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Environmental constraints can explain clutch size differences between urban and forest blue tits (*Cyanistes caeruleus*): insights from an egg removal experiment



Mark Pitt · B.Sc. Hons Ecology ·

Submitted in fulfilment of the requirements for the Degree of Master of Science (R) Ecology

School of Biodiversity, One Health, and Veterinary Medicine
College of Medical, Veterinary and Life Sciences
University of Glasgow

Supervisors: Dr Davide Dominoni, Dr Jelle Boonekamp, Dr Pablo Capilla-Lasheras

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Abstract

Urban environments are expanding globally, presenting novel ecological challenges to which species might not be well adapted. Understanding whether species responses to urban living are adaptive or maladaptive is critical to predicting the future impacts of urbanisation on biodiversity. Urban breeding birds exhibit reduced reproductive investment (clutch size) compared to neighbouring non-urban populations. However, whether this reduction is an adaptive response or a result of physiological constraints is unclear. Here, I investigated the ability of urban and forest blue tits (*Cyanistes caeruleus*) to lay new eggs following an egg removal manipulation. Consistent with the constraint hypothesis, egg removal did not induce urban females to lay replacement eggs. Meanwhile, forest birds laid approximately two replacement eggs after egg removal. Additionally, I found that the size of the replacement eggs from forest females declined over the lay sequence. Hatchlings from experimental nests had a smaller body mass in both habitats, with smaller hatchlings having a reduced probability of survival. Furthermore, as urban blue tits did not lay replacement eggs, egg removal resulted in a brood reduction in the city and nestlings from urban experimental nests had higher survival than those from urban control nests. Overall, my results suggest cities place constraints on egg production in urban birds. Urban females may experience energetic or nutrient limitations that restricts egg formation and/or exacerbates the trade-off between survival and egg production. Additionally, females may be misjudging urban habitat quality, due to time constraints when laying, and produce a clutch too large to be sustained in the city.

Key words: Urbanisation · Clutch size · Breeding constraints · Adaptation

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1. Introduction

Urban land coverage is rapidly expanding, increasing from 450.97 thousand km² in 1990 to 747.05 thousand km² in 2010, and now represents nearly 1% of the global land surface (Liu *et al.*, 2018; Chen *et al.*, 2020). Urban environments have a distinct set of ecological features, characterised by increased ambient temperatures (Kim, 1992; Hibbard *et al.*, 2017), a high abundance of non-native species (McKinney, 2008; Blouin *et al.*, 2019), increased habitat fragmentation (van Bohemen, 1998; Concepción *et al.*, 2015), increased pollution (light, chemical, and noise) (Dorado-Correa *et al.*, 2016; Isaksson, 2018) and changes in the quality, composition, and availability of food (Fehlmann *et al.*, 2021; Fenoglio *et al.*, 2021; Jensen *et al.*, 2022). Urban environmental conditions create novel ecological and evolutionary pressures to which species might not be well adapted, potentially compromising the persistence of wildlife globally. Understanding adaptive and/or maladaptive biological changes associated with urbanisation is, therefore, crucial to determine the current and future impact of urbanisation on biodiversity.

Studies on birds, which are among the best-studied organisms in urban ecology and evolution, provide robust evidence for a reduction in reproductive investment (i.e., clutch size) and success in urban breeding populations compared to their non-urban counterparts (Chamberlain *et al.*, 2009; Wawrzyniak *et al.*, 2015; de Satgé *et al.*, 2019; Capilla-Lasheras *et al.*, 2022). For example, in great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*), the clutch sizes of urban breeding birds tend to be 10-20% smaller than those nesting in neighbouring forests (Gładalski *et al.*, 2015; Bailly *et al.*, 2016; Branston *et al.*, 2021). Meta-analyses that include multiple species also provide evidence for smaller clutch sizes in urban populations compared to neighbouring non-urban populations (Chamberlain *et al.*, 2009; Sepp *et al.*, 2018; Capilla-Lasheras *et al.*, 2022). However, it remains unclear whether the small clutches of urban birds represent a constraint imposed upon females by the urban environment when laying or reflect an adaptation to city living.

Cities could impose constraints on birds during egg production, limiting the female's ability to invest in a larger clutch (constraints hypothesis; Perrins, 1970; Nur, 1987; Ramsay and Houston, 1997; Mänd *et al.*, 2007; Shultz *et al.*, 2009). Egg

production is metabolically demanding, with the energy required for egg-laying ranging between 13-41% above the basal metabolic rate in passerines (Carey, 1996; Ward, 1996; Nilsson and Råberg, 2001). For example, the basal metabolic rate of great tits increases by 22% during ovarian follicle development (Vezina and Williams, 2002). In small passerines that produce large clutches, the resources required for egg production far exceed what females can store endogenously (Perrins, 1996; Graveland, 1996; Mejer and Drent, 1999). Therefore, birds need to source the energy and nutrients for egg formation (including proteins, antioxidants, omega-3 polyunsaturated fatty acids, and calcium), from their diet when laying, with invertebrates often being the most nutrient-rich food items (Ramsay and Houston, 1988; Graveland and Drent, 1997; Biard *et al.*, 2005; Andersson *et al.*, 2015). Habitat fragmentation, non-native plant species, pollution, and increased pesticide use in the city reduces the quality and quantity of invertebrate prey (Narango *et al.*, 2018; Fenoglio *et al.*, 2021; Jensen *et al.*, 2022). City birds may attempt to compensate for the reduced availability of invertebrates by exploiting the abundant and predictable human-provisioned food from refuse and bird feeders (Pollock *et al.*, 2017). However, anthropogenic food, despite being energy-rich, is nutritionally poor and contains limited proteins, antioxidants, and omega-3 polyunsaturated fatty acids (Toledo *et al.*, 2016; Isaksson, 2018). Thus, nutrient-constrained urban birds may be already laying at their maximum, being unable to source sufficient resources to invest in the formation of additional eggs that result in viable offspring. Under the constraint hypothesis, the observed clutch size does not maximise the female's fitness payoffs (via maximising offspring recruitment and female survival). If females were freed from the constraint, they would immediately upregulate egg production (Mänd *et al.*, 2007; Shultz *et al.*, 2009).

Alternatively, the small clutches of urban birds may be an adaptive response to city living. Here, rather than being constrained by the available resources when laying, birds may be using environmental cues (e.g., food availability) when making clutch size decisions to predict the conditions the brood will experience later in the reproductive cycle (adaptive hypothesis; Lack, 1954; Ewald and Rohwer, 1982; Perrins, 1991; Arnold, 1994; Nager *et al.*, 1997; Millon *et al.*, 2008). Therefore, the small clutch sizes of urban birds may be an adaptive response to match the number of young the parents can adequately provision given the

reduced high-quality food available in the local environment (Lack, 1947; Lack, 1954; Senar *et al.*, 2021). Previous research reveals that urban birds have smaller broods and fledge fewer offspring than their non-urban counterparts (Peach *et al.*, 2008; Chamberlain *et al.*, 2009; Baily *et al.*, 2016). Small clutch sizes could be an adaptation to city living, allowing urban parents to invest more resources into fewer nestlings, thereby maximising the number of offspring recruited (Sepp *et al.*, 2018). Although large clutches result in more offspring, this may increase sibling competition over access to resources (Nilsson and Svensson, 1996; Nilsson and Gårdmark, 2001). Therefore, offspring from large urban broods could be more likely to be malnourished, with limited survival prospects (Godfray *et al.*, 1991). If the small urban clutch sizes maximises the female's fitness payoffs in a given habitat, any deviation from this observed clutch size should result in fewer offspring recruited into the population (Haywood, 1993; Kennedy, 1991).

To determine whether clutch size is constrained by the environment when laying or is adapted to maximise the number of offspring recruited, previous studies have removed eggs from the clutch and observed the re-laying ability of females. These egg removal experiments reveal that some species (known as indeterminate layers) can replace eggs in response to egg removal (Kennedy, 1991). In indeterminate layers, if the observed clutch size is adapted to maximise the number of offspring recruited, then females should lay replacement eggs following egg removal in an attempt to match the optimal clutch size for the habitat (Haywood, 1993b; Monaghan and Nager, 1997). Alternatively, if laying females are constrained during laying (e.g., by diet quality as explained above), then this should limit the number and size of replacement eggs produced in response to experimental egg removal (Visser and Lessells, 2001; Stevenson and Bryant, 2000; Williams and Miller, 2003; Mänd *et al.*, 2007). Only a few studies have compared if females differentially respond to egg removal between environments and none have done so in the urban habitat.

Here, I investigate the constraint and adaptive strategy hypotheses to explain differences in clutch size between urban and forest populations of blue tits. Blue tits evolved as a cavity-nesting forest species but have readily colonised urban environments (Cramp and Perrins, 1993; Stenning, 2018). Due to their prevalence in cities, willingness to use provisioned nest boxes, and ability to tolerate human

disturbance, blue tits are an ideal study system for investigating how urbanisation influences reproductive decisions. Blue tits are moderately indeterminate layers, tending to lay replacement eggs in response to egg removal (Kennedy, 1991; Haywood, 1993a; Stenning, 2018; Bründl *et al.*, 2019). Thus, the number of eggs that females produce can be experimentally manipulated by removing eggs from the clutch before the female initiates incubation. Previous research on great tits reveals females lay two replacement eggs following the removal of the first four eggs (Oppliger *et al.*, 1996; Visser and Lessells, 2001). Blue tits are close relatives of great tits and, if they are not immediately constrained, should exhibit a similar response to egg removal (Kennedy, 1991; Haywood, 1993b).

Specifically, I removed the first four eggs laid in city and forest breeding blue tit nests and observed how this manipulation affected the number and quality of eggs laid. Subsequently, I examined how egg removal influenced offspring quality and their fledging probability. If differences in clutch size between habitats are caused by constraints on egg production, then I predict that, following the egg removal manipulation, urban females should lay fewer and smaller additional eggs (and, therefore, produce smaller hatchlings) than forest females. However, fledging probability and growth rates should be higher in experimental nests than in control nests if females do not replace the removed eggs, as, here, egg removal would also result in a brood reduction. Under the adaptive hypothesis, I predict that urban and forest females should lay a similar number of additional eggs of equal size following the egg removal manipulation, with no difference in hatchling body mass, nestling growth rates, and fledging probability between treatment groups and habitats. However, I predict that the number of offspring fledged will be higher in control nests in both habitats if the observed clutch size reflects an adaptive strategy.

