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Early-life exposure to artificial light at night elevates physiological stress in free-living songbirds☆☆

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ABSTRACT

Artificial light at night (ALAN) can disrupt adaptive patterns of physiology and behavior that promote high fitness, resulting in physiological stress and elevation of steroid glucocorticoids (corticosterone, CORT in birds). Elevated CORT may have particularly profound effects early in life, with the potential for enduring effects that persist into adulthood. Research on the consequences of early-life exposure to ALAN remains limited, especially outside of the laboratory, and whether light exposure affects CORT concentrations in wild nestling birds particularly remains to be elucidated. We used an experimental setup to test the hypothesis that ALAN elevates CORT concentrations in developing free-living birds, by exposing nestling great tits (*Parus major*) to ALAN inside nest boxes. We measured CORT in feathers grown over the timeframe of the experiment (7 nights), such that CORT concentrations represent an integrative metric of hormone release over the period of nocturnal light exposure, and of development. We also assessed the relationships between feather CORT concentrations, body condition, nestling size rank and fledging success. In addition, we evaluated the relationship between feather CORT concentrations and telomere length. Nestlings exposed to ALAN had higher feather CORT concentrations than control nestlings, and nestlings in poorer body condition and smaller brood members also had higher CORT. On the other hand, telomere length, fledging success, and recruitment rate were not significantly associated with light exposure or feather CORT concentrations. Results indicate that exposure to ALAN elevates CORT concentrations in nestlings, which may reflect physiological stress. In addition, the organizational effects of CORT are known to be substantial. Thus, despite the lack of an effect on telomere length and survivorship, elevated CORT concentrations in nestlings exposed to ALAN may have subsequent impacts on later-life fitness and stress sensitivity.

1. Introduction

Novel disturbance factors experienced in urbanized areas create the possibility for maladaptive or adaptive responses, which can be mediated through behavioral plasticity or rapid evolutionary change (Marzluff, 2001; Sih et al., 2011, 2013; Atwell et al., 2012; Sol et al., 2013). Thus, urban environments are increasingly becoming model systems for studies of how free-ranging organisms adjust behavior and physiology to changing environmental conditions (Hu and Cardoso, 2009; Stillfried et al., 2017; Morelli et al., 2018). Artificial light at night (ALAN) is one anthropogenic disturbance factor that is ubiquitous within the urban matrix, and is affecting a growing proportion of the planet (Hölker et al., 2010; Gaston et al., 2013; Swaddle et al., 2015). Introduction of ALAN into the environment by humans lacks a strong parallel in natural systems, and thus has the potential to disrupt biological systems and result in physiological stress. Indeed, organisms have evolved with the periodicity of natural light-dark cycles, such that light plays an integral role in coordinating adaptive daily and seasonal patterns of physiology and behavior (Gwinner and Brandstätter, 2001; Dominoni et al., 2013).

Exposure to ALAN may disrupt the internal clock controlled by the suprachiasmatic nucleus of the hypothalamus, with consequent

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effects on production of hormones, activity, sleep and recovery cycles, and health (Navara and Nelson, 2007; Dickmeis, 2008; Figueiro and Rea, 2010). One hormonal system that helps synchronize internal conditions and external stimuli and which may be particularly sensitive to exposure to ALAN, is the hypothalamus-pituitary-adrenal (HPA) axis (Ouyang et al., 2018; Dickmeis, 2008). The HPA axis helps mediate diurnal and seasonal cycles of behavior in vertebrates, with peaks in hormone production coinciding with the onset of daily activity and the nadir in hormone concentrations occurring at night in diurnal species (Breuner et al., 1999; Ouyang et al., 2018). The effect of the HPA axis on activity levels may interact with patterns of release of melatonin, a pineal hormone which is both light sensitive and which has been shown to be affected by glucocorticoid concentrations in complex ways (Persengiev et al., 1991; Navara and Nelson, 2007). The HPA axis also primes organisms to cope with predictable life-history challenges, such as reproduction and molt (Sapolsky et al., 2000; Romero, 2002; Landys et al., 2006; Romero et al., 2009), and mediates the vertebrate response to acute, unpredictable stressors (Wingfield et al., 1998; Romero et al., 2009; Angelier and Wingfield, 2013). Furthermore, elevation of glucocorticoid hormones (GCs; corticosterone, CORT, in birds) via the HPA axis plays complex and adaptive functions in glucose metabolism and energy balance, flight responses, and the immune system (Sapolsky et al., 2000; Romero, 2004; Wingfield and Romero, 2001), whereas prolonged elevation of GCs may result in chronic stress, deterioration in health status and fitness declines (McEwen and Wingfield, 2003). Thus, disruption of the HPA axis by anthropogenic disturbance factors such as ALAN could have wide-reaching effects.

