



## Parenting in the city: effects of urbanization on incubation behaviour and egg temperature in great tits, *Parus major*

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With 68% of the world's population expected to live in cities by 2050, it is crucial to understand how animals respond to urbanization. Urban areas are associated with changes in microclimate, light, noise and human activity, which can affect animal physiology, behaviour and fitness. In particular, urbanization may affect how parents allocate time and energy between parental care and self-maintenance. In birds, a critical aspect of parental care is incubation, where parents must maintain egg temperatures for successful offspring development. However, incubation is energetically costly, and anthropogenic disturbances may affect parental decisions related to this cost. Determining whether urbanization affects incubation is crucial for understanding the differences between urban and nonurban populations in their reproductive performance and fitness. We measured incubation behaviour and egg temperature in one urban and one forest population of great tits, a uniparental incubator, using temperature loggers. We found that, compared to forest females, urban females spent more time incubating, took shorter off-bouts and ended their last daily off-bouts at a later hour. Furthermore, time spent off the nest, the number and duration of off-bouts and daily start of activity were related to ambient temperature; start of activity was related to clutch size; and time spent off the nest was related to the day of incubation. Despite urban females spending more time on the nest, eggs of both forest and urban females experienced relatively similar average egg temperatures, and urban eggs tended to experience greater variability in egg temperature. Furthermore, there were no differences between sites for any fitness-related offspring variable. Our results suggest that urbanization can influence the thermal environment of developing avian offspring by causing urban adults to be inefficient incubators through increased activity off the nest in the evening, differences in nest insulation and/or suboptimal incubation behaviour compared to forest counterparts.

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With 68% of the world's population expected to live in urban areas by 2050 (United Nations, 2019), it is crucial to understand how animal populations respond to urbanization. Urbanization is associated with altered food quality, differences in microclimate, pollution, noise, light and human activity (Chamberlain et al., 2009; Lancaster & Rees, 1979; Longcore & Rich, 2004; Parlow, 2011; Rizwan et al., 2008; Seress & Liker, 2015), which can lead to population declines and a loss of biodiversity (Chace & Walsh, 2006). Subsequently, the urban environment can trigger changes in animal behaviour (Abolins-Abols et al., 2016; Ditchkoff et al., 2006), physiology (Biard et al., 2017; Meillère et al., 2015), phenology (Deviche & Davies, 2014; Hope et al., 2016) and life history strategies (Sepp et al., 2018). These changes can either allow animals to

cope with urbanization and even exploit urban resources, or be maladaptive and lead to fitness costs (Chace & Walsh, 2006; French et al., 2018; Lahr et al., 2018; Russo & Ancillotto, 2015; Shochat et al., 2006).

One important way in which urbanization can affect animals is through influencing how parents allocate their time and energy between parental care and self-maintenance. Urbanization may impose an energetic constraint, making self-maintenance more costly for parents and, thus, leaving less time and energy available for parental care. This may lead to fitness costs if the reduction in parental care negatively affects offspring survival. For example, in birds, some studies have shown that, compared to rural parents, urban parents feed their nestlings less often (song sparrows, *Melospiza melodia*; Foltz, 2015) or have lower parental coordination when feeding their nestlings (house wrens, *Troglodytes aedon*; Baldan & Ouyang, 2020). This lower parental performance may explain why some studies find that urban nestlings are lighter than

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rural nestlings (Baldan & Ouyang, 2020; Biard et al., 2017). In addition, urban nestlings are likely lighter than rural nestlings because of lower food quality in urban habitats (Seress et al., 2020). However, urban parents may also be able to make adaptive behavioural changes that compensate for urban environmental conditions. For example, parents in a population of urban great tits fed their nestlings at a faster rate than their rural counterparts, which appeared to compensate for the lower nutritional quality of the food source (i.e. lower carotenoid content in caterpillars) compared to the rural area (Isaksson & Andersson, 2007). Similarly, another study found that great tits fed their nestlings more, but smaller, prey in cities than in forests (Sinkovics et al., 2021). Yet, even when urban parents appear to behaviourally compensate and exert more energy on parental care than their rural counterparts, urbanization may constrain their effectiveness. For example, one study found that blue tits, *Cyanistes caeruleus*, from urban areas travelled farther during chick provisioning (i.e. expended more energy) to search high-quality food, but their increased effort did not translate into more high-quality food (i.e. caterpillars) for their offspring and, as a result, their offspring were lighter than those in the forest population (Jarrett et al., 2020).

One of the most important aspects of avian parental care is incubation, but the effects of urbanization on incubation behaviours remain understudied. Avian parents must maintain their eggs within a small temperature range to ensure hatching success (Deeming & Ferguson, 1991). On top of this, small changes in egg temperature have large effects on offspring quality (DuRant, Hopkins, Hepp, & Walters, 2013; Hepp et al., 2015; Hope et al., 2021; Nord & Nilsson, 2011) and even long-term survival (Berntsen & Bech, 2016; Hepp & Kenamer, 2012; Nord & Nilsson, 2016). However, incubation is energetically costly and time-consuming for parents (Nord & Williams, 2015; Tinbergen & Williams, 2002), and parental incubation behaviour can vary among individuals due to ambient temperature, parental experience and clutch size (Aldrich & Raveling, 1983; Coe et al., 2015; Conway & Martin, 2000; Haftorn & Reinertsen, 1985; Hope et al., 2020). In turn, egg temperatures can vary both among and within nests (Boulton & Cassey, 2012; Coe et al., 2015; Hope et al., 2021). Ambient temperature, in particular, is an important factor for avian incubation because it can affect parental incubation behaviour (Amininasab et al., 2016; Conway & Martin, 2000; Londoño et al., 2008), and thus indirectly affect egg temperature (Coe et al., 2015), and can also directly affect egg temperature when parents are both on and off the nest (Boulton & Cassey, 2012).

Although studies have shown that some factors that are associated with urbanization, such as higher ambient temperatures (i.e. urban heat island; Heppner & Ouyang, 2021), noise (Williams et al., 2021), light at night (van Dis et al., 2021) and human activity (McGowan & Simons, 2006; Verhulst et al., 2001), can affect avian incubation behaviour, comparisons among the incubation behaviours of urban and rural bird populations are lacking. To date, only one study has investigated whether urbanization as a whole may affect avian incubation behaviour (Heppner & Ouyang, 2021), which found that house wrens in urban areas spent less time incubating per day than their rural counterparts. Another important aspect of behaviour that urbanization can affect is the timing of daily activities. Factors such as light at night and noise in urban areas can cause animals to start their daily activities at an earlier hour and end their daily activities at a later hour (Dominoni et al., 2014, 2020; Sanders et al., 2021). For example, compared to rural birds, urban birds begin their daily activities earlier (Dominoni et al., 2013), begin singing earlier in the morning (Bermúdez-Cuamatzin et al., 2020) and sing more at dusk to avoid urban noise (Fuller et al., 2007). However, no study has yet tested whether daily rhythms of incubation behaviour are affected by urbanization.

