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Source: Zoological Science, 42(1) : 144-152

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zs240063

ZOOLOGICAL SCIENCE **42**: 144–152 (2025)

It Pays to Sit Tight: Stable Night-Time Incubation Increases Hatching Success in Urban and Forest Great Tits, *Parus major*

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Animals organize their time so that their behaviors do not conflict with each other and align well with environmental conditions. In species with parental care, adults must also accommodate offspring needs into their temporal allocation of resources and activities. Avian parents face harsh constraints on their time budget during incubation, when they must sustain themselves but also transfer heat to eggs. During day-time, their shuttling between incubating and foraging is well studied. At night, birds usually rest on the nest and provide stable incubation. However, the stability of night rest depends on parental physiology and environmental conditions, and its patterns and consequences are poorly understood. We propose that stable parental night rest enhances the chances of embryos to hatch and might shorten incubation time, but that, in an urbanizing world, night rest may be compromised. We recorded nocturnal incubation restlessness, defined as variation in nest temperature, by placing thermal loggers into nest boxes of urban (25 clutches) and forest (70 clutches) great tits, where only females incubate. We found that with increasing nocturnal restlessness, hatching success dropped by ca. 60% per unit of increase in incubation restlessness in both habitats, despite higher hatching success in the forest. One putative driver of unstable incubation was artificial light at night, which for urban nest boxes was associated with increased nocturnal restlessness. Restlessness did not affect time to hatching. We conclude that sitting tight at night provides fitness pay-offs for incubating birds, but is influenced by environmental conditions, including those shaped by human activities.

Key words: incubation restlessness, artificial light, bird, urban ecology, great tit

INTRODUCTION

Reproduction in oviparous species is extremely vulnerable to environmental conditions because of the external development of embryos (Hepp et al., 2006; Nord and Nilsson, 2011). As a consequence, natural selection has generated parental brood care strategies that partly buffer developing embryos from the external environment. Parental strategies entail, amongst others, building nests, protection from predators, brood hygiene, thermal regulation of the nest or active incubation of the eggs (extensively covered in [Bloch, 2010; Deeming and Reynolds, 2015]). Developing embryos are highly sensitive to changes in temperature, whereby high and low temperatures can impact embryonic development, modify the incubation period and increase embryonic mortality (Hepp et al., 2006; Nord and Nilsson, 2011; Gwinner et al., 2018; Ton et al., 2021). Consequently, incubation commonly provides far-reaching parental protection of the embryo from challenging thermal conditions (Webb, 1987). Such behaviors can put high demands on parents and often compromise their survival and future reproductive prospects. Parental brood care strategies thereby involve trade-offs between self-maintenance and successful rearing of offspring (Conway and Martin, 2000a; Williams, 2012; Hepp et al., 2015).

Brood care is embedded in the rhythmic organization of animal life. Animals generally organize their diel and annual cycles to avoid overlap of conflicting processes, such as activity and rest, or reproduction and migration (Foster and

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doi:10.2108/zs240063

Kreitzman, 2009; Helm et al., 2017). Their surrounding environments also change rhythmically, captured in the term “timescape” which refers to temporal phases “that exhibit fitness-relevant heterogeneity in biotic and/or abiotic factors of interest” (Gilbert et al., 2023). Thus, when animals compartmentalize their activities, they commonly allocate them to specific times of day or year when they best align with environmental conditions (Kronfeld-Schor and Dayan, 2003). This allocation can be affected by the need to care for eggs or offspring (Bloch et al., 2013). For example, to provide brood-care, social bees alter their diel rhythmicity on levels ranging from behavior to molecules (Bloch, 2010; Beer and Bloch, 2020). In bees and other species including humans, such altered rhythms for the sake of offspring care can entail a temporary loss of sleep (Bloch et al., 2013; Nagari et al., 2019).

In birds, brood care can also conflict with the rhythmic organization of parental behavior, in particular with the diel alternation between activity and rest (Bulla et al., 2016; Helm et al., 2017). Avian parents meet the demand for heat transfer to the developing offspring by allocating time to incubation on the nest (i.e., on-bouts), alternatingly with activities away from the nest (i.e., off-bouts), such as foraging, movement and self-maintenance (Cooper and Voss, 2013; Hepp et al., 2015; Gwinner et al., 2018). The trade-off between self-maintenance and offspring care often involves fine coordination of schedules between the parents (Bulla et al., 2016; Huffeldt et al., 2020). In species with uniparental incubation, trade-offs are exacerbated by substantial temporal and energetic constraints (Conway and Martin, 2000a; Cooper and Voss, 2013; Hepp et al., 2015). During day-time, when the incubating parent typically shuttles between on-bouts and off-bouts, trade-offs between daytime allocations have been studied in detail. Parental behavior that reduced heat transfer to the eggs came at costs of hatching success and nestling quality (Cooper and Voss, 2013; Hepp et al., 2015; Bueno-Enciso et al., 2017; Sieving et al., 2024). Conversely, at night-time, incubating birds are thought to constantly transfer heat to eggs while resting on the nest, but variation in night-time incubation and its consequences for embryo development are less commonly studied (but see [Indykiewicz et al., 2021; van Dis et al., 2021; McGlade et al., 2023]).