Previous studies have demonstrated that exposure to ALAN can eliminate the natural circadian rhythm of GC release or elevate GC concentrations, both extensively in the laboratory (Scheving and Pauly, 1966; Mohawk et al., 2007; Fonken et al., 2012; Bedrosian et al., 2013; Martynhak et al., 2017; Alaasam et al., 2018; Emmer et al., 2018), and to a limited extent in free-living animals (Ouyang et al., 2015). For instance, Siberian hamsters (*Phodopus sungorus*) and rats exposed to ALAN in the laboratory displayed increased cortisol concentrations (Scheving and Pauly, 1966; Bedrosian et al., 2013), with the rats also displaying an altered diurnal pattern of hormone release. In addition, in the first study to explore the effects of ALAN in a wild animal, Ouyang et al. (2015) reported that adult great tits (*Parus major*) exposed to white light at night had elevated CORT concentrations relative to control birds and birds exposed to red or green light.

However, a critical deficit in knowledge exists regarding whether exposure to ALAN results in elevated GC concentrations (or otherwise altered HPA activity) in developing organisms. Elevation of GCs may have particular potent effects during development, when the phenotype remains sensitive to organizational programming effects (Metcalfe and Monaghan, 2001; Spencer et al., 2009). For instance, past research has demonstrated that elevated GC concentrations early in life can be correlated with heightened sensitivity to stressors later in life (Chung et al., 2005; Cottrell and Seckl, 2009; Spencer et al., 2009; Banerjee et al., 2012; although the reverse has also been reported; Love and Williams, 2008; Zimmer et al., 2013), declines in the expression of sexually selected traits (Spencer et al., 2003; Husak and Moore, 2008; Spencer and MacDougall-Shackleton, 2011; Schmidt et al., 2014; Dupont et al., 2019), and reduced life expectancy (Monaghan et al., 2012; Grace et al., 2017), with effects potentially being mediated by life-history tradeoffs (Hau et al., 2010; Vitousek et al., 2018).

The effects of early-life elevation of GCs on later-life fitness may, in part, be mediated through effects on telomere dynamics (Monaghan, 2014; Angelier et al., 2018). Telomeres are conserved repeats of nucleotides in organisms that cap the ends of chromosomes and protect DNA from damage and malfunction (Haussmann et al., 2005, 2012; Monaghan and Haussmann, 2006; Monaghan, 2014), and accelerated telomere loss has been linked to reduced longevity and disease (Blackburn et al., 2006). Telomere shortening is especially rapid early in life, and has been shown to be accelerated by stressful conditions and elevation of GCs (Haussmann et al., 2012; Herborn et al., 2014; Reichert and Stier, 2017; Angelier et al., 2018). In addition, both exposure to ALAN and elevation of GCs may elevate oxidative stress. ALAN may result in higher oxidative stress by suppressing the concentrations of melatonin, a potent antioxidant, and through down-stream effects of sleep deprivation and heightened activity on the production of pro-oxidants and the concentrations of antioxidants (Navara and Nelson, 2007). GCs may increase metabolic rate and alter patterns of activity, thus increasing the generation of reactive oxygen species (Angelier et al., 2018). Telomeres are especially sensitive to damage by reactive oxygen species due to a high guanine content, and although its importance has been debated, elevated oxidative stress has been linked to increased rates of telomere shortening (Reichert and Stier, 2017).

We experimentally investigated the effect of ALAN on great tit nestlings, using a system of LEDs to produce light inside of nest boxes. In an earlier publication, we report that this manipulation had no effect on nestling telomere length, but that body condition changed differently in control and experimental nestlings over the course of the experiment (significant interaction term), with control nestlings tending to gain condition and experimental nestlings tending to experience condition declines (Grunst et al., 2019). Here we incorporate new data from hormonal assays to explore the effects of exposure to ALAN on CORT concentrations. Our study differs from the earlier Ouyang et al. (2015) study, also on the effect of ALAN on CORT release in great tits, in that we focused on nestlings instead of adults. We also used a lower light intensity than Ouyang et al. (2015) (1 lux relative to ~8 lux), but since nestlings are not mobile they are also less able to avoid the light manipulation than are free-ranging adults. Unlike Ouyang et al. (2015), we did not consider the effects of different wavelengths of light.

We exposed nestlings to light for 7 nights, constituting a substantial proportion of the nestling cycle, and assessed CORT release using hormone concentrations from feathers (feather CORT; Bortolotti et al., 2008; Lattin et al., 2011; Jenni-Eiermann et al., 2015; Romero and Fairhurst, 2016). CORT is deposited in feathers over the period of feather growth, and the period over which these feathers grew corresponded to the period of light exposure. Thus, feather CORT concentrations provide an integrative metric of CORT release during the timeframe of the experiment. We predicted that CORT concentrations would be elevated in experimental nestlings, due to disruptive effects of ALAN on physiology and behavior (Swaddle et al., 2015; Ouyang et al., 2018). To our knowledge, this is the first study to investigate the effect of exposure to ALAN on CORT concentrations in a developing, free-living bird. In addition, there may be a component of variation in GCs concentrations that is independent of exposure to ALAN, based energetic state, competitive environment, or genetics (Wada et al., 2008; Jenkins et al., 2014). Thus, we also assessed whether CORT concentrations could independently predict telomere length, body condition, or fledging success, and whether CORT concentrations interacted with exposure to ALAN to predict these variables. We predicted that nestlings with higher feather CORT concentrations would be in poorer body condition, have shorter telomeres, and have reduced fledging success. With respect to the predicted negative relationship between body condition and CORT concentrations, the causality is not necessarily unidirectional. Elevated CORT might act to reduce body condition (although high CORT concentrations might also facilitate maintenance of body condition in some cases), but low body

condition could also lead to higher CORT concentrations. With respect to the interaction between CORT and ALAN, we hypothesized that relationships might differ in directionality or magnitude in the two treatment groups, since CORT might play different functions given exposure to ALAN. Finally, to gain additional insight into potential fitness ramifications, we evaluated whether exposure to ALAN or feather CORT concentrations were negatively related to rates of recruitment into the population.

