > 0.1, ***p* < 0.01. CI, confidence interval.

from the natural patterns can be induced by manipulation of nest temperature (Bryan & Bryant, 1999) and light inside the nest-box (McGlade et al., 2023). This has been shown in the response to ALAN as a deviation from a naturally advancing activity offset

throughout incubation but not for the delaying activity onset (McGlade et al., 2023). We, however, exposed our females only for 5 days instead of the full incubation phase. Our light treatment also changed the natural incubation patterns and seemed to inhibit the natural delay in onset and offset in our study. In particular, our morning-treated females did not delay their activity onset and our evening-treated females did not delay their offset. To facilitate the direct comparison to other studies whose controls did not modify activity patterns, our ALAN effects can be rephrased as change relative to the controls' changing activity patterns. So in other words, morning-treated females advanced their activity onset relative to the morning-controls and evening-treated females their offset relative to the evening-controls.

An advanced onset in response to ALAN has been shown in several avian species (Sanders et al., 2021). In studies carried out with great tits under controlled conditions, advanced activity onsets were even visible at very low light intensities such as 0.05 lux and this response was increasing with higher intensities used (de Jong et al., 2016a; Dominoni et al., 2022; Spoelstra et al., 2018). Also, when ALAN exposure was reduced to the early morning, an advance can be induced (Kumar et al., 2021; Santema & Kempenaers, 2023; Schlicht et al., 2014). In our study, onset was advanced in the morning-treated females compared to their controls, but not in the evening-treated females. Thus, our females were unresponsive in their activity onset towards the light in the early evening. This is in line with a study on

TABLE 2 Model statistics for female weight using stepwise model reduction, and thereafter *post hoc* tests.

Weight [g]	n = 36											
Fixed	F	df1	df2	p								
Treatment	7.06	2	24.44	<0.01**								
Tarsus	4.81	1	29.87	0.04*								
Time	1.71	1	27.61	0.20								
Time ²	0.23	1	28.46	0.63								
Hatch day	2.30	1	30.93	0.14								
Random	Chisq	df	p									
Light set	0.49	1		0.48								
Post hoc	Treatment				Tarsus				Light Set			
	F	df1	df2	p	F	df1	df2	p	Chisq	df	p	
Combined controls—Morning-treated	10.23	1	15.02	<0.01**	6.74	1	22.75	0.02*	0.44	1	0.51	
Combined controls—Evening-treated	11.28	1	16.07	<0.01**	2.87	1	18.87	0.11	0.97	1	0.33	
Morning-treated—Evening-treated	0.01	1	9.30	0.94	1.00	1	16.66	0.33	0.09	1	0.76	

Note: Sample size is included as the number of females.

* $p < 0.05$; ** $p < 0.01$.

captive redheaded buntings (*Emberiza bruniceps*) that were exposed to 4 h of light in the early night (Kumar et al., 2021).

Similarly, our morning-treated females did not shift their activity offset as also seen in the redheaded buntings when treated with ALAN in the late night (Kumar et al., 2021). In contrast, the activity offset was affected by the light treatment in our evening-treated group. Though, the direction of the shift was contrary to our expectation that the females would delay their end of active day. Such a delay was seen in the buntings exposed to light in the early night (Kumar et al., 2021). We, however, observed an advance of the offset relative to the activity pattern observed in the controls. Note that there was a marginal difference in the offset between evening-treated group and evening-controls already before the treatment started. In studies where ALAN was provided all night, findings are inconsistent ranging from non-responsive to delayed offset (de Jong et al., 2016a; Raap et al., 2015; Ulgezen et al., 2019). In our study, however, females seemed to be attracted to enter the nest-box before the light turned off. This fits with most, but not all studies, that show preferences for brighter roosting (Ulgezen et al., 2019, but see Raap et al., 2015) and breeding sites (i.e., preference for nest-boxes, that had a side window; Podkowa & Surmacki, 2017).

