Title:

An analysis of passerine egg traits across the city mosaic: Urbanisation does not affect egg size and pigmentation patterns

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ABSTRACT

1. Rapid urbanisation provides remarkable opportunities to study how sudden, extreme changes impact wildlife. Compared to natural areas, cities are characterised by factors affecting both abiotic (e.g. climate, pollution, habitat fragmentation) and biotic (e.g. Normalized Difference Vegetation Index (NDVI), species composition, phenology) components of the ecosystem, ultimately changing the ecological and evolutionary dynamics of those habitats. Analogously to many other taxonomic groups, urban birds differ from rural birds in morphology, behaviour and reproductive patterns. Yet potential links between urbanisation and avian egg traits — a key aspect of the avian life-cycle — remain under-researched. Given the limited availability of primary natural calcium sources (snails) in cities, eggs from heavily urbanised areas were expected to be smaller and more pigmented, indicating thinner shells and lower overall egg quality.
2. To better understand how urbanisation affects eggs, data on 718 great tit (*Parus major*) eggs from 90 clutches, spread across eight study sites in a city mosaic, were collected for two breeding seasons. All clutches were photographed using a standardised approach, and further analysed using digital imaging and visual scoring to assess egg volume and pigmentation patterns. Urbanisation was quantified as the percentage of Impervious Surface Area (ISA) in the vicinity of each clutch via satellite imagery.
3. In line with some of the earlier studies conducted on semi-natural bird communities, egg volume covaried with lay date and female body condition, while for both egg volume and egg pigmentation (*spots percentage*) — a year effect was detected.

However, in contrast with predictions, there was no association between urbanisation and the examined egg traits.

4. While urban clutches are consistently smaller, this study shows that eggs as such are similar to those found in rural habitats in terms of volume and pigmentation patterns. These results suggest that urban-driven environmental pressures may not be as strong or directional during the egg laying phase as they are at later stages of reproduction, and highlight the complexities of urban ecological and evolutionary dynamics.

Key words: environmental constraints, calcium, egg pigmentation, egg volume, great tit, protoporphyrin IX, reproduction, urbanisation

INTRODUCTION

Cities provide remarkable opportunities to study how extreme environmental changes can impact organismal biology, giving us both a better understanding of eco-evolutionary processes and consequences that anthropogenic pressures may have (Rivkin et al., 2019). Among other factors, urbanisation is characterised by a large amount of impervious surfaces (Szulkin et al., 2020) which translates into highly fragmented habitats, higher average temperatures (known as urban heat island effect; Oke, 1973), lower capacity for water retention, and fewer biologically active areas. Ultimately, such changes are known to affect eco-evolutionary dynamics, species composition, abundance, interactions (Des Roches et al., 2020), and even genetic makeup (Bosse et al., 2017).

Due to its common presence in both rural habitats and cities (Bańbura & Bańbura, 2012), the great tit (*Parus major*) is a valuable model organism for urban biology research. Over the years, much work has demonstrated the impact of urbanisation on a large number of this passerine's traits, including changes in morphology (Thompson et al., 2022), behaviour (Corsini et al., 2022), survival (Corsini et al., 2020; Corsini & Szulkin 2025) and reproduction (Charmantier et al., 2017). Thus, urban tits start laying their clutches earlier, often have fewer eggs (Charmantier et al., 2017), hatchlings and fledglings (Corsini & Szulkin, 2025) relative to their rural conspecifics. However, only a few studies to date investigated the potential associations between urbanisation and egg size (Hõrak et al., 1995; Bańbura et al., 2010; Bailly et al., 2016; Hargitai et al., 2016a) or pigmentation (Hargitai et al., 2016a). More generally, great tit egg traits have been shown to be associated with the immediate environment - specifically, calcium availability (Mänd et al., 2000; Gosler et al., 2005, Gosler & Wilkin, 2017) and were proposed as predictors of clutch quality (Sanz & García-Navas, 2009). Namely, egg size has been shown to positively covary with calcium availability (Mänd et al., 2000), hatching success, nestling development and survival (meta-analysis, Krist, 2011), while pigmentation patterns were found to correlate with calcium availability, eggshell thickness (Gosler et al., 2005), incubation period length, hatching success, and nestling morphology (Sanz & García-Navas, 2009).

Great tits lay one egg per day, with typically 5 to 12 of them in a clutch. Each egg weighs *c.*1.2-2.0 g (Gosler et al., 2005), and has a volume of 1.50 ± 0.12 cm^3 (Encabo et al., 2001). The eggshell is white with reddish spots (the pigment is protoporphyrin IX, produced by females in the biosynthesis of blood haem (Burley & Vadehra, 1989)). The primary component of the eggshell is calcium carbonate (Romanoff & Romanoff, 1949). A single blue tit (*Cyanistes caeruleus*) clutch can contain more calcium than the entire female skeleton (Perrins & Birkhead, 1983). Since tits do not store calcium in their bones for egg laying (Graveland & Gijzen, 1994), it must be obtained daily from the environment, making it a limiting resource in egg formation (Graveland & Gijzen, 1994, Graveland & Berends, 1997, Graveland & Drent, 1997). Hence, breeding in calcium-poor habitats can have detrimental consequences for bird reproduction (Reynolds & Perrins, 2010). The main source of calcium for great tits in rural environments (such as woodlands and forests) are small snails (Graveland, 1996), which may be negatively affected by urbanisation both in terms of species diversity (Čiliak et al., 2024) and abundance (Perez et al., 2021), although abundance patterns are species-specific (Saeki et al., 2020).

Snail abundance correlates strongly with soil-calcium (Jubb et al., 2006). In tits, calcium availability in the immediate environment has been shown to be reflected in both egg size and pigmentation patterns in non-urban habitat. Thus, eggs in calcium-poor areas are smaller (Mänd et al., 2000; Hargitai et al., 2013; Bańbura et al., 2020), with pigment being darker and more aggregated around the broad end of the egg (Gosler et al., 2005; Gosler & Wilkin, 2017; Briggs

& Mainwaring, 2017; see Fig.2 for an example of pigment variation within and between clutches).

While previous researchers did not find urban-driven differences in great tit egg size (Hõrak et al., 1995; Bańbura et al., 2010; Bailly et al., 2016; Hargitai et al., 2016a) or pigmentation patterns (Hargitai et al., 2016a), the studies were methodologically limited. Thus, in earlier studies urbanisation was defined using habitat dichotomy (urban vs. rural), an approach that does not necessarily reflect the ecological structure of the urban mosaic (Szulkin et al., 2020). Most importantly, to date, only one study examined pigmentation patterns in an urban-rural contrast, and was based on a small sample size (Hargitai et al., 2016a; a total of 37 eggs from 37 clutches) — thus, finer scale environmental data and larger sample sizes are needed to uncover potential urban-driven variation in egg traits.

The aim of this study is to quantify the impact of urbanisation on egg size and pigmentation patterns (proposed indicators of clutch quality), which is currently overlooked in the field of urban ecology. To achieve this goal, data were collected over two years on 718 eggs, from 90 clutches, spread across eight diverse study sites within the urban mosaic of Warsaw, Poland. Importantly, urbanisation was quantified at high resolution with the use of remote sensing imagery, thus acknowledging the fine-scale environmental variability occurring in cities. Considering the amount of impervious surface area (ISA) in the city and the well documented detrimental impact of urbanisation on nestling development (e.g. Chamberlain et al., 2009; Bailly et al., 2016; Corsini et al., 2020), we predicted that eggs found in highly urbanised areas would be smaller and more pigmented, indicating lower clutch and habitat quality.

METHODS

Study sites

This study is part of a long-term research project investigating the ecology and evolution of passerine birds in the urban mosaic. Initiated in 2016, the project consists of 500 woodcrete nestboxes (Schwegler, type 1b with 32 mm entrance) arranged in a grid of 50 meters at eight study sites across the Capital City of Warsaw, Poland, each varying in urbanisation levels (see Fig.1). For this study, data were collected over two breeding seasons, in 2021 and 2022. Brief descriptions of each study site can be found in the Supplementary Materials (Text S1).