2. Methods

2.1. Study population and general methods

Our study population of great tits breeds in the immediate vicinity of the University of Antwerp's Campus Drie Eiken (Wilrijk, Belgium; 51°9′44″N, 4°24′15″E), and consists of >120 resident breeding pairs (e.g. Van Duyse et al., 2000, 2005; Rivera-Gutierrez et al., 2010, 2012; Raap et al., 2015, 2016b, 2016a, 2017a, 2017b, 2018a, 2018b; Vermeulen et al., 2016). We checked nest boxes every other day beginning in late March, and continuing through the end of the breeding season in mid-May, to determine laying date, hatching date, brood size, and fledging success. Nestlings were considered to have recruited into the population, which is extensively monitored, if they were re-sighted or recaptured on the study sight in subsequent years. This study was approved by the ethical committee of the University of Antwerp (ID number: 2017−90) and conducted in accordance with Belgian and Flemish laws.

2.2. Experimental design

Details of the experimental design are given in Grunst et al. (2019). In brief, we exposed nestlings to ALAN from day 8 to day 15 of the nestling stage (hatch day = day 1) using a system of 4 small LED lights (Diameter: 5 mm, Cree® Round LED C535A-WJN, Durham, North Carolina, USA) that produce broad-spectrum white light (see Grunst et al., 2019 for color spectrum specifications). The LED system was fitted under the nest box lid, and standardized to produce 1 lux at the average nest height of great tits (8 cm above the nest box bottom; ILM 1335 light meter, ISO-TECH, Northamptonshire, UK). We used a timer inside a home-made enclosure to turn light systems on at 1900 in the evening and off at 0700 in the morning. In addition, to reduce chances of nest abandonment, the system was turned off during the night from 2400 to 0200. Control nest boxes were fitted with LED systems, but no electronics. The experiment was completed between April 20 and May 8, 2017 on first nesting attempts (N = 26 nest boxes; 12 ALAN, 14 CTR; 206 nestlings; 93 ALAN, 113 CTR). Control and experimental nests did not differ significantly in hatching date ($t_{21} = -0.231$, $P = 0.820$) or brood size ($t_{19} = -0.384$, $P = 0.705$). Broods included in the experiment had an average (mean ± SE) of 7.96 ± 0.296 (range: 5 to 12) nestlings, and had hatching dates between April 13 and April 23, 2017. For one control nest box, feather samples were of insufficient mass for use in the CORT assay.

2.3. Field sampling

To assess the effect of ALAN on CORT concentrations, we gently removed ≈15−20 contour (breast) feathers from each nestling on day 15 of the nestling stage, following the last night of light exposure. Feathers were stored in small envelops in a dark and dry location.

To assess the effect of ALAN on telomere length and body condition, we used a repeated measures design, detailed in Grunst et al. (2019). In brief, blood samples and body measurements were taken on day 8 and 15 of the nestling stage, and were completed between 0800 and 1230. Body condition was calculated as the residuals of a regression predicting body mass from tarsus length (Schulte-Hostedde et al., 2005). On day 8, we uniquely marked nestlings with a metal ring or color band.

2.4. Laboratory assays

2.4.1. Feather CORT radioimmunoassay

Following feather collection, we used a high precision balance (Mettler Toledo XS205 Dual Range) to weigh feather samples, with a target mass of ≈20 mg. Masses of feather samples ranged from 19.1 to 20.7 mg, and sample mass was not related to CORT concentrations (mean ± SE: −0.197 ± 0.153, $t_{157} = -1.29$, $P = 0.200$). A non-linear relationship between feather mass and the amount of CORT/mg detected may occur, especially at low feather masses (<20 mg), and using similar feather masses avoids this problem (Kennedy et al., 2013; Grunst et al., 2015). Feather samples were transported to the Centre d'Etudes Biologiques de Chizé, where radioimmunoassay was performed.

We extracted CORT from feathers by adding 10 ml of methanol (HPLC grade) to each sample, placing samples in a sonicating water bath at room temperature for 30 min, and incubating at 50 °C in a shaking water bath overnight. Feathers were very small, so it was unnecessary to pulverize samples before the extraction process. We separated methanol from feather residue using filtered syringes (see details in Meillère et al., 2016), dried extracts under air in a 50 °C water bath placed in a fume hood, and reconstituted extract residues in a small volume of the phosphate buffer system (PBS; 0.05 m, pH 7.6). We used previously described radioimmunoassay techniques to determine CORT concentrations in reconstituted extracts (Lormée et al., 2003). We confirmed the linearity of the assay with respect to nestling great tit feathers before running the assay. Samples were run in 5 assays, with all samples assayed in duplicate. The intra- and inter-assay coefficients of variation were 7.96% and 15.6%, respectively. Although the inter-assay coefficient of variation is somewhat high, samples were randomly distributed between assays, so we do not expect this to bias bias our results.