The expression of avian incubation behavior, and the allocation of time and energy to offspring, is sensitive to environmental conditions and parental physiology (Conway and Martin, 2000b; Deeming and Reynolds, 2015; Marasco and Spencer, 2015). Birds modify the fine temporal organization of their daytime shuttling between incubation and self-maintenance according to body state and ambient conditions (White and Kinney, 1974; Conway and Martin, 2000b; Londoño et al., 2008; Hepp et al., 2015; Marasco and Spencer, 2015; Diehl et al., 2020; Nord and Cooper, 2020). In turn, environmental conditions can modify the effects of incubation behavior on offspring. For example, warm ambient temperatures can facilitate incubation even in the absence of a parent, while cold and hot temperatures may threaten embryonic development (Webb, 1987; Haftorn, 1988; Griffith et al., 2016; Ton et al., 2021). So far, few studies have linked nocturnal brood care behavior to parental physiology and possible consequences for offspring. Great tits (*Parus major*) whose nests were infested with fleas shortened their night rest considerably during nestling provisioning (Christe et al., 1996), but no effects on brood care were detected. European starlings (*Sturnus vulgaris*) that incubated in nests experimentally amended with beneficial, aromatic herbs also ended their night rest earlier than conspecifics incubating in herb-less nests (Gwinner et al., 2018). The steadier incubation in herb-nests compared to herb-less nests was associated with a shortened incubation period and higher nestling weight. The specific timing of parental activity patterns has also been shown to be associated with fitness. In great tits, the partitioning of activity and rest on the nest differed by maternal chronotype, i.e., by consistent time patterns of an individual relative to conspecifics (Helm et al., 2017; Womack et al., 2023). Great tit female incubation chronotype was associated with the number of young that subsequently fledged. At matched urban and forest sites, females in both habitats that started their activities earlier in the day raised more offspring than late risers (Womack et al., 2023).

Human-mediated changes of environmental conditions can also affect diel rhythms of animals (Gaynor et al., 2018; Sanders et al., 2021; Gilbert et al., 2023; Helm et al., 2024). Changes in the timescape, for example by nocturnal disturbance due to artificial light at night (ALAN), impinge on many aspects of behavior and physiology, including incubation (McGlade et al., 2023) and brood-care (Raap et al., 2016; Remacha et al., 2016; Aulsebrook et al., 2018; van Dis et al., 2021; Sieving et al., 2024; Straus et al., 2024). Among the most commonly reported responses of animals are shifts in activity timing (Sanders et al., 2021), as well as impaired quality of rest (Ouyang et al., 2017). During brood care, great tits that were briefly exposed to artificial light at night (ALAN) greatly reduced their night rest (Raap et al., 2016). Females fell asleep much later, woke up much earlier, and decreased the frequency of their sleep bouts. Nonetheless, in this experimental study no fitness effects were detected (Raap et al., 2016). Similarly, urbanization (including effects of ALAN and noise) can affect the quality of incubation and modify its timing (van Dis et al., 2021; Hope et al., 2022; McGlade et al., 2023; Sieving et al., 2024; Strauß et al., 2024). Some changes in diel incubation patterns may be mediated by reduced sleep and increased restlessness of incubating parents, but to our knowledge, no fitness consequences of nocturnal incubation restlessness have so far been reported (Diehl et al., 2020; van Dis et al., 2021; Hope et al., 2022; McGlade et al., 2023).

We here investigate nocturnal incubation restlessness and its consequences for embryo development in a well-established study model for ecology and biological timekeeping, the hole-nesting great tit (Christe et al., 1996; Helm and Visser, 2010; van Dis et al., 2021; Hope et al., 2022; McGlade et al., 2023; Strauß et al., 2024). We quantified night-time incubation restlessness by recording variation in night-time incubation temperature with loggers inserted in nest boxes while keeping track of hatching success of the offspring. To identify possible anthropogenic effects, we studied great tits in urban and forest environments, and measured ALAN intensity at their nest sites. Regarding consequences of nocturnal incubation behavior for embryonic development, we expected hatching success to decrease, and time to hatching (i.e., the incubation period) to lengthen with increasing night-time restlessness. We based this expectation on evidence that ALAN typically directly reduces the quality of rest, although alternative scenarios are also conceivable. Regarding environmental effects on incubation, we expected females breeding in the city to show increased night-time restlessness due to disturbance from human activities. We further investigated whether ALAN (partly or fully) explained urban effects on incubation restlessness.

MATERIALS AND METHODS

The field methods to generate the dataset used in this study have been described in detail in studies that did not investigate consequences of night-time incubation restlessness (McGlade et al., 2023; Womack et al., 2023). The data in our present manuscript comprise data from a subset of nest boxes studied by McGlade et al. (2023) and Womack et al. (2023), for which no experimental manipulations were conducted. They include multiple-day information from a total of 95 occupied nest boxes (25 in an urban setting, 70 in a forest setting; see Supplementary Table S1 for details). Here, we briefly describe the methods and refer readers to (McGlade et al., 2023; Womack et al., 2023) for details. Conversely, the statistical analyses employed to address the aims of this study are explained in detail. All research was carried out under permits issued to B. Helm.

