

RESEARCH ARTICLE

Urbanisation impacts plumage colouration in a songbird across Europe: Evidence from a correlational, experimental and meta-analytical approach

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Funding information

Marie Curie Career Integration Grant FP7-CIG, Grant/Award Number: 322217; Vetenskapsrådet, Grant/Award Number: C0361301

Handling Editor: Albert Phillimore

Abstract

1. Urbanisation is accelerating across the globe, transforming landscapes, presenting organisms with novel challenges, shaping phenotypes and impacting fitness. Urban individuals are claimed to have duller carotenoid-based colouration, compared to their non-urban counterparts, the so-called 'urban dullness' phenomenon. However, at the intraspecific level, this generalisation is surprisingly inconsistent and often based on comparisons of single urban/non-urban populations or studies from a limited geographical area.
2. Here, we combine correlational, experimental and meta-analytical data on a common songbird, the great tit *Parus major*, to investigate carotenoid-based plumage colouration in urban and forest populations across Europe.
3. We find that, as predicted, urban individuals are paler than forest individuals, although there are large population-specific differences in the magnitude of the urban-forest contrast in colouration. Using one focal region (Malmö, Sweden), we reveal population-specific processes behind plumage colouration differences, which are unlikely to be the result of genetic or early-life conditions, but instead a consequence of environmental factors acting after fledging.
4. Finally, our meta-analysis indicates that the urban dullness phenomenon is well established in the literature, for great tits, with consistent changes in carotenoid-based plumage traits, particularly carotenoid chroma, in response to anthropogenic disturbances.
5. Overall, our results provide evidence for uniformity in the 'urban dullness' phenomenon but also highlight that the magnitude of the effect on colouration depends on local urban characteristics. Future long-term replicated studies, covering a wider range of species and feeding guilds, will be essential to further our understanding of the eco-evolutionary implications of this phenomenon.

KEYWORDS

biomarker, carotenoid, colouration, pigmentation, plumage, urbanisation

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1 | INTRODUCTION

Rapid expansion of global urban development, in parallel with high human population growth, is expected to result in an almost tripling of urban land cover by 2030 when compared with the year 2000 (Seto et al., 2012; United Nations, 2018). This unprecedented increase in area of urban landscapes strongly affects the environmental conditions encountered by organisms and poses a major challenge for wildlife across the globe (Hendry et al., 2017). Despite urban habitats being relatively young ecosystems, their novel selective pressures can dramatically shape the phenotypic and genotypic variation of populations living in them (e.g. Salmón et al., 2021; Santangelo et al., 2022; Thompson et al., 2022). In recent decades, research across the globe has demonstrated divergence in multiple phenotypic traits between urban and non-urban populations, across a variety of taxa (Alberti et al., 2017; Capilla-Lasheras et al., 2022).

Even though one of the most well-known examples of natural selection on a phenotypic trait arose due to urban industrial pollution—the case of the peppered moth (Steward, 1977)—animal colouration has been surprisingly little studied in the context of urbanisation (reviewed in Leveau, 2021). Colour traits play significant roles in sexual signalling, predator deterrence and camouflage and can therefore strongly influence survival and reproductive success (Hill, 2006). The evidence, to date, suggests that urban organisms tend to present higher levels of melanisation ('*urban melanism*') and duller carotenoid-based traits ('*urban dullness*', Leveau, 2021). However, these results are largely based on examples from one or a few populations and/or a limited geographical area (Leveau, 2021), and there has been no attempt yet to comprehensively evaluate the extent to which these effects can be generalised across larger spatial scales. Given that the scale and attributes of urban environmental change can strongly depend on local characteristics (Alberti et al., 2020), it is especially important to understand the uniformity of widely asserted urban impacts.

Carotenoid-based colouration (i.e. yellow, orange and red colours) is central to widespread and conspicuous ornamental traits in animals (Blount & McGraw, 2008). In birds, approximately 30% of species are estimated to deposit carotenoids in their feathers (40% of passerine and 13% of non-passerine species; Thomas et al., 2014), and over half of non-passerine species also express carotenoids in other integumentary structures (e.g. skin, beak; Davis & Clarke, 2022). Carotenoid-based colouration is often shown to be under strong sexual selection (Svensson & Wong, 2011). Importantly, vertebrates cannot synthesise carotenoids *de novo* and must obtain them from the diet (Goodwin, 1986), making them a limited resource. Moreover, carotenoids serve not only as a pigment, but also as an antioxidant and immune system enhancer. Consequently, carotenoid-based colouration has received much attention in the literature because of its sensitivity to environmental factors (e.g. parasite prevalence, Martínez-Padilla et al., 2007; food availability and predation risk, Ruell et al., 2013), roles in life-history trade-offs and links to individual quality (e.g. Weaver et al., 2018).

Aspects of the urban environment, such as air pollution and the predominance of non-native trees in urban parks, could alter animal carotenoid-based colouration via changes in the bioavailability of carotenoids in the diet (Eeva et al., 1998; Jensen et al., 2022; Sillanpää et al., 2008). For example, primary producers and the caterpillars that feed upon them have been shown to have lower carotenoid levels in polluted and urban areas, compared with unpolluted non-urban areas (Isaksson & Andersson, 2007; Sillanpää et al., 2008). Indeed, the great tit—a model system in avian urban ecology and common across Europe—is known to exhibit *duller* plumage colouration in some urban populations (Biard et al., 2017; Hörak et al., 2000; Isaksson et al., 2005), possibly reflecting carotenoid-deficient diets. Additionally, since carotenoids are often regarded as key immunomodulators and antioxidants (Pérez-Rodríguez, 2009; but see e.g. Koch et al., 2018), observed differences in carotenoid-based colouration between urban and non-urban populations could be the result of resource allocation trade-offs between ornamentation and health, whereby increased pathogen exposure and/or higher levels of oxidative stress in urban environments demands greater allocation of carotenoids to immune and oxidative defences (e.g. Bortolotti et al., 2003; Isaksson et al., 2005). The expression of carotenoid-based colour traits is not purely the result of deposition of carotenoid pigments in tissues, but it can also be influenced by feather structure and reflectance of carotenoid-containing tissues (McCoy et al., 2021; Shawkey & Hill, 2005). It has been demonstrated that the urban environment can impact feather structure (e.g. Sándor et al., 2022), and thus structural changes could also be an important source of variation in carotenoid-based colouration in urban-dwelling birds (McCoy et al., 2021).