2.4.2. Telomere qPCR and nestling sex determination

We determined telomere length and molecularly sexed nestlings (Griffiths et al., 1998) using DNA extracted from blood samples using the Macherey-Nagel NucleoSpin® blood kit. We measured the concentration and purity of DNA using a Nanodrop (2000c; Thermo Scientific; Merelbeke, Belgium). Samples were of high purity, with 260/280 and 260/230 ratios close to recommended values (mean ± SE: 1.90 ± 0.01 and 2.14 ± 0.36, respectively) (Desjardins and Conklin, 2010). We determined telomere length using a relative real-time qPCR assay modified from Criscuolo et al. (2009), and developed for the great tit by Atema et al. (2013), which measures telomere length relative to a single copy reference gene (in our case glyceraldehyde-3-phosphate dehydrogenase (GAPDH)). See Grunst et al. (2019) for details on the telomere assay.

2.5. Statistical analyses

We performed all statistical analyses in R 3.4.1 (R Core Team, 2017). We used linear mixed effects models (LMMs) in R package lme4 (Bates et al., 2015) to investigate the effect of exposure to ALAN on feather CORT concentrations, with Satterthwaite approximations for degrees of freedom (R package lmerTest; Kuznetsova et al., 2016). We used the scale function in R to center and

standardize all continuous predictor variables to a mean of zero and a standard deviation of one. Performing this operation aids in interpretation of beta estimates, especially when interaction terms are included in the model (Schielzeth, 2010). We report statistics from global models in all cases. We predicted feather CORT concentrations (log-transformed) from treatment (ALAN, control), with nest ID as a random effect, and brood size, size rank within a brood (largest nestling = size rank 1 on the basis of day 15 mass), nestling sex and hatching date as fixed-effect covariates. To assess the possibility of size- or sex-dependent effects of ALAN on feather CORT concentrations, we also included the two-way interactions between treatment and sex and treatment and size rank. We used a separate LMM to test whether body condition was associated with feather CORT. We did not include body condition in the initial model because body condition is reduced at day 15 in nestlings exposed to ALAN (see Grunst et al., 2019). Thus, in the analysis presented here, treatment and body condition were collinear in the model predicting feather CORT.

Second, we tested whether RTL (log-transformed) or body condition were associated with feather CORT concentrations. We predicted RTL from the interaction between feather CORT and treatment, with nestling age (day 8 or day 15) and size rank as covariates, and Nest ID, nestling ID, and qPCR assay number as random effects. We used the same fixed and random effects in the model predicting body condition, with the exception of additionally including the interaction between nestling age and treatment (we previously found that this interaction was significant for body condition, but not for telomere length; Grunst et al., 2019), and excluding the assay number random effect. We also previously found that sex had no effect on telomere dynamics or body condition in nestling great tits (Grunst et al., 2019).

Finally, we assessed whether fledging success or recruitment rates were associated with exposure to ALAN, feather CORT concentrations or body condition at day 15. To this end, we used general linear models with a binomial error structure to predict whether or not a nestling fledged or recruited (both 1, 0). We did not test the interaction between feather CORT and treatment in these models since the number of nestlings that died before fledging (28/206) and recruited (20/206) were limited, and we wanted to avoid over-fitting. We included nest ID as a random effect.

3. Results

3.1. Feather CORT concentrations

Feather CORT concentrations ranged from 2.91 to 24.0 pg/mg (mean ± SE = 8.16 ± 0.25) and were significantly higher in nestlings exposed to ALAN than in nestlings in the control group (Table 1; Fig. 1). Nestlings that were smaller than their brood mates (higher size rank) had higher feather CORT (Table 1; Fig. 2a), whereas nestlings in better body condition had lower ($\beta \pm SE = -0.081 \pm 0.020$, $t_{134} = -3.97$, $p < 0.001$, N = 159 nestlings, 25 nest boxes; Fig. 2b), feather CORT. The interactions between treatment and size rank and treatment and sex were non-significant.

Brood size and hatching date were also unrelated to feather CORT concentrations (Table 1).

3.2. Telomere length and body condition

Relative telomere length was not associated with treatment or nestling feather CORT concentrations, and the interaction between light exposure and feather CORT was also non-significant (Table 2). Nestlings that were smaller than their brood mates (which are also in poorer body condition) had shorter telomeres, and nestling telomere length also declined with age, although this effect was not significant in this model (Table 2).