2. Methods

2.1 Study populations

I monitored one urban and one forest nest-box population of blue tits in Scotland during the breeding season of 2022 (Figure 1a; breeding season: April 1st to June 30th; nest-box: made with woodcrete, 260H × 170W × 180D, hole diameter: 32mm, Schwegler, Germany). The urban site is a city centre park in Glasgow (Figure 1b;

Kelvingrove Park, N=28 nest-boxes included in the current study, coordinates = [55.869, -4.2851]). In Kelvingrove, the environment contains open land, and small shrubs, with tree species comprising non-native species, including Turkey oak (*Quercus cerris*), laurel (*Laurus nobilis*), and ornamental cherry (*Prunus spp.*). The forest site is located at Loch Lomond, 40km north of Glasgow, and is an ancient native oak woodland (Figure 1c; Scottish Centre for Ecology and the Natural Environment (SCENE), N=33 nests included in the current study, coordinates = [56.129, -4.6145]). The dominant tree species at SCENE include oak (*Quercus spp.*), birch (*Betula spp.*), and sycamore (*Acer pseudoplatanus*) (Capilla-Lasheras *et al.*, 2017; Pollock *et al.*, 2017; Jarett *et al.*, 2020).

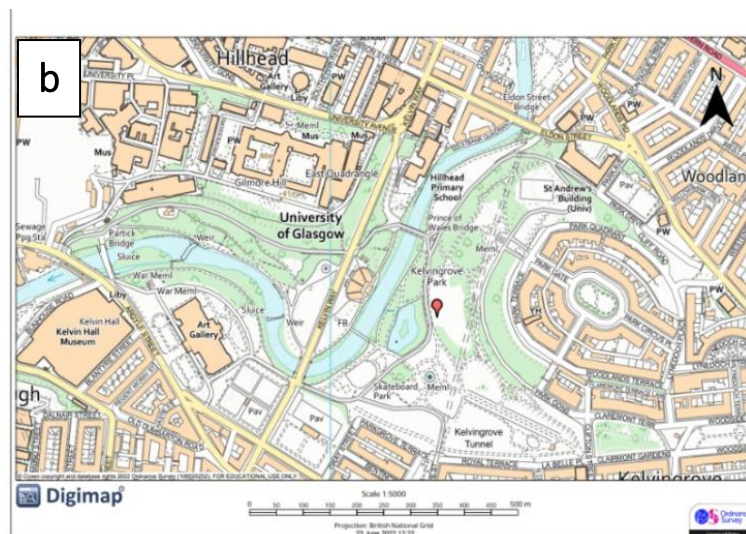
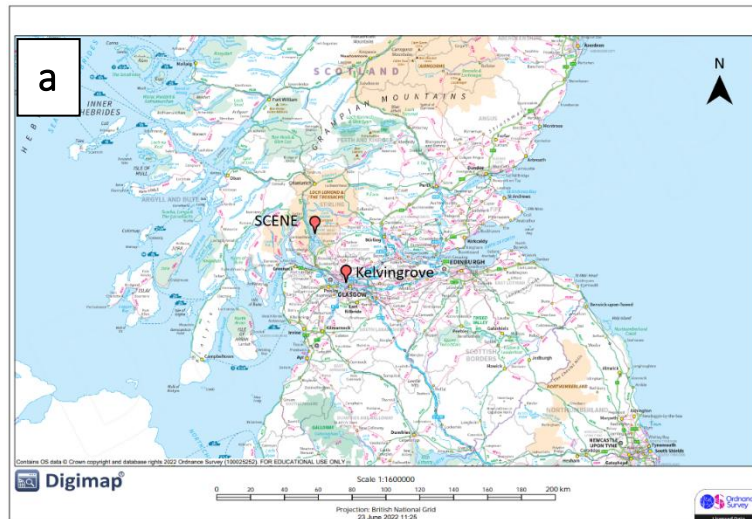


Figure 1. a) Map of Scotland, United Kingdom showing the location of the sampling sites (red tags). The sampling locations included b) Kelvingrove park, Glasgow, and c) SCENE, Loch Lomond. Maps from Digimap.edina.co.uk.

2.2 Experimental design

2.2.1 Assignment of treatment groups

From April 1st, I visited nest-boxes twice weekly to monitor nest-building activity. I increased the frequency of visits to every two days once the blue tits started lining the nest-cup to ensure I accurately recorded the first egg date. I visited nests after 11:00 to ensure sufficient time for the blue tit to lay (in passerines, most egg-laying occurs shortly after sunrise (McMaster *et al.*, 2004)). Once a new clutch was detected, it was alternatively assigned to the control or experimental group, following a 1:1 ratio to reduce the risk of any differences in phenology occurring between treatment groups. For nests included in the study, I found no difference in the first egg-laying date between treatment groups ($\chi^2_{df=1}=0.124$, $P=0.725$). In all nests, once a new egg was found, I marked eggs using a non-soluble marker pen, with a number corresponding to the eggs position in the laying sequence. I photographed every egg (used to calculate egg volume; see section 2.2.3) in both control and experimental nests, including a measuring chart in each photograph. Control nests had no eggs removed, with the photographing and marking of eggs being the only time I disturbed control females during egg production (Figure 2). In experimental nests, I removed the first four eggs from the nest on the morning they were laid. Eggs removed from the experimental nests were also weighed ($\pm 0.010g$) using digital scales. At the urban site, I included 14 control and 14 experimental clutches. At the forest site, I included 17 control and 16 experimental clutches.

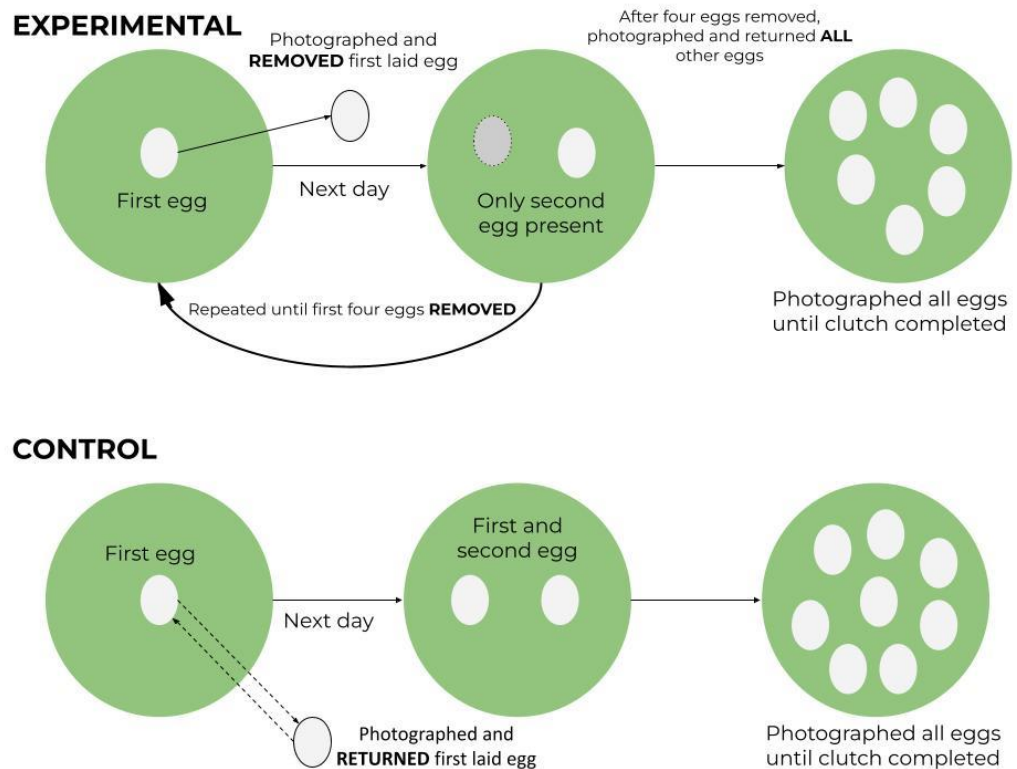


Figure 2. Overview of the treatment groups. The first four eggs were photographed, marked, and removed from experimental nests on the day they were laid. After the fourth egg was removed, all subsequent laid eggs remained in the nest. No eggs were removed from control nests, with eggs only being handled when photographed and marked.

When the female stopped laying for two days, and the eggs were warm to touch, I assumed the female had completed the clutch and started incubating (Womack *et al.*, 2022). Assuming a minimum incubation length of 14 days from clutch completion (Nord and Nilsson, 2011), 13 days after clutch completion, I started nest-box visits every two days to record the exact hatch date.

2.2.2 Cross-fostering of nestlings

I cross-fostered nestlings among nest-boxes two-days after the nestlings had hatched, within and between habitats and treatment groups, to account for variation in nestling body mass and survival stemming from genetic differences or early-life maternal effects, and the environment (Salmón *et al.*, 2018). I paired nests based on their earliest hatching date and brood size. As the mean brood size differed across habitats and treatment groups, I prioritised pairing nests across habitats by hatch date. Where the brood sizes in a pair of nests differed, I swapped the number of nestlings corresponding to half of the smaller brood. For example, if a forest nest had seven nestlings and the urban nest had five, I swapped two

nestlings. To select the nestlings to be cross-fostered, I placed all nestlings in the brood in a plastic box. Nestlings to cross-foster were then blindly selected to remove observer bias. Observer bias was still apparent and cross-fostered nestlings were heavier than their foster siblings ($\chi^2_{df=1} = 13.995$, $P < 0.001$). However, this observer bias was habitat independent (habitat \times cross foster group interaction: $\chi^2_{df=1} = 1.465$, $P = 0.226$). I marked the flanks and thighs of all two-day old nestlings with non-toxic marker pens to identify individuals until they were fitted with a ring (on day six of their life). I placed the nestlings to be cross-fostered in a plastic box (19.3cm \times 13.2cm \times 6cm) lined with cotton wool, underneath which there were three small (6 \times 9cm) heat packs (HotHands®, KOBAYASHI, Osaka, Japan) to keep the nestlings warm during transit. I activated the heat packs 60 minutes before cross-fostering, as the temperature of the heat packs peaked 60 to 240 minutes after activation. I placed the plastic box containing nestlings into a thermal bag to carry nestlings during transportation. The time taken to cross-foster chicks between habitats never exceeded more than 90 minutes. I cross-fostered 16 nest-boxes between habitats and ten nest-boxes within habitats (four within city cross-fosters and six within forest cross-fosters). Nestlings were weighed (± 0.1 g) on days two (before cross-fostering), six, and 12 after hatching. On day six, nestlings were fitted with a British Trust for Ornithology (BTO) metal ring with a unique ID number to establish individual identities. I also measured tarsus and wing lengths (± 0.1 mm) 12 days after hatching. Nest-boxes were checked >21 days after hatching to identify and record dead and fledged nestlings.