Lastly, although the temperature at which eggs are incubated is crucial for offspring development (DuRant, Hopkins, Hepp, & Walters, 2013), no study has investigated whether egg temperature differs between urban and rural bird populations. Exploring how urbanization may affect incubation behaviour, the timing of daily activity and egg temperature will provide insight into how urban development can impact avian parental care and offspring quality.

Here, we investigated whether urbanization influences avian incubation by measuring incubation behaviour, timing of daily incubation activity and egg temperature in one urban and one forest population of great tits, a species in which only the female incubates, by placing artificial egg temperature loggers in nests during the 7 days before hatch. Due to the various factors associated with urbanization that can disturb incubating females and affect parental investment (e.g. light at night, noise, human disturbance, altered food quality, etc.), we hypothesized that urbanization would lead to a change in incubation behaviour. Specifically, we predicted that urban females would take more and longer off-bouts and spend more total time off the nest (i.e. lower incubation constancy). Furthermore, we predicted that this would lead to lower and more variable egg temperatures in urban nests compared to forest nests. We also hypothesized that urbanization influences the timing of daily activity of incubating females, due to differences in light and noise. Specifically, we predicted that, compared to forest females, urban females would begin their first daily off-bout at an earlier hour and end their last daily off-bout at a later hour (Sanders et al., 2021). Lastly, because both ambient temperature and clutch size are known to influence avian incubation behaviour and egg temperature (Amininasab et al., 2016; Boulton & Cassey, 2012; Coe et al., 2015; Conway & Martin, 2000; Hope et al., 2021), we included these important covariates in our analyses. Specifically, we predicted that ambient temperature would be positively related to the amount of time that females spent off the nest (Diez-Méndez et al., 2021; Matysioková & Remeš, 2010) and positively related to egg temperature (Coe et al., 2015; Hope et al., 2021). We also predicted that, because incubating large clutch sizes is energetically costly (DuRant, Hopkins, Hepp, & Romero, 2013; Moreno et al., 1991), and thus females should need to spend more time off the nest to replenish energy reserves, clutch size would be positively related to the amount of time that females spent off the nest (Haftorn & Reinertsen, 1985; Wiebe & Martin, 2000). Because we predicted that females with large clutch sizes would spend more time off the nest, we predicted that, as a result, clutch size would be negatively related to egg temperature (Hope et al., 2021; Nord et al., 2010). There is also some evidence that larger clutch sizes retain more heat during off-bouts (Boulton & Cassey, 2012), and thus it is also possible that clutch size could be positively related to egg temperature.

## METHODS

### *Study Species and Sites*

The great tit is a widespread and common passerine throughout Europe and lives in both urban and natural areas. Great tits are secondary cavity nesters and nest readily in nestboxes. Females lay one egg per day, with final clutch sizes of 5–12 eggs (Gosler et al., 2020). On average, incubation begins on the day that the last egg is laid (Álvarez & Barba, 2014). Only the females incubate, but males feed the female on the nest (Gosler et al., 2020). The incubation period usually lasts 12–15 days (Gosler et al., 2020). During the nesting period, parents travel within a foraging area of up to 4500 m<sup>2</sup> (Naef-Daenzer, 2000).

We studied two populations of great tits, one in an urban area (Niort, France) and the other in a nearby forest (Chizé Forest, Villiers en Bois, France). Niort (46°19'33"N, 0°27'38"W) is a small city in western France, with an area of 68.20 km<sup>2</sup>, an average elevation of 28 m and a human population of 58 707. The Chizé Forest is a deciduous forest that spans across 34.35 km<sup>2</sup> in the commune of Villiers en Bois in western France, has an elevation ranging from 47 to 101 m and is located 20 linear kilometres from the urban area. Our study site was located in the biological reserve surrounding the Centre d'Etudes Biologiques de Chizé (46°08'44"N, 0°25'35"W; elevation = 87 m), where access is limited to authorized personnel (i.e. researchers, managers) and thus there is very little human activity. Habitat characteristics and urbanization scores of Niort and Villiers en Bois are reported in Meillère et al. (2017) and Beaugéard et al. (2019). These two studies show that Villiers en Bois has the lowest urbanization score out of all sites, while Niort is within the top three urbanized sites (30 sites in Meillère et al., 2017; 11 sites in Beaugéard et al., 2019). Niort is a small city, with an urbanization score lower than that of Paris (Beaugéard et al., 2019) but greater than that of the most urbanized sites in some other European studies of songbirds (e.g. Budapest, Hungary: Bókonyi et al., 2012; Liker et al., 2008). We considered the urban site and the forest site to be separate populations because the distance of 20 km between the two sites is >20 times greater than the average natal dispersal distance of great tits (e.g. 0.498–0.643 km: Dingemans et al., 2003; 0.528–0.788 km: Szulkin & Sheldon, 2008), and another study in France found low but significant genetic differentiation among urban and rural populations of great tits located 20 km apart (Perrier et al., 2018).

#### *Nest and Incubation Monitoring*

We checked nestboxes three times per week during March–June 2018. If we found a nest with more than one egg, we assumed that one egg was laid per day to determine the nest initiation date. Then, we waited 8 days after nest initiation to check whether egg laying was complete, and then checked every day until clutch completion. We considered that a clutch was complete on the first day that an additional egg was not laid, and we considered the start date of incubation to be the date that the last egg was laid.

After egg laying was complete, we installed a temperature logger to record incubation behaviour and egg temperature. We did not install the loggers earlier than this (i.e. during egg laying) to avoid nest abandonment. We used HOBO® temperature data loggers (Onset® Corporation, Bourne, MA, U.S.A.), which have two thermal probes. We attached one probe to the inside of the nestbox, about 15 cm above the nest (in the same position in each nest), to record the ambient temperature in the box. The other probe was modified with a thermistor and fitted into a 3D printed artificial great tit egg, which was painted to closely mimic the pattern of great tit eggs and filled with wire-pulling lubricant (ClearGlide, Ideal Industries, Sycamore, IL, U.S.A.) to allow the egg temperature to be maintained and mimic actual temperatures that an egg would experience, as opposed to an empty egg (Coe et al., 2015; Cooper & Voss, 2013; Hope et al., 2020). We threaded this probe through the nesting material so that only the egg was visible. The wire was flexible, allowing the egg to be moved slightly by the females but remain within the nest cup. We attached the body of the logger to the pole/tree of the nestbox, which allowed data to be downloaded without opening the nestbox. The logger recorded egg temperature and ambient temperature simultaneously every 30 s. Ten days after clutch completion, we checked nests daily for hatching. After the hatch date (day 0) was determined (i.e. at least one nestling hatched), we checked nests 3 days later (nestling day 3) to determine how many nestlings had hatched. We also measured body

mass of nestlings on nestling day 3 and calculated the average nestling body mass for each nest.