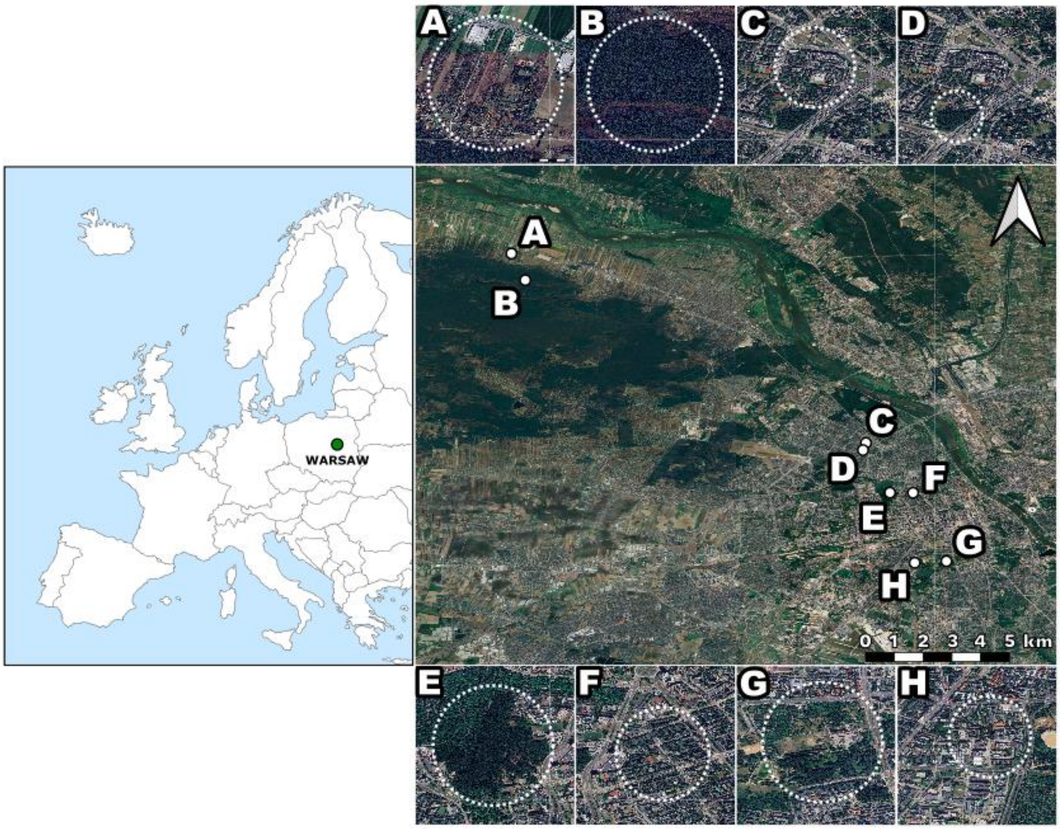

Figure 1. Map of study sites located in a gradient of urbanisation in the capital city of Warsaw, Poland. These include: a suburban village (A), a national park (B), two residential areas (C and F), two urban woodlands (D and E), an urban park (G) and a university campus (H). Satellite images acquired from Google Maps (2024).

Quantifying urbanisation

The definition of urbanisation is not agreed unanimously among researchers (Szulkin et al., 2020). Urbanisation is increasingly quantified using continuous parameters, such as land-cover classes, specific environmental variables or pollution gradients, which allows for finer inference than qualitative urban-rural assessments (Szulkin et al., 2020). Here, urbanisation was quantified using the amount of Impervious Surface Area (ISA) as a proxy - that is all built-up areas, such as infrastructural networks and buildings, around the nestbox vicinity. ISA is a reliable and repeatable indicator of urbanisation worldwide (Szulkin et al. 2020; Murray-Stoker et al., 2025). ISA also covaries positively with other urban features, such as temperature, sound or noise pollution, and negatively covaries with NDVI (Normalized Difference Vegetation Index, a measure of vegetation greenness) and tree cover (Szulkin et al. 2020). The percentage of ISA was measured in a 100 m radius for each nestbox using the open source software QGIS (version 3.40), with a 20-m-pixel resolution extrapolated via satellite imagery from 2015 (Copernicus Land Monitoring Services, https://land.copernicus.eu/sitemap) as described in Szulkin et al. 2020. The 100 m radius also corresponds to a conservative estimate of distance typically flown from the nest by great tits to forage for food for their chicks in urban settings (Seress et al., 2025).

Life history data collection and bird measurements

Starting from the last week of March, all nestboxes across the study sites were visited weekly to monitor occupancy. Once incubation started, hatching date was estimated (12 days from clutch completion) (Álvarez & Barba, 2014). Nestboxes were visited on the expected hatching date (±1 day) and every other day thereafter until hatching (hatching day=day 1) or until the nest was considered deserted. Each parent bird was caught once between chick day 10 and 15, at the nestbox with traps or by manually blocking the nestbox entrance. All birds were ringed using standard-numbered metal rings supplied by the Polish Ringing Centre (Museum and Institute of Zoology, Polish Academy of Sciences). All recaptures from previous years were noted. Adult biometric measurements were collected as follows: birds were sexed and aged as first year breeders or older (two age levels) according to plumage characteristics (Demongin, 2016), wing length was measured to the nearest 1.0 mm with a wing-ruler, tarsus to the nearest 0.1 mm with a manual calliper, and weight was recorded to the nearest 0.1 g with a digital scale. In this study, only data on great tit first broods, defined as clutches that started within 30 days from the recording of the first egg at a given site and year (Van Balen, 1973), were included.

Egg photography

To collect data on egg characteristics, clutches were photographed during the incubation phase, around 10 days after clutch completion. Pictures of eggs were taken using a Canon EOS 1100D reflex camera with a Canon EF-S 18-55 mm f/3.5-5.6 IS II lens, set on a tripod. Eggs were put in shallow pits on a custom-made tray. The tray was fixed to a small tripod and equipped with grey colour standards (X-Rite ColorChecker Classic Mini, X-Rite, Grand Rapids, USA) and a scale bar (5 cm with 1 mm divisions) (Fig. 2). Using a round spirit level, both the camera and the tray were aligned perpendicularly to the ground. All clutches were photographed between 9:00 AM and 4:15 PM, when the sun was not covered by clouds. To avoid overexposure, equipment was set with a photographer casting a shadow on the whole tray with eggs (following Troscianko et al., 2016). Aperture f/8, ISO 400 and focal length of 18 mm were used. Only shutter speed was manipulated to adjust the duration of light exposure. Exposure bracketing with ±1 step was used. All images were saved in RAW format. To generate a full record of egg speckling, eggs were first photographed from one side, then turned 180° sideway and photographed again. Furthermore, eight clutches (61 eggs) were photographed on two separate days to check for data collection repeatability. Eggs that were to be photographed again were numbered, so that they could be identified later. To test for a potential effect of the placement of the eggs on the tray on the measurements, they were arranged differently (i.e. put in different pits) between the trials and the order was recorded for further repeatability estimates.

Figure 2. Example of great tit clutches differing in pigmentation patterns. Top line - a lightly pigmented clutch with mostly medium and small sized spots, laid in an *Urban park*. Bottom line - heavily pigmented clutch with a lot of large spots laid in a *Natural forest*.

Digital imaging

Pictures were processed using the ImageJ software (version 1.54d) with MICA Toolbox plug-in designed for research in the field of visual ecology (Troscianko & Stevens, 2015a). Using the Photo Screening tool, the brightest and simultaneously not over-exposed images were selected for both sides of the eggs for all clutches. Second, images were calibrated with respect to the darkest and the brightest grey standards (3.22% and 90.03% of light reflectance respectively) and a 10 mm scale bar was selected to set the scale.