Despite increasing interest in urban ecology research, in recent decades, our current knowledge of the impact of urbanisation on animal colour traits is based on a few comparative studies involving single urban/non-urban populations, without replication, even for ecologically well-studied species (Leveau, 2021). Moreover, studies often use a single methodological approach, and no study to date has combined correlative, experimental and synthesised data. These limitations hinder our ability to generalise about urban effects on colouration across larger spatial and temporal scales. For instance, methodological differences across studies could increase between-site variance. In addition, the key factors influencing carotenoid-based traits, such as food availability or plant diversity and abundance, are expected to differ locally across urban environments (Szulkin et al., 2020). By bringing together correlative data at a large geographical scale, field experiments and a research synthesis, it would be possible to deliver a sound assessment of the uniformity of the '*urban dullness*' phenomenon on a large spatial scale and derive causal relationships, ultimately providing a holistic picture of how urbanisation affects organismal colouration.

Here, we aim to quantify, for the first time, the spatial uniformity of the '*urban dullness*' phenomenon to gain understanding of the possible underlying causes. Using a case study of the great tit, an ecologically relevant avian model in Europe whose characteristic yellow feathers are a well-known example of a carotenoid-based

ornament (Broggi & Senar, 2009; Evans & Sheldon, 2012, 2013), we combine three complementary approaches, utilising correlational data at a continental scale, a cross-fostering experiment and a meta-analysis. In doing so, we can verify the uniformity of the 'urban dullness' phenomenon and confirm causal relationships. Previous work in this widely distributed bird species within Eurasia has shown dramatic phenotypic shifts in response to urbanisation across multiple traits (e.g. Branston et al., 2021; Caizergues et al., 2018; Charmantier et al., 2017; Hardman & Dalesman, 2018; Sprau et al., 2017), including carotenoid-based colouration and its physiological basis (see in Table S3; e.g. Biard et al., 2017; Hörak et al., 2000). However, other studies have also found no differences in carotenoid-based plumage traits (e.g. Isaksson et al., 2007), suggesting inconsistency in the pattern.

Firstly, we test if urbanisation is consistently correlated with breast carotenoid-based colouration in adult birds, using a paired sampling design of urban and forest populations across five urban centres in Europe (Figure 1a). Secondly, in a reciprocal cross-fostering experiment within one of the population pairs (Malmö, Sweden), we quantify the relative contribution of environmental versus genetic/parental influence on carotenoid-based colour variation of chicks. In the great tit, carotenoid-based plumage colouration is fully established in the second year of life, and it is highly repeatable across moult cycles thereafter (Evans & Sheldon, 2013). However, little is known about how urban habitats might influence the acquisition of adult plumage colouration. Therefore, thirdly, again using focal urban and forest populations in Malmö, we investigate how colouration varies with age in relation to urbanisation. Finally, ecotoxicology

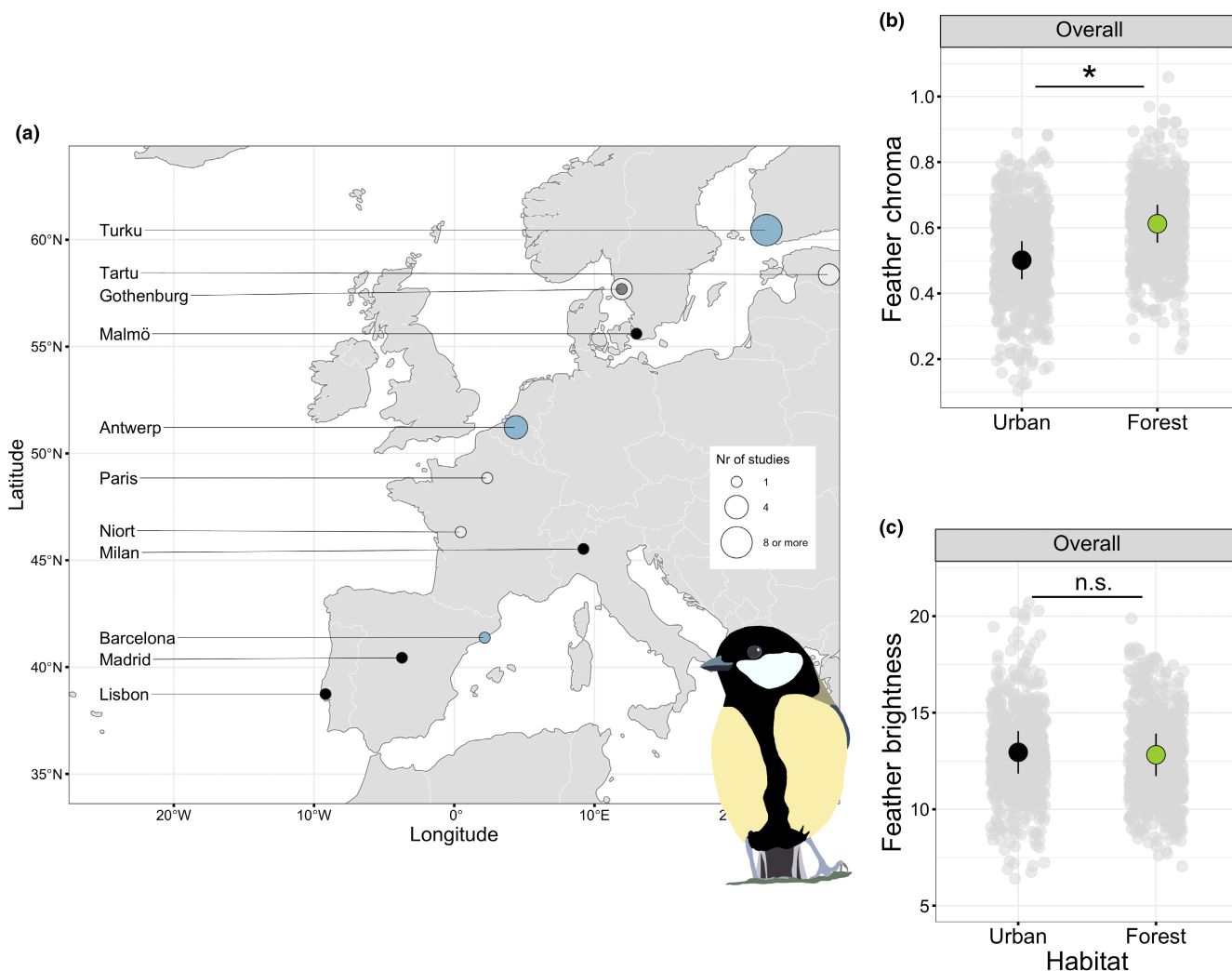


FIGURE 1 (a) Locations of great tit *Parus major* populations across Europe included in this study. Black circles: urban/forest population pairs sampled in the empirical study in which differences in breast plumage colour traits were compared (see Table S1 for details); white and blue circles: populations from the literature on effects of urbanisation (white) or pollution (blue) on colour traits and included in the meta-analysis (see Table S3 for details). For adult birds (1 and 2+ years) sampled in the empirical study, that is black circles in (a): (b) chroma reflects the amount of pigment in the feathers (i.e. carotenoids) and (c) brightness reflects the structural quality of feathers. Sample sizes (urban/forest): Gothenburg (11/11), Malmö (41/41), Milan (16/18), Madrid (16/20), and Lisbon (18/16). Asterisks denote significant differences between urban and forest habitats with $p < 0.05$ (habitat main term see Table S5). n.s., non-significant differences. Model mean values (solid-coloured circles) with 95% confidence intervals are plotted, along with raw data (grey circles, 6 technical measurements per individual).