#### *Incubation Behaviour, Timing of Activity and Egg Temperature*

We collected temperature data from 10 forest nests and 14 urban nests. Before we began collecting data, we allowed birds to settle for at least 1 day after we disturbed the nest to install loggers. To standardize our data among nests, we only analysed the 7 days of incubation before the hatch date, because we had collected at least this quantity of data from each nest. To analyse female behaviour, we used Rhythm and RAVEN software (Cooper & Mills, 2005) to determine when the female was off the nest (off-bout). An off-bout was defined as a drop in temperature of at least 1.5 °C that lasted at least 4 min (Coe et al., 2015). We validated our method by videorecording three nests for a total of 8.3 h and comparing off-bouts observed in the video with off-bouts that had already been selected using the temperature data. Out of 12 off-bouts that lasted >4 min in the videos, 11 had been correctly identified by using the temperature data. The one off-bout that was not correctly identified lasted only 4.2 min.

For each nest, we analysed each of the 7 days of incubation separately to determine standard behaviours (Coe et al., 2015; Hope et al., 2020). After selecting off-bouts, we determined three incubation behaviours for each day: the total time spent off the nest (i.e. not incubating), the number of off-bouts taken and the average length of the off-bouts taken that day. Furthermore, we determined two behavioural end points related to the timing of daily activity: the time of the start of the first off-bout ('start of activity') and the end of the last off-bout ('end of activity'). Then, to examine egg temperature, we calculated the average egg temperature and the standard deviation in egg temperature for each day. Lastly, we calculated average ambient temperature for each day.

#### *Statistical Analyses*

In total, we collected data from 10 forest nests and 14 urban nests, with data during 7 days of incubation for each nest. However, there were two nests (both urban) in which the temperature loggers experienced technical problems, which made it difficult to select off-bouts or to be confident in the temperature readings. Thus, we excluded these two nests from the analyses investigating the 'time spent off the nest', 'number of off-bouts', 'off-bout duration', 'average egg temperature' and 'standard deviation in egg temperature'. However, we included these nests in the analyses investigating the 'start' and 'end of activity' because the distinctly different temperature patterns of night-time and daytime incubation activity were always evident despite technical problems.

We conducted all statistical analyses using R v.3.5.1 (R Core Team, 2018) and used the 'lme4' package (Bates et al., 2015) to build our models. We verified that our data met all model assumptions by visually inspecting normal quantile plots, predicted versus residuals plots, and histograms of model residuals. We used stepwise backward elimination to remove nonsignificant ( $P > 0.05$ ) factors, starting with interaction terms, and all full and reduced models are reported in the Results. We report  $P$  values using type III Wald chi-square tests, using the 'Anova' function in the 'car' package (Fox & Weisberg, 2011). For all models, we verified that independent variables were not correlated by calculating the variance inflation factor (VIF; 'car' package; Fox & Weisberg, 2011). All VIFs were <4 in final models (James et al., 2013; Vittinghoff et al., 2012). Furthermore, to decrease multicollinearity, we centred continuous independent variables when used as part of an interaction term. Day of year was not included in any model because it was highly correlated with average ambient temperature ( $P < 0.001$ ), and we

chose ambient temperature over day of year because of its greater biological relevance to our questions pertaining to incubation behaviour and egg temperature. We first included the interaction between days before hatch and ambient temperature in all models but it was nonsignificant in all models ( $P > 0.10$ ), and thus, we excluded it from all models for simplicity. We also examined plots of the relationships among all dependent variables to ensure that they were not correlated. However, we found that 'time spent off nest', 'number of off-bouts' and 'off-bout duration' were correlated, and thus, we used a Bonferroni correction for these three models ( $\alpha = 0.05/3 = 0.0167$ ). For all other models, significance was set at  $P < 0.05$ .

To examine whether incubation behaviours differed between urban and forest females, we built three linear mixed effects models. The dependent variables were the daily time spent off the nest, the daily number of off-bouts and the daily average off-bout duration. For all models, habitat (urban versus forest), daily average ambient temperature, clutch size and days before hatch (where 1 indicates the last day of incubation before the hatch date) were the independent variables, along with the interactions between habitat and ambient temperature, between habitat and clutch size and between habitat and days before hatch. Time spent off the nest and off-bout duration were ln-transformed to meet model assumptions. The model for the number of off-bouts was a generalized linear mixed model with a Poisson distribution for count data. Nest identity (ID) was included as a random effect to account for repeated measures (i.e. 7 days of incubation per nest).

Next, to investigate whether the timing of daily activity differed between urban and forest females, we built two linear mixed effects models. The dependent variables were the daily start of activity and the daily end of activity. The independent variables and random effect were the same as described in the first set of models. One extreme and influential (>20 times the mean Cook's distance) outlier was excluded from the 'start of activity' model (1 out of 168 total data points; data point = 0133 hours).

Furthermore, to investigate whether egg temperature differed between urban and forest nests, we built two linear mixed effects models. Daily average egg temperature and daily standard deviation in egg temperature were the dependent variables, and the independent variables and random effect were the same as described in the first set of models. Standard deviation in egg temperature was ln-transformed to meet model assumptions.

Last, we investigated whether fitness-related variables differed between urban and forest populations. We built five simple linear models with habitat as the independent variable and clutch size, incubation period (the number of days between the date of the last laid egg and hatch date), hatch success (proportion of nestlings that hatched out of the total number of eggs), the number of nestlings on nestling day 3 and the average mass of nestlings on nestling day 3 as dependent variables.

#### Ethical Note

All procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee (protocol number 18-046) and the DREAL Nouvelle-Aquitaine.