Using the multipoint tool, each egg was outlined using at least eight anchor points along the egg's edges, which also provided data on egg *volume* (Troscianko, 2014). Afterwards, the Scale Bar Calculator tool was used to obtain the minimum px/mm factor for the set of pictures (10.7012 px/mm rounded down to 10.5 px/mm). This factor is needed to make pattern measurements comparable between images and should always be rounded down (Troscianko & Stevens, 2015b). Finally, colour and pattern were automatically measured using custom-written scripts for the MICA Toolbox that segmented the egg image into spots and eggshell background (Szala and Šulc, in prep). The following settings were used for the segmentation: 50 px radius for thresholding (Phansalkar et al., 2011), a difference of 128 px Gaussian blur (Gómez et al., 2018) and the outline was shrunk by 10% of egg width to avoid measuring shadows occurring at the egg's edges. Egg spot colour and the extent to which spots cover the egg area (in %) were shown to best correlate with the concentration of protoporphyrin in great tit eggshells (Wegmann et al., 2015; Hargitai et al., 2016b). For this reason, these two features of eggshell coloration were measured: spot colour was calculated as the reflectance in the red channel divided by the sum of reflectance in red, green and blue channels (*spot red chroma* hereafter) and *spot percentage* as the eggshell area covered by spots divided by the whole surface area of the egg side.

After normalisation, a significant portion of the eggs (N=*c.*320) were identified as overexposed near the pointed end ('top'). To retain as large a sample size as reliably possible and exclude overexposed regions, measurements were restricted to a sub-area of eggs. This sub-area comprised 85% of every egg's width, 30% of its length and was positioned at the 0.6 of its length (with 0 being the 'top' and 1 being the 'bottom' edge of the egg) and constituted c. 27% (minimum=26.02%, median=26.82%, maximum=27.87%) of one side of the egg surface. Overexposed pixels were counted in the sub-area and eggs with >0.5% of overexposed pixels were excluded from further analyses (N = 29). Using pictures of clutches that were not overexposed (N = 382 eggs from 46 clutches), the repeatability of pigmentation characteristics between the whole egg surface and its sub-area was tested. Given good repeatability (R>0.820; see Results), measurements of eggshell pigmentation (*spot red chroma* and *spot percentage*) based on the sub-area of eggs were used in further analyses.

Data from clutches in which no egg was overexposed from either side (N=359 eggs from 43 clutches) were extracted first and repeatability tests of egg traits between two sides were performed (see Methods, Statistical analysis). The repeatability of egg volume was above 0.9 and the repeatabilities of pigmentation characteristics were above 0.8 (see Results). Therefore, the rest of the clutches were imaged using just one picture (side) instead of two, and data from only one side of each egg were used in the models.

Visual scoring of egg pigmentation

To retain comparability with earlier studies of egg pigmentation a method of visual egg pigmentation scoring, following Gosler et al. (2000) was also applied. Each egg was visually scored in three categories: pigment *intensity* (scored in 0.5 increments from 1 for palest to 5 for the darkest), *distribution* (scored in 0.5 increments from 1 for > 90% of spots concentrated at one end, to 5 for an evenly distributed) and *spot-size* (scored in 0.5 increments from 1 for small spots, to 3 for large spots). All eggs were scored by the same observer, with the scores being assigned using pictures of both sides of the egg. To test for within-observer repeatability of scoring, the observer assessed one egg from each brood again, in a randomized order. To learn more about different methods of assessing eggshell pigmentation and their limitations see Brulez et al. (2014) and Stevens (2011).

Statistical analysis

All statistical analyses were performed in R (version 4.1.2). To test for repeatability of egg *volume* and pigmentation between (1) sides of eggs, (2) between trials (i.e. same clutches photographed twice on different days), (3) between two visual scoring sessions and (4) between whole eggshell side and sub-area, the *rpt* function in the *rptR* package (version 0.9.22, Stoffel et al., 2016, 2017) was used. *Egg ID* was used as a grouping factor for all tests. The confidence interval was set to 0.95, number of bootstrap samples to 500, number of permutations to 0 and Gaussian distribution was used. Repeatability reliability categories, based on R value, were assigned following Koo & Li (2015).

To test for an association between egg traits (egg *volume* and pigmentation characteristics: *spot red chroma, percentage, intensity, distribution* and *size*), urbanisation and life-history traits, Generalized Linear Mixed Models (GLMMs) were fitted using Template Model Builder (glmmTMB) (Brooks et al., 2017). For continuous predictors, mean-centered values of *ISA* (%), *lay date* (date of the first egg laid in the clutch; starting from 1st of April recorded as 1), *clutch size* and *female body condition* (calculated following Peig & Green, 2009) were used. In terms of categorical variables, *year* was fitted as a fixed effect with two levels. To avoid pseudoreplication, *clutch ID*, *site ID* and *female ID* were included as random effects, accounting for eggs sampled within the same clutch and site and for females sampled in both years, respectively. To detect potential multicollinearity issues, the *check_collinearity* function was used (*performance* package, Fox & Weisberg, 2019): all VIF (variance inflation factor) values were below 2 and therefore no continuous predictors were excluded. To verify model fits and assumptions, the *DHARMAa* package was used (see Fig.S1) (Hartig, 2020). Data were visualised using *ggplot2* (Wickham, 2011). Environmental categories used in Fig. 3 were defined based on the median value of ISA for all clutches, i.e. *Low ISA*<median, *High ISA*>median (see Results for details).

RESULTS

Sample size and Impervious Surface Area

Following digital processing, the final dataset included measurements from 718 eggs from 90 broods. Final GLMMs included 568 eggs from 74 broods as data were unavailable for 16 females that were not caught or escaped before all measurements were performed. The amount of Impervious Surface Area ISA (in %) in a 100 m radius around each clutch used in this study varied from a minimum of 0.00% to a maximum of 60.73%, with an average at 12.73% and median at 4.73 %. For details see Table S1.

Repeatability of egg traits

The results of repeatability tests summarised in Table 1 were categorised following Koo & Li, 2015, i.e. R, less than 0.5 — poor, 0.5-0.75 — moderate, 0.75-0.9 — good, greater than 0.9 — excellent. The tests confirmed that egg traits have excellent (*volume*) or good (*spot red chroma* and *percentage*) repeatability between egg sides. Importantly, the reliability (defined as consistency of results when measurements are repeated under similar conditions) of both digital and visual quantification methods were confirmed with good repeatability. Additionally, both *spot red chroma* and *percentage* had good repeatability between the whole side of an egg and its sub-area, which allowed for inclusion of eggs that were partially overexposed in the analysis (for details, see Methods, Repeatability).

Table 1

Repeatability of egg traits regarding quantification and data collection methods: (1) sides of eggs, (2) between trials (i.e. same clutches photographed twice on different days), (3) between two visual scoring sessions and (4) between whole eggshell side and sub-area. Significance levels are indicated **in bold**: ***p < 0.05, **p < 0.01, ***p < 0.001.**

Egg sides **N=359 x 2**	**R**	**SE**	**CI**	**p-value**
Volume	0.98	0.00	[0.98, 0.98]	**<0.001*****
Spot red chroma	0.83	0.02	[0.79, 0.85]	**<0.001*****
Spot percentage	0.83	0.02	[0.79, 0.86]	**<0.001*****
Trials **N=61 x 2**	**R**	**SE**	**CI**	**p-value**
Volume	0.79	0.05	[0.67, 0.87]	**<0.001*****
Spot red chroma	0.78	0.05	[0.66, 0.86]	**<0.001*****
Spot percentage	0.83	0.04	[0.73, 0.89]	**<0.001*****
Visual scoring **N=90 x 2**	**R**	**SE**	**CI**	**p-value**
Intensity	0.88	0.03	[0.82, 0.92]	**<0.001*****
Distribution	0.87	0.03	[0.80, 0.91]	**<0.001*****
Spot size	0.79	0.04	[0.70, 0.86]	**<0.001*****
Sub-area **N=382 x 2**	**R**	**SE**	**CI**	**p-value**
Spot red chroma	0.86	0.02	[0.79, 0.85]	**<0.001*****
Spot percentage	0.83	0.02	[0.79, 0.86]	**<0.001*****

Covariation between urbanisation, egg traits and life-history traits

Urbanisation modelled as the amount of *ISA* in the nest vicinity was not associated with egg *volume* (Table 2) or egg pigmentation traits (*spot red chroma*, *spot percentage*) as quantified with digital imaging (Fig. 3, top line; Table S2). In contrast to a lack of urban-driven effects on egg traits, egg *volume* covaried positively with *lay date* and *female body condition* (Table 1). Egg traits also varied between years: eggs were larger and less covered in spots in 2022 than in 2021 (Table 1 & Table S2). Finally, *spot red chroma* was not associated with any of the explanatory variables (Table S2).