studies suggest a link between certain urban stressors, such as pollutants, and great tit colouration (Eeva et al., 1998). Therefore, to better understand the overall effect of urbanisation on colouration, fourthly, we conduct a meta-analysis of the literature concerning changes in breast colour in response to urbanisation and pollution in great tits. Overall, our multi-analytical approach provides a comprehensive understanding of the repeatability of the 'urban dullness' phenomenon across a large spatial scale and insight into the possible driving mechanisms.

2 | MATERIALS AND METHODS

2.1 | General fieldwork and cross-fostering design

During the years 2014 and 2015 (breeding, i.e. spring, and post breeding, i.e. summer, seasons), we captured a total of 208 adult great tits (aged 1 and 2+ years) in five paired urban-forest habitats across Europe ($n=5$ urban/5 forest populations; see Salmón et al. (2021); Figure 1a; Tables S1 and S2). Adult individuals were captured using mist nets (Lisbon, Madrid, and Milan) or caught in nest-boxes during breeding (Gothenburg and Malmö; see Table S1). In all cases, we sampled each urban and forest pair within the same season and year. All urban sites were located in built-up areas or parks within city boundaries, see Salmón et al., 2021 for details about Gothenburg, Milan, Madrid and Lisbon, and Andersson et al., 2015; Salmón et al., 2016, 2017 for details about Malmö. Forest locations were natural/semi-natural forests in areas of very low human habitation. Each pair of study locations were separated by 33.6 ± 5.37 km (mean \pm SD; range: 25–39 km), a distance far exceeding the mean adult and natal dispersal distance of this species (mean \pm SD: adult, 2.5 ± 12.3 km and natal, 5.3 ± 17.9 km, see Paradis et al. (1998)).

In 2013, we performed a reciprocal cross-fostering of nestlings between the urban and forest populations in Malmö (Salmón et al., 2016). Briefly, when nestlings were 2 days old (hatching day = 0 day), we exchanged half of the nestlings from a brood in the urban habitat with the same number of nestlings from a nest of the same age and similar brood size (± 1 nestling) in the forest study population ($n=10$ nest pairs: 72 nestlings; see Table S2 for sample sizes for each sex). Where brood sizes differed, we swapped the number of chicks corresponding to half the number of the smaller brood. Cross-fostered chicks were marked by clipping the outermost tip of their claws. Nestlings were individually ringed, and breast feathers were collected at 15 days.

Feathers were collected from birds using a standardised methodology, in which a total of eight yellow feathers were pulled from both sides of the upper part of the breast; all feathers were stored in paper envelopes, protected from the light, in the same location until measurement. These either represent the feathers grown during the nestling period (nestlings), autumn post-juvenile partial moult (1 year) or autumn post-breeding complete moult (2+ years). Birds were sexed

either visually (adults) or molecularly, using primers P2 and P8 (nestlings, Griffiths et al., 1998). There was no difference in sex ratio in the dataset between habitats across population pairs (urban-forest, Table S2): adults (GLMM, $\chi^2_1=0.04$, $p=0.842$; males: $n=108$; females: $n=100$), nestlings (GLMM, $\chi^2_1=0.13$, $p=0.712$; males: $n=37$; females: $n=34$). For adults, only feathers from individuals whose age category could be determined based on plumage characteristics were used in this study, following Svensson (1992). The collection of feather samples (all study sites), cross-fostering and toe-clipping (only Malmö) were approved by the relevant local regulatory bodies: Malmö-Lund Animal Ethics Committee (permit no. M454 12:1), the CEMPA and Portuguese Ministry of Environment (permit nos. 40/2014 and 164/2014), the Ministry of the Environment, Housing and Territorial Planning of Madrid (permit nos. 10/103329.9/14, 10/169940.9/13, 10/045383.9/14, 10/127641.9/14 and 10/055393.9/14), the Institute for Environmental Protection and Research (ISPRA, licence nos. 15510 and 15944) and the Lombardy Region (permit no. 3462).

2.2 | Feather colouration measurements

Feather colouration was measured by a single person (D.L.-I.) using a spectrophotometer (AVASPEC-2048, Avantes BV, Apeldoorn, Netherlands) and a deuterium-halogen light source (AVALIGHT-DH-S lamp, Avantes BV) covering the range 300–700 nm and kept at a constant angle of 90° from the feathers (Fargevieille et al., 2017). For each bird, reflectance spectra were measured six times, with three measurements of each of two separate sets of four breast feathers arranged to mimic their natural position in the plumage (López-Ildiáquez et al., 2022; Quesada & Senar, 2006). Based on these spectra, we computed three colour variables: yellow chroma, brightness and hue using the R package PAVO (Maia et al., 2019). Specifically, we computed yellow chroma as (R700-R450)/R700 (hereafter chroma), with higher values of chroma being associated with higher carotenoid content in plumage (Isaksson et al., 2008). Mean brightness, linked to the feather structural quality, was calculated as the area under the reflectance curve divided by the width of the interval at 300–700 nm (hereafter brightness). Finally, hue was computed as the wavelength at which the slope of the spectra reaches its maximum; however, hue was excluded from analyses due to low variance and correlation with chroma (see Figure S1). To estimate the technical repeatability of our colouration measurements, we repeated measurements on a subset of 30 adults (~50:50 1 and 2+ years) and 15 nestlings twice (on different days). Our measures were highly repeatable (adjusted for sex, age and habitat) for the analysed colour traits and age groups (yellow chroma: $R_{\text{adults}}=0.68$, 95% CI [0.631, 0.731], $R_{\text{nestlings}}=0.67$, 95% CI [0.593, 0.753]; yellow brightness: $R_{\text{adults}}=0.82$, 95% CI [0.788, 0.852], $R_{\text{nestlings}}=0.70$, 95% CI [0.633, 0.783]; all $p < 0.001$). Chroma and brightness were not correlated in our data, neither in adults ($r=0.06$, 95% CI [-0.076, 0.194]) nor in nestlings ($r=0.01$, 95% CI [-0.221, 0.241]).