## RESULTS

Incubation behaviours were related to habitat, ambient temperature and days before hatch. There was an interactive relationship of habitat and days before hatch with the time spent off the nest (Table 1); urban females spent less time off the nest than forest females earlier in the incubation period but behaviours converged as the hatch date approached (Fig. 1a). For example, 4 days before

hatching (day 4), forest females spent an average of 3.6 h off the nest, while urban females only spent an average of 2.7 h off the nest. Furthermore, there was an interactive relationship of habitat and ambient temperature with the time spent off the nest (Table 1), where urban females spent less time off the nest than forest females when ambient temperature was cooler but behaviours converged between populations at warmer temperatures (Fig. 1b). There was also an interactive relationship of ambient temperature and habitat on the number of off-bouts (Table 1), where urban females took more off-bouts on warmer days but the number of off-bouts that forest females took was not related to ambient temperature (Fig. 1c). Lastly, off-bout duration was related to both habitat (Table 1) and ambient temperature (Table 1); average off-bout duration increased as ambient temperature increased, and urban females took shorter off-bouts than forest females (Fig. 1d). No incubation behaviour variable was related to clutch size, and neither the number of off-bouts nor off-bout duration were related to days before hatch (Table 1).

The timing of daily activity was related to habitat, ambient temperature and clutch size. Females started their activity at an earlier hour when days were warmer (Table 1, Fig. 2a), and females with larger clutch sizes started their activity at a later hour than those with smaller clutch sizes (Table 1, Fig. 2b). Furthermore, urban females ended their activity at a later hour than forest females (Table 1, Fig. 2c) where, on average ( $\pm$ SE), forest females ended their activity at 1920 hours  $\pm$  4.6 min and urban females ended their activity at 1943 hours  $\pm$  5.2 min. The start of activity was not related to habitat or days before hatch, and the end of activity was not related to ambient temperature, clutch size or days before hatch (Table 1).

Egg temperatures were related to habitat, ambient temperature and days before hatch. Average egg temperature increased in both populations as ambient temperature increased (Table 1, Fig. 3a). Furthermore, there was an interactive relationship of habitat and days before hatch with average egg temperature (Table 1), where egg temperature tended to be higher in the urban habitat than in the forest habitat during days further away from the hatch date, but this relationship was reversed during the days just before hatch (Fig. 3b). Additionally, the standard deviation in egg temperature tended to be greater in the urban habitat than in the forest habitat (Fig. 3c), although the relationship was only marginally significant (Table 1).

We did not find any significant differences in fitness-related variables between the two populations in this study (Table 2). However, we found that daily ambient temperatures inside our nestboxes tended to be 2.7 °C warmer in the urban habitat than in the forest habitat (mean  $\pm$  SE: forest: 14.94  $\pm$  0.44 °C; urban: 17.64  $\pm$  0.39 °C), although the difference only approached statistical significance (simple linear mixed effects model with nest ID as random effect:  $\chi^2 = 3.81$ ,  $P = 0.051$ ).

## DISCUSSION

In this study, we found that urbanization was related to changes in a crucial aspect of avian parental care – incubation. Urban females spent less time off the nest (i.e. higher incubation constancy), took shorter off-bouts and ended their activity (i.e. ended their last daily off-bout) later, compared to forest females. We found that the differences in incubation constancy between populations were sometimes apparent only at cool ambient temperatures or during earlier days of incubation, which may suggest that urban females modulate their behaviour in response to external cues more readily than forest females. However, despite urban females spending more time on the nest than forest females, average egg temperatures were similar between populations, and the standard

**Table 1**

Results of full and reduced linear mixed effects models investigating whether parental incubation behaviour and egg temperature of great tits are related to habitat (urban versus forest), ambient temperature and/or clutch size

Term	Time spent off the nest <sup>a</sup> ( $N_{\text{urban}} = 12$ , $N_{\text{forest}} = 10$ ) <sup>b</sup>		Average off-bout duration <sup>a</sup> ( $N_{\text{urban}} = 12$ , $N_{\text{forest}} = 10$ ) <sup>b</sup>		Number of off-bouts <sup>c</sup> ( $N_{\text{urban}} = 12$ , $N_{\text{forest}} = 10$ ) <sup>b</sup>		Start of activity ( $N_{\text{urban}} = 14$ , $N_{\text{forest}} = 10$ ) <sup>b</sup>		End of activity ( $N_{\text{urban}} = 14$ , $N_{\text{forest}} = 10$ ) <sup>b</sup>		Average egg temperature ( $N_{\text{urban}} = 12$ , $N_{\text{forest}} = 10$ ) <sup>b</sup>		Standard deviation in egg temperature <sup>a</sup> ( $N_{\text{urban}} = 12$ , $N_{\text{forest}} = 10$ ) <sup>b</sup>	
	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
<i>Full models</i>														
Habitat	6.69	<b>0.01</b>	5.21	<b>0.02</b>	0.26	0.61	0.82	0.36	3.63	0.057	0.62	0.43	2.34	0.13
Ambient temperature	6.49	<b>0.01</b>	12.1	<b>0.0005</b>	0.04	0.85	2.73	0.098	0.75	0.39	17.8	<b>&lt;0.0001</b>	0.10	0.76
Clutch size	0.38	0.54	0.003	0.95	0.24	0.62	0.07	0.79	0.13	0.72	0.21	0.65	<0.001	>0.99
Days before hatch	0.55	0.46	0.001	0.98	0.12	0.73	0.003	0.95	1.69	0.19	4.14	<b>0.042</b>	0.04	0.84
Habitat × ambient	12.9	<b>0.0003</b>	0.03	0.87	6.16	<b>0.013</b>	0.33	0.57	1.78	0.18	0.34	0.56	1.02	0.31
Habitat × clutch	0.83	0.36	0.06	0.81	0.35	0.55	3.26	0.07	0.04	0.84	0.18	0.67	0.11	0.74
Habitat × days	13.8	<b>0.0002</b>	1.89	0.17	1.99	0.16	0.82	0.37	1.62	0.20	12.6	<b>0.0004</b>	1.49	0.22
<i>Reduced models</i>														
Habitat	9.77	<b>0.002</b>	6.34	<b>0.012</b>	0.70	0.40	NA		3.84	<b>0.04997</b>	0.47	0.50	2.97	0.085*
Ambient temperature	11.0	<b>0.0009</b>	49.7	<b>&lt;0.0001</b>	0.06	0.80	4.61	<b>0.032</b>	NA		61.3	<b>&lt;0.0001</b>	NA	
Clutch size	NA		NA		NA		10.0	<b>0.002</b>	NA		NA		NA	
Days before hatch	0.39	0.53	NA		NA		NA		NA		6.18	<b>0.013</b>	NA	
Habitat × ambient	13.2	<b>0.0003</b>	NA		6.52	<b>0.011</b>	NA		NA		NA		NA	
Habitat × clutch	NA		NA		NA		NA		NA		NA		NA	
Habitat × days	13.3	<b>0.0003</b>	NA		NA		NA		NA		12.9	<b>0.0003</b>	NA	

\*Marginal significance ( $0.05 < P < 0.1$ ). Bold indicates significance ( $P < 0.05$ ).