Consistently with models using egg traits data quantified with digital imaging, models using data based on visual egg pigmentation scoring (*spot intensity, distribution and size*) were not influenced by urbanisation (*ISA*) (Fig. 3, bottom line; Table S2). The only association found in all three models was a negative one, between *female body condition* and *spot intensity* (Table S2).

Table 2

GLMMs with Gaussian distribution testing the association between egg *volume*, urbanisation and life-history traits, N_{eggs}=568. Continuous predictors were mean-centered (mc). Significance levels are indicated **in bold: *p < 0.05, **p < 0.01, ***p < 0.001.**

<table>
<tr><td colspan="5">Family: Gaussian, Random = Site ID (N=8) + Clutch ID (N=74) + Female ID (N=66)</td></tr>
<tr><td colspan="5">Model structure (glmmTMB): volume ~ ISA_{mc} + Lay $date_{mc}$ + Clutch $size_{mc}$ + Female body $condition_{mc}$ + Year</td></tr>
<tr><td>Volume</td><td>Estimate</td><td>SE</td><td>z-value</td><td>p-value</td></tr>
<tr><td>Intercept</td><td>1536.07</td><td>29.17</td><td>52.76</td><td><0.001***</td></tr>
<tr><td>ISA</td><td>-0.25</td><td>1.02</td><td>-0.25</td><td>0.804</td></tr>
<tr><td>Lay date</td><td>6.72</td><td>2.82</td><td>2.38</td><td>0.017 *</td></tr>
<tr><td>Clutch size</td><td>5.51</td><td>9.40</td><td>0.59</td><td>0.558</td></tr>
<tr><td>Female body condition</td><td>32.65</td><td>15.10</td><td>2.16</td><td>0.03 **</td></tr>
<tr><td>Year</td><td>90.84</td><td>24.19</td><td>3.76</td><td><0.001***</td></tr>
</table>

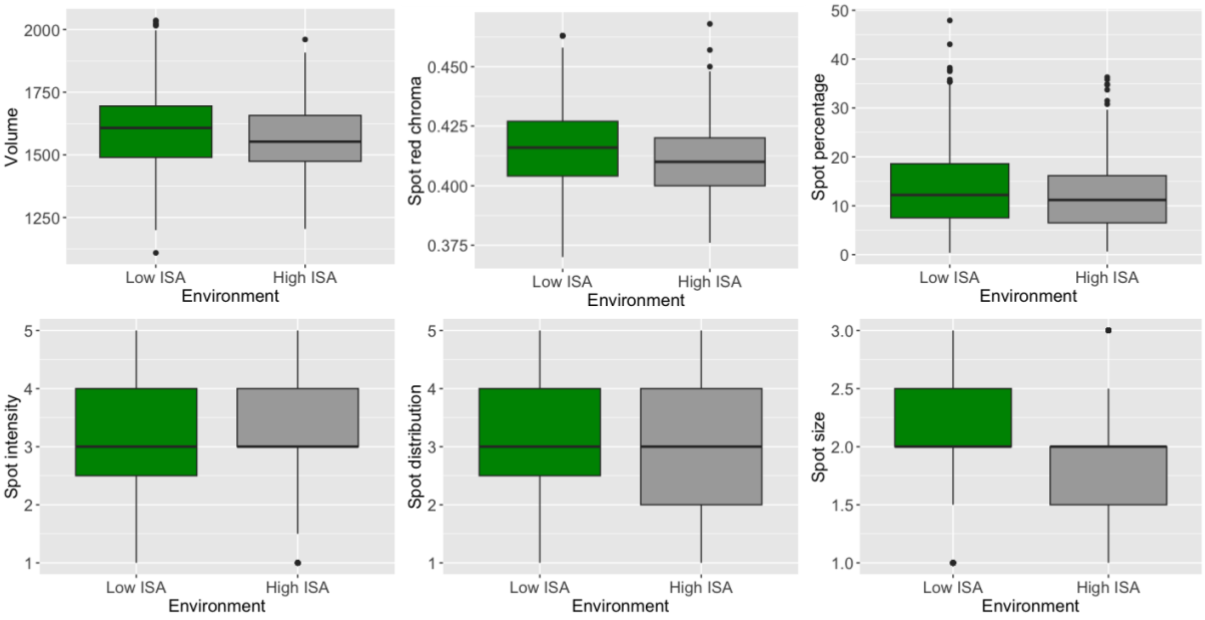

Figure 3. Comparison of six great tit egg traits between *Low ISA* and *High ISA* environments, when using digital quantification (top line) and visual scoring (bottom line); N_{eggs}=568. No significant effect of urbanisation was noted in either digital quantification of trait variation (egg *volume*, *spot red chroma*, *spot percentage*; see Table 2 and Table S2 for models) or visual scoring (egg spot *intensity*, *distribution* or *size*; see Table S2 for models).

DISCUSSION

Based on a two-year data set containing 568 eggs from 74 clutches, spread across 8 study sites differing in urbanisation levels, this study reports that while great tit egg *volume* and pigmentation patterns covary with life history variables (i.e. *lay date*, *female body condition* and *year*), these egg traits are not affected by urbanisation. Although literature for egg trait variation is available from rural habitats (see Introduction), knowledge about urban-driven variation is limited (Hõrak et al., 1995; Bailly et al., 2016; Hargitai et al., 2016a; Bańbura et al., 2010). Moreover, to the best of the authors' knowledge, this is the first study on egg trait variation that incorporates a fine-scale spatial quantification of urbanisation: in this study, all analyses were based on the amount of impervious surface area in close nest vicinity, as opposed to using dichotomical approaches (rural vs urban), commonly used in urban ecology and evolution research. While analysing egg trait data, both digital and visual quantification methods were applied in parallel. To confirm that the methods of data collection and analysis were reliable, four repeatability tests regarding different aspects of the methodology were performed, and confirmed a reliable quantification of egg trait variation (Table 1). Therefore, this study offers valuable insight into the scope for detecting the potential effect of urbanisation on eggs, a key aspect of bird reproduction.

Based on selected literature, Table 3 presents a summary of findings regarding egg size and pigmentation and associated factors in great tits and blue tits. What emerges from the current study and earlier research, is the strong heterogeneity in variables associated with egg traits between studies. Thus, while some studies report specific associations, other studies fail to report the same biological signal. Such inconsistencies in earlier research outcomes on egg size have readily been pointed out by Encabo et al. (2001), as different trends could be found in the same population in different years of study. Therefore, if possible, research on egg traits should rely on large samples, spanning multiple years. Moreover, obtained results should be interpreted cautiously.

Table 3

Summary of associations between great tit and blue tit egg traits and explanatory variables reported in selected literature. Associations (or lack thereof) as tested in this study are indicated in **bold**. As some studies used either egg volume or mass (which are highly correlated, R=0.97, Van Noordwijk et al., 1981) the collective category *egg size* was used. *Female condition* refers to either *body mass* or *tarsus length*, while *body condition* was used in this study.