2.3 | Statistical analysis

Firstly, we investigated differences in chroma and brightness in adult birds from the five urban regions, across Europe, using linear mixed models (LMMs). Models included habitat (urban or forest), sex, age (1 or 2+ years) and all two-way interactions as fixed effects. Sampling location ($n=10$), urban region ($n=5$) and bird ID ($n=208$; to account for repeated measures of reflectance spectra) were included as random effects. The season when feathers were collected, that is breeding or post-breeding (see Table S1), did not influence chroma ($F_{1,8.00}=0.01$, $p=0.932$) or brightness ($F_{1,7.68}=1.37$, $p=0.276$); thus, season was omitted from models to avoid overparameterisation. In addition, we conducted two separate post hoc LMMs for urban and forest populations, respectively, to explore habitat variation in chroma colouration across populations within each habitat type (i.e. percentage explained by the region ID). These models had the same structure as described above but without the fixed effect of habitat.

Secondly, using LMMs, we analysed the variation due to the environment (i.e. habitat—urban or forest) and early maternal/genetic effects on chroma and brightness in nestlings from the cross-fostering experiment. Models included the rearing habitat (urban or forest), habitat of origin (urban or forest) and their interaction, together with sex as fixed effects. As our experimental broods included nestlings of mixed origin (i.e. partial cross-fostering), we included the nest of origin (to account for genetic and maternal effects) and the nest of rearing (to account for the effects of the common environment) as random effects, together with bird ID. Thirdly, to further investigate the age-dependent variation in colouration between urban and forest habitats, we used LMMs to cross-sectionally analyse the habitat effect using one focal urban region (Malmö), combining data included in the first (adult) and second (nestlings) analyses for that specific region, as described above. We analysed variation in chroma and brightness across three age categories: nestlings, 1 and 2+ years. Data were included from two consecutive years (2013 and 2014) and collected during breeding. We only included birds ringed as nestlings in the urban habitat (i.e. only those of confirmed urban origin), to understand how the environmental conditions during the first (nestling to 1 year) and subsequent (1 to 2+ years) moult cycles influenced individual colouration. Models included habitat, sex, age and all two-way interactions as fixed effects and territory (i.e. nest-box) and bird ID as random effects.

All statistical analyses were performed in R 4.1.3 (R Core Team, 2021). The significance of model terms was estimated using F -tests based on the Satterthwaite approximation for the denominator degrees of freedom in $LMERTEST$ (Kuznetsova et al., 2017). In all cases, the distribution of model residuals was inspected visually and did not show marked deviations from normality. Significant two-way interactions were further dissected using pairwise planned comparisons adjusted by Tukey HSD in $EMMEANS$ (Lenth et al., 2018). The explanatory power of the models was calculated as marginal (R^2_m) and conditional (R^2_c) values using the R package $R2GLMM$ (Jaeger et al., 2017).

LMMs were fitted using the $LME4$ R package (Bates et al., 2014), and we used the 'sim' function in the ARM package to simulate posterior distributions of the model parameters, based on 5000 simulations, and calculate means and 95% CIs (Gelman, 2011).

2.4 | Meta-analysis: Literature search, data extraction and effect size calculation

To synthesise and assess the current knowledge of urbanisation and pollution on coloration in great tits, we systematically searched the peer-reviewed scientific literature. We searched six databases: A&HCI 1975–present; BKCI-S 2005–present; BKCI-SSH 1990–present; ESCI 2015–present; SCI-EXPANDED 1900–present; SSCI 1900–present, within Web of Science (search conducted on August 2nd, 2021) using the following search string: TS = ('urban*' OR 'pollut*') AND ('color*' OR 'colour*' OR 'carat*') AND ('Parus major' OR 'great tit' OR 'tit'). This search produced 81 papers, which were complemented by five studies identified as potentially suitable by the authors and estimates from the new empirical data presented in this paper (Figure S2). We read the title and abstract of these 86 papers to determine their suitability for further inspection and inclusion in this meta-analysis. Papers were included in our meta-analysis if they reported the effect of urbanisation or chemical pollution on traits related to yellow colouration of great tit plumage (e.g. brightness or chroma; see full details in Tables S3 and S4). We also included studies on urbanisation and pollution that measured carotenoid levels in feathers or plasma as these have been shown to reflect the plumage colouration of interest (Isaksson et al., 2008). We did not include studies that assessed habitat differences between natural/semi-natural populations (i.e. non-urban linked). This resulted in the inclusion of a total of 22 studies (23 studies including the data presented in the current study) and 128 effect sizes in the meta-analysis. All effect sizes were extracted by one author, PS. We extracted correlation coefficients between pollutants and carotenoid-based colour ($n=8$ studies; $k=44$ effect sizes) and mean values for population comparisons (either urban/forest or polluted/control; $n=20$ studies; $k=82$ effect sizes). In the latter case, we calculated point-biserial correlation coefficients (Tate, 1954) as a standardised effect size for comparisons between urban and non-urban populations. Standardised effect sizes (r) and their sampling variances were calculated using the R function 'escal' in the R package METAFOR 3.0-2 (Viechtbauer, 2010).

2.5 | Meta-analysis: Statistical analysis and detection of publication bias

We performed multilevel meta-analytic mixed-effect models using METAFOR v3.0-2 in R v.4.1.1. (R Core Team, 2021). In each model, we used the standardised effect size (r) as the response variable. We first fitted an intercept-only model to estimate the overall mean

effect (i.e. meta-analytic mean) for the association between anthropogenic disturbance (i.e. urbanisation and pollution) and all the great tit yellow breast colouration traits obtained from the literature search (see Section 2.3). We then built individual meta-analytic models to investigate potential drivers of differences in coloration between urban/polluted and non-urban/unpolluted populations using only the effect sizes of the same colouration traits as evaluated in the present study, that is chroma and brightness, together with carotenoid concentration. Models aimed to evaluate the effect of our chosen moderators (see Table S4 for details) on the association between carotenoid-based colouration and anthropogenic disturbance, based on (i) the type of plumage trait, that is chroma, brightness or carotenoid concentration (feathers and plasma); (ii) the developmental stage, that is adult or nestling and (iii) the type of anthropogenic disturbance, that is urbanisation or pollution. In all models, we included study geographical location and study ID as random effects, as well as a term for residual variance. We present model estimates with their 95% confidence intervals (CIs) throughout. We calculated total heterogeneity (i.e. the total amount of variation after accounting for sampling variance, I^2_{total}), the amount of among-location variation ($I^2_{location}$; Figure 1a), the amount of among-study variation (I^2_{study}) and amount of residual variation ($I^2_{residual}$) using the function the R function 'i2_ml' in METAFOR. We also estimated the percentage of variation explained by each moderator in our models using $R^2_{marginal}$ (Nakagawa & Schielzeth, 2013). In the text, we present meta-analytic model means and 95% CIs.