2.2.3 Egg volume measurements

I used egg volume as a proxy of egg size, which reflects the level of pre-natal investment into the young before incubation and may dictate offspring quality at hatching (Williams, 1994; Krist, 2011). I used an Olympus TG-6 digital camera to photograph eggs in the field. I photographed eggs on a 20 \times 20 cm measuring chart at a 90° angle to the egg's long axis, and there were no adjustments to lens distortion. I photographed each egg four times, rotating the egg along its long axis between photographs. As blue tits exhibit substantial intra-specific variation in egg shape (Nilsson and Svensson, 1993), commonly used methods to estimate volume, such as Hoyt's (1976) equation ($Volume = k \times length \times breadth^2$), are difficult to generalise across different study populations (Boersma and Rebstock,

2010). Hoyt's equation uses k (a species-specific variable), egg length, and breadth to estimate egg volume but assumes that the shape of the measured egg exactly matches that of the test population (Bridge *et al.*, 2007). Therefore, to calculate egg volume, I used IMAGEJ and the egg measurement plugin developed by Troscianko, (2014). For a given length along the eggs long axis, IMAGEJ calculated the radius (r) of the egg using equation 1.

$$1) \quad r = \frac{ae^{\frac{-l^2}{2b^2} + \frac{cl}{b^2} - \frac{c^2}{2b^2}} \sqrt{1-l\sqrt{l}}}{\pi b}$$

Where a is the width (mm) of the egg, b is the spread of distribution (the standard deviation in a normal distribution), c is where the peak of the distribution is located along the egg's length (the mean in a normal distribution), and l is the length of the egg (mm). Changing b alters the spread of the egg's shape, with smaller values creating a more pointed egg. Altering c changes where the widest part of the egg is located, with a value of 0.5 placing the widest part of the egg along the centre of the long axis. The plugin uses a least-squares function to fit the model to the shape of the egg and requires anchor points around the outer edge of the egg in the image. Using the multipoint selection tool in IMAGEJ, I selected 12 anchor points around the edge of the eggs surface, making sure I selected the tip and base of the egg (Figure 3). For each egg, I calculated volume from three separate images, producing three volume measurements per egg. In total, 1677 images of 559 eggs were analysed. For each egg, I took the mean of the three volume measurements, resulting in one mean volume measurement per egg. I used the R package *rptR* v.0.9.22 (Stoffel *et al.*, 2017) to quantify the repeatability of the volume measurements calculated in IMAGEJ (see 2.3.3.2). The repeatability of the volume measurements between images was low (*repeatability [95% Confidence Interval 'CI'] = 0.347 [0.279, 0.434]*). However, individual females exhibited consistent egg size within clutches (*repeatability [95% CI] = 0.527 [0.414, 0.616]*) and mean egg volume across the three images per egg was strongly correlated with egg mass (using removed eggs, $N = 86$ eggs, Pearson's

correlation coefficient r [95% CI] = 0.748 [0.637, 0.828]), confirming that our measurement of egg volume was capturing biologically relevant variation.

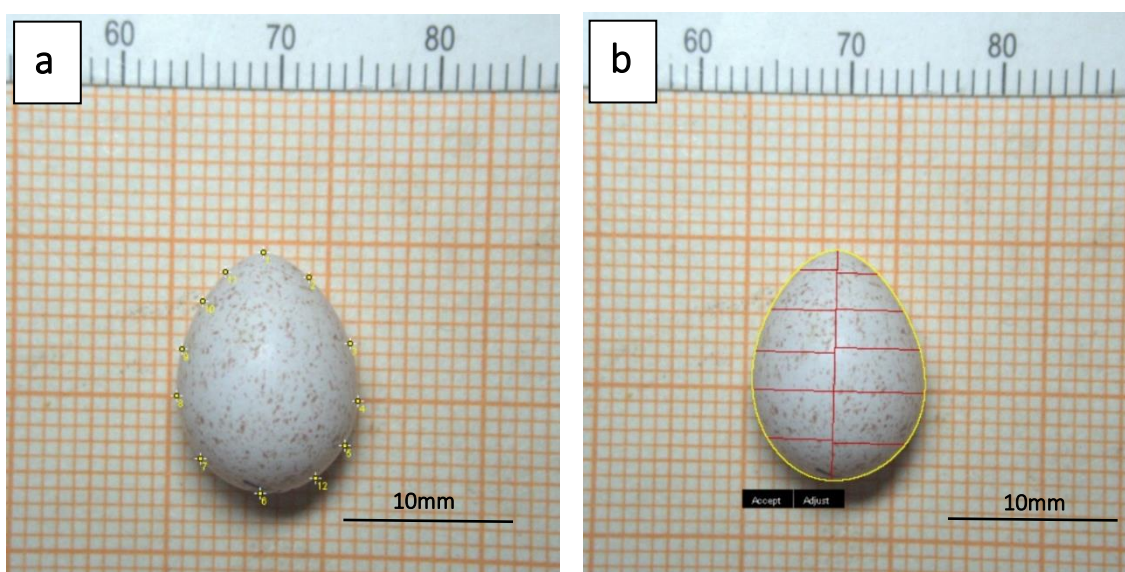


Figure 3. Egg shape fitting. a) 12 anchor points were placed around the edge of the blue tit egg using the multipoint selection tool in IMAGEJ. b) The egg shape model generated from the anchor points.

2.3 Data analysis

2.3.1 General modelling procedure

I performed all statistical analysis and data visualisation in Rstudio v.1.4.1106 (RStudio Team, 2022) using R v.4.2.0 (R core Team, 2022). I used the R package *lme4* v.1.1 (Bates *et al.*, 2015) to build linear models and generalised and linear mixed models to explain variation in the investigated reproductive traits (see below). I initially built a global model for each reproductive trait, containing all explanatory variables and interactions with the potential to explain variation in that trait. Using the package *lmtest* v.0.9 (Zeileis and Hothorn, 2002), I used likelihood-ratio tests (LRTs) to determine the statistical significance of each model predictor. To test the statistical significance of each term in the global model, I sequentially dropped predictors from the model and compared the model without the focal predictor against the global model using LRTs. I then derived final models by backwardly eliminating non-significant interactions. I did not apply model simplification of single effect predictors. In several models (see 2.3.2), I mean centred continuous predictors. In all models, date (either hatch date, first egg date, or laying date) was expressed as the number of days since the 1st of January. Date was initially fitted as both a quadratic and linear term before

quadratic terms were dropped from all the models if not significant. I used the R package *performance* v.0.9.1 (Lüdtke *et al.*, 2021) to assess the normality of residuals, homogeneity of variance, and the collinearity between fixed effects. I used the package *ggplot2* v.3.3.6 (Wickham, 2016) to visualise the model predictions and raw data.

2.3.2 Global models

2.3.2.1 Number of eggs laid

I used linear models to explain variation in the number of eggs laid by a female, including the first egg-laying date (mean-centred) and the habitat (2-level factor, “urban” versus “forest”) × treatment group (2-level factor, “experimental” versus “control”) interaction as fixed effects. A total of 61 clutches were initially included in the analyses, with nine clutches removed from the final dataset because the female abandoned the clutch before incubation and I could not determine if the clutch was completed. I then created additional post-hoc within-habitat models for the city and forest, including the first egg-laying date (the day the first laid egg was recorded in the nest; mean-centred) and treatment group as fixed effects. There were 20 additional clutches external to the experiment in the forest (hereafter referred to as “super controls”) which could be added as controls to the first analysis to increase the model’s ability to detect an interaction effect. However, unlike the control nests which were visited daily during the laying period, the super control nests were only visited weekly when the female was laying. Therefore, to determine whether the level of disturbance caused any differences in clutch size between super control and control groups, I created another within-habitat model, including the treatment group (2-level factor: “control”, or “super controls”) and first egg-laying date as fixed effects. In the city, all blue tit clutches were included in the experiment and there were no additional nests available to use as super controls. Finally, as there were no differences in clutch size between super control and control nests in the forest, I created a post-hoc model where the super control and control treatments were grouped into one control group. The first egg-laying date (mean-centred) and the habitat × treatment group (“super control + control”, or “experimental”) interaction were included as fixed effects in the final post-hoc model.

2.3.2.2 Repeatability of the egg volume measurements

I used the R package *rptR* v.0.9.22 to quantify the repeatability of the volume measurements calculated in IMAGEJ. To estimate repeatability, I used a mixed effects model framework, including habitat as a fixed effect. Clutch ID (a 70-level factor) and the unique identifier given to each egg (egg ID: a 559-level factor) were included as random effect intercepts. Here, repeatability was calculated for egg ID (consistency of the volume measurements per egg) and clutch ID (consistency of the volume measurements per clutch [i.e., nest-box], equivalent to how consistent egg size was within an individual female). For experimental nests, where the first four eggs laid were weighed, I used Pearson's correlation coefficient r to test the strength of the relationship between egg mass and egg volume.

2.3.2.3 Egg volume

To determine whether there were differences in egg volume between habitats and treatment groups, I initially built a linear mixed model with egg volume as a response variable and the following explanatory variables: the total number of eggs laid by each female (mean-centred), egg-laying date (the exact day each egg was laid; mean-centred), and the interaction between habitat and treatment group. Clutch ID (a 70-level factor) was included as a random effect intercept. Additionally, I tested whether the egg removal treatment differentially affected the total volume of eggs produced by a female between habitats. I calculated the total volume of egg produced by a female by taking the mean egg volume per nest box and multiplying it by the number of eggs laid. I then used a linear model to analyse the relationship between the total volume of egg material produced and the following explanatory variables: first egg laying date (mean-centred), and the interaction between habitat and treatment group. Finally, I created within-habitat models for the city and forest, with the total volume of eggs produced as the response variable and the first egg-laying date (mean-centred) and treatment group as fixed effects.