Feather CORT concentrations positively interacted with treatment to predict body condition, reflecting that feather CORT concentrations were negatively related to body condition only within the control group ($\beta \pm SE = -0.227 \pm 0.108$, $t_{170} = -2.11$, $p = 0.037$, N = 187 observations, 93 nestlings, 13 nest boxes) and not among nestlings exposed to ALAN ($\beta \pm SE = 0.101 \pm 0.088$, $t_{122} = 1.148$, $p = 0.253$, N = 129 observations, 66 nestlings, 12 nest boxes) (Fig. 3). There was also a negative interaction between feather CORT concentrations and nestling age, reflecting that feather CORT concentrations were only significantly negatively related to body condition in day 15 ($\beta \pm SE = -0.104 \pm 0.048$, $t_{148} = -2.16$, $p = 0.032$, N = 159 nestlings, 25 nest boxes), and not in day 8 ($\beta \pm SE = -0.045 \pm 0.037$, $t_{152} = -1.21$, $p = 0.230$, N = 157, 24 nest boxes), nestlings. Nestlings that were smaller than their brood mates (higher size rank) were also in poorer body condition (Table 3). The interaction between treatment and nestling age was not significant in this model (Table 3).

Table 1
Linear mixed effect model predicting feather CORT concentrations from treatment and covariates. Significant p-values appear in bold.

	Estimate (β ± SE)	Df	T	P > |t|
Intercept	2.00 ± 0.09	29.6	21.5	**< 0.001**
Treatment[a]	0.209 ± 0.10	38.8	2.09	**0.043**
Size rank	0.096 ± 0.03	129	2.99	**0.003**
Sex[b]	0.063 ± 0.062	133	1.02	0.310
Brood size	0.035 ± 0.042	23.0	0.842	0.408
Hatching date	−0.063 ± 0.076	25.5	−0.83	0.414
Treatment × size rank	0.039 ± 0.048	136	0.82	0.413
Treatment × sex	−0.054 ± 0.098	141	−0.55	0.582

Random effects	Variance	SD	N
Nest	0.035	0.186	25
Residual	0.060	0.246	151

[a] ALAN relative to control nestlings.
[b] Males relative to females.

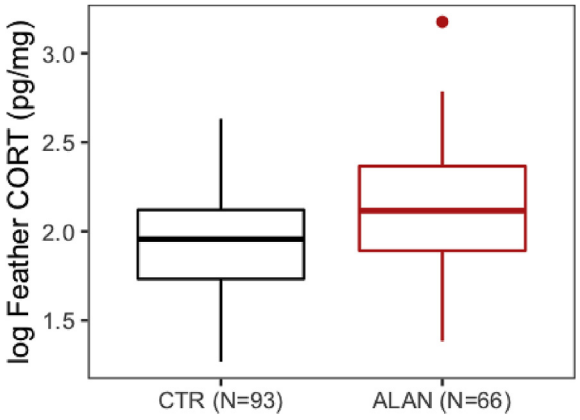

Fig. 1. Box plot of feather CORT concentrations in the control (CTR) and light (ALAN) treatment groups. CORT concentrations were significantly higher in nestlings exposed to ALAN ($\beta \pm SE = 0.209 \pm 0.100$; $t_{39} = 2.09$; $p = 0.043$). N = the number of nestlings in each treatment group. Whiskers extend from the first and third quartiles to the highest value within 1.5 times the interquartile range.

3.3. Fledging success and recruitment rate

Exposure to ALAN did not affect fledging success ($\beta \pm SE$: -1.15 ± 1.18, $z = -0.969$, $p = 0.333$, N = 159 nestlings, 25

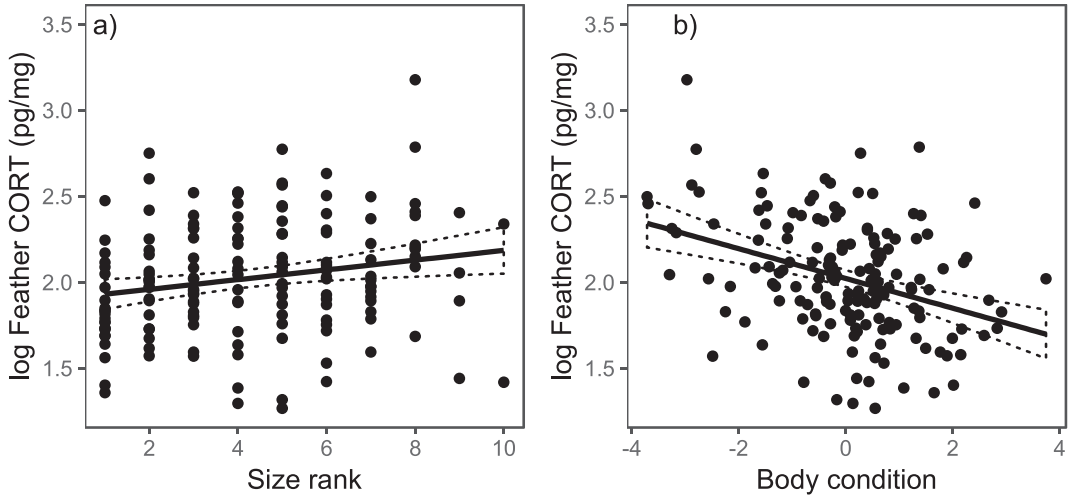

Fig. 2. Relationships between feather CORT concentrations and (a) size rank and (b) body condition. Dotted lines show 95% confidence intervals.

Table 2
Linear mixed effect model predicting relative telomere length from treatment, feather CORT concentrations and covariates. Significant p-values appear in bold.