<sup>a</sup> Dependent variable was ln-transformed to meet model assumptions.

<sup>b</sup> Sample sizes are number of nests, but 7 days of incubation data were used per nest and nest identity (ID) was included as a random effect to account for repeated measures.

<sup>c</sup> Generalized linear mixed model with a Poisson distribution for count data.

deviation in egg temperature even tended to be greater in urban nests compared to forest nests. Thus, overall, it appears that urban females may be expending more time and energy to maintain a similar average, but more variable, thermal environment for their developing embryos. However, we did not find any effects on fitness-related offspring traits (i.e. hatch success, nestling mass, etc.), and thus, there were no apparent immediate effects on offspring. More work is needed to determine whether differences in incubation behaviour or egg temperature could have other effects on offspring phenotype similar to those observed in other study systems (reviewed in DuRant, Hopkins, Hepp, & Walters, 2013; Hope et al., 2021). Furthermore, although our results provide insight into how urbanization affects avian parental care and reproduction, our study was conducted on only one urban and one forest site; thus, we cannot completely exclude the possibility that our results are due to a site/population-specific effect rather than an effect of urbanization. Thus, future studies that compare multiple sites/populations are also needed to verify our results.

#### Differences Between Urban and Forest: Incubation Behaviour

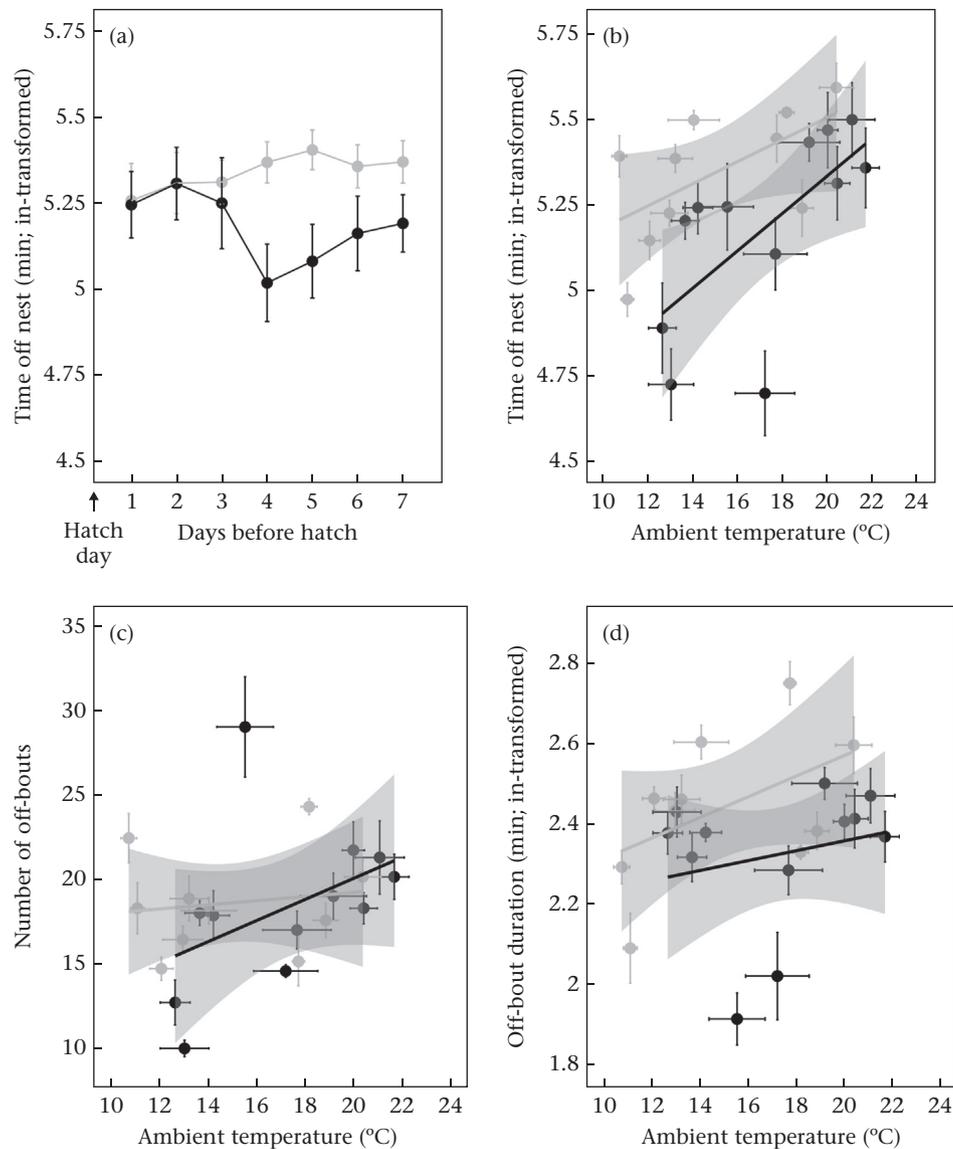
Contrary to our prediction, urban females spent less time off the nest and took shorter off-bouts compared to the forest females. Our result disagrees with the one other study that investigated urbanization on incubation behaviour in another songbird species, which found that urban house wrens had a lower incubation constancy compared to rural birds (Heppner & Ouyang, 2021). It is possible that the effects of urbanization are species dependent and context specific. For example, Heppner and Ouyang (2021) compared house wren populations in a city park and an agricultural farm, which differs from our study comparing great tits in a city and a forest. Additional studies are needed to identify how specific habitat differences may differentially influence avian incubation behaviour and how this might vary among species and/or populations.

Our observation that urban females spent less time off the nest compared to forest females suggests that urban females may be less

energetically constrained during incubation, which could be due to multiple factors. First, urban females may leave the nest for shorter periods to forage because it may be easier to access certain types of food, such as seeds, in urban areas compared to the forest. Indeed, food predictability may be high in urban areas due to bird feeders or food scraps (Robb et al., 2008). It is also possible that urban females can forage quickly because animal territories in urban areas tend to be smaller than in the forest (Juárez et al., 2020; Lowry et al., 2013). A second, nonmutually exclusive possibility is that urban males invest more time and energy into feeding the female during incubation, compared to forest males. Indeed, male feeding behaviour varies due to factors such as ambient temperature, predation and food supply (Amininasab et al., 2016; Martin & Ghalambor, 1999; Matysioková & Remeš, 2010), although we did not measure male feeding behaviour in this study. More male investment could allow females to stay on the nest longer and/or maintain a better body condition than those in the forest, although there is no evidence for differences in body condition between urban and forest great tits (Caizergues et al., 2021).