Study populations and field protocols

Great tits were studied in five nest box populations in Scotland (Fig. 1) in the breeding seasons of 2016, 2017, and 2018 (April to June). Three of these study populations were located in ancient deciduous forests (56°11′N, 4°57′W; 56°7′N, 4°36′W; 56°6′N, 4°34′W). The other two populations were situated in an urban park (55°52′N, 4°16′W) and a suburban park (55°54′N, 4°19′W) in the city of Glasgow (UK) (Fig. 1; for further details, see Jarrett et al. [2020], Branston et al. [2021]). All nest boxes were checked weekly from 1 April for signs of nest-building and egg laying. We calculated the date of clutch completion from the number of eggs present between two consecutive nest box visits (assuming that females laid one egg per day). We assumed a minimum incubation length of 14 days from the date of clutch completion (Gosler, 1993) and checked nest boxes thereafter every other day to accurately determine date of hatching. Incubation length was then calculated as the time difference between the date the last egg of the clutch was laid (i.e., incubation start) and the date the first egg of the clutch hatched. We defined hatching success as the proportion of eggs that hatched for all completed clutches (i.e., where egg laying had been finished and incubation had started). Daily (i.e., 24 h) mean temperatures for the breeding seasons of 2016, 2017 and 2018 were obtained from the U.K. Met Office close to our forest sites (Tyndrum; 56°25′N, 4°42′W) and city sites (Bishopton; 55°54′N, 4°30′W). They were used as predictors for incubation behavior during entire nights whose start fell into a daily temperature window.

As we were specifically interested in whether Artificial light at Night (i.e., ALAN) affected incubation restlessness, rather than in effects of the overall light environment, we quantified ALAN in winter around new moon, when no measurable natural light was present at night and vegetation that was possibly interfering with ALAN was still sparse. Our study thus provides a rough proxy for the maximum contribution of ALAN to the birds' nocturnal light environment during incubation. We measured ALAN for a subset of nest boxes in two field seasons, one in March 2019 (data set 1), and the other between January and February of 2024 (data set 2). In total, ALAN data were available for 56 nest boxes, including 435 repeated measurements of variation in night-time incubation temperature across 61 breeding events. However, due to very low variation in ALAN in forest nests (see Supplementary Figure S1), only the 21

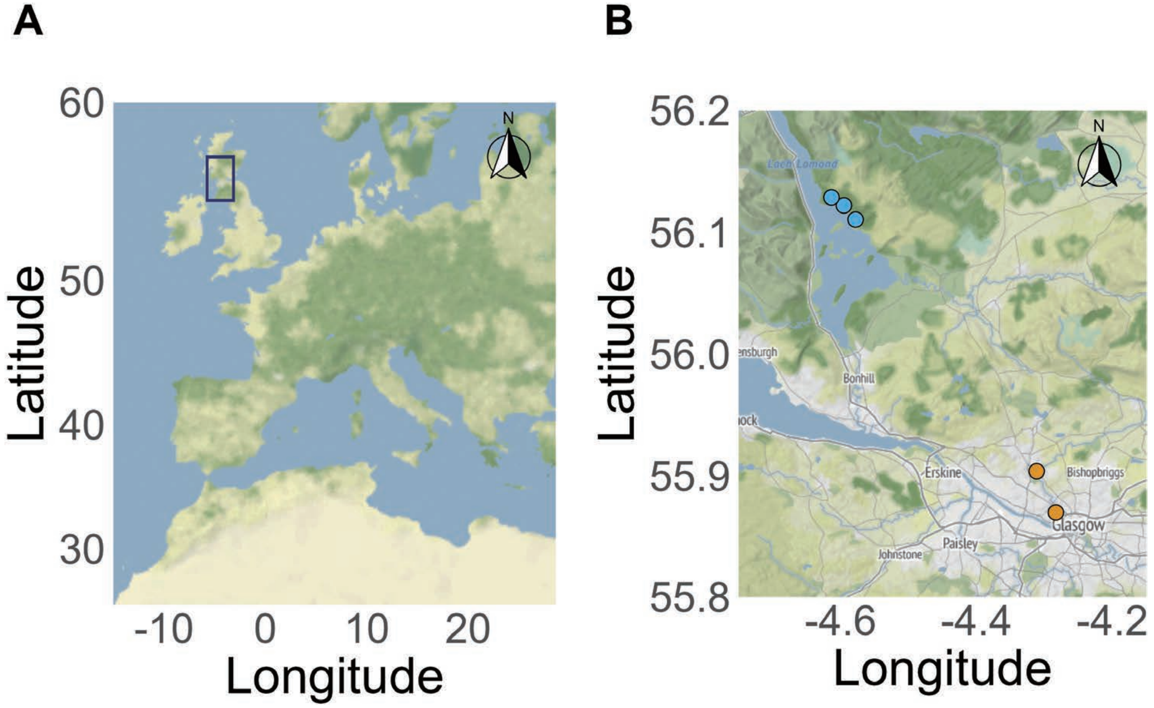

Fig. 1. Location of the study sites. **(A)** General location of the study sites in Europe, indicated by a dark blue rectangle in Scotland. **(B)** Zoom of the area within the dark blue rectangle in **(A)** providing the location of the forest (blue dots) and city (orange dots) study sites in and around the city of Glasgow. Maps produced in R using ggmap and Stadia Maps. Colors in maps illustrate hill shading and the color of natural vegetation ('Stamen Terrain' map style).