	Estimate (β ± SE)	Df	T	P > |t|
Intercept	0.458 ± 0.095	16.8	4.82	<0.001
Treatment	0.034 ± 0.051	25.9	0.67	0.510
Feather CORT	0.055 ± 0.037	112	1.48	0.141
Nestling age	−0.038 ± 0.020	153	−1.87	0.064
Size rank	−0.020 ± 0.01	202	−2.18	**0.026**
Treatment × CORT	−0.077 ± 0.047	120	−1.65	0.100
CORT × age	−0.019 ± 0.021	157	−0.90	0.371

Random effects	Variance	SD	N
Individual ID	0.0003	0.018	159
Nest box	0.003	0.059	25
qPCR Plate	0.061	0.248	11
Residual	0.123	0.351	295

[a]ALAN relative to control nestlings.

Table 3
Linear mixed effect model predicting body condition from treatment, feather CORT concentrations and covariates. Significant p-values appear in bold.

	Estimate (β ± SE)	Df	T	P > |t|
Intercept	0.609 ± 0.236	29.7	2.58	0.015
Treatment[a]	−0.448 ± 0.315	21.5	−1.43	0.168
Feather CORT	−0.221 ± 0.103	308	−2.13	**0.034**
Nestling age	0.169 ± 0.063	283	2.65	**0.008**
Size rank	−0.104 ± 0.022	299	−4.54	**<0.001**
Treatment × CORT	0.338 ± 0.127	307	2.66	**0.008**
Treatment × age	−0.143 ± 0.103	286	−1.39	0.167
CORT × age	−0.167 ± 0.051	284	−3.31	**0.001**

Random effects	Variance	SD	N
Individual ID	0	0	159
Nest box	0.535	0.731	25
Residual	0.735	0.857	316

[a] ALAN relative to control nestlings.

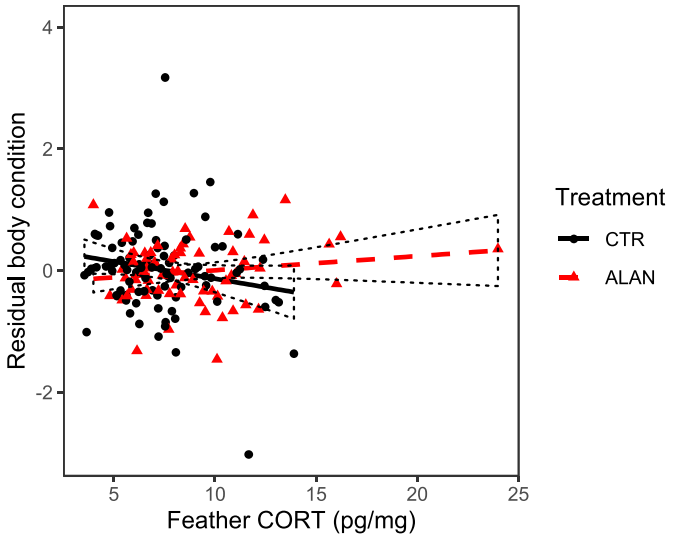

Fig. 3. Relationship between body condition (mass-size residuals) and feather CORT concentrations in the two treatment groups. The values used for body condition are residuals that control for the other variables included in the model. Dotted lines show 95% confidence intervals.

nest boxes). Of the nestlings that reached day 15 and had feather CORT measured, 4/93 and 5/66 nestlings did not fledge, in the control versus ALAN group, respectively. Feather CORT concentrations (β ± SE = −0.754 ± 0.450, z = −1.68, p = 0.094), and body condition at day 15 (β ± SE = 0.336 ± 0.344, *z* = 0.975, p = 0.330) were also unrelated to fledging success, although the coefficient estimate for feather CORT was negative. Exposure to ALAN was unrelated to recruitment rate (β ± SE = −0.627 ± 0.862, z = −0.728, p = 0.467). Of the nestlings that reached day 15 and had feather CORT measured, 11/93 and 9/66 nestlings recruited, in the control versus ALAN group, respectively. Feather CORT (β ± SE = 0.746 ± 0.442, z = 1.69, p = 0.091) was also unrelated to recruitment into the population, with the coefficient estimate in this case being positive. However, nestlings that were in better body condition at day 15 were more likely to recruit (β ± SE = 0.686 ± 0.319, z = 2.15, p = 0.032).

4. Discussion

We used an experimental setup to expose free-living songbird nestlings to ALAN. We demonstrate that exposure to ALAN elevates feather CORT concentrations in these developing organisms. In the context of our study, feather CORT concentrations reflect an integrative metric of hormone release over both the period of light