#### Behavioural Plasticity of Urban Birds

Urban females appeared to change their behaviour more in relation to ambient temperature, as compared to forest females, which may indicate that they have greater behavioural plasticity. For example, the number of off-bouts and the time spent off the nest increased as ambient temperature increased, but more so in the urban population. This relationship with ambient temperature agrees, in general, with other studies on great tits (Diez-Méndez et al., 2021; Matysioková & Remeš, 2010) and may help to preserve incubation temperatures when ambient temperatures are cold (i.e. fewer off-bouts; less time spent off the nest), while allowing females to conserve their energy when temperatures are warm. In this way, urban females may be able to 'take advantage' of warmer ambient temperatures (i.e. when eggs will not cool down as quickly) to relieve themselves from some incubation effort, while



**Figure 1.** Factors related to female incubation behaviours in an urban population (black symbols) and a forest population (grey symbols) of great tits. Relation between the time that females spent off the nest each day and (a) day of incubation and (b) ambient temperature. Relation between ambient temperature and (c) the number of off-bouts taken per day and (d) average daily off-bout duration. Dependent variables are represented as mean  $\pm$  SE.  $N_{\text{urban}} = 12$  nests and  $N_{\text{forest}} = 10$  nests, and each nest was studied during 7 days of incubation.

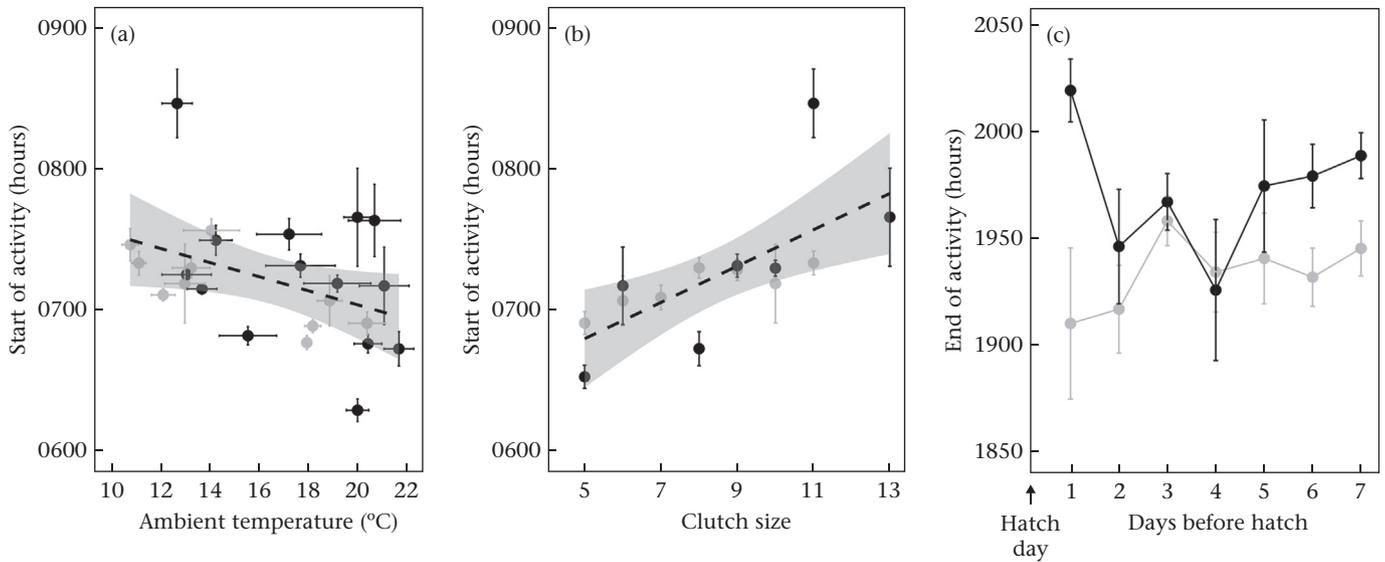
forest females maintain a similar incubation effort regardless of ambient temperature. This behavioural flexibility could be beneficial for urban females. Indeed, behavioural plasticity is expected to be important for the colonization and survival of urban-dwelling animals (Lowry et al., 2013; Wong & Candolin, 2015).

Urban females also appeared to display more behavioural plasticity in relation to the day of incubation than forest females. While forest females spent relatively the same amount of time off the nest regardless of the day of incubation, urban females increased the amount of time off the nest as the hatch date approached. However, this is the opposite of what we would expect. In general, birds should increase their incubation effort as the hatch day approaches and embryos become more sensitive to temperature changes (Cooper & Voss, 2013), but urban females in our study seemed to be doing the opposite. There are multiple possible explanations for this relationship. First, it is possible that urban females spend too much energy early in incubation and then are not able to maintain their incubation behaviour at the end of

incubation. Second, it is possible that, at the end of incubation, female investment shifts to focus on searching for foraging areas that are rich in insects (i.e. to prepare for nestlings), and thus females leave the nest more often. This may be especially important in urban areas if females primarily feed on seeds (i.e. bird feeders) or food scraps during incubation, and then must provision their nestlings with insects, which are usually less abundant in cities (Seress et al., 2018). Third, it is possible that the male spends less time feeding the female during the end of incubation, which would be consistent with a lower parental coordination in urban birds (Baldan & Ouyang, 2020), and thus, this would force the female to leave the nest more often to forage.