measurements from urban nests were used for further analysis. For measuring ALAN, each nest box was visited once after astronomical twilight. We took three (data set 2) or four (data set 1) light measures per nest box as the sensor records light in a narrow field. We took measurements with a Li-cor LI-250A Light Meter with a LI210R Photometric Sensor from a distance of 1 m, facing the front of the nest box, and recorded light measurements in lux. To take measurements, the sensor was held above the recorder's head facing upward for the first measurement, then facing left for the second, and then right for the third. In 2019, we also measured the light intensity birds would experience when looking outward from the nest box entrance. To gain a more representative value of light pollution surrounding each nest box, the mean of these measures was then used for further analysis.

Quantification of night-time incubation restlessness

To quantify incubation behavior and temperature in female great tits, we deployed small temperature loggers (iButtons DS1922L-F5, ± 0.0625°C, Thermochron, www.thermochron.com) that recorded temperature inside nests every 3 minutes. The resulting incubation temperature time-series were visually inspected to retain only data recorded inside the nest cup (e.g., some individuals removed iButtons from the nest cup and pushed them to the side of the nest box) (Womack et al., 2023). From these incubation temperature time-series and using the R package incR (v1.1.0; Capilla-Lasheras [2018]), we computed for every 24-hour period the night-time variance in incubation temperature (i.e., night-time incubation restlessness) for further analysis. To capture night-time throughout the breeding season in Scotland, where nights are short in late spring, we used the core night between 22:00 and 03:00 hours. In total, we calculated this variable for 628 days of incubation across 95 nest boxes (for sample sizes per analysis, population and habitat, see Supplementary Table S1).

Statistical analysis

General statistical methods

Statistical models and plots were carried out in R (version 4.3.1; [R Core Team, 2024]). We use generalized linear mixed models (GLMM) to analyze variation in nocturnal incubation temperature, hatching success, and length of the incubation period. A full model containing all explanatory variables and interactions of interest for each trait was built. We used likelihood ratio tests (LRTs) to assess the statistical importance of each model predictor. Nonsignificant interactions were removed from the initial full models to ease biological interpretation of single effect predictors. We did not apply model simplification beyond nonsignificant interactions and present the resulting full model outputs. When quadratic effects were present in a model, the corresponding linear effects were always also present. Random effects were present in every model as specified for the analysis of each response variable. All statistical models were performed using the R package lme4 (v1.1.35.1; [Bates et al., 2015]). Residuals were visually inspected to check the assumption of normality using the R package performance (v0.10.9; [Lüdecke et al., 2021]) for Gaussian model, and the R package DHARMa (v0.4.6; [Hartig, 2018]) for non-Gaussian models.

Table 1. Analysis of night-time incubation restlessness. Full model output of the Gaussian generalized linear mixed model for variation in night-time nest temperatures. The non-significant interactions between habitat and days to hatching^2 ($\chi^2_1 = 2.12$, $P = 0.145$) and habitat and days to hatching ($\chi^2_1 = 3.33$, $P = 0.068$), and the quadratic effect of days to hatching^2 ($\chi^2_1 = 3.78$, $P = 0.052$) were removed from the final model to ease interpretation of single effect predictors. Random effect standard deviations (in log°C): nestbox ID = 0.064, year = 0.040, study site = 0.000).

Fixed effect	Estimate	SE[A]	95% CI[A]	χ^2	df	*P*
Intercept	−2.558	1.193	−4.896, −0.220			
Incubation start date	0.141	0.074	−0.005, 0.287			
Incubation start date^2	−0.002	0.001	−0.004, 0.000			
Days before hatching	−0.014	0.004	−0.023, −0.005	9.58	1	0.002
Mean daily temperatures	−0.02	0.006	−0.032, −0.008	10.51	1	0.001
Clutch size	0.002	0.017	−0.032, 0.036	0.01	1	0.905
Habitat						
City	—	—	—			
Forest	7.77	1.81	4.223, 11.317			
Incubation start date × Habitat				15.33	1	< 0.001
Incubation start date × Forest	−0.419	0.103	−0.620, −0.218			
Incubation start date^2 × Habitat				13.89	1	< 0.001
Incubation start date^2 × Forest	0.006	0.001	0.003, 0.009			

[A] SE = Standard Error, CI = Confidence Interval

What drives night-time incubation restlessness?

To understand what explains night-time incubation restlessness in great tits, we fitted a GLMM, with Gaussian residual error distribution, with night-time incubation temperature variation as the response variable (log transformed to meet assumption of residual normality). This model included, as single effect predictors, habitat (urban versus forest), mean daily (24 h) temperature, clutch size, days before hatching and incubation start date. Quadratic and linear effects of days before hatching and incubation start date were included as these have been found to correlate with a wide range of avian incubation traits (e.g., Cooper and Voss, 2013; Gwinner et al., 2018; McGlade et al., 2023; Womack et al., 2023). We also included the interactions of habitat by days before hatching, and of habitat by incubation start date (for both quadratic and linear terms) to test whether habitat modulated temporal effects on night-time incubation restlessness. Year, site, and nest box identity were included as random effects to account for among year, site, and nest box variation. This analysis included the full data set of 628 incubation days in 95 nest boxes (see Supplementary Table S1). To further understand environmental impact on night-time incubation restlessness, we subsequently analyzed effects of ALAN. Because there was very little variation in ALAN in forest nests we only used urban nests in this complementary analysis. We used a subset of the urban data for which ALAN measurements were available (21 nest boxes, 173 observations of night-time incubation temperature). We included ALAN per nest box in the model described above and also tested for 'data set' to control for potential differences between the two measurement series.