exposure and of development. Nestlings with higher feather CORT concentrations were also in poorer body condition, and smaller brood members had higher feather CORT, both of which are consistent with higher feather CORT being indicative of physiological stress (Bortolotti et al., 2008). Feather CORT concentrations were not significantly associated with fledging success (in contrast to Lodjak et al., 2015) or recruitment rate, such that we have no evidence of longer-term effects of high CORT on fitness. However, our power to detect these effects was limited, and past work suggests that nestlings in poorer condition are less likely to survive the juvenile period, and to recruit into the population (Perrins, 1979; Tinbergen and Boerlijst, 1990; Naef-Daenzer et al., 2001; Perrins and McCleery, 2001; Rodríguez et al., 2016; Vermeulen et al., 2016). Indeed, nestlings in better body condition were also more likely to recruit in this study. Moreover, even if unrelated to fledging success and recruitment rates, elevated CORT concentrations during development may have deleterious effects on other phenotypic traits important to fitness, such as song learning and acquisition of sexual coloration (Spencer et al., 2003; Husak and Moore, 2008; Spencer and MacDougall-Shackleton, 2011; Schmidt et al., 2014), and may affect the function of the HPA axis during adulthood, with implications for stress-sensitivity (Chung et al., 2005; Cottrell and Seckl, 2009; Spencer et al., 2009; Banerjee et al., 2012).

Exposure to ALAN could result in higher CORT concentrations via a number of mechanisms. Light exposure during the night can disrupt the natural circadian rhythm of the HPA axis (Scheving and Pauly, 1966; Ouyang et al., 2018). Indeed, light exposure can directly activate the adrenal glands, resulting in higher CORT secretion (Ishida et al., 2005) and photoreceptors are also present in the hypothalamus (Ouyang et al., 2018). Behavioral alterations arising from exposure to ALAN may be mediated by elevated CORT, and may also potentially feedback to promote higher HPA activity. For instance, in an earlier study we found that ALAN increases the nighttime activity levels (begging) of nestlings, which could reflect sustained CORT secretion into the nighttime period (Raap et al., 2016a), and other studies have also found increases in nighttime activity levels, and parallel increases in CORT concentrations, in adult birds (Alaasam et al., 2018). Since CORT supports glucose metabolism, increased activity could in turn enforce elevated release of CORT. ALAN could also induce changes in parental behavior that couldaffect patterns of CORT release in nestlings (e.g. Stracey et al., 2014). For instance, parents may reduce nestling provisioning rates prior to fledging to encourage nestlings to leave the nest, potentially reducing body mass and elevating CORT concentrations (Kern et al., 2001; Lodjak et al., 2015). ALAN may have expediated or enhanced this late-nestling stage reduction of feeding rates, or could have reduced provisioning rates for the duration of the experiment.

A number of past studies have linked conditions of elevated stress, or disturbance, during development to increases in feather CORT (Lodjak et al., 2015; Johns et al., 2018; Beaugeard et al., 2019). For instance, great tit nestlings in enlarged broods had higher feather CORT concentrations in a poorer-quality coniferous forest habitat (Lodjak et al., 2015), and nestling house sparrows (*Passer domesticus*) in an urban environment had higher feather CORT than rural counterparts (Beaugeard et al., 2019). Since a number of different hypotheses can explain elevation of CORT, different or similar pathways could be leading to elevated feather CORT in these studies. However, this body of work suggests that elevated feather CORT concentrations may reflect a mechanism via which organisms cope with a number of different natural and anthropogenic developmental stressors, with potential implications for life-history trajectories.

Somewhat unexpectedly, although body condition was negatively related to feather CORT concentrations, this relationship was stronger, and only statistically significant among control nestlings. This result arose despite the fact that feather CORT concentrations were higher in nestlings exposed to ALAN. Why feather CORT would more strongly predict body condition in control nestlings is unclear. One possible explanation is that elevated feather CORT in nestlings exposed to ALAN is not merely a reflection of pathology, but may actually aid nestlings in coping with ALAN ALAN-associated stress, thus dampening the relationship between low body condition and elevated CORT in comparison to in the control group. In addition, CORT exerts biological effects via two classes of receptors, mineralocorticoid receptors, which bind the hormone at lower concentrations, and glucocorticoid receptors, which bind the hormone at higher concentrations (Sapolsky et al., 2000; Romero, 2004). Thus, differences in receptor activation patterns could explain the stronger relationship between feather CORT concentrations and body condition in control versus experimental nestlings.

We also found that body condition was only related to feather CORT concentrations in 15-day old, and not in 8-day old nestlings. In great tit nestlings, feathers are just beginning to grow around day 8, and in the case of our study, the experimental ALAN exposure occurred beginning on day 8 and continuing through day 15. Thus, it is logical that differences in feather CORT between nestlings would be more closely related to body condition at day 15.

Finally, with respect to our conclusions regarding body condition, in Grunst et al. (2019) we found a significant interaction term between nestling age and treatment. This interaction reflects a tendency for control nestlings to gain condition and experimental nestlings to lose condition between day 8 and day 15. In the current analysis, this interaction term was not significant, although the relationship is in the same direction. We noted in our previous publication that the effect of exposure to ALAN on body condition was modest and did not translate into a significant difference in body condition at day 15. Thus, it is not surprising that this interaction term is not significant when other variables are included in the model. In short, we can conclude that exposure to ALAN had only a minimal impact on nestling body condition in our study. Thus, it seems probable that different pathways underlie the positive relationship between exposure to ALAN and CORT concentrations and the negative relationship between body condition and CORT concentrations.