#### *Differences Between Urban and Forest: Timing of Activity*

Our study is the first that we are aware of that has investigated the timing of daily activity rhythms of incubating birds in response to urbanization. As predicted, we found that the end of daily

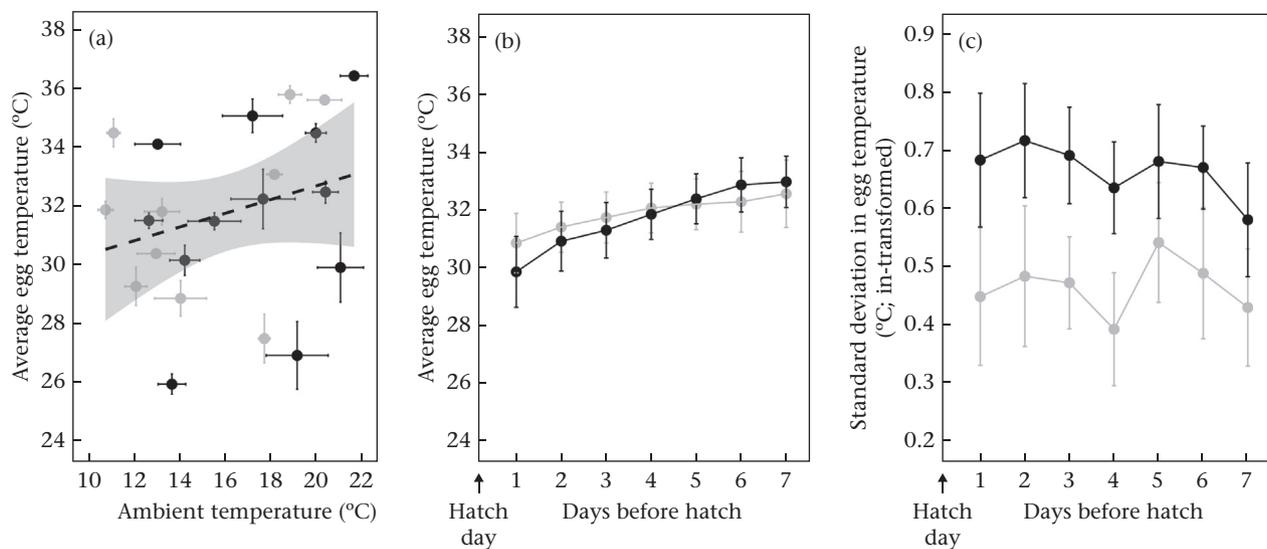


**Figure 2.** Factors related to timing of daily incubation activity in an urban population (black symbols) and a forest population (grey symbols) of great tits. Relation between the start of daily activity and (a) ambient temperature and (b) clutch size. (c) Relation between the end of activity and day of incubation. Dependent variables are represented as mean  $\pm$  SE. Trendlines in (a) and (b) were calculated using pooled forest and urban females because there was no statistical difference between populations.  $N_{\text{urban}} = 14$  nests and  $N_{\text{forest}} = 10$  nests, and each nest was studied during 7 days of incubation. For the start of activity (a, b), one extreme and influential data point (1 day, one nest: start of activity at 0133 hours) was not included in the plot nor in the model (see [Methods](#)).

activity (i.e. end of last daily off-bout) was later in urban females compared to forest females. Urban females may spend more time active at night due to several, nonmutually exclusive factors. First, light at night in the urban area may disrupt sleep or allow individuals more time to forage at night ([Dominoni et al., 2013](#); [Sanders et al., 2021](#)). Second, anthropogenic noise may affect the time at which females are active during the day ([Fuller et al., 2007](#)). Third, the tendency for warmer ambient temperatures (2.7 °C) inside nestboxes in the urban area may stimulate females to be more active at night ([Lehmann et al., 2012](#)). Lastly, because predation risk is generally lower in urban areas ([Ibáñez-Álamo & Soler, 2010](#); [Møller & Ibáñez-Álamo, 2012](#); [Sorace & Gustin,](#)

[2009](#)), urban females may be able to be more active at dusk, whereas forest females would experience too great of a predation risk at this time.

Contrary to our prediction, the daily start of activity did not differ between habitats. This was surprising considering that other studies show that anthropogenic noise and light at night are related to an earlier start of daily activity in birds (e.g. singing, foraging; [Arroyo-Solís et al., 2013](#); [Dominoni et al., 2014](#); [Sanders et al., 2021](#)), although these studies did not investigate activity rhythms related to incubation behaviour. However, the daily start of activity (i.e. start of first daily off-bout) was negatively related to ambient temperature and positively related to clutch size in both urban and



**Figure 3.** Factors related to egg temperature in an urban population (black symbols) and a forest population (grey symbols) of great tits. Relation between average egg temperature and (a) ambient temperature and (b) day of incubation. (c) Relation between the standard deviation in egg temperature and day of incubation. In (a), the trendline was calculated using pooled forest and urban nests. Dependent variables are represented as mean  $\pm$  SE.  $N_{\text{urban}} = 12$  nests and  $N_{\text{forest}} = 10$  nests, and each nest was studied during 7 days of incubation.

**Table 2**  
Means ( $\pm$  SE) and ranges of fitness-related variables measured in a forest and urban population of great tits

Variable	Forest			Urban			$P^a$
	Mean $\pm$ SE	Range	$N$	Mean $\pm$ SE	Range	$N$	
Clutch size	7.90 $\pm$ 0.59	5–11	10	9.07 $\pm$ 0.62	5–13	14	0.20
Incubation period (days)	12.8 $\pm$ 0.39	11–15	10	13.0 $\pm$ 0.59	9–18	14	0.80
Hatch success	0.72 $\pm$ 0.06	0.29–0.90	10	0.65 $\pm$ 0.07	0.18–0.90	14	0.49
Number of nestlings 3 days after hatch	5.4 $\pm$ 0.81	0–9	10	5.5 $\pm$ 0.72	0–9	14	0.93
Mass of nestlings when 3 days old (g) <sup>b</sup>	3.82 $\pm$ 0.19	2.88–4.70	9	3.63 $\pm$ 0.20	2.17–4.98	13	0.52

<sup>a</sup> Calculated using simple linear models with habitat as the independent variable.

<sup>b</sup> Average mass of all nestlings in each nest 3 days after hatch.

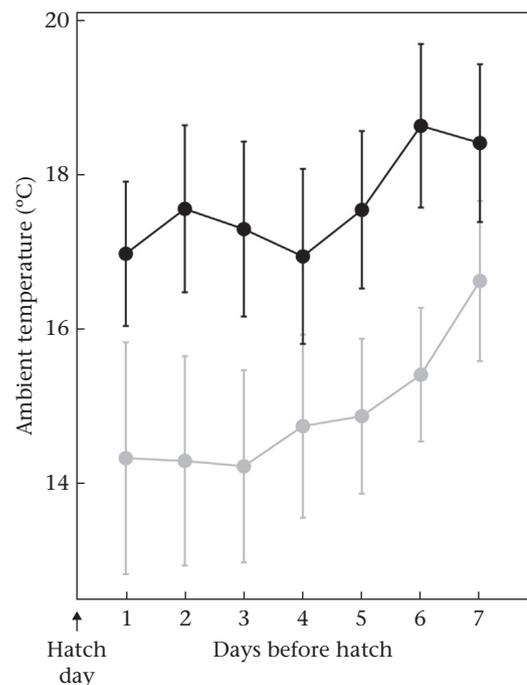
forest populations. The negative relationship with ambient temperature may be because, when temperatures are cold, females need to stay on the nest later in the morning to protect eggs from cold temperatures (Diez-Méndez et al., 2021; Matysioková & Remeš, 2010). The positive relationship with clutch size may be because individuals with large clutch sizes are also of better individual quality and, in turn, may be able to invest more in incubation (Gorman & Nager, 2003). Alternatively, females with large clutches may need to invest more time into incubation because larger clutches are more difficult to heat efficiently compared to smaller clutches (DuRant, Hopkins, Hepp, & Romero, 2013; Hope et al., 2021). Thus, overall, we found evidence that urbanization influenced the timing of the end of daily incubation activity in great tits but not the start of daily activity.