In both behavioral responses described above, the difference between light-treated and dark controls only became clear by looking at the within-individual differences from before to during the treatment. Accordingly, our effect sizes were small compared to the general variation in activity onset and offset across the whole dataset. However, the 17-min advance in onset of the morning-treated females is comparable with the findings from other ALAN studies that looked at female tits during the breeding season

(14–20 min advance in activity onset; McGlade et al., 2023; Raap et al., 2015; Schlicht et al., 2014, but see 118 min in Raap et al., 2016b). This seems mostly consistent despite differences in exposure duration (h per night and number of nights) or light intensity (1.5–300 lux) and sample size (11–73 ALAN females) between these studies. Behavioral responses towards ALAN in the wild might be relatively weak due to factors other than natural and artificial light influencing activity patterns. For example, weather conditions have been shown to alter incubation activity patterns (Coe et al., 2015) as well as activity onset and offset throughout the year (Schlicht & Kempenaers, 2020). Our study population is located on an island in the North Sea and was, therefore, particularly exposed to the wet weather condition in May 2021, leading to many laying and incubation delays as well as desertions during incubation and chick provisioning (own observations). While we did not observe systematic effects of the harsh weather on the ALAN effects, these conditions might have caused additional noise in our activity data throughout the experimental phases.

In our study, light-treated individuals had lower body weights during the subsequent chick-rearing period, and these differences were consistent for the morning- and evening-treated groups. It is possible that females might have reduced body weight as anti-predator strategy (Krams, 2000) as they were disturbed at the nest once or twice more often during incubation compared to dark controls. However, such responses of females to nest-box study disturbance have to our knowledge not been previously reported. Conversely, we had predicted possible negative effects on females due to the ALAN treatments, which could be explained by one or multiple of the following causes. First, we observe not just a shift the

activity parameters of light-treated individuals but also that they modified their active day due to asymmetrical changes in onset and offset. In particular, morning-treated females likely shortened their nocturnal attendance inside the nest-box while evening-treated females extended it compared to the general decrease visible in the dark controls. As we only measured nest temperature, which is an approximation for leaving and entering the nest-box, we can just speculate about the actual onset and offset of activity inside the nest-box, or offset and onset of sleep. Other studies, however, looked into the details of sleep parameters of great tits that were exposed to ALAN. Video recordings inside the nest-box revealed a later sleep onset and earlier awakening and lower sleep quality for females under continuous ALAN (Raap et al., 2015, 2016b). Under ALAN, variation in nest temperature during incubation increased in another study (McGlade et al., 2023), which could indicate restlessness inside the nest-box. Indeed, also great tits roosting outside the nest-box increased nocturnal activity closer to the light sources (Ouyang et al., 2017). Those ALAN effects on sleep are likely mediated through suppression of the sleep hormone melatonin. Melatonin is expressed rhythmically, with high levels during sleep and mediated through the internal clock, so that ALAN could shift the melatonin expression rhythm depending on the time of exposure (Dominoni, 2015; de Jong et al., 2016a; Kumar et al., 2021).

Associated with melatonin suppression and sleep deprivation, continuous ALAN also seems to modify the metabolic system and thus increase body weight (Nelson & Chbeir, 2018; Svechkina et al., 2020). Increased body mass and fat has been shown for various model organisms such as rodents (Cissé et al., 2017; Shuai et al., 2023) and songbirds (Batra et al., 2019). In these studies, individuals shifted feeding times and sometimes also increased their food intake under *ad libitum* food. This might be associated with changes in the diel rhythm of metabolism, the fat storage in the liver as well as the gut microbiome (Batra et al., 2019; Nelson & Chbeir, 2018). Similar results were also found under part-night illumination in the context of seasonal fattening for a migratory bird (Kumar et al., 2021). Interestingly, we found the opposite, i.e., our light-treated females were about 0.7 g lighter than the controls. So far ALAN effects on body weight in wild birds has only been studied for great tit nestlings with inconclusive results on the body mass growth (Raap et al., 2016a; Ziegler et al., 2021). In a wild setting, food availability and foraging possibilities can be scarce and fluctuate unpredictably, while energy expenses can be elevated under ALAN exposure (Ulgezen et al., 2019). Therefore, individuals could have lost weight instead of gained weight. This difference can be consequential for retaining sufficient energy during the night when feeding is not possible (McNamara et al., 1994), especially if energy expenses potentially increases under ALAN (Ulgezen et al., 2019). In the early morning during chick provisioning, females need to trade-off refilling of their own energy reserves against those of their chicks (Haftorn, 1989; Pagani-Núñez & Senar, 2013). However, we could not find carry-over effects of the light treatments on the breeding success or chick condition. Similarly, these fitness traits were generally unaffected by diel timing in this population (Strauß et al., submitted). Despite the

limited number of broods that survived until fledging in 2021, females might maintain good parental care and invest less into self-maintenance under the potential carry-over effect from our ALAN treatments.