2.3.2.4 Variation in egg volume throughout the laying sequence

I then created two additional models testing the effect of laying order (fitted either as a categorical or continuous variable) on egg volume. In small, income-breeding birds, laying order influences egg composition and size (Ojanen *et al.*, 1981; Slagsvold *et al.*, 1984), and egg investment is therefore functionally biased within the clutch (D'Alba *et al.*, 2010). Therefore, to account for the effect of laying order on egg volume, I included eggs one to nine in the laying sequence, as only three city birds produced a clutch larger than nine eggs. I first divided eggs into two laying order groups: eggs one to three; or eggs four to nine. Evidence suggests blue tits acquire the resources for egg formation daily (Ramsay and Houston, 1997; Meijer and Drent, 1999); however, in small passerines the yolk rings for the first three eggs develop two to three days before the first egg is laid (Schifferli, 1980). Therefore, females may not recognise the effect of the egg removal treatment until the fourth egg in the lay sequence, after having synthesised the yolks for the first three eggs before the experimental manipulation. The global linear mixed model for the effect of laying order group on egg volume included the following explanatory variables: egg laying date (mean-centred), the total number of eggs laid by each female (mean-centred), and the three-way interaction between laying order group (2-level factor), habitat, and treatment group. I then created an additional model testing the effect of laying order (fitted as a continuous variable) on egg volume to determine if the investment into egg quality changes over the lay sequence. The global linear mixed model contained egg volume as the response variable and egg laying date (mean-centred), the total number of eggs laid by each female (mean-centred), and the three-way interaction between the position of the egg in the lay sequence, habitat, and treatment group as fixed effects. I included Clutch ID (a 70-level factor) as a random effect in both volume models.

2.3.2.5 Nestling body mass

I used a linear mixed model to determine whether egg removal differentially affected nestling body mass between habitats two-days after hatching. As previous research suggests there may be a relationship between egg size and nestling body mass at hatching (Williams, 1994; Smith and Bruun, 1998), the habitat or treatment group with the largest eggs should also produce heavier

nestlings at hatching. I included nestling body mass two days after hatching (the first-time nestlings were weighed) as the response variable with the number of siblings (equivalent to the number of hatched eggs; mean-centred), hatch date (mean-centred), time of day (2-level factor: “morning” or “afternoon”), and the two-way interaction between habitat and treatment group as fixed effects. Clutch ID (a 58-level factor) was included as a random effect.

I also investigated whether nestling body mass later in the nestling-rearing phase depended on the treatment group, habitat, and nestling’s age (2-level factor: “six” and 12-days after hatching). I created a linear mixed model with nestling body mass as the response variable and the following explanatory variables: the number of siblings (mean-centred), hatch date (mean-centred), time of day, the three-way interaction between habitat of rearing, treatment group in the nest of rearing, and nestling age, the site of hatching (before cross-fostering), and the treatment group in the nest of hatching (before cross-fostering). The nestlings ring number (a 354-level factor), the clutch ID of hatching (a 58-level factor), and the clutch ID of rearing (a 58-level factor) were included as random effect intercepts.

2.3.2.6 The effect of hatchling body mass on nestling survival

To assess whether nestling body mass two days after hatching influenced the probability of a nestling surviving until fledging, the nestling’s survival probability (i.e., the probability that a nestling was alive or dead at each measured time-point; 2-level factor: “fledged” or “dead”) was included as a response variable in a binomial generalised linear mixed model. I included the following explanatory variables: hatch date (mean-centred), number of siblings (mean-centred), time of day, and the three-way interaction between the treatment group, nestling body mass two days after hatching, and habitat. I included the clutch ID of hatching (a 58-level factor) and clutch ID of rearing (a 58-level factor) as random effect intercepts.

2.3.2.7 Nestling survival

To test the effect of habitat, treatment group, and age on nestling survival, I created two separate models: one with the number of nestlings as the response variable, the other with the proportion of nestlings over brood size as the response

variable. I used a linear mixed model to determine whether the effect of the treatment group on the number of nestlings depended on habitat and nestling age (4-level factor: “day two”, “six”, “12”, or “fledged”). I included the number of nestlings as the response variable while the number of eggs incubated (mean-centred), hatch date (mean-centred), and the three-way interaction between nestling age, habitat, and treatment group were included as fixed effects. I modelled the probability of nestling survival (i.e., the probability a nestling was either alive or dead at each measured time-point) using a binomial generalised linear mixed model. Survival probability was included as the response variable while hatch date (mean-centred) and the three-way interaction between nestling age, habitat, and treatment group were included as fixed effects. Clutch ID (a 58-level factor) was included as a random effect intercept in both models.

3. Results

3.1 The effect of egg removal on the female’s ability to replace eggs

Initially, I found no differential effect of egg removal on the female’s ability to re-lay eggs between habitats, represented by the absence of a location \times treatment group interaction ($\chi^2_{df=1}=2.069$, $P=0.150$; Appendix 1). However, visually, there was a clear difference in the ability of experimental and control females to replace the removed eggs in the urban and forest habitats (Appendix 2), suggesting that the lack of statistical support of the interaction may have been due to the small sample size. Following this, I created within-habitat models for the city and forest. In the city, treatment group had no effect on the number of eggs laid (model estimate \pm SE=0.472 \pm 0.594 eggs, $\chi^2_{df=1}=0.701$, $P=0.403$; Appendix 3). In the forest, birds in the experimental group laid more eggs than those in the control group (model estimate \pm SE=1.632 \pm 0.600 eggs, $\chi^2_{df=1}=7.267$, $P=0.007$; Appendix 4). I then compared the number of eggs laid between control, experimental, and super control nests external to the experiment in the forest. I found no difference in the number of eggs produced by control and super control nests (model estimate \pm SE=-0.681 \pm 0.450 eggs, $\chi^2_{df=1}=2.409$, $P=0.121$; Appendix 5). As there were no differences in the number of eggs produced between control and super control nests, I grouped super controls as controls in the forest. After grouping, I found a significant interaction between treatment group and location ($\chi^2_{df=1}=4.132$, $P=0.050$; Table 1, Figure 4). Here, forest experimental females laid

approximately two more eggs than forest control females (Figure 4). Meanwhile, experimental and control females did not differ in the number of eggs they laid in the city (Figure 4). In both habitats, the number of eggs laid declined over the breeding season ($\chi^2_{df=1}=24.498$, $P<0.001$; Table 1).

Table 1. a) Likelihood-ratio tests (LRT) for the following predictors explaining the number of eggs produced: the treatment group (“control + super control” or “experimental”) × habitat interaction and first egg date. Significant P-values are highlighted in bold. “df” = degrees of freedom for the LRTs. Superscripts “1” and “2” refer to linear and quadratic terms, respectively. **b)** Global model coefficients from the linear mixed model of the effects of the following predictors on the number of eggs laid: habitat × treatment group interaction and first egg date. The standard errors and 95% confidence intervals (CI) are provided for each model coefficient. N=81 clutches.

Number of eggs produced				
a) Likelihood-ratio test results				
<i>Predictors</i>	χ^2	<i>df</i>	<i>P-value</i>	
Habitat	-	-	-	
Treatment group	-	-	-	
1st egg laying date¹	24.498	1	<0.001	
1 st egg laying date ²	0.000	1	0.996	
Treatment group × Habitat	4.132	1	0.050	
b) Model coefficients from global model				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Control group in the urban habitat)	8.106	0.424	7.261	8.950
Treatment group: <i>Experimental</i>	0.532	0.589	-0.641	1.701
Habitat: <i>Forest</i>	0.819	0.492	-0.161	1.800
1st egg laying date¹	-0.161	0.031	-0.223	-0.099
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i>	1.509	0.757	0.001	3.017

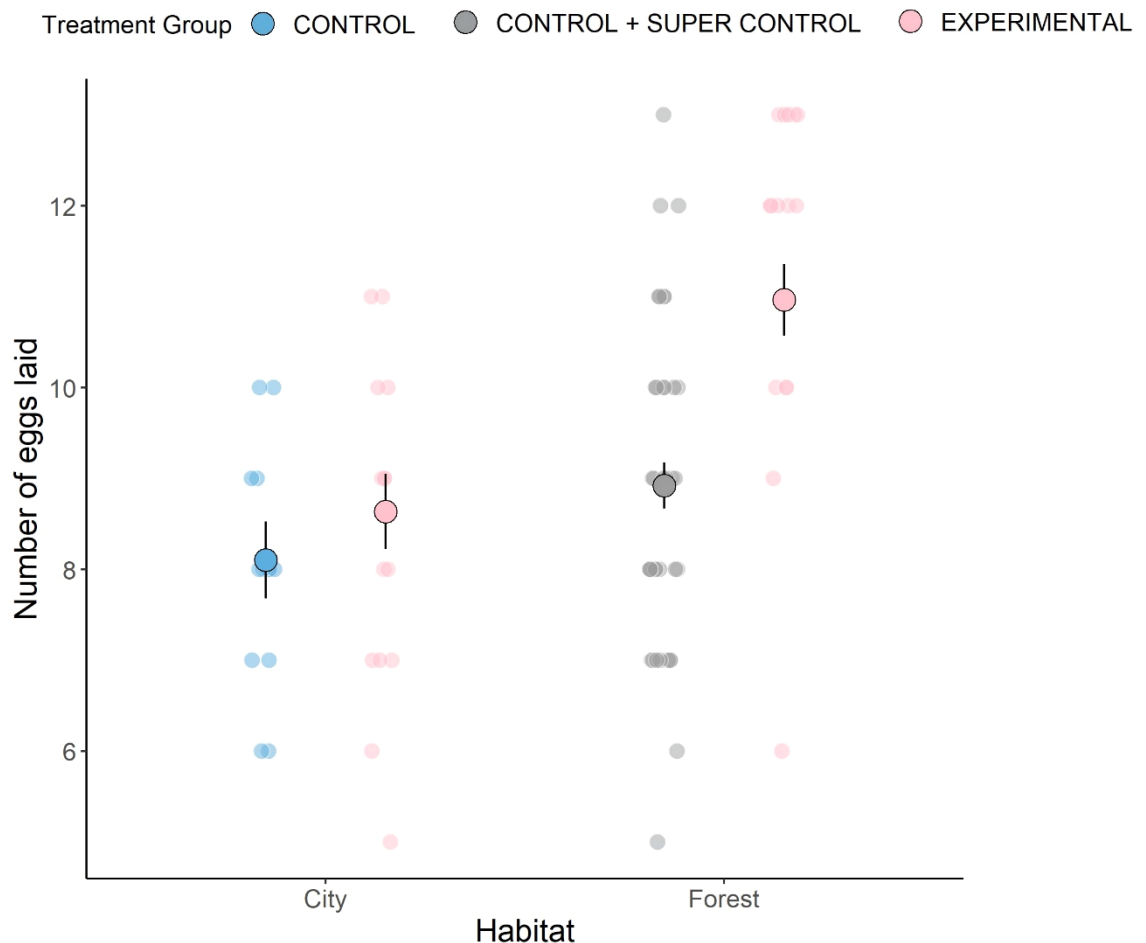


Figure 4. The effect of egg removal on the number of eggs laid by the females in the city and the forest. Large dots represent model predictions \pm standard error. Small dots are the raw data points. N=81 clutches.