Does night-time incubation restlessness predict hatching success or incubation length?

To investigate whether variation in night-time incubation temperature impacted reproductive success of great tits in urban and non-urban environments, we first fitted a GLMM, with binomial residual error distribution, with the proportion of hatched eggs per nest as the response variable. We also fitted a second binomial GLMM for the probability of at least one egg hatching per clutch (‘1’) versus no egg hatched per clutch (‘0’). Then, we fitted a third GLMM, with Gaussian error structure, to explain variation in incubation length. These three models included, as single effect predictors, mean variation in night-time incubation temperatures over the incubation period, habitat (urban versus forest), mean daily temperature and incubation start date (as a quadratic and a linear effect). We also included the interactions of habitat by variation in night-time incubation temperature, and of habitat by incubation start date, to test whether nocturnal incubation restlessness had differential affects across habitats on hatching success, or whether habitat modulated the seasonal effects on hatching success. Year, site, and nest box identity were included as random effects to account for among year, site, and nest box variation (see sample sizes for these analyses in Supplementary Table S1).

RESULTS

What drives night-time incubation restlessness?

Variation in night-time incubation temperature of great tits was overall similar for urban and forest sites (Fig. 2A), but seasonality affected incubation restlessness differently in the two habitats (Table 1, interaction between incubation start date and habitat). Restlessness decreased over the incubation period ($\chi^2_1 = 9.58$, $P = 0.002$; Table 1; Fig. 2B) and varied more markedly throughout the season in the forest than in urban habitats (Fig. 2C). In the forest, night-time incubation restlessness decreased in the early breeding season and later increased again, but this pattern was reversed in the urban habitat (Fig. 2C; Table 1). Cooler days were associated with increased night-time incubation restlessness ($\chi^2_1 = 10.51$, $P = 0.001$). We did not detect associations of night-time incubation restlessness with clutch size (Table 1). In a complementary analysis, we detected a significant effect of ALAN in the urban habitat on night-time incubation restlessness: restlessness increased in nest boxes with greater exposure to ALAN ($\chi^2_1 = 11.81$, $P = 0.001$; see Supplementary Table S2, Supplementary Figure S2).

Does night-time incubation restlessness predict hatching success or incubation length?

Females that kept a more constant incubation temperature over incubation hatched a greater proportion of eggs of