While captive studies are highly controlled for other external factors, they also focus on males (e.g., Dominoni et al., 2022; de Jong et al., 2016a; Kumar et al., 2021; Spoelstra et al., 2018, but see Batra et al., 2019). However, for the application to wildlife conservation we need to consider potential sex differences in the response to ALAN. Great and blue tits alter their activity patterns under natural conditions across the year with increasing differences between the sexes in the breeding season (Kluijver, 1950; Schlicht & Kempenaers, 2020; Stuber et al., 2015). In particular, breeding females seem more vulnerable to ALAN exposure, but their response can vary between individuals (Raap et al., 2016b). We exposed our great tit females during the incubation phase, when females are spending most of their time warming the eggs to enable the embryo to develop (Cooper et al., 2005). They were unable to avoid light exposure due to the experimental setup. Roosting males, that are less bound to the nest-box, are more able to avoid ALAN exposure resulting in more variable responses to ALAN inside the nest-box (Santema & Kempenaers, 2023). If wild great tits can largely avoid direct ALAN exposure under natural conditions while roosting inside a cavity such as a nest-box (de Jong et al., 2016b; Raap et al., 2018), they are behaviorally unresponsive (Titulaer et al., 2012; Welbers et al., 2017). Therefore, light pollution might be more problematic for open cup-nesting species, especially for incubating individuals that are exposed to light pollution at the nesting sites.

5 | CONCLUSION

Incubating great tits showed a small behavioral response to ALAN in the morning and in the evening. Light-treated females and their dark controls differed in their within-individual activity changes across the incubation phase. This resulted in an advanced activity onset and extended length of the active day for morning-treated females, and an advanced activity offset and shortened day for evening-treated females compared to their dark controls. Both treatments caused a different short-term behavioral response depending on the time of exposure. The body condition during chick provisioning, however, was equally affected indicating a carry-over effect for both treatments. Even a briefly disturbed sleep pattern or shift, independent of the time of exposure, might have caused an energy-consuming mismatch between behavior and internal clock. Additionally, females seem to be more vulnerable to ALAN during the breeding season than males because they are often closely bound to the nesting site and, therefore, less flexible in avoiding ALAN exposure. Thus, future research should also investigate physiological consequences for female birds. They might have paid a cost in their body condition while maintaining good parental care. In our case, light schedules could not mitigate negative effects on the bird's physiology. Therefore, ALAN should be avoided and spatially reduced

in general to decrease effects on wildlife and health (Davies & Smyth, 2018).

AUTHOR CONTRIBUTIONS

Aurelia F. T. Strauß and Barbara Helm formulated the idea for the study and all authors were involved in designing the experiment. Aurelia F. T. Strauß and Lies Bosma collected the data, and Aurelia F. T. Strauß processed the data and led the statistical analyses and writing. Marcel E. Visser and Barbara Helm advised on the statistics and the writing.

ACKNOWLEDGMENTS

AFTS was supported by the Adaptive Life programme of the University of Groningen, the Netherlands. We would like to thank Theo Jurriens for the development of the LED setup and the programming of the custom-made timer as well as Gabriel Charvalakis for measuring the light spectrum of the LED. We are also grateful to Henri Bouwmeester and Pradyut Rao for intensive help in the field during the exceptional breeding season in 2021, and to Simon Verhulst and Barbara Tomotani for stimulating discussions.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at <https://doi.org/10.6084/m9.figshare.24961596>. Raw data needed for the data processing pipeline can be found separately (<https://doi.org/10.6084/m9.figshare.24973095>).

ETHICS STATEMENT

Brood monitoring and experimental procedures were according to the Dutch Experiments on Animals Act and approved by the national Animal Ethics Committee (CCD Permit AVD80100 2017 831).

PREVIOUS SUBMISSION STATEMENT

This manuscript has not been submitted previously to another journal.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Strauß, A. F. T., Bosma, L., Visser, M. E., & Helm, B. (2024). Short-time exposure to light at night affects incubation patterns and correlates with subsequent body weight in great tits (*Parus major*). *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 341, 364–376. <https://doi.org/10.1002/jez.2787>