3.2 The effect of egg removal on egg size and total egg investment

Egg volume did not depend on the treatment group \times habitat interaction when laying order was not included in the model ($\chi^2_{df=1}=2.154$, $P=0.341$; Appendix 6). Additionally, when fitted as single effects (in contrast to when they were included in the interaction), habitat ($\chi^2_{df=1}=3.460$, $P=0.063$; Table 2, Figure 5a) and treatment group ($\chi^2_{df=1}=0.016$, $P=0.899$; Table 2, Figure 5a) did not affect egg volume when laying order was not included in the model. The total volume of egg material produced (mm^3) did not depend on the interaction between location and treatment group ($\chi^2_{df=1}=1.149$, $P=0.284$; Table 3, Figure 5b, Appendix 7). However, as per analysis 3.1, this may be an artefact of the small sample size reducing statistical power. When I created within-habitat models, there was no difference in the total volume of eggs produced between the city treatment groups ($\chi^2_{df=1}=0.578$, $P=0.447$; Appendix 8). Meanwhile, experimental females produced

a greater total volume of eggs than forest control females in the forest ($\chi^2_{df=1}=5.310$, $P=0.021$; Appendix 9).

Table 2. a) Likelihood-ratio tests (LRT) of following predictors explaining egg volume: the treatment group \times habitat interaction, number of eggs laid, and egg laying date. Clutch ID was included as a random effect. Significant P-values are highlighted in bold. **b)** Model coefficients from the minimal linear mixed model of the effects of the following predictors on egg volume: habitat, treatment group, the number of eggs laid, and egg laying date. The standard errors and 95% confidence intervals (calculated by running 500 parametric bootstrap simulations of the model) are provided for each model coefficient. N=460 eggs.

Egg volume (mm ³)				
a) Likelihood-ratio test results				
<i>Predictors</i>	χ^2	<i>df</i>	<i>P-value</i>	
Habitat	3.460	1	0.063	
Treatment group	0.016	1	0.899	
Number of eggs laid	1.377	1	0.241	
Egg laying date¹	9.713	1	0.001	
Egg laying date ²	1.658	1	0.198	
Habitat \times Treatment group	2.154	1	0.341	
b) Model coefficients				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Control group in the urban habitat)	1379.235	28.031	1328.813	1434.894
Treatment group: <i>Experimental</i>	3.200	25.139	-43.492	48.566
Habitat: <i>Forest</i>	-56.522	29.914	-114.639	0.747
Number of eggs laid	-8.266	6.989	-21.415	3.645
Egg laying date¹	-4.130	1.316	-6.410	-1.728
Random effects				
<i>Groups</i>	<i>Variance</i>	<i>Standard deviation</i>		
Clutch ID	8022	89.570		
Residual	6427	80.170		

Table 3. a) Likelihood-ratio tests (LRT) for the minimal model of the following predictors explaining the total volume of egg produced by a female: treatment group × habitat interaction, and first egg date. Significant P-values are highlighted in bold. **b)** Minimal model coefficients from the minimal linear model of the effects of the following predictors on total volume of egg: habitat, treatment group, and first egg date. The standard errors and 95% confidence intervals are provided for each model coefficient. N=61 clutches.

Total volume of egg (mm ³) produced by a female				
a) Likelihood-ratio test results				
<i>Predictors</i>	<i>x²</i>	<i>df</i>	<i>P-value</i>	
Habitat	9.110	1	0.003	
Treatment group	5.513	1	0.019	
1 st egg laying date ¹	11.763	1	0.001	
1 st egg laying date ²	0.392	1	0.531	
Treatment group × Habitat	1.149	1	0.284	
b) Model coefficients				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Control group in the urban habitat)	10944.660	569.510	9802.850	12086.462
Treatment group: <i>Experimental</i>	1397.430	602.230	190.038	2604.823
Habitat: <i>Forest</i>	1954.800	645.044	661.576	3248.024
1 st egg laying date ¹	-245.460	67.430	-380.649	-110.272

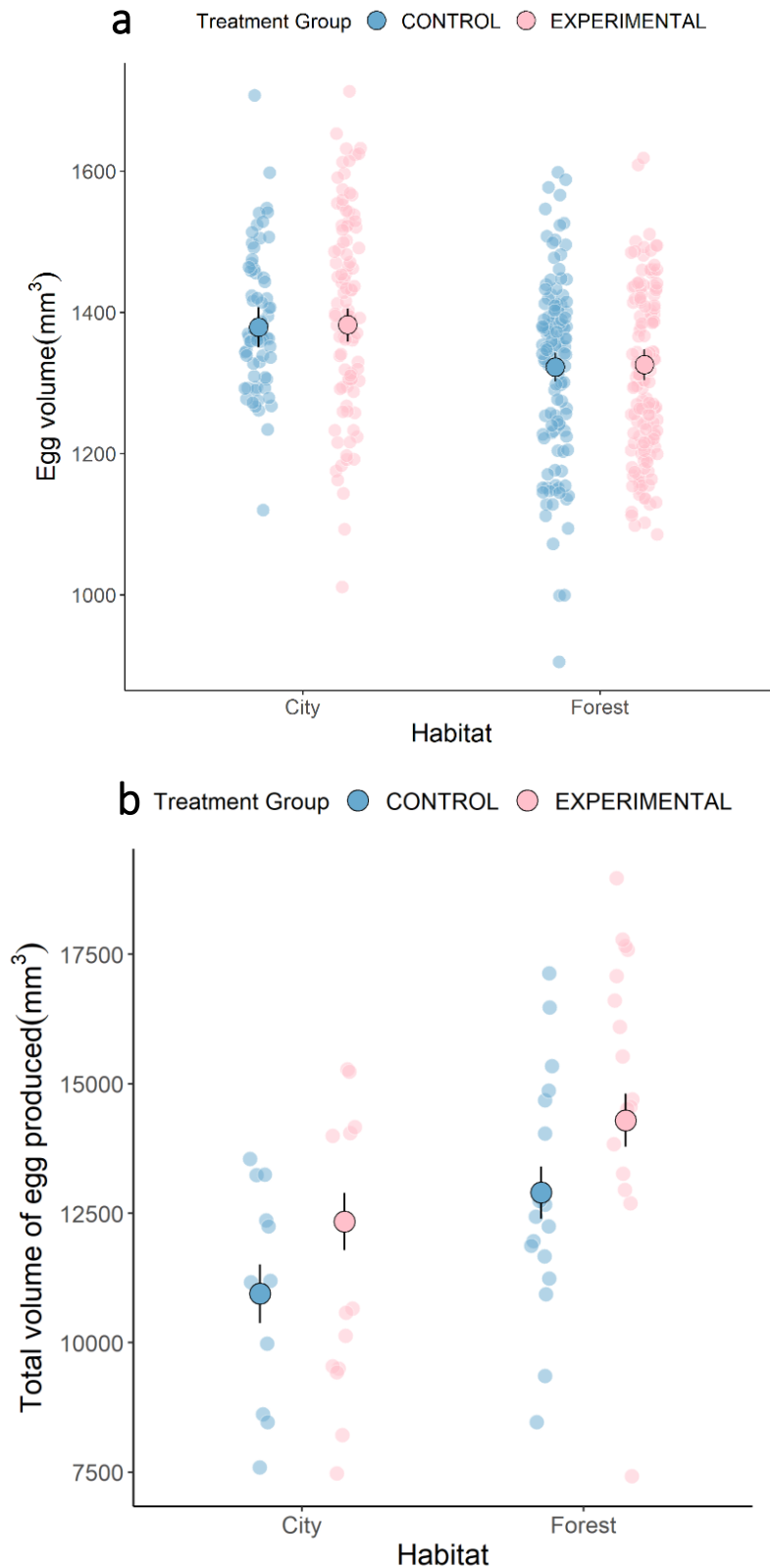


Figure 5. a) The effect of the treatment group on egg volume in the forest and city. Large dots represent model predictions from the minimal model \pm standard error. Small dots are the raw data points. $N=460$ eggs. **b)** The effect of habitat and treatment group on the total volume of egg material produced by a female. Large dots represent minimal model predictions \pm standard error. Small dots are the raw data points. $N=61$ clutches.

3.3 The effect of laying order on egg size

When eggs were grouped according to their laying order position (1. eggs one to three or 2. four to nine), there was no three-way interaction between the laying order group, treatment group, and habitat ($\chi^2_{df=1}=3.167$, $P=0.075$; Appendix 10). However, I found that egg volume depended on the two-way interactions between location and laying order ($\chi^2_{df=1}=10.435$, $P=0.001$; Table 4) and treatment group and laying order ($\chi^2_{df=1}=4.234$, $P=0.040$; Table 4). There was no difference in volume between habitats for eggs one to three in the lay sequence. However, eggs four to nine in the forest had a smaller mass than those in the city (Figure 6a). Additionally, eggs one to three were heavier in experimental nests than control nests. Egg volume for eggs four to nine in the lay sequence did not differ between treatment groups (Figure 6a).

I then further investigated the effect of laying order on egg volume by fitting laying order as a continuous variable. When laying order was fitted continuously, I found a three-way interaction between laying order, treatment group, and habitat ($\chi^2_{df=1}=3.923$, $P=0.047$; Table 5, Figure 6b). There was no difference in egg volume across the lay sequence and between treatment groups in the city, the same holds for control nests in the forest. Meanwhile, egg volume declined over the lay sequence in experimental nests from the forest (Figure 6b).