#### Differences Between Urban and Forest: Egg Temperature

Although urban females spent more time incubating than forest females, and despite urban ambient temperatures tending to be warmer, urban eggs experienced relatively similar average egg temperatures, and even tended to experience a greater standard deviation in egg temperature, compared to forest eggs. In other words, urban embryos do not appear to accrue additional thermal benefits from the increased incubation effort of their mothers, nor do the embryos experience the positive thermal influence of higher ambient temperatures. This was surprising because a higher incubation constancy and shorter off-bouts are usually associated with higher and less variable egg temperatures (Coe et al., 2015). There are multiple possible explanations for this inconsistency between incubation behaviour and egg temperature. First, the most likely explanation for this is, because urban females end their daily activity later than forest birds, they likely spend more time off the nest at night when ambient temperatures are lower. Indeed, between 1830 and 2030 hours, which is the range of hours in which females ended their daily activity (Fig. 2c), urban females took more off-bouts than forest females ('lmer' with Poisson distribution:  $\chi^2 = 5.04$ ,  $P = 0.02$ ). Because ambient temperatures are usually lower at night, the magnitude of the temperature drops during off-bouts should be greater in urban nests. Indeed, although the daily maximum egg temperature did not differ between habitats ('lmer' with ambient temperature as covariate: habitat:  $\chi^2 = 0.03$ ,  $P = 0.9$ ), the daily minimum egg temperature tended to be 1.1 °C lower in the urban habitat compared to the forest habitat ('lmer' with ambient temperature as covariate: habitat:  $\chi^2 = 3.65$ ,  $P = 0.056$ ). Lower daily minimums should then, in turn, increase the standard deviation in egg temperature, especially in the evening. Indeed, between 1830 and 2030 hours, urban eggs experienced, on average, a significantly higher standard deviation in egg temperature than forest eggs ('lmer':  $\chi^2 = 8.51$ ,  $P = 0.004$ ). Furthermore, the difference in the standard deviation of egg temperature between urban and forest nests was 58% greater between 1830 and 2030 hours than during the course of the entire day (average daily SD:

forest = 1.67; urban = 2.03; average SD during 1830–2030 hours: forest = 1.28; urban = 1.85). This suggests that being active later in the day may have caused egg temperatures to be more variable in the urban population, and that greater activity later at night may lead to negative consequences despite a higher incubation constancy. A second, nonmutually exclusive explanation could be that urban eggs may experience greater temperature variation, despite their mothers' high incubation constancy, due to differences in nest insulation between habitats. Indeed, nest composition varies among urban and rural sites in blue tits (Reynolds et al., 2016), although we did not measure this in our study and did not observe any noticeable differences in nest material between our sites. Lastly, it is possible that urban females exhibit suboptimal incubation behaviour while they are inside the nestbox due to anthropogenic disturbances, where they may move enough within the nest cavity to vary the nest temperature but not meet the criteria to qualify as off-bout in our behavioural analyses.

Average egg temperature also varied in response to ambient temperature and the days before hatch. As predicted, average egg temperature was positively related to ambient temperature (Coe et al., 2015; Hope et al., 2021). In relation to the number of days before hatch, average egg temperature decreased slightly but



**Figure 4.** Relation between days before hatch and ambient temperature (mean  $\pm$  SE) measured at urban nestboxes (black symbols) and forest nestboxes (grey symbols).  $N_{\text{urban}} = 14$  nests and  $N_{\text{forest}} = 10$  nests.

remained relatively constant across days in the forest population, then decreased more drastically as the hatch date approached in the urban population, as supported by the significant interaction between days before hatch and habitat (Table 1, Fig. 3b). This is the opposite of what we would expect, and it is especially surprising because, on average, urban ambient temperatures tended to be warmer than forest temperatures. It is likely that the low egg temperatures that we observed close to hatch day were caused by ambient temperatures being, on average, warmer on the day farthest from the hatch date (day 7) than on the day before hatch date (day 1; Fig. 4), which is the opposite of what we would expect based on calendar dates. This is likely because many nests in our study began in April 2018, which began as a very hot month in our study area but ended with an unusual cold snap. Note, however, that this weather phenomenon likely does not explain the relationship between days before hatch and the time spent off the nest (Fig. 1a), because the time spent off the nest was positively related to ambient temperature in the urban population (Fig. 1b).

### Implications of Urbanization

Overall, our study suggests that the urban habitat poses different challenges than the forest habitat, but both sets of challenges appear to be within the breadth of conditions that this species seems capable of tolerating. Although urban females appeared to invest more time and energy into incubation behaviour than forest females, egg temperatures were similar between habitats and there were no differences in any of the fitness-related offspring traits that we measured. Thus, it is possible that the behavioural differences that we observed represent different behavioural strategies that are tailored to the particular habitat and, ultimately, lead to the same outcomes for the offspring. However, it is still possible that urban living has other long-term consequences that we did not measure, such as effects on offspring phenotype, long-term offspring or mother survival, or the future reproductive success of the mother. For example, urban females may be able to efficiently incubate their eggs due to high food availability (i.e. bird feeders, food scraps), only to experience a shortage of suitable food for nestlings (i.e. insects: Catto et al., 2021; Seress et al., 2018). Furthermore, some cues in urban areas, such as light at night, could trigger higher female activity in the evening when the temperature drops, even if it is disadvantageous for embryonic development (i.e. leads to greater variation in incubation temperature). These ecological traps may make it appear that urban birds are thriving, while in the long-term, there may be negative consequences. Thus, additional studies are needed to investigate the effects of urbanization on avian nesting ecology and reproductive success.

### Author Contributions

S.F.H. and F.A. collected the data. S.F.H. conducted statistical analyses and wrote the first draft of the paper. F.A. and W.A.H. provided supervision. All authors conceptualized the study, provided funding and resources, provided comments on the manuscript and agreed on its final version.

### Data Availability

Data will be made available on request.

### Declaration of Interest

We have no conflicts of interest.

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