	Egg size	Spot red chroma	Spot percentage	Spot Intensity	Spot distribution	Spot size
Lay date	**Positive**: **This study** Wilkin et al., 2009 Negative: Pitt et al., 2024 None: Eeva, 1995 Encabo et al., 2001 Hargitai et al., 2016c Järvinen & Pryl, 1989 Szigeti et al., 2007	Positive: Negative: **None:** **This study**	Positive: Negative: Hargitai et al., 2016c **None**: **This study** Martínez-de La Puente et al., 2007	Positive: Negative: Gosler et al., 2000 Hargitai et al., 2016c **None**: **This study** Sanz & García-Navas, 2009	Positive: Gosler et al., 2000 Negative: **None**: **This study** Hargitai et al., 2016c Sanz & García-Navas, 2009	Positive: Negative: Gosler et al., 2000 **None**: **This study** Hargitai et al., 2016c
Clutch size	Positive: Encabo et al., 2001 Negative: Bańbura et al., 2018 You et al., 2008 **None:** **This study** Eeva, 1995 Järvinen & Pryl, 1989	Positive: Negative: Hargitai et al., 2016b **None**: **This study** Malinowska et al., 2023	Positive: Malinowska et al., 2023 Negative: **None**: **This study** Hargitai et al., 2016b Martínez-de La Puente et al., 2007	Positive: Negative: **None:** **This study** Gosler et al., 2000 Hargitai et al., 2016b Sanz & García-Navas, 2009	Positive: Sanz & García-Navas, 2009 Negative: **None:** **This study** Gosler et al., 2000 Hargitai et al., 2016b	Positive: Negative: **None:** **This study** Gosler et al., 2000 Hargitai et al., 2016b
Female condition	**Positive**: **This study** Järvinen & Pryl, 1989 Ojanen et al., 1979 Negative: None: Szigeti et al., 2007	Positive: Negative: **None:** **This study** Hargitai et al., 2016b	Positive: Negative: Martínez-de La Puente et al., 2007 **None**: **This study** Hargitai et al., 2016b	Positive: Sanz & García-Navas, 2009 **Negative:** **This study** None: Hargitai et al., 2016b	Positive: Negative: **None**: **This study** Hargitai et al., 2016b Sanz & García-Navas, 2009	Positive: Negative: **None:** **This study** Hargitai et al., 2016b
Year effect	**Present**: **This study** Encabo et al., 2001 Szigeti et al., 2007 Not present: Bańbura et al., 2018 Eeva, 1995 Järvinen & Pryl, 1989	Present: **Not present**: **This study**	**Present**: **This study** Not present: Hargitai et al., 2016b	Present: Gosler et al., 2000 Hargitai et al., 2016b **Not present**: **This study** Sanz & García-Navas, 2009	Present: Gosler et al., 2000 **Not present:** **This study** Hargitai et al., 2016b Sanz & García-Navas, 2009	Present: Gosler et al., 2000 **Not present:** **This study** Hargitai et al., 2016b

None of the investigated egg traits were associated with urbanisation. This is in line with previous studies on great tits, reporting that neither egg size nor pigmentation was associated with urban habitat (Hõrak et al., 1995; Bańbura et al., 2010; Bailly et al., 2016; Hargitai et al., 2016a). Moreover, two of the studies incorporated direct eggshell thickness measurements and also reported no differences between urban and rural habitats (Bailly et al., 2016), or only marginal differences (Hargitai et al., 2016; $F_{1,35} = 4.53$, $P = 0.040$; mean ± SD: woodland: 79.1 ± 3.0 µm; urban: 81.6 ± 3.8 µm). In contrast, urban-driven differences in egg size were found in blue tits, with larger eggs found in the urban habitat than in rural habitat (Bailly et al., 2016; Bańbura et al., 2010), indicating a species-specific response in egg trait variation in response to urbanisation. Therefore, results from this study and earlier studies in the topic demonstrate that great tit eggs do not differ in terms of size and pigmentation patterns between urban and rural environments.

Given that both egg size and pigmentation patterns were previously proposed as clutch quality indicators as they are associated with hatching success (Sanz & García-Navas, 2009; Krist, 2011), this study suggests that urbanisation does not necessarily affect egg quality in great tits. Indeed, research conducted on the same population as this study, revealed that there are no urban-driven differences in hatching success or in chick mass 1 day after hatching (Corsini et al. 2020, Corsini & Szulkin 2025, Table S3). However, Bailly et al., (2016) and Charmantier et al. (2017), did find a slightly lower hatching success in cities. Interestingly, urban clutches have consistently fewer eggs, a trend that was reported both in this population (Corsini & Szulkin, 2025, Supplementary Information), as well as in others (see meta-analysis by Chamberlain, 2009).

A strong argument that urban females are environmentally constrained by nutrient availability was suggested by Pitt et al. (2024). An egg removal experiment conducted on blue tits revealed that in comparison to forest birds, urban birds have lower capacity to compensate for missing eggs (i.e., approximately 2 vs 0.36 new eggs laid in forest vs urban environments). Therefore, it is likely that birds in urban environments have an ability to cope with limited food and nutrient availability while laying the eggs, but only to some extent - which is translated into eggs of similar characteristics, but with a smaller number of them in each clutch.

In terms of coping mechanisms, it is likely that calcium-seeking urban females either increase their search efforts for calcium and/or that they supplement their diet with other resources. In calcium-poor natural forests, great tits nesting near human settlements were compensating for the lack of snails with anthropogenic resources such as chicken eggshells (Graveland, 1996), while some other suggestions include plaster, ash and calcium leaching from concrete (Eeva & Lehikoinen, 2004). Moreover, urban tits are known to have higher exploration rates (Charmantier et al., 2017) and to travel further while seeking food for their chicks (Jarrett et al., 2020; Seress et al., 2025). At the same time, females which were experimentally deprived of calcium were seeking it actively, doubling the time spent on searching and resorting to eating sand, small stones or even their own eggs (Graveland & Berends, 1997). Lastly, it is worth noting that a smaller clutch size does not necessarily change the final reproductive success of urban tits as they are strongly constrained by the urban environment at later stages of reproduction, which results in fewer fledglings leaving the nest (Charmantier et al., 2017; Corsini & Szulkin, 2025), meaning that urban females lay more eggs than they can successfully rear (Pitt et al., 2024).

Conclusions

This study highlights the complexities of urban phenotypic egg trait variation. While it was expected that urbanisation would affect egg traits negatively, as such trends were reported in calcium-poor natural habitats, and for other avian reproductive traits in cities, no such pattern was found, despite using a large dataset and fine-scale quantification of urban habitat. Instead, urban birds lay eggs which are similar to those found in more natural habitats in terms of *volume* and pigmentation patterns, but often have fewer of them in a clutch. Given the environmental constraints arising in the urban habitat and associated, consistently lower fledging success, investing additional resources into larger clutches is not likely to benefit their reproduction and could instead further hinder it.

Although this paper broadens our understanding of egg trait variation in the urban space, future long-term research on other avian species, and in cities with different socio-geographical settings, is needed. Moreover, further studies quantifying the urban availability of calcium, as well as experimental approaches incorporating calcium supplementation in urban and natural populations should be conducted to confirm the mechanistic links underlying the findings presented in this study.

Ethical statement

This research was carried out with a permit from the Regional Directorate for Environmental Protection in Warsaw, Poland. Permissions for bird ringing were granted by the Polish Ringing Centre (Museum and Institute of Zoology, Polish Academy of Sciences).

Acknowledgements

We thank all the field workers who took part in data collection and the site managers for enabling research in the study areas. This work was supported by the University of Warsaw (Excellence Initiative Research University IV.2.2 grant number 501-D114-20-0004410 awarded to Ignacy Stadnicki) Polish National Science Centre (NCN-Sonata bis grant number 2014/14/E/NZ8/00386, OPUS grant 2021/41/B/NZ8/04472, both awarded to Marta Szulkin, and NCN-Preludium grant number 2017/25/N/NZ8/02852, awarded to Michela Corsini).

Author contributions

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Conflicts of interest statement

The authors declare no conflicts of interest.

Data sharing and accessibility

Data and codes for this manuscript will be made available on Mendeley Data Repository in the near future.