Table 4. a) Likelihood-ratio tests (LRT) for the following predictors explaining egg volume: the treatment group × habitat × laying order interaction, the number of eggs laid, and egg laying date. Clutch ID was included as a random effect. Significant P-values are highlighted in bold. b) Minimal model coefficients from the linear mixed model of the effects of the following predictors on egg volume: Habitat × laying order interaction, treatment group × laying order interaction, the number of eggs laid, and egg laying date. The standard errors and 95% confidence intervals are provided for each model coefficient. N=460 eggs.

Egg volume (mm ³)				
a) Likelihood-ratio test results				
<i>Predictors</i>	<i>x²</i>	<i>df</i>	<i>P-value</i>	
Habitat	-	-	-	
Treatment group	-	-	-	
Lay order group	-	-	-	
Number of eggs laid	1.308	1	0.253	
Egg laying date¹	14.835	1	<0.001	
Egg laying date ²	2.016	1	0.156	
Habitat × Lay order group	10.435	1	0.001	
Lay order group × Treatment group	4.234	1	0.040	
Habitat × Treatment group	0.224	1	0.636	
Habitat × lay order group × Treatment group	3.167	1	0.075	
b) Model coefficients				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Eggs 1-3 from the control group in the urban habitat)	1352.928	31.495	1274.162	1398.480
Treatment group: <i>Experimental</i>	44.034	29.647	-10.129	100.749
Habitat: <i>Forest</i>	-6.964	32.533	-70.808	57.376
Number of eggs laid	-8.415	7.314	-22.456	4.806
Egg laying date¹	-4.200	1.988	-8.113	-0.514
Lay order group: <i>Eggs 4-9</i>	35.642	14.149	15.660	94.948
Habitat: <i>Forest</i> × Lay order group: <i>Eggs 4-9</i>	-64.187	19.717	-102.952	-20.563
Treatment group: <i>Experimental</i> × Lay order group: <i>Eggs 4-9</i>	-40.355	19.557	-78.701	-2.362
Random effects				
<i>Groups</i>	<i>Variance</i>	<i>Standard deviation</i>		
Clutch ID	8366	91.470		
Residual	6218	78.850		

Table 5. a) Likelihood-ratio tests (LRT) for the following predictors explaining egg volume: the treatment group × location × laying order (fitted linearly) interaction, number of eggs laid, and egg laying date. Clutch ID was included as a random effect. Significant P-values are highlighted in bold. b) Global model coefficients and confidence intervals from the linear mixed model of the effects of the following predictors on egg volume: treatment group × location × laying order interaction, the number of eggs laid, and egg laying date. The standard errors and 95% confidence intervals (calculated by running 500 parametric bootstrap simulations of the model) are provided for each model coefficient. N=460 eggs.

Egg volume (mm ³)				
a) Likelihood-ratio test results				
<i>Predictors</i>	<i>x²</i>	<i>df</i>	<i>P-value</i>	
Habitat	-	-	-	
Treatment group	-	-	-	
Lay order	-	-	-	
Number eggs laid	1.214	1	0.271	
Egg laying date ¹	2.623	1	0.105	
Egg laying date ²	2.652	1	0.103	
Habitat × lay order × Treatment group	3.923	1	0.047	
b) Model coefficients				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (1 st laid egg in the control group in the urban habitat)	1370.765	31.116	1302.674	1419.384
Treatment group: <i>Experimental</i>	29.706	39.425	-43.373	100.577
Habitat: <i>Forest</i>	-43.267	38.702	-114.244	25.948
Number of eggs laid	-8.753	7.911	-23.959	4.230
Egg laying date ¹	-4.751	2.921	-10.754	0.025
Lay order	6.038	5.889	-5.904	15.780
Habitat: <i>Forest</i> × Lay order	-3.040	5.871	-14.330	6.857
Lay order × Treatment group: <i>Experimental</i>	2.282	6.183	-10.174	12.212
Habitat: <i>Forest</i> × Treatment group: <i>Experimental</i>	-22.915	51.531	-136.433	57.664
Habitat: <i>Forest</i> × Lay order × Treatment group: <i>Experimental</i>	-15.439	7.749	-30.467	-1.546
Random effects				
<i>Groups</i>	<i>Variance</i>	<i>Standard deviation</i>		
Clutch ID	9655	98.260		
Residual	6200	78.740		

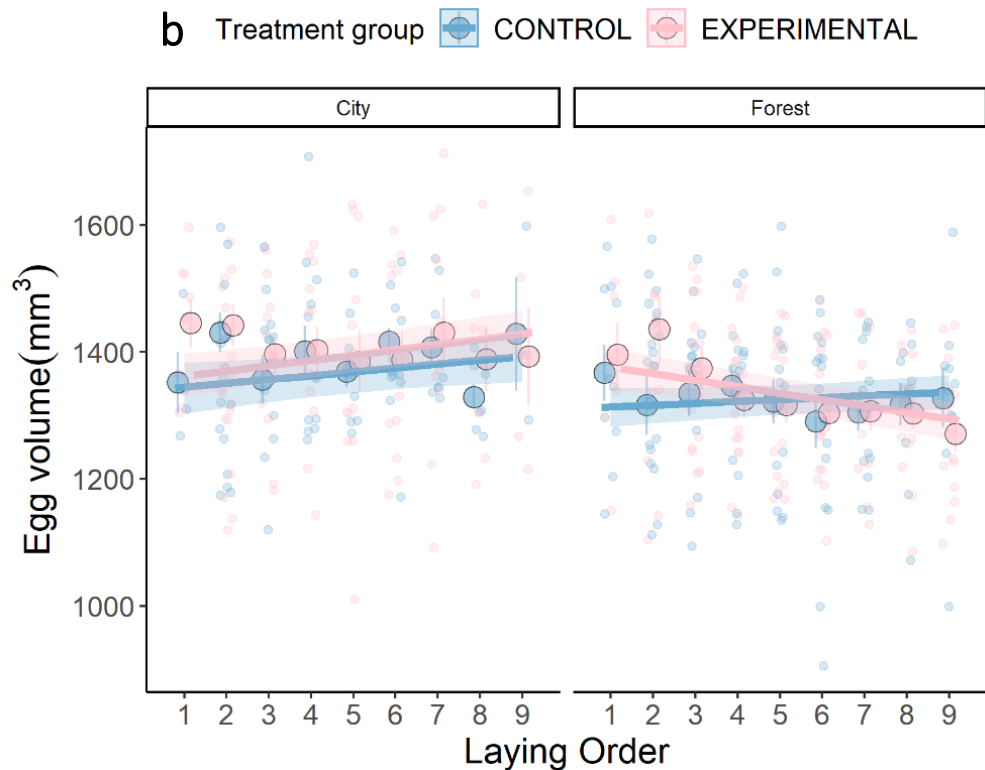
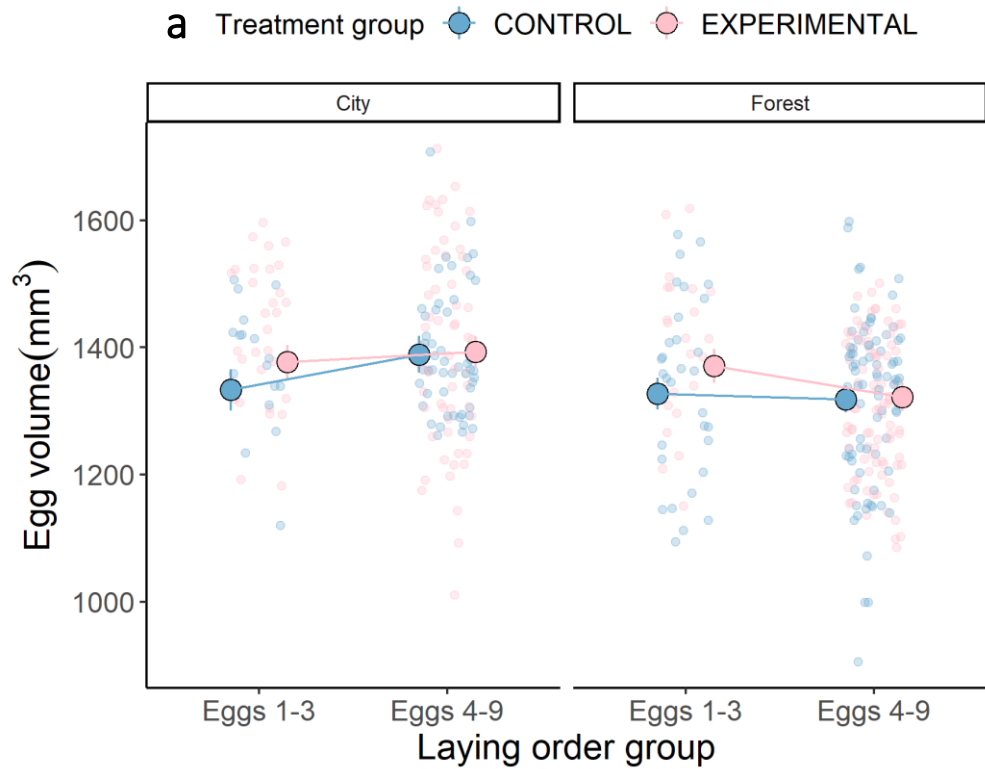


Figure 6. a) The effect of habitat, treatment group, and laying order group (1. eggs one to three or 2. eggs four to nine) on egg volume in the forest and city. Large dots represent model predictions from the minimal model \pm standard error. Small dots are the raw data points. $N=460$ eggs. **b)** The effect of the treatment group and laying order (eggs one to nine) on egg volume in the forest and city. Large dots represent raw means \pm standard error. Straight lines and ribbons represent model predictions \pm standard error. Small dots are the raw data points. $N=460$ eggs.