We reported earlier that exposure to ALAN had no effect on telomere dynamics in great tit nestlings (Grunst et al., 2019). Here we additionally show that CORT concentrations are not related to telomere length. Elevated CORT, and associated increases in oxidative stress, have been proposed as mechanisms that mediate accelerated telomere shortening (Angelier et al., 2018; Casagrande and Hau, 2019). However, in a previous study we found that there was no effect of exposure to ALAN on oxidative stress in great tit nestlings (Raap et al., 2016c), and in the current study, telomere length appeared resistant to early-life exposure to ALAN and elevation of CORT. As we suggested in our previous publication, it is possible that ALAN induces a unique cascade of physiological and behavioral responses that combine to cause no overall effect on telomere length (Grunst et al., 2019). A past study on great tits also reported no effect of ALAN on telomere shortening (Ouyang et al., 2017; but see Raap et al., 2017b), despite higher CORT concentrations in adults exposed to white light (Ouyang et al., 2015). Although not in the context of light exposure, a number of other studies have also found no relationship between CORT concentrations and telomere dynamics, or have reported positive relationships (see Angelier et al., 2018 for a review). On the other hand, several past studies have associated conditions of elevated stress and/or elevated CORT concentrations with reduced telomere length

(Herborn et al., 2014; 6Quirici et al., 2016; Pegan et al., 2019; Angelier et al., 2019). As for the relationship between CORT concentrations and fitness, the relationship between CORT concentrations and telomere dynamics is likely to be complex and species- and context-dependent (Angelier et al., 2018). Methodological differences between studies, such as when and how GC concentrations and telomere length were measured and the life-history stage of focus, could also in part explain the mixed results of past studies. Further research and meta-analyses will be necessary to resolve this complexity into overarching patterns.

Regarding our specific methodology, and as discussed in greater length in our earlier publication (Grunst et al., 2019), great tits have two classes of terminal telomeres, and attrition of the shorter class cannot be detected via the qPCR technique (Atema and Verhulst, 2019). We were able to detect biologically meaningful patterns with respect to telomere dynamics, including shortening between day 8 and 15 and reduced telomere length in nestlings in poor condition (Grunst et al., 2019). Although the relationship between nestling age and telomere length was not significant in the analysis presented in this paper, it was only marginally non-significant ($p = 0.064$). However, it remains possible that presence of these two classes of telomeres could have affected our ability to detect an effect of ALAN and elevated CORT concentrations on telomere dynamics.

In addition, details of our experimental design could be implicated in our failure to detect an effect on telomere dynamics. In particular, we included a dark period in the middle of the night, with the aim of preventing complete mortality. Including this dark period could have resulted in recovery of melatonin levels, thus dampening effects on the oxidative status of nestlings. We also used a relatively low light intensity, and we would predict that a higher light intensity would have more pronounced phenotypic effects. Thus, in the future, examining the effects of lighting through the entire night, or exposing nestlings to a higher light intensity, could prove informative, although employing these regimes could also result in total nest abandonment.

Great tits serve as a convenient organism to test the effects of ALAN on nestlings since they readily occupy nest boxes, allowing for experimental manipulation and facilitating sampling. Thus, our experimental design holds promise for more detailed studies of the ramifications of early life exposure to ALAN. Future experiments could further investigate the effect of different light intensities, wavelengths and durations of exposure on physiological and behavioral endpoints and fitness outcomes. However, since great tits are cavity nesters, nestlings may be relatively buffered against ALAN in natural situations, although some cavities may be less impervious to light than man-made nest boxes. Indeed, our past research suggests that nest boxes buffer sleeping adults from ambient ALAN (Raap et al., 2018a), and effects of ambient ALAN on nestlings in nest boxes are similarly low (Casasole et al., 2017; Raap et al., 2017a). However, our results are transferable to real-life situations since the nestlings of open-cup bird species are likely to experience light levels comparable to those used in our experiment. In addition, nestlings and adult great tits may experience comparable light levels when sleeping outside of nest boxes, and consequently experience elevated CORT, as indeed suggested by the work on adult great tits cited earlier (Ouyang et al., 2015).

5. Conclusions

In conclusion, our results suggest that exposure to ALAN induces physiological stress in developing songbirds, as indicated by elevated feather CORT concentrations. Elevation of CORT could arise via a number of mechanisms, including direct effects on the circadian rhythmicity of the HPA axis, disruption of sleep and activity patterns of nestlings, and altered parental behavior. These mechanisms are not mutually exclusive. Regardless of mechanistic underpinnings, elevated CORT concentrations could have effects on physiology and fitness that persist into adulthood. In a world where true darkness is increasingly difficult to find, more research is urgently needed to elucidate the extent to which ALAN affects physiology, behavior, and fitness, and whether animals can develop adaptive avoidance or mitigation strategies.

Data availability

Data will be available in the Dryad Digital Repository.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

Melissa L. Grunst: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Thomas Raap:** Investigation, Methodology, Writing - review & editing. **Andrea S. Grunst:** Conceptualization, Investigation, Writing - review & editing. **Rianne Pinxten:** Project administration, Supervision, Writing - review & editing. **Charline Parenteau:** Methodology. **Frédéric Angelier:** Methodology, Writing - review & editing. **Marcel Eens:** Project administration, Project administration, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113895.

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