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SUPPORTING INFORMATION

Title:

An analysis of passerine egg traits across the city mosaic: Urbanisation does not affect egg size and pigmentation patterns

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ABSTRACT

1. Rapid urbanisation provides remarkable opportunities to study how sudden, extreme changes impact wildlife. Compared to natural areas, cities are characterised by factors affecting both abiotic (e.g. climate, pollution, habitat fragmentation) and biotic (e.g. Normalized Difference Vegetation Index (NDVI), species composition, phenology) components of the ecosystem, ultimately changing the ecological and evolutionary dynamics of those habitats. Analogously to many other taxonomic groups, urban birds differ from rural birds in morphology, behaviour and reproductive patterns. Yet potential links between urbanisation and avian egg traits — a key aspect of the avian life-cycle — remain under-researched. Given the limited availability of primary natural calcium sources (snails) in cities, eggs from heavily urbanised areas were expected to be smaller and more pigmented, indicating thinner shells and lower overall egg quality.
2. To better understand how urbanisation affects eggs, data on 718 great tit (*Parus major*) eggs from 90 clutches, spread across eight study sites in a city mosaic, were collected for two breeding seasons. All clutches were photographed using a standardised approach, and further analysed using digital imaging and visual scoring to assess egg volume and pigmentation patterns. Urbanisation was quantified as the percentage of Impervious Surface Area (ISA) in the vicinity of each clutch via satellite imagery.
3. In line with some of the earlier studies conducted on semi-natural bird communities, egg volume covaried with lay date and female body condition, while for both egg volume and egg pigmentation (*spots percentage*) — a year effect was detected.

However, in contrast with predictions, there was no association between urbanisation and the examined egg traits.

4. While urban clutches are consistently smaller, this study shows that eggs as such are similar to those found in rural habitats in terms of volume and pigmentation patterns. These results suggest that urban-driven environmental pressures may not be as strong or directional during the egg laying phase as they are at later stages of reproduction, and highlight the complexities of urban ecological and evolutionary dynamics.

Key words: environmental constraints, calcium, egg pigmentation, egg volume, great tit, protoporphyrin IX, reproduction, urbanisation

TABLE OF CONTENTS

Text S1

Descriptions of study sites set in a gradient of urbanisation in Warsaw, Poland. Descriptions are ordered from the most northern to the most southern sites. For more details see Corsini et al. (2020).

A. Suburban village (20°46'48.9748" E - 52°22'11.3382" N, *c.* 95 ha., 47 nestboxes). Palmiry village is located *c.* 21 km northwest from Warsaw city borders, next to Kampinos National Park. It is characterised by residential homes with gardens, crop fields and plots of unused land covered by grass, shrubs or trees.

B. Natural forest (20°47'14.3867" E - 52°21'22.5409" N, *c.* 38,500 ha., 110 nestboxes). Kampinos National Park is located *c.* 20 km northwest from Warsaw city border. The large mixed-coniferous forest, with a predominance of pine trees (*Pinus sp.*) and oaks (*Quercus sp.*), is divided into strictly and partially protected zones and is connected with Warsaw city by a green corridor.

C. Residential area I (20°57'39.37097" E - 52°16'23.71883" N, *c.* 19 ha., 52 nestboxes). Olszyna Residential Area is located in a north-western district of Warsaw. It is characterised by blocks of flats, public facilities (e.g. schools, grocery stores) and green spaces.

D. Urban woodland I (20°57'33.93652" E - 52°16'10.55093" N, *c.* 3.4 ha., 21 nestboxes). Olszyna woodland, adjacent to Residential area I, is a small urban wet forest with a predominance of common alders (*Alnus glutinosa*), birches (*Betula sp.*) and oaks (*Quercus sp.*).

E. Urban woodland II (20°58'23.44285" E - 52°14'52.45584" N, c. 33 ha., 91 nestboxes). Jewish Cemetery, located in the midtown of Warsaw, is a historical site, which was partially destroyed during World War II. A portion of the area has been in renovation since 1990s, however it mostly consists of moss-covered tombstones and a wild urban forest, composed of both native and foreign species, mainly oaks (*Quercus sp.*), birches (*Betula sp.*), elms (*Ulmus sp.*) and Norway maples (*Acer platanoides*).

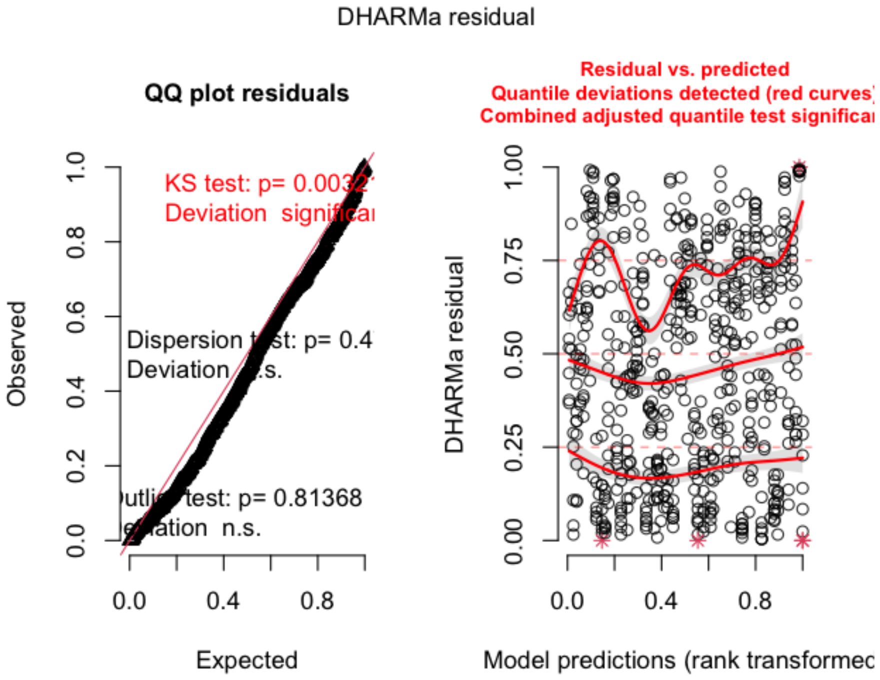
F. Residential area II (20°59'5.74332" E - 52°14'52.17925" N, *c.* 36 ha., 46 nestboxes). Muranów Residential Area is located next to Urban woodland II. Similarly to Residential Area I, it is characterised by blocks of flats, public facilities and green spaces.

G. Urban park (21°0'6.98321" E - 52°12'46.66874" N, *c.* 65 ha., 105 nestboxes). Mokotów Field, located in the south-central district of Warsaw, is a large urban park characterised by a mosaic of flowerbeds, grass, trees as well as impervious surfaces. It also offers dining places, playgrounds, an outdoor gym and a water pond, making it a popular recreational area among city dwellers.

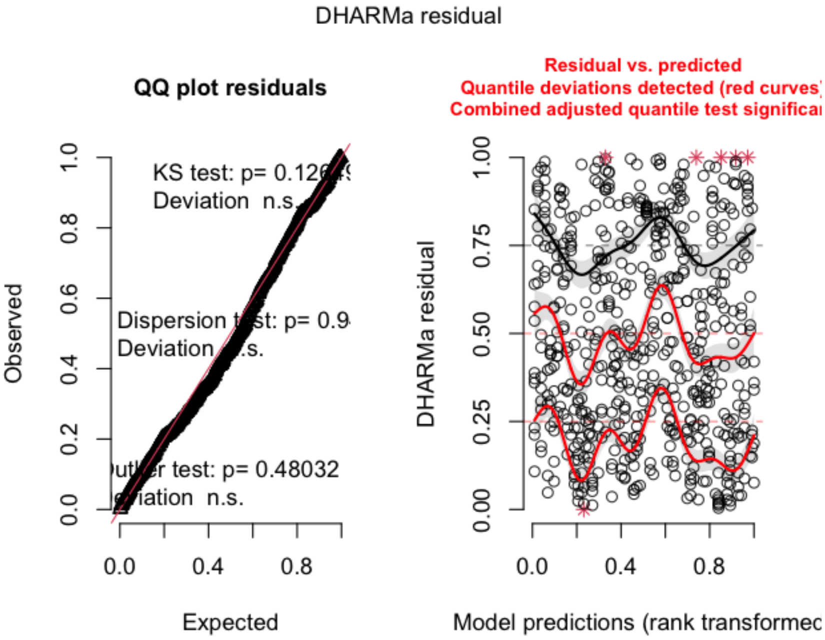
H. University campus (20°59'8.85224" E - 52°12'43.77676" N, c. 9 ha., 28 nestboxes). Ochota Campus, adjacent to the Urban park, is an office area consisting of university faculties, research centres, student dormitories and scarce greenery.