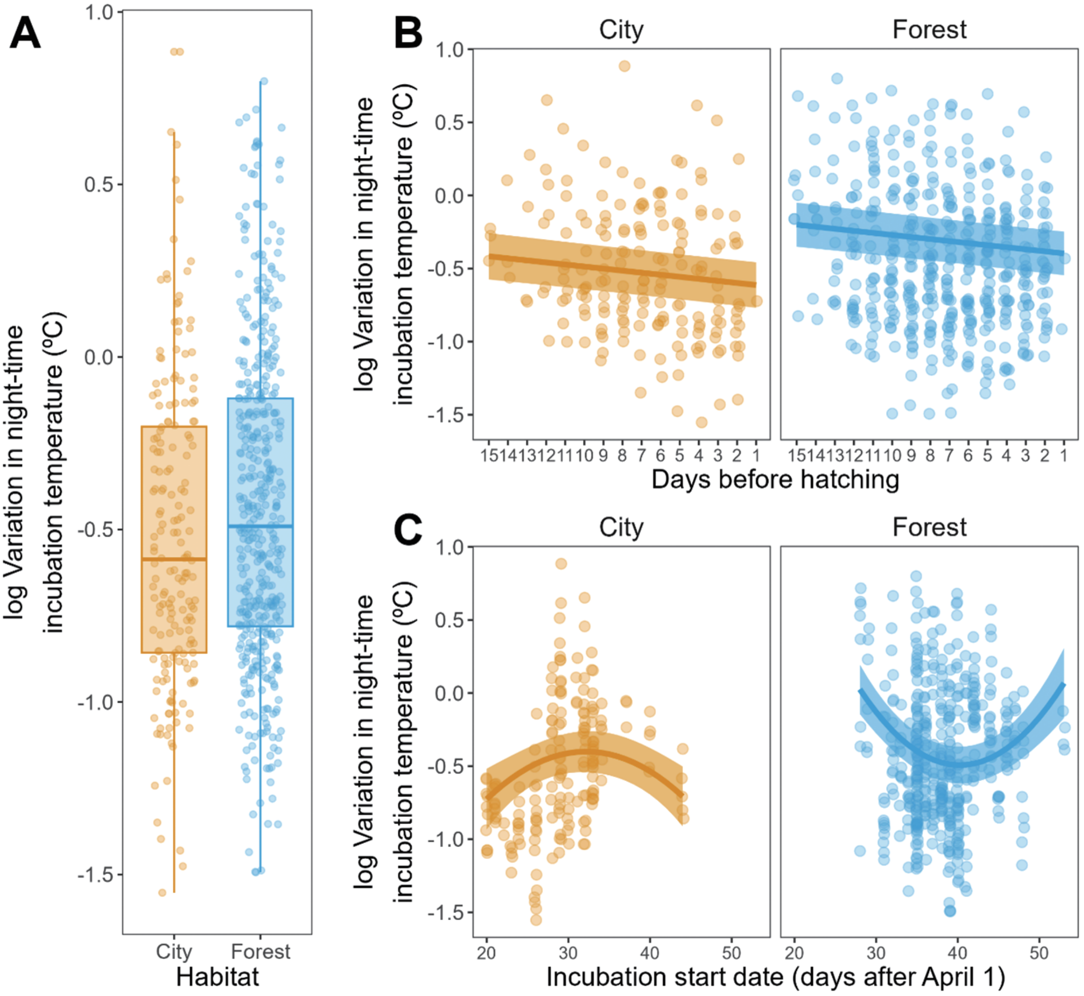

Fig. 2. Analysis of night-time incubation restlessness in great tits across habitats and across time. **(A)** Variation in night-time incubation temperature did not differ overall between urban and forest habitats; **(B)** and decreased throughout the incubation period in both habitats. **(C)** The seasonal pattern of variation differed between habitats. Translucid dots illustrate raw data points; box plots in **(A)** provide median, and 1st and 3rd quantile values; regression lines and shaded areas in **(B)** and **(C)** represent mean model predictions ± one standard error.

Table 2. Analysis of hatching success. Full model output of binomial generalised linear mixed model explaining variation in hatching success (proportion of eggs that hatched). The interactions between habitat and incubation start date^2 (χ^2_1 = 0.07, *P* = 0.785), habitat and incubation start date (χ^2_1 = 0.61, *P* = 0.435), and habitat and night-time incubation restlessness (χ^2_1 = 2.24, *P* = 0.134), as well as the quadratic effect of incubation start date (χ^2_1 = 0.13, *P* = 0.717), were not significant and were removed from the final model to ease interpretation of single effect predictors. Random effect standard deviations (in log°C): nestbox ID = 1.535, year = 0.000, study site = 0.000).

Fixed effect	Estimate	SE^A	95% CI^A	χ^2	df	*P*
Intercept	−0.563	0.97	−2.460, 1.333			0.56
Variation in night-time incubation temperature	−0.936	0.30	−1.515, −0.357	9.75	1	0.002
Incubation start date	−6.621	2.17	−10.868, −2.375	8.43	1	0.004
Mean daily temperatures	0.059	0.08	−0.092, 0.209	0.57	1	0.449
Habitat				12.47	1	< 0.001
City	—	—	—			
Forest	2.321	0.60	1.145, 3.496			

^{A} SE = Standard Error, CI = Confidence Interval

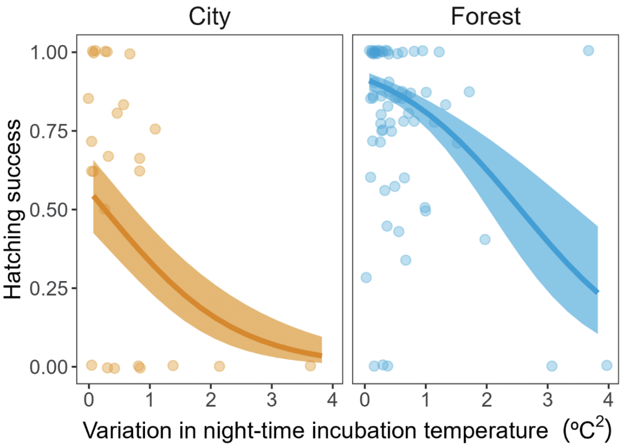

Fig. 3. Analysis of hatching success. Effect of night-time incubation restlessness (variation in night-time temperature) over the incubation period on hatching success (i.e., proportion of eggs hatched in the clutch) in urban and forest habitats. Translucid dots illustrate raw data points, with regression lines and shaded areas representing mean model predictions ± one standard error.

their clutch (i.e., higher hatching success) than females with greater night-time incubation restlessness (χ^2_1 = 9.75, *P* = 0.002; Table 2; Fig. 3). After back-transforming the estimated coefficient for effects of variation in incubation restlessness (Table 2), hatching success was reduced by 60% per unit increase in variation in night-time incubation temperature (Fig. 3; i.e., per °C^2). This reduction was the case both in the urban and in the forest habitat (interactive effect habitat by variation in night-time incubation temperatures: χ^2_1 = 2.24, *P* = 0.134). We found very similar results when we analyzed the probability of at least one egg hatching per clutch (see Supplementary Table S3). In our analysis of the proportion of eggs hatched, hatching success was higher in the forest than in the urban habitat (χ^2_1 = 12.47, *P* < 0.001; Table 2; Fig. 3) and decreased over the season, with early clutches having higher hatching success (χ^2_1 = 8.43, *P* = 0.004; Table 2; Fig. 3). Forest clutches took on average 1.88 days longer to hatch than urban clutches (χ^2_1 = 8.26, *P* = 0.004; see Supplementary Table S4). Incubation length decreased over the season (χ^2_1 = 12.05, *P* = 0.001; see Supplementary Table S4) but was not affected by night-time incubation restlessness nor by daily mean ambient temperatures (see Supplementary Table S4).