To check for possible publication bias, we performed an extra multilevel meta-regression with the same random structure as above but using the moderators of (i) sampling variance (for small-study effects; see eq. 21 in Nakagawa et al. (2022)); and (ii) mean-centred year of study publication to test time-lag bias (Koricheva & Kulinskaya, 2019).

3 | RESULTS

3.1 | Adults: Urbanisation and colouration across Europe

Urban and forest adult great tits differed in the chroma of the yellow breast colouration, but not in brightness, across the five studied urban regions (Figure 1b,c; Figure S3; Table S5). Adult urban birds had consistently lower chroma than forest birds (Figure 1b). However, the magnitude of the negative urban effect on chroma seemed to vary across cities (Figure S3a), although we did not test specifically for it. Nonetheless, the post hoc analysis treating urban and forest populations in separate models reinforced this observation. In cities, the percentage of variation in chroma explained by among-population differences was 4% (95% CI: 1.3, 9.8), which was much lower than the percentage of among-population variance in chroma in forests (29%, 95% CI: 11.6, 45.7).

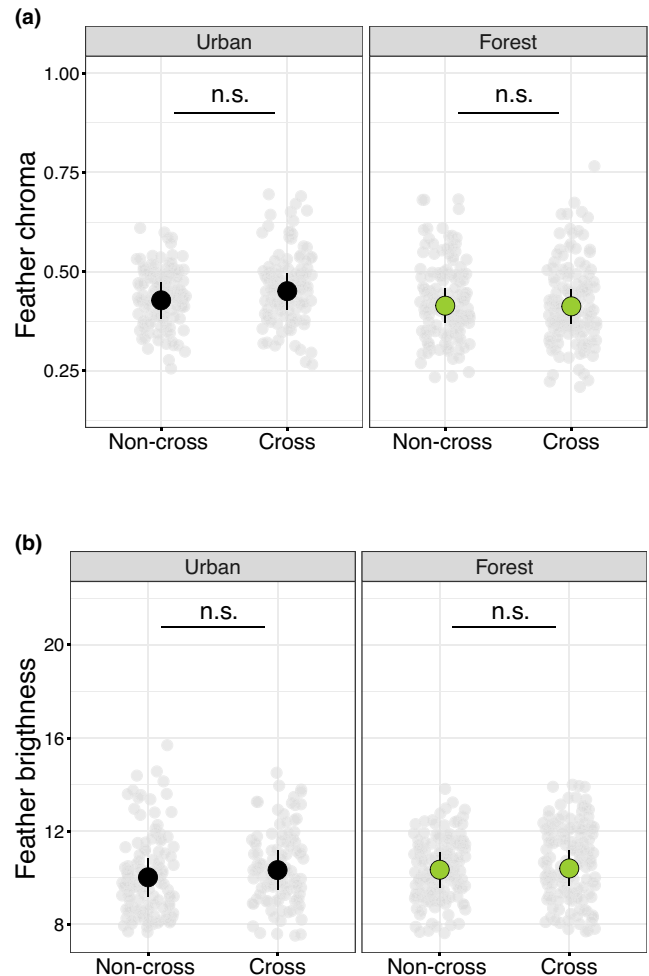


FIGURE 2 Variation in breast plumage colouration in 15-day-old nestlings, reared in urban (left) or forest (right) habitat following a partial cross-fostering between habitats ($n=10$ nest pairs; 72 nestlings). (a) Chroma reflects the amount of pigment in the feathers (i.e. carotenoids) and (b) brightness reflects the structural quality of feathers. Non-cross: same origin and rearing habitat (17 urban, 20 forest nestlings); Cross: different origin and rearing habitat (15 urban, 20 forest nestlings). Model mean values (solid-coloured circles) with 95% confidence intervals are plotted, along with raw data (grey circles, 6 technical measurements per individual). n.s., non-significant differences.

3.2 | Nestlings: Urbanisation and colouration in a cross-fostering experiment

In contrast to our findings for adults, great tit nestlings reared in the urban habitat did not differ in yellow breast colouration (chroma or brightness) from their forest counterparts in the Malmö population pair (Figure 2a,b; 'Habitat of rearing' in Table S6). There were no differences in colour traits between cross-fostered and non-cross fostered nestlings, indicating neither habitat of origin nor habitat of rearing influenced colouration (Table S6). Our results indicate that a high proportion of colour variation among nestlings was explained by the family of rearing independently from the rearing habitat per

se, in particular for chroma (approx. 64% of the total variation; mean: 0.64, 95% CI: 0.34, 0.99).

3.3 | Age-dependent habitat differences in colouration

While we found no effects of urbanisation on yellow colouration in nestling great tits, in Malmö, when we investigated plumage colouration after fledging, in juvenile birds and into adulthood, we found that habitat explained a significant proportion of the variation in yellow chroma, but not in brightness, in an age-dependent manner (Figure 3; 'Habitat \times Age' in Table S7). Consistent with the cross-fostering experiment in 3.2, we found no differences in chroma between urban

and forest nestlings ('Nestling' in Figure 3a). However, habitat differences emerged with increasing age: first year urban birds (1 year) had lower yellow chroma than their forest counterparts (post hoc Tukey test: $t_{127}=3.43$, $p<0.001$; Figure 3a), though habitat differences disappeared when comparing urban and forest birds of 2+ years (post hoc Tukey test: $t_{109,4}=0.61$, $p=0.541$; Figure 3a).

3.4 | Meta-analysis on colouration differences in response to urbanisation and other anthropogenic disturbances

We performed a meta-analysis on 128 effect sizes from 23 studies that assessed anthropogenic effects (i.e. urbanisation and