Figure S1. DHARMa diagnostics of models testing for the association between egg traits, urbanisation and life-history traits (see Methods).

(a)



(b)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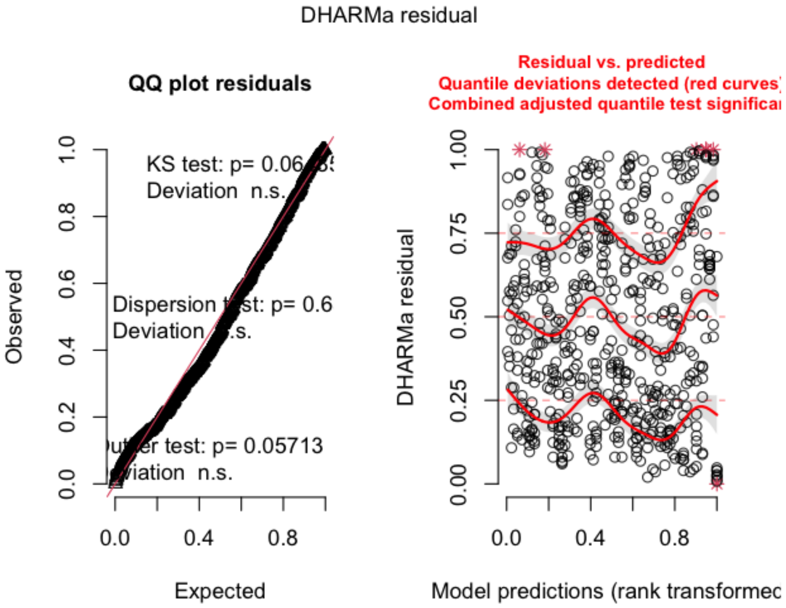
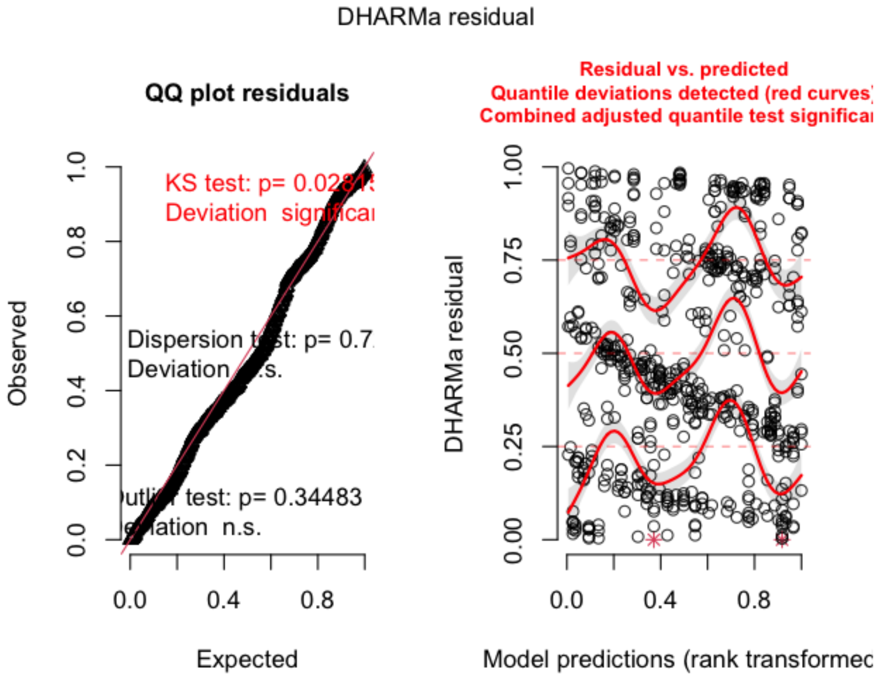
QQ plot residuals
KS test: p= 0.126
Deviation n.s.
Dispersion test: p= 0.9
Deviation n.s.
utlier test: p= 0.48032
eviation n.s.
Observed
Expected
0.0
0.2
0.4
0.6
0.8
1.0
Residual vs. predicted
Quantile deviations detected (red curves
Combined adjusted quantile test significa
DHARMa residual
0.00
0.25
0.50
0.75
1.00
Model predictions (rank transforme

(c)


DHARMa residual
QQ plot residuals
KS test: p= 0.06
Deviation n.s.
Dispersion test: p= 0.6
Deviation n.s.
utlier test: p= 0.05713
viation n.s.
Observed
Expected
0.0
0.2
0.4
0.6
0.8
1.0
Residual vs. predicted
Quantile deviations detected (red curves
Combined adjusted quantile test significa
DHARMa residual
0.00
0.25
0.50
0.75
1.00
Model predictions (rank transforme

(d)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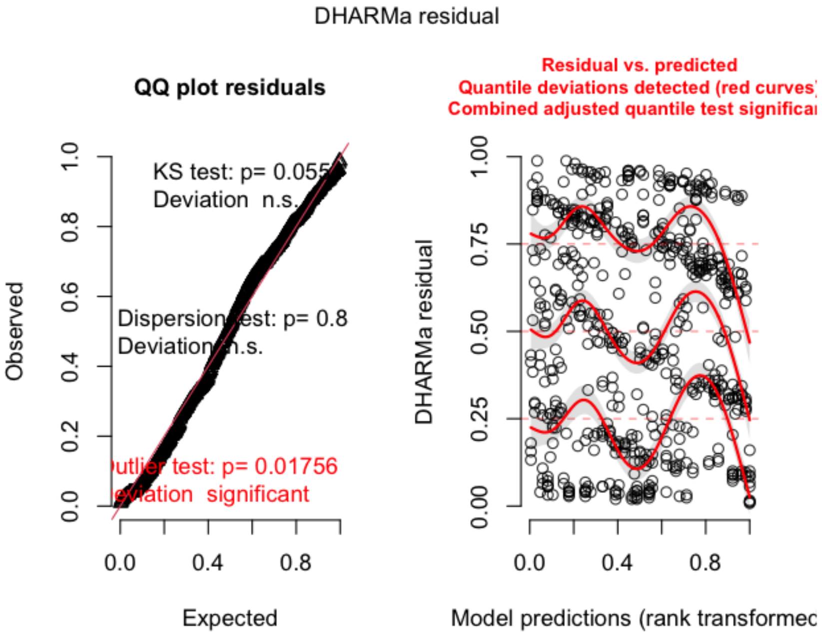
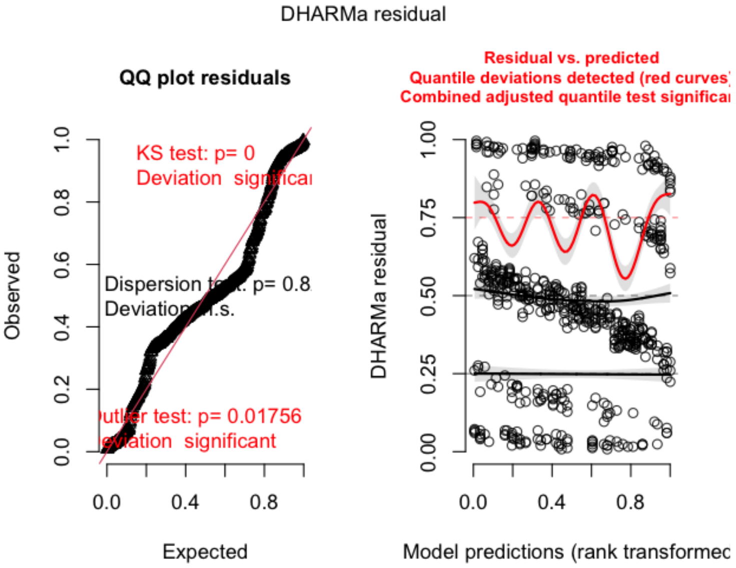
QQ plot residuals
KS test: p= 0.028
Deviation signi
Dispersion test: p= 0.7
Deviation n.s.
tlier test: p= 0.34483
viation n.s.
Observed
Expected
0.0
0.2
0.4
0.6
0.8
1.0
Residual vs. predicted
Quantile deviations detected (red curves)
Combined adjusted quantile test significa
DHARMa residual
0.00
0.25
0.50
0.75
1.00
Model predictions (rank transformed