DISCUSSION

Our findings strongly support the importance of steady nocturnal incubation for the developing offspring. With increasing variation in nocturnal incubation temperature, hatching success dropped by roughly half in both the urban and forest habitats. Contrary to our predictions, we detected no lengthening of the incubation period with increasing night-time incubation restlessness. Our evidence for environmental effects on incubation is mixed. In contrast to our expectations, urban conditions were not generally associated with increased nocturnal restlessness. Although we only analyzed effects of ALAN in the urban habitat, night-time incubation restlessness increased notably with increasing illumination. In both habitats, time to hatching and ambient temperature affected nocturnal incubation, whereas date of incubation start had habitat-specific effects.

The most important aspect of our results is the finding of substantial effects of night-time incubation behavior on hatching success (Fig. 3). Females that sat tightly on their clutch considerably improved the prospects of developing embryos. Previous research has shown that incubation behavior during daytime, such as changes in the length, timing, and frequency of on-bouts, can affect survival of the developing offspring (Hepp et al., 2015; Bueno-Enciso et al., 2017). Studies that experimentally modified incubation temperature over several days also reported effects on the developing embryos, but relative contributions of daytime and night-time incubation could not be separated, and thus, the night component was not estimated (Nord and Nilsson, 2011). To our knowledge, our data are the first to demonstrate a clear link between the steadiness of nocturnal incubation and hatching success.

The second predicted consequence of stable nocturnal incubation, a shorter incubation period due to increased heat transfer to eggs (Hepp et al., 2006; Hepp et al., 2015; Bueno-Enciso et al., 2017), was not supported by our findings. A shorter incubation period can facilitate higher offspring fitness through reduced risk of nest predation, but the benefits of leaving the nest earlier may be relatively small in hole-nesting birds (Bueno-Enciso et al., 2017). We do not know why these two effects on embryonic development, which in some other studies occurred in conjunction, were uncoupled in our study (Nord and Nilsson, 2011; Ton et al., 2021). We speculate that the main negative effect for embryos was the highly temperature-variable incubation regimes which may have induced mortality, even if the overall heat-transfer might have been independent of nocturnal restlessness (Webb, 1987; Hepp et al., 2015; Hope et al., 2022). To better understand the overall consequences of nocturnal incubation restlessness, detailed, visual information on the incubating birds' behavior would be useful (Hepp et al., 2015; Raap et al., 2016; Indykiewicz et al., 2021). So far, we do not know what behaviors cause variation in nocturnal nest temperature. For example, females could be physically moving away from the nest cup, or they could be restlessly shifting on the nest, thereby interrupting contact between the eggs and the females' brood patch (Williams, 2012). We also do not know whether restlessness on the nest reflects the sleep state of incubating birds, but improving methods of measuring sleep in wild animals might provide clarity in the future (Aulsebrook et al., 2018; van Hasselt et al., 2020). Regardless of the mechanism, our results suggest strong effects of night-time incubation restlessness on embryo survival and highlight the need for future studies to understand such mechanisms.

For the subset of urban nest boxes for which light measurements were available, we found a clear increase in nocturnal restlessness with increasing ALAN (see Supplementary Figure S2). This increase aligns with widely observed or experimentally induced nocturnal restlessness in response to ALAN in a diverse group of species (Aulsebrook et al., 2018; Davies and Smyth, 2018; Gilbert et al., 2023). For example, adult great tits in late winter were restless at night under ALAN, both within nest boxes and when roosting in natural vegetation (Raap et al., 2016; Ouyang et al., 2017). Similarly, nocturnal restlessness, reduced sleep, and modified physiology under bright nights were recorded in geese and swans kept in human enclosures (Aulsebrook et al., 2020; van Hasselt et al., 2021). During the incubation stage, one study showed increased nocturnal restlessness in some experimentally ALAN-exposed birds (McGlade et al., 2023), and in that and another experimental study, birds also altered the timing of night rest in response to ALAN (McGlade et al., 2023; Strauß et al., 2024). Under ALAN of specific wave lengths, nocturnal nest temperatures were also lower than under dark nights (van Dis et al., 2021).