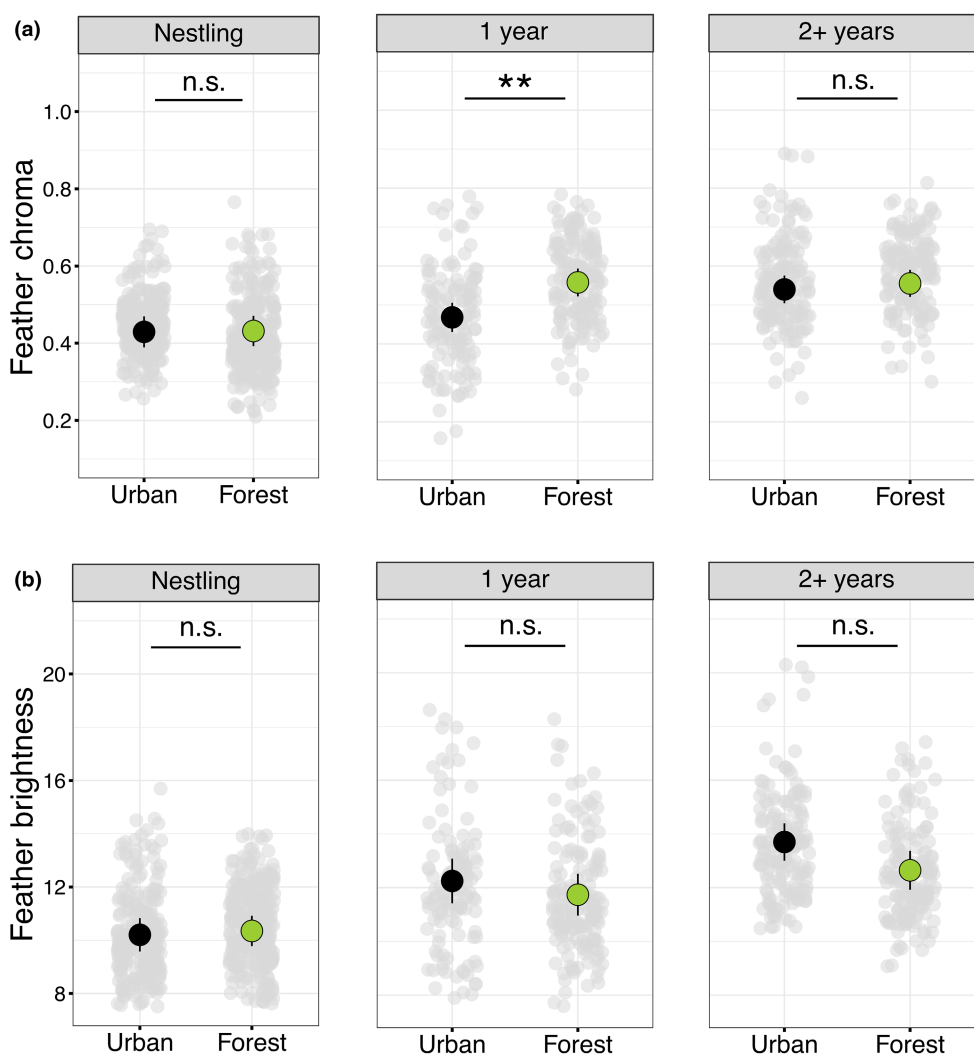


FIGURE 3 Variation in breast plumage colouration in nestlings and adults in relation to habitat (urban or forest) and age (nestling; first year: 1 year; and second or more year: 2+ years) in Malmö, southern Sweden. (a) Chroma reflects the amount of pigment in the feathers (i.e. carotenoids) and (b) brightness reflects the structural quality of feathers. Note the design is cross-sectional, although nestlings and 1 year were born in the same year (2013). Sample sizes (urban/forest): nestling (32/40), 1 year (17/19), 2+ years (24/22). Urban: black circles; forest: green circles. Model mean values (solid-coloured circles) with 95% confidence intervals are plotted, along with raw data (grey circles, 6 technical measurements per individual). Asterisks denote significant differences between urban and forest habitats with $**p<0.01$ (Tukey's HSD; see Table S6 for main effect). n.s., non-significant differences.

pollution) on great tit yellow breast colouration traits (Table S3). This revealed that, overall, anthropogenic factors were associated with a decrease in the intensity of plumage colouration of this species (meta-analytic mean: -0.22 [-0.373 , -0.067]; Figure 4a). This model provided evidence for high heterogeneity ($I^2_{\text{total}} = 94.7\%$) (Table S8). Geographical location explained 34.0% of the total variation in effect sizes, possibly reflecting among-population variation in the response to anthropogenic disturbances, while 45.5% of the variation was explained by study ID and 15.2% of the total variation was residual variation (Table S8). Further investigation into each of the retrieved plumage traits from the literature supported our findings across the five European regions, and—among the evaluated traits—the anthropogenic effect on great tit breast coloration was strongest for chroma (-0.21 [-0.330 , -0.096]; Figure 4b; Table S8).

Interestingly, our meta-analysis also reveals that the observed negative anthropogenic effects on colouration were similar in nestlings and adults (Figure 4c; Table S8). Negative anthropogenic effects on colouration were of a similar magnitude for both urbanisation and pollution, although there was higher uncertainty for pollution, and the effect was not significant (Urbanisation: -0.19 [-0.371 , -0.018], Pollution: -0.16 [-0.382 , 0.066]; Figure 4d; Table S8). Adjusting our initial meta-analysis estimate to account for potential small-study effects still provided support for an overall negative anthropogenic effect on great tit coloration (adjusted meta-analytic mean: -0.27 [-0.431 , -0.102]; see eq. 22 in Nakagawa et al. (2022)) and revealed a significant positive small-study effect (slope: 6.04 [2.568 , 9.522]); that is the higher the sampling variance, the less evidence for a meta-analytic mean different from zero. We did not find evidence