3.4 The effect of egg removal on nestling body mass

There was no differential effect of the egg removal treatment on nestling body mass two days after hatching between habitats, as represented by the absence of a treatment group \times habitat interaction ($\chi^2_{df=1}=0.132$, $P=0.717$; Appendix 11). However, I found that nestlings in experimental nests were smaller than those in control nests two days after hatching in both habitats ($\chi^2_{df=1}=4.117$, $P=0.042$; Table 6, Figure 4a). I found that the nestlings hatch date did not affect body mass ($\chi^2_{df=1}=0.182$, $P=0.670$; Table 6). Additionally, there were no differences in hatch date across habitats ($\chi^2_{df=1}=0.190$, $P=0.663$) and between treatment groups ($\chi^2_{df=1}=0.180$, $P=0.184$). On days six and 12 after hatching, there was an interaction between nestling age, treatment group, and habitat ($\chi^2_{df=1}=18.895$, $P<0.001$; Appendix 12). In the forest, there were no differences in nestling body mass between treatment groups on days six and 12, the same holds for both treatment groups in the city on day six. Meanwhile, in the city, nestlings in experimental nests were heavier than those in control nests 12 days after hatching. I found that the habitat and treatment group the nestling originated from (before cross-fostering on day two) did not affect nestling body mass (Habitat of hatching: $\chi^2_{df=1}=0.071$, $P=0.789$, Treatment group of hatching: $\chi^2_{df=1}=0.895$, $P=0.344$; Appendix 12). Additionally, nestlings weighed in the afternoon were heavier than those weighed in the morning ($\chi^2_{df=1}=20.035$, $P<0.001$; Appendix 12).

Table 6. a) Likelihood-ratio tests (LRT) for the following predictors explaining nestling body mass two days after hatching: the treatment group × habitat interaction, number of siblings in the nest, time of day, and hatch date. Clutch ID was included as a random effect. Significant P-values are highlighted in bold. **b)** Minimal model coefficients from the linear mixed model of the effects of the following predictors on nestling mass two days after hatching: treatment group, habitat, number of siblings in the nest, time of day, and hatch date. The standard errors and 95% confidence intervals (calculated by running 500 parametric bootstrap simulations of the model) are provided for each model coefficient. N=372 nestlings.

Nestling mass (g) two days after hatching				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Habitat	1.730	1	0.188	
Treatment group	4.117	1	0.042	
Hatch date ¹	0.182	1	0.670	
Hatch date ²	0.219	1	0.640	
Number of siblings	0.681	1	0.409	
Time of day	1.803	1	0.179	
Treatment group × Habitat	0.132	1	0.717	
b) Model coefficients from the minimal model				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Control group in the urban habitat in the morning)	1.912	0.091	1.749	2.059
Treatment group: Experimental	-0.252	0.121	-0.479	-0.045
Habitat: <i>Forest</i>	0.153	0.115	-0.057	0.324
Number of siblings	-0.023	0.028	-0.086	0.025
Hatch date	0.006	0.014	-0.021	0.028
Time of day: <i>Afternoon</i>	0.143	0.104	-0.086	0.318
Random effects				
Groups	Variance	Standard deviation		
Clutch ID	0.098	0.312		
Residual	0.156	0.395		

3.5 The effect of hatchling body mass on survival

I found no differential effect of nestling mass on survival two days after hatching between treatment groups and habitats, as represented by the absence of a three-way interaction between nestling body mass two days after hatching, treatment group, and habitat ($\chi^2_{df=1}=0.256$, $P=0.611$; Appendix 13). In both treatment groups and habitats, the likelihood of a nestling surviving until fledging increased with body mass two days after hatching ($\chi^2_{df=1}=10.647$, $P=0.001$; Table 7; Figure 7b).

Table 7. a) Likelihood-ratio tests (LRT) from the minimal model for the following predictors explaining the nestlings fate: the nestling body mass 2 days after hatching × treatment group × habitat interaction, number of siblings, time of day, and hatch date. Clutch ID of hatching and rearing were included as random effects. Significant P-values are highlighted in bold. **b)** Minimal model coefficients from the binomial generalised linear mixed model of the effects of the following predictors on the nestling's fate: nestling body mass 2 days after hatching, the treatment group, habitat, number of siblings, time of day, and hatch date. The standard errors and 95% confidence intervals are provided for each model coefficient. N=364 nestlings.

Fate (Dead or Fledged)				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Mass (g) at day 2	10.647	1	0.001	
Habitat	17.562	1	<0.001	
Treatment group	2.542	1	0.111	
Number of siblings	1.708	1	0.191	
Hatch date ¹	4.222	1	0.039	
Hatch date ²	2.296	1	0.130	
Time of day	3.537	1	0.060	
Mass (g) at day 2 × Habitat	1.631	1	0.202	
Habitat × treatment group	0.147	1	0.702	
Mass (g) at day 2 × treatment group	3.233	1	0.199	
Mass (g) at day 2 × treatment group × Habitat	0.256	1	0.611	
b) Model coefficients from the minimal model				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Control group in the urban habitat)	-4.123	0.573	-10.082	-1.223
Mass (g) at day 2	1.987	0.689	0.637	3.337
Treatment group: <i>Experimental</i>	3.196	2.005	-0.734	7.126
Habitat: <i>Forest</i>	7.660	2.491	2.777	12.543
Hatch date ¹	-0.522	0.262	-1.036	-0.007
Number of siblings	0.588	0.435	-0.219	1.600
Time of day: <i>Afternoon</i>	-3.285	1.762	-6.737	0.168
Random effects				
Groups	Variance	Standard deviation		
Clutch ID of hatching	5.764	2.401		
Clutch ID of rearing	21.369	4.623		

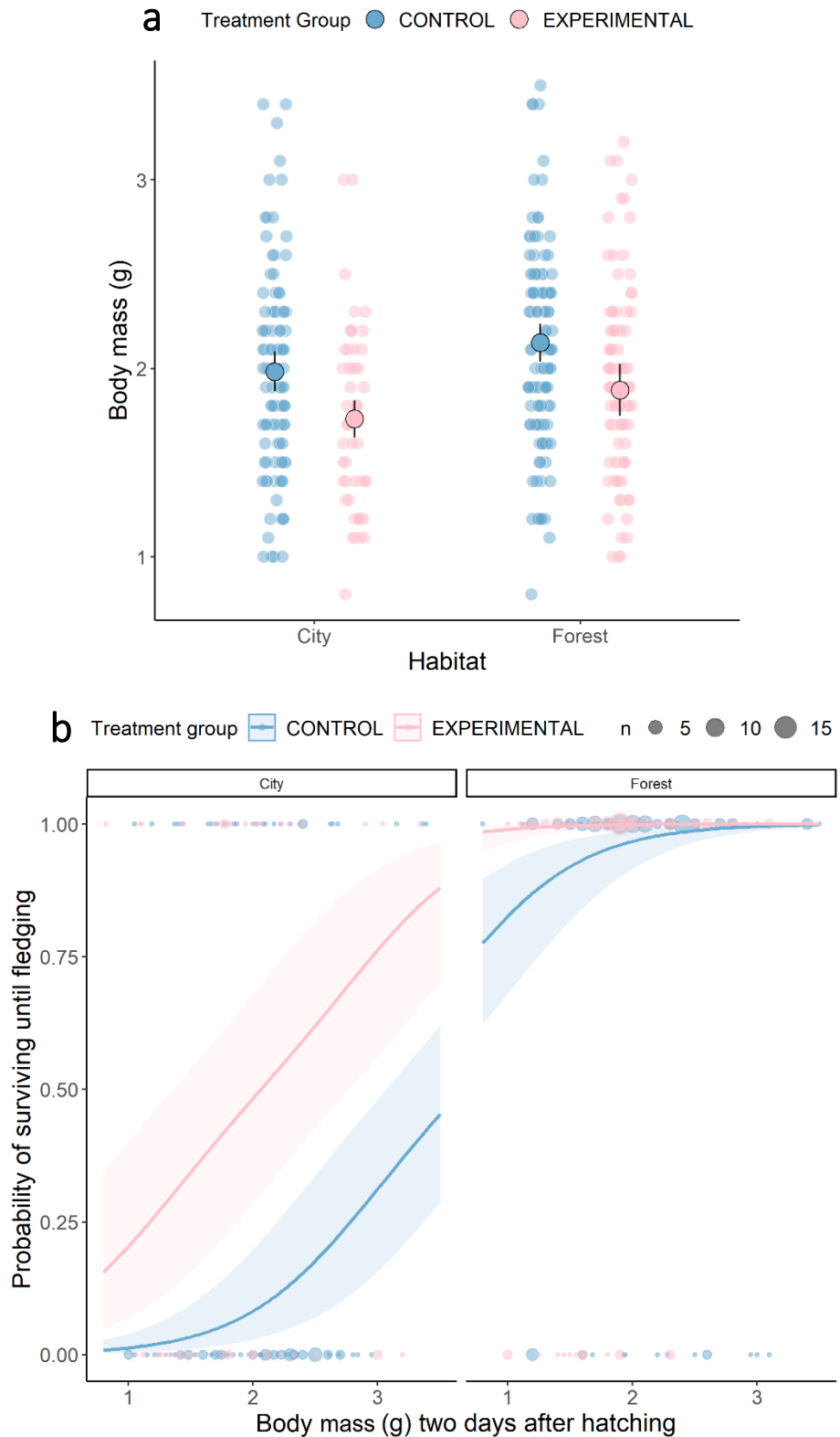


Figure 7.a) The effect of the habitat and treatment group on nestling mass (g) two days after hatching. Large dots represent minimal model predictions \pm standard error. Small dots are the raw data points. N=372 nestlings. **b)** The effect of nestling body mass two days after hatching on the probability of survival until fledging in both treatment groups and habitats of rearing. Lines and associated ribbons represent the minimal model predictions \pm standard errors. Small dots represent raw data points. Dot size indicates the number of nestlings of each body mass. N=364 nestlings.

3.6 The effect of egg removal on nestling survival

I found that the number of nestlings in the brood depended on the three-way interaction between treatment group, habitat, and nestling age ($\chi^2_{df=3}=18.530$, $P<0.001$; Table 8, Figure 8a). Control broods in the forest had more nestlings than experimental broods at each measured time point. Meanwhile, in the city, on days two and six after hatching, control broods had more nestlings in the brood than experimental nests. However, on day 12 and fledging, there was no difference between treatment groups in the number of nestlings in the city. Additionally, I found that nestling survival depended on the three-way interaction between treatment group, habitat, and nestling age ($\chi^2_{df=3}=8.761$, $P=0.034$; Table 9, Figure 8b). There was no difference in nestling survival between treatment groups across all time points in the forest. In the city, on days two to six after hatching, there was no difference in nestling survival. Meanwhile, on day 12 and fledging, nestling survival was higher in experimental nests than in control nests in the city.