In contrast to the demonstrated ALAN-effects on nocturnal incubation in city birds, our findings do not support a predicted overall higher night-time restlessness in urban than forest great tits (Fig. 2). This was surprising because ALAN levels were elevated in the city (see Supplementary Figure S1), suggesting less stable nocturnal incubation in the urban population. Furthermore, disruption linked to urban environments and altered timescapes more broadly associates with restless nights and potentially negative impact (Gaynor et al., 2018; Gilbert et al., 2023; Sieving et al., 2024). For example, experiments on eastern bluebirds, *Sialia sialis*, revealed that daytime play-back of human noise increased daytime restlessness of incubating females and reduced hatching success (Sieving et al., 2024). We can only speculate about the reasons for the generally stable incubation of great tits in the city. It is possible that our selection of the core night between 22:00 and 03:00 hours excluded nocturnal restlessness linked to disruptive human activities at other times (Remacha et al., 2016), but at least regarding ALAN, we would expect effects of this time-restriction to be small. Potentially, urban great tits may be less sensitive to a generally brighter night-environment, while still being impacted by bright ALAN. An apparently greater sensitivity to ALAN of forest great tits in our population is supported by an experiment that exposed incubating forest and urban females to ALAN in their nest boxes (McGlade et al., 2023). Forest females responded by elevated night-time restlessness compared to control females, while no such response was detected in urban females (McGlade et al., 2023). Such habitat differences in sensitivity could be a genetic adaptation of urban great tits to the brighter night-environment, as discovered in moths (Altermatt and Ebert, 2016). Evolutionary change might be conceivable based on evidence for subtle changes in genes linked to neuronal processing between urban and non-urban great tits (Salmón et al., 2021). Alternatively, or additionally, a reduced sensitivity of city great tits to ALAN and other urban disruptors could have also arisen through phenotypic plasticity or habituation (Dominoni et al., 2013; Hope et al., 2022). Likewise, forest great tits might have been nocturnally restless for other reasons, such as a higher parasite load (Christe et al., 1996) or heightened predation risk (Indykiewicz et al., 2021). At present, we cannot distinguish between these and possible further explanations.

Further detectable environmental influences on nocturnal incubation restlessness included ambient temperature, time to hatching, and habitat-specific effects of date of incubation start. Our findings of increased night restlessness under cooler nights is counter to general behavioral patterns, whereby birds incubate more tightly when nights are cold (White and Kinney, 1974; Conway and Martin, 2000b; Marasco and Spencer, 2015; Sieving et al., 2024). High night restlessness under cooler nights is possibly due to the fact that incubation temperature drops more rapidly when environmental temperature is low, increasing incubation temperature variation from small movements of the incubating bird. The remaining detected environmental influences were closer to expectations. Dynamic changes in incubation behavior with increasing embryonic age are well described (White and Kinney, 1974; Webb, 1987; Cooper and Voss, 2013). While patterns can vary, the gradual decline in nocturnal restlessness we observed during incubation was similar to patterns reported for starlings and great tits (Gwinner et al., 2018; McGlade et al., 2023). Date effects on incubation behavior have also been widely reported and could be linked, for example, to seasonal changes in the length of the night (Nord and Cooper, 2020) or in parental physiology or environmental factors (Conway and Martin, 2000b; Londoño et al., 2008; Hepp et al., 2015; Marasco and Spencer, 2015; Diehl et al., 2020; Nord and Cooper, 2020). Our findings of habitat-specific effects of date (Fig. 2) make the latter explanations more likely.

In conclusion, we report that night-time incubation restlessness strongly predicts hatching success, and is in turn sensitive to environmental conditions, including ALAN. These findings highlight the importance of understanding environmental effects on incubation behavior. We speculate that human-induced restlessness of incubating birds is widespread, and we encourage further study of this phenomenon. Anthropogenic changes, such as modified timescapes and the global increase in temperatures, could impose new constraints or relax previous limits on incubation behavior. Incubating individuals are faced with a delicate time allocation tradeoff between investing in incubation or self-maintenance. Changes in environmental conditions are likely to affect the optimal resolution of such tradeoffs, with possibly harmful consequences for avian reproduction. In greater perspective, we interpret our findings as highlighting the importance of nuanced, rhythmic partitioning of biological processes. We thus call for greater efforts to mitigate against consequences of altered timescapes for living organisms, including humans.

ACKNOWLEDGMENTS

We wish to thank other members of the 2018 City Clocks group at the University of Glasgow for helping with fieldwork, especially Sam Collins, Crinan Jarrett, Ophélie Le Cloirec, Kate Griffiths, Paul Baker, and Thomas McGillavry. The work was funded by the University of Glasgow. PC-L, CJB, and DMD were funded by a NERC Highlight Topics grant to DMD (grant number: NE/S005773/1). PC-L and BH were additionally funded by internal funds of the Swiss Ornithological Institute. PC-L was also funded by the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101150591. RJW received funding from a MVLS DTP studentship.

COMPETING INTERESTS

None.

AUTHOR CONTRIBUTIONS

PC-L, RJW, DMD, and BH conceived the study; RJW, CLOM, and BH collected incubation and reproductive success field data; CJB and DMD measured light at night; PC-L performed the statistical analysis; PC-L and BH wrote the initial version of the manuscript and all authors contributed to the final text.

DATA AVAILABILITY

All R scripts and data sets needed to reproduce the analyses presented in this paper are available at DOI 10.5281zenodo. 14264610.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: https://doi.org/10.2108/zs240063)

Supplementary Table S1. Summary of sample sizes used in the analyses.

Supplementary Table S2. Analysis of effects of light at night on variation in night-time incubation temperatures.

Supplementary Table S3. Analysis of the probability of at least one egg hatching per clutch.

Supplementary Table S4. Analysis of effects of variation in night-time incubation temperature on incubation length.

Supplementary Figure S1. Differences in lights at night between city and forest nest boxes.

Supplementary Figure S2. Effects of light at night on variation in night-time incubation temperatures.

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(Received July 7, 2024 / Accepted December 2, 2024 /
Published online January 31, 2025)