(e)


DHARMa residual
QQ plot residuals
KS test: p= 0.055
Deviation n.s.
Dispersion test: p= 0.8
Deviation n.s.
utlier test: p= 0.01756
eviation significant
Observed
Expected
0.0
0.2
0.4
0.6
0.8
1.0
Residual vs. predicted
Quantile deviations detected (red curves)
Combined adjusted quantile test significa
DHARMa residual
0.00
0.25
0.50
0.75
1.00
Model predictions (rank transformed

(f)



(a) *Model structure:* **Volume** ~ ISA$_{mc}$ + Lay date$_{mc}$ + Clutch size$_{mc}$ + Female body condition$_{mc}$ + Year, Random = Site ID (N=8) + Clutch ID (N=74) + Female ID (N=66)

(b) *Model structure:* **Spot red chroma** ~ ISA$_{mc}$ + Lay date$_{mc}$ + Clutch size$_{mc}$ + Female body condition$_{mc}$ + Year, Random = Site ID (N=8) + Clutch ID (N=74) + Female ID (N=66)

(c) *Model structure:* **Spot percentage** ~ ISA$_{mc}$ + Lay date$_{mc}$ + Clutch size$_{mc}$ +Female body condition$_{mc}$ + Year, Random= Site ID (N=8) + Clutch ID (N=74) + Female ID (N=66)

(d) *Model structure:* **Spot intensity** ~ ISA$_{mc}$ + Lay date$_{mc}$ + Clutch size$_{mc}$ + Female body condition$_{mc}$ + Year, Random = Site ID (N=8) + Clutch ID (N=74) + Female ID (N=66)

(e) *Model structure:* **Spot distribution** ~ ISA$_{mc}$ + Lay date$_{mc}$ + Clutch size$_{mc}$ + Female body condition$_{mc}$ + Year, Random = Site ID (N=8) + Clutch ID (N=74) + Female ID (N=66)

(f) *Model structure:* **Spot size** ~ ISA$_{mc}$ + Lay date$_{mc}$ + Clutch size$_{mc}$ + Female body condition$_{mc}$ + Year , Random = Site ID (N=8) + Clutch ID (N=74) + Female ID (N=66)

Table S1

Summary of ISA values for clutches for all study sites.

Site	$N_{clutches}$	Minimum	Average	Median	Maximum
Suburban village	18	0.00	1.97	0.85	13.60
Natural forest	16	0.00	0.00	0.00	0.00
Residential area I	2	32.38	42.06	42.06	51.74
Urban woodland I	2	24.19	24.19	24.19	24.19
Urban woodland II	3	10.24	18.99	16.65	30.07
Residential area II	5	35.62	42.91	43.68	47.17
Urban park	36	0.00	10.22	7.21	38.08
University campus	8	29.04	29.04	38.28	60.73
Summary	90	0.000	12.73	4.74	60.73

Table S2

GLMMs with Gaussian distribution testing the association between egg pigmentation traits, urbanisation and life-history traits, N_{eggs}=568. Continuous predictors were mean-centered (mc). Significance levels are indicated **in bold: *p < 0.05, **p < 0.01, ***p < 0.001.**

Family: Gaussian, Random = Site ID (N=8) + Clutch ID (N=74) + Female ID (N=66)				
Model structure (glmmTMB): Egg trait ~ ISA_{mc} + Lay date_{mc} + Clutch size_{mc} + Female body condition_{mc} + Year				
Spot red chroma	Estimate	SE	z-value	p-value
Intercept	0.41	<0.01	164.53	**<0.001*****
ISA	<0.001	<0.001	-0.37	0.714
Lay date	<0.001	<0.001	0.29	0.773
Clutch size	<0.001	<0.01	-0.15	0.883
Female body condition	<0.001	<0.01	-0.52	0.603
Year	<0.001	<0.01	0.31	0.754
Spot percentage				
Intercept	17.65	0.92	19.29	**<0.001*****
ISA	<0.01	0.03	0.14	0.887
Lay date	0.02	0.12	0.19	0.848
Clutch size	0.51	0.40	1.26	0.207
Female body condition	-0.33	0.66	-0.50	0.618
Year	-7.66	1.18	-6.51	**<0.001*****
Spot intensity				
Intercept	3.26	0.14	23.45	**<0.001*****
ISA	<0.01	0.01	0.63	0.526
Lay date	-0.01	0.02	-0.30	0.764
Clutch size	0.12	0.06	1.85	0.064
Female body condition	-0.26	0.11	-2.37	**0.018***
Year	-0.03	0.16	-0.20	0.840
Spot distribution				
Intercept	3.36	0.16	21.58	**<0.001*****
ISA	-0.01	0.01	-0.81	0.420
Lay date	-0.02	0.02	-0.73	0.463
Clutch size	0.10	0.07	1.45	0.146
Female body condition	0.15	0.12	1.26	0.207
Year	-0.31	0.17	-1.82	0.069
Spot size				
Intercept	2.16	0.08	27.70	**<0.001*****
ISA	<0.001	<0.01	0.20	0.840
Lay date	0.01	0.01	0.91	0.362

Clutch size	0.05	0.04	1.32	0.186
Female body condition	-0.01	0.06	-0.21	0.84
Year	-0.15	0.08	-1.92	0.06

Table S3. Linear Mixed Effect Models (LMMs) testing the association between Impervious Surface Areas (%) measured in a 100m radius around each nestbox, and the number of hatched offspring in great tits and blue tits. Continuous predictors were scaled and mean-centered (mc). Significance levels are indicated **in bold: *p < 0.05, **p < 0.01, ***p < 0.001.** Data and details regarding methods can be found in Corsini & Szulkin (2025).

Family: Gaussian, Random = Site (N=8)

Model structure (LMMs): Number of hatched offspring ~ ISA$_{mc}$ + Clutch size$_{mc}$ + Female body mass$_{mc}$ + Lay date$_{mc}$ + Year

				Great tit (n = 252)	
Variable		Estimate	se	t-value	p-value
(Intercept)		7.78	0.20	38.28	**<0.001*****
ISA		<0.01	<0.01	0.13	0.899
Clutch size		0.95	0.05	17.87	**<0.001*****
Female body mass		-0.07	0.09	-0.80	0.426
Lay date		-0.01	0.01	-1.00	0.320
Year $_{2017}$					
	Year $_{2018}$	0.11	0.27	0.40	0.689
	Year $_{2019}$	-0.19	0.25	-0.76	0.446
	Year $_{2020}$	-0.50	0.25	-1.96	0.051
	Year $_{2021}$	-0.58	0.29	-1.97	0.050
				Blue tit (n = 305)	
(Intercept)		8.62	0.21	42.02	**<0.001*****
ISA		-0.01	0.01	-2.12	0.052
Clutch size		0.95	0.06	15.39	**<0.001*****
Female body mass		-0.29	0.16	-1.86	0.06
Lay date		-0.01	0.02	-0.56	0.57
Year $_{2017}$					
	Year$_{2018}$	0.40	0.28	1.43	0.154
	Year $_{2019}$	0.25	0.28	0.89	0.373
	Year $_{2020}$	-0.34	0.28	-1.22	0.222
	Year $_{2021}$	0.18	0.29	0.63	0.503