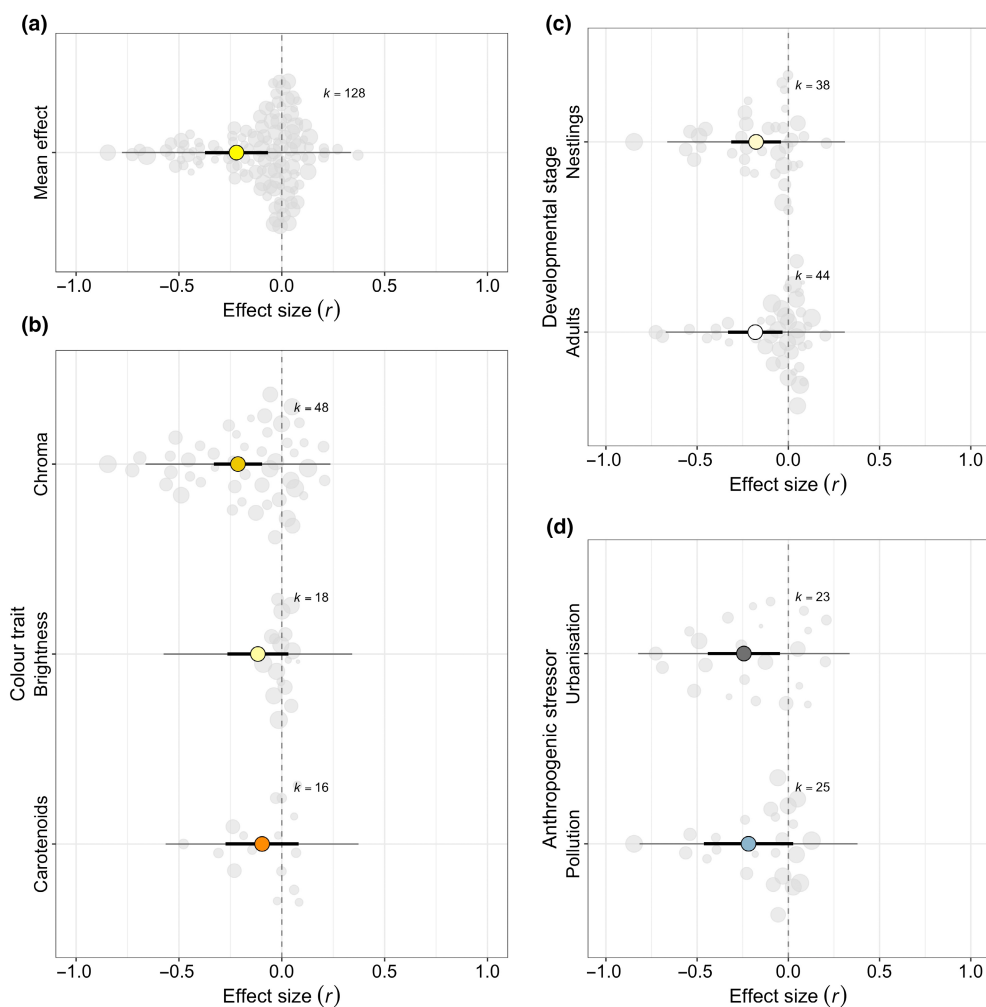


FIGURE 4 Effect sizes from meta-analysis of the anthropogenic effects on great tit breast plumage coloration. (a) Overall mean effect of urbanisation and pollution on great tit breast feather coloration traits ($n=23$ studies; $k=128$ effects); (b) type of colour trait (feather chroma, feather brightness and feather and plasma carotenoid levels); (c) developmental stage (adult versus nestling) and (d) type of anthropogenic disturbance (pollution versus urbanisation). In all cases, negative values represent 'duller' yellow colouration/brightness/or lower carotenoid concentration in response to urbanisation or exposure to pollution. (b–d) Data only corresponds to effect sizes for chroma, brightness and carotenoid levels ($n=20$ studies; $k=82$ effects). See Table S4 for a detailed description of each moderator levels. Plots show model means and 95% confidence intervals (thick whisker), 95% precision intervals (thin whisker) and individual effect sizes (grey circles; size is scaled to illustrate the sample size from which they were estimated, for example, the larger the point, the bigger the sample size). Vertical dashed line drawn at an $x=0$.

for time-lag effects in the dataset used in our meta-analysis (slope: 0.01 [-0.008, 0.023]).

4 | DISCUSSION

It has been long observed that human actions, in particular urbanisation and pollution, can alter wildlife colouration (e.g. Steward, 1977). However, the current knowledge is mostly derived from single paired site comparisons or geographically confined studies, and there has been no effort to quantify and investigate the spatial replicability of observed patterns at the intraspecific level. Here, using a well-known wild model system in ecology, evolution and environmental sciences, we empirically demonstrate the overall significant influence of anthropogenic stressors on yellow plumage colouration, supporting previous claims of the 'urban dullness' phenomenon. Nonetheless, despite the overall dulling effect of urbanisation on plumage colouration, we also reveal spatial variation in the magnitude of the differences in yellow colouration between urban and forest populations, among distinct localities spanning the continent of Europe.

Across Europe, the yellow breast plumage of urban great tits was, on average, paler (i.e. lower chroma) than forest birds, a difference potentially discriminable by conspecifics and thus possibly impacting the functional role of carotenoid-based colouration in signalling (e.g. Baldassarre et al., 2022; see Figure S4 and Supporting Information for further details on the visual models). In contrast, plumage brightness did not differ between urban and non-urban populations, indicating that the differences in colouration between areas was caused by differential pigment deposition rather than differences in feather structure (Shawkey & Hill, 2005). This disparity in the sensitivity to anthropogenic impacts of different plumage colour traits is also supported by our meta-analysis and has been previously described for two other great tit urban populations (Biard et al., 2017). While we found an overall negative effect of anthropogenic disturbances on plumage colouration, among the analysed traits, chroma was the only one showing a significant negative effect. Yellow chroma not only reflects circulating carotenoid levels and diet quality during moult (Biard et al., 2006; Eeva et al., 2008, 2009; e.g. Isaksson et al., 2007; Peters et al., 2011), but also the ability to incorporate carotenoids into the feathers (Peneaux et al., 2021).

Changes in colour traits have often been suggested as indicators of pollution exposure in wildlife (Lifshitz & St Clair, 2016). It is well established that certain common urban pollutants, such as cadmium or lead, can reduce carotenoid synthesis in plants (Cenkci et al., 2010; Rai et al., 2005), subsequently reducing bioavailability in invertebrate prey (Eeva et al., 2010; Isaksson & Andersson, 2007). Ultimately, this manifests in a paler plumage for birds, such as great tits, that feed predominantly on invertebrates during spring and summer (Eeva et al., 2008). However, lower carotenoid bioavailability does not necessarily reflect the direct action of pollution but could be the result of changes in phenology and invertebrate biomass in polluted habitats (Eeva et al., 2010; Sillanpää et al., 2008). At the European level, it has been shown that the phenology and spatial

distribution of insect prey biomass, in particular caterpillars, differs between urban and non-urban populations (Jensen et al., 2022; Pollock et al., 2017; Seress et al., 2018). Therefore, a diet low in carotenoids, either due to diet quality or quantity, during plumage development (i.e. either during postnatal development in young or post-fledging/breeding moult in juveniles/adults) could contribute to the observed plumage *dullness* across urban great tits.

Physiological constraints, imposed by a trade-off between investment in carotenoid display versus homeostasis, could also lead to differences in the display of carotenoid-based colouration (but see Koch et al., 2018). In our study, we did not measure any of these proximate mechanisms, but studies in the same populations suggest higher levels of oxidative stress in great tits in relation to urban environmental pollution (Isaksson et al., 2005; Salmón et al., 2018). Furthermore, our meta-analysis indicates consistent trends between the effects of both pollution and urbanisation on yellow breast colouration. Further research, including experimental work, is needed to disentangle the contribution of multiple urban-associated stressors, including pollution and diet quantity and quality on the observed differences in plumage colouration.