Table 8. a) Likelihood-ratio tests (LRT) for the following predictors explaining the number of nestlings alive: the treatment group × location × age interaction, clutch size, and hatch date. Clutch ID was included as a random effect. Significant P-values are highlighted in bold. b) Global model coefficients from the linear mixed model of the effects of the following predictors on the number of nestlings alive: the treatment group × location × age interaction, clutch size, and hatch date. The standard errors and 95% confidence intervals (calculated by running 500 parametric bootstrap simulations of the model) are provided for each model coefficient. N=60 broods.

Number of nestlings alive				
a) Likelihood-ratio test results				
<i>Predictors</i>	χ^2	<i>df</i>	<i>P-value</i>	
Habitat	-	-	-	
Treatment group	-	-	-	
Age	-	-	-	
Clutch size	32.388	1	<0.001	
Hatch date ¹	0.642	1	0.423	
Hatch date ²	0.000	1	0.985	
Habitat × treatment group × Age	18.530	3	<0.001	
b) Model coefficients				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Control group in the urban habitat two days after hatching)	6.431	0.494	5.437	7.237
Treatment group: <i>Experimental</i>	-0.259	0.810	-1.844	1.029
Habitat: <i>Forest</i>	0.858	0.690	-0.539	2.086
Clutch Size	0.767	0.116	0.506	0.978
Hatch date	-0.041	0.051	-0.146	0.052
Age: <i>day 6</i>	-0.857	0.478	-1.762	0.033
Age: <i>day 12</i>	-3.786	0.478	-4.791	-2.869
Age: <i>fledged</i>	-5.214	0.478	-6.036	-4.414
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i>	-0.115	0.978	-2.068	1.442
Habitat: <i>Forest</i> × Age: <i>day 6</i>	0.607	0.655	-0.662	1.698
Habitat: <i>Forest</i> × Age: <i>day 12</i>	3.411	0.655	2.13	4.449
Habitat: <i>Forest</i> × Age: <i>Fledged</i>	4.180	0.661	2.783	5.194
Treatment group: <i>Experimental</i> × Age: <i>day 6</i>	0.011	0.689	-1.311	1.217
Treatment group: <i>Experimental</i> × Age: <i>day 12</i>	2.709	0.689	1.442	3.761
Treatment group: <i>Experimental</i> × Age: <i>Fledged</i>	3.291	0.689	2.059	4.289
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i> × Age: <i>day 6</i>	-0.361	0.943	-2.299	1.186
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i> × Age: <i>day 12</i>	-2.934	0.943	-4.869	-1.296
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i> × Age: <i>Fledged</i>	-3.313	0.953	-5.376	-1.521
Random effects				
<i>Groups</i>	<i>Variance</i>	<i>Standard deviation</i>		
Clutch ID	1.747	1.322		
Residual	1.603	1.266		

Table 9. a) Likelihood-ratio tests (LRT) for the following predictors explaining the proportion of nestlings alive: the treatment group × location × age interaction, clutch size, and hatch date. Clutch ID was included as a random effect. Significant P-values are highlighted in bold. b) Model coefficients from the binomial generalised linear mixed model the effects of the following predictors on nestling survival: the treatment group × location × age interaction, clutch size, and hatch date. The standard errors and 95% confidence intervals are provided for each model coefficient. N=60 broods.

Survival (Alive or Dead)				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Habitat	-	-	-	
Treatment group	-	-	-	
Age	-	-	-	
Hatch date ¹	0.375	1	0.540	
Hatch date ²	0.061	1	0.805	
Number of hatched eggs	8.154	2	0.017	
Habitat × treatment group × Age	8.761	3	0.034	
b) Model coefficients				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Control group in the urban habitat two days after hatching)	2.466	0.537	1.413	3.520
Treatment group: <i>Experimental</i>	1.056	0.910	-0.728	1.659
Habitat: <i>Forest</i>	0.066	0.813	-1.528	2.840
Hatch date ¹	-0.037	0.059	0.153	0.062
Number of hatched eggs	0.372	0.134	0.110	0.080
Age: <i>day 6</i>	-0.944	0.408	-1.744	-0.139
Age: <i>day 12</i>	-3.189	0.426	-4.025	-2.341
Age: <i>fledged</i>	-4.334	0.462	-5.240	-3.414
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i>	1.083	1.230	-1.327	3.426
Habitat: <i>Forest</i> × Age: <i>day 6</i>	0.544	0.607	-0.646	1.736
Habitat: <i>Forest</i> × Age: <i>day 12</i>	2.625	0.613	1.423	3.826
Habitat: <i>Forest</i> × Age: <i>Fledged</i>	3.032	0.627	1.802	4.262
Treatment group: <i>Experimental</i> × Age: <i>day 6</i>	-0.571	0.689	-1.327	0.762
Treatment group: <i>Experimental</i> × Age: <i>day 12</i>	1.352	0.698	-0.017	2.696
Treatment group: <i>Experimental</i> × Age: <i>Fledged</i>	1.416	0.728	-0.011	2.813
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i> × Age: <i>day 6</i>	-0.521	1.026	-2.532	1.493
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i> × Age: <i>day 12</i>	-2.278	1.028	-4.295	-0.252
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i> × Age: <i>Fledged</i>	-2.337	1.042	-4.379	-0.294
Random effects				
Groups	Variance	Standard deviation		
Clutch ID	2.452	1.566		

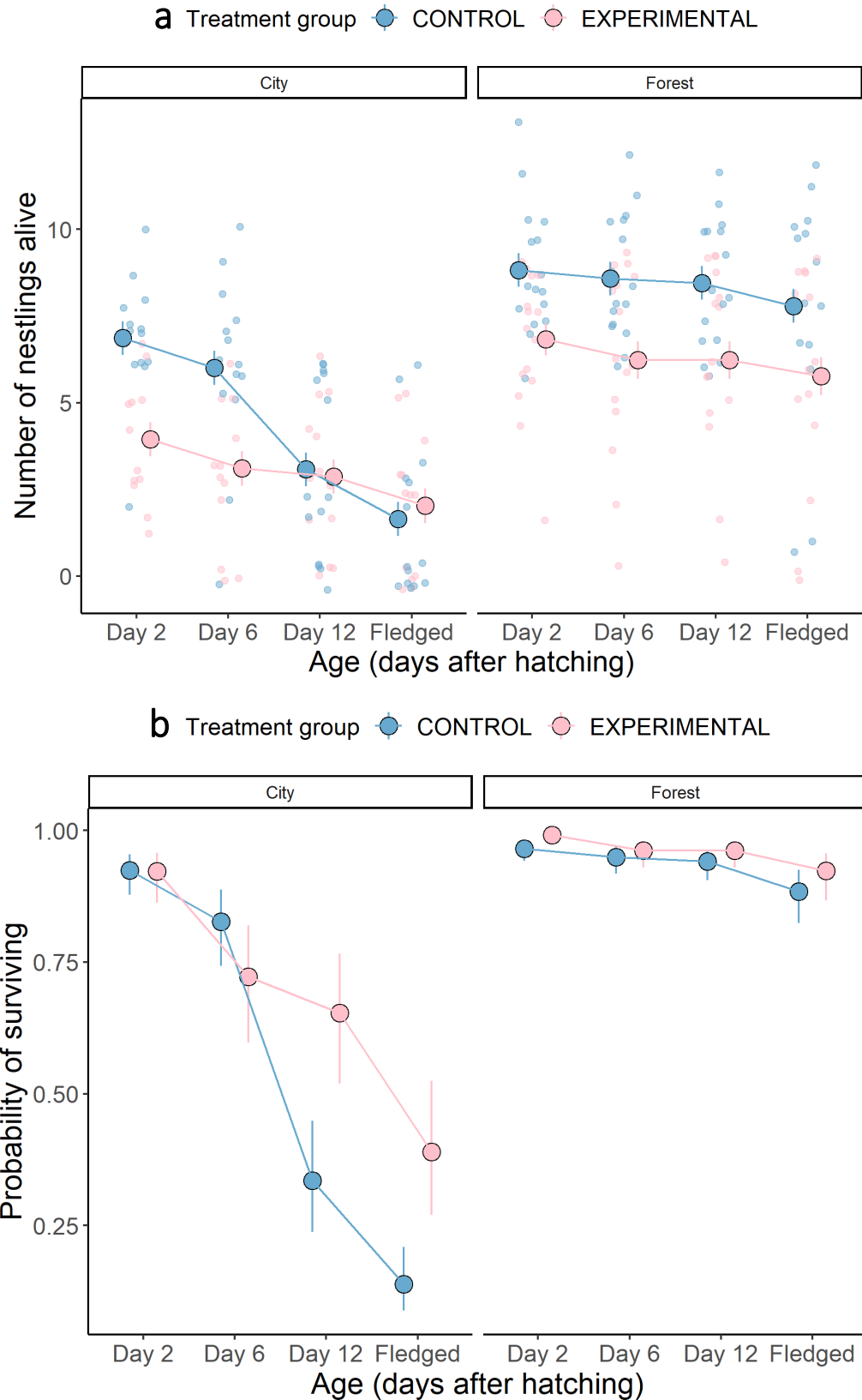


Figure 8. a) The effect of treatment group, habitat, and age on the number of offspring alive. Large dots and associated bars represent model predictions \pm standard errors. Small dots represent raw data points. N=60 broods. **b)** The effect of treatment group, habitat, and age on nestling survival. Large dots represent model predictions \pm standard error. N=60 broods.

4. Discussion

To test if the small clutches of urban birds reflected constraints imposed upon females by the urban environment when laying or represented an adaptation to urban living, I investigated the ability of females to replace eggs following egg removal in both the city and the forest. If the differences in clutch size between habitats were due to constraints on egg production, I predicted urban females would produce fewer or smaller eggs (and, subsequently, have offspring with a smaller body mass two days after hatching) than forest females following egg removal. Additionally, if females did not replace the removed eggs (with egg removal resulting in a brood reduction), nestling survival and growth rates should be higher in experimental than control nests, with this difference being more pronounced in the city. Meanwhile, under the adaptive hypothesis, the number and size of additional eggs (and offspring two days after hatching) produced after egg removal should have been similar between urban and forest females. Furthermore, there should be no difference in fledgling probability and growth rates between treatment groups and across habitats. However, the number of offspring fledged should be