Our meta-analysis demonstrates that the *dullness* phenomenon in response to anthropogenic disturbance is consistent in nestling and adult great tits, which could suggest that the same mechanism drives the paler phenotype despite the different timing of feather development and moult, respectively, in these two different life stages. However, this pattern contrasts with our cross-fostering study in one focal population pair (Malmö), where we found differences in chroma between urban and non-urban adults but not at the nestling stage. Siblings raised in the city and forest neither differed in chroma nor brightness of the yellow breast plumage, regardless of habitat of origin, which suggests that carotenoid availability was not a constraint during the nestling period, at least not during the year of study. Variation in nestling colouration was largely driven by the nest of rearing, which suggests that variation in parental provisioning ability, presumably linked to individual quality, was the main source of colour variation at the nestling stage. Thus, habitat-specific differences in carotenoid metabolism or physiological constraints during the nestling period—as previously suggested (Hörak et al., 2000)—might not explain the observed habitat differences in colouration (specifically chroma) in 1-year birds. Rather, it is likely that the habitat differences arise after fledging, that is during the post-fledging moult in autumn and reflect temporal variation in environmental conditions (diet and carotenoid availability) during that period. We cannot discount the possibility that observed differences in chroma between urban and forest 1-year birds are the result of differences in population demographics (Hörak et al., 2001). Indeed, in the same population, we have previously found evidence for selective disappearance of 'low-quality' birds between fledging and recruitment (Salmón et al., 2017).

Nonetheless, juvenile birds usually moult faster than adults and are less efficient in absorbing or utilising dietary carotenoids (Ferns & Hinsley, 2008; Hill, 2002). While this could lead to paler plumage in birds in their first year, compared with older birds, in both urban

and forest habitats, the effect might be exacerbated in the urban environment where carotenoid availability is limited compared to the forest and resources often remains less abundant after the breeding season (Jensen et al., 2022; Pollock et al., 2017; Seress et al., 2018). Although we found habitat differences in colouration in 1-year birds, these did not persist among birds of 2 years or older. Adult birds could outcompete juveniles (e.g. Dingemanse & de Goede, 2004) and secure sufficient resources even if scarce, thus relaxing habitat differences in colouration in older birds (Krams et al., 2010). However, the lack of differences in this age group could easily be blurred by age-related changes, since it is not possible to determine exact age of birds beyond their first year.

In conclusion, our multi-analytical study—combining empirical data and a meta-analysis—exemplify that a core chromatic trait of carotenoid-based colouration is consistently paler in response to urbanisation in a model system in avian urban ecology. Yet, as previously predicted (Szulkin et al., 2020), our study confirms that spatial and temporal differences exist among, and within, populations, likely the result of heterogeneity in the urban landscape, as well as the variable contribution of distinct urban stressors such as pollution. Furthermore, the observed differences in colouration seem to be driven by factors acting after fledging, rather than conditions experienced in the nest. Carotenoid signals are proposed as reliable biomarkers of population health (e.g. in birds, Peneaux et al., 2021), although their link with individual quality is often species specific (Weaver et al., 2018). Despite some attempts to establish the nature of the relationship between carotenoids (and other pigment colouration) and individual quality (Hörak et al., 2001; Rodewald et al., 2011), we still lack replicated studies linking plumage and individual performance in urban scenarios. Future longitudinal work should seek to understand if habitat variation in colouration results from processes such as selective disappearance of certain colour phenotypes or between-year changes in availability of carotenoid resources. Furthermore, there is a clear bias in the literature regarding the species and feeding guilds in which the 'urban dullness' phenomenon has been tested (Leveau, 2021), and studies across more diverse taxa and feeding guilds, beyond insectivorous birds, are required to address this bias. However, carotenoid levels in invertebrates seem to be reliable proxies of carotenoid levels in primary producers (Isaksson, 2009), and therefore it is likely that the links between carotenoid limitation and the urban dullness phenomenon would equally apply across frugivorous and granivorous birds. Indeed, although primarily insectivorous in spring and summer, great tits supplement their diet with seeds and fruit, yet urban-dwelling great tits still have dull carotenoid-based plumage traits. We believe that these present exciting avenues for future research to further our understanding of the eco-evolutionary implications of the 'urban dullness' phenomenon.

AUTHOR CONTRIBUTIONS

David López-Idiáquez, Pablo Salmón and Hannah Watson conceived the study. Caroline Isaksson, Javier Pérez-Tris, Pablo Salmón and Hannah Watson collected the feather samples. David López-Idiáquez

analysed the feather colouration. Data analysis was carried out by Pablo Salmón, Pablo Capilla-Lasheras conducted the meta-analysis, assisted by David López-Idiáquez and Pablo Salmón. Pablo Salmón and Hannah Watson drafted the initial manuscript with input from all other authors.

ACKNOWLEDGEMENTS

We thank everyone who assisted during the field work and provided logistical support, especially, Sweden: Andreas Nord; Madrid: ringers from La Herrería and Jose Ignacio Aguirre de Miguel; Milan: Michelangelo Morganti and the Lago di Pusiano ringers; Lisbon: Vítor Encarnação and the Pardal family. Also, we thank Claire Doutrelant for access to the spectrophotometer.

CONFLICT OF INTEREST STATEMENT

All the authors declare not having any competing interest.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.70rxwcd1v> (Salmón et al., 2023).

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SUPPORTING INFORMATION

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

Supplementary methods. Avian visual model.

Figure S1. Variation in breast plumage hue colouration.

Figure S2. Preferred reporting items for systematic reviews and meta-analyses (PRISMA).

Figure S3. Variation in breast plumage colouration among adult great tits in five urban and forest population pairs.

Figure S4. Chromatic contrasts (dS) of the breast plumage colouration of urban and forest great tit.

Table S1. Summary of sampling locations.

Table S2. Summary of sample sizes for each analysis.

Table S3. Summary table of the systematic review of studies linking breast plumage colouration and anthropogenic disturbances, including direct or indirect actions, e.g., pollution, urbanisation, or habitat fragmentation, on great tits (*Parus major*) and used in the meta-analysis.

Table S4. Description of moderators used in the meta-analytic models testing for the association between anthropogenic disturbance (i.e., urbanisation and pollution) and great tit breast plumage colouration and carotenoids.

Table S5. Summary of linear mixed models exploring the variation in breast plumage colouration among adult great tit in relation to habitat.

Table S6. Summary of linear mixed models exploring the variation in breast plumage colouration among nestling great tit in a between-habitat cross-fostering experiment in a single urban/forest population pair (Malmö).

Table S7. Summary of linear mixed models exploring the variation in breast plumage colouration across great tit age categories in relation to habitat in a single urban/forest population pair (Malmö).

Table S8. Summary of the multilevel meta-analytic mixed-effect models.

How to cite this article: Salmón, P., López-Idiáquez, D., Capilla-Lasheras, P., Pérez-Tris, J., Isaksson, C., & Watson, H. (2023). Urbanisation impacts plumage colouration in a songbird across Europe: Evidence from a correlational, experimental and meta-analytical approach. *Journal of Animal Ecology*, 92, 1924–1936. <https://doi.org/10.1111/1365-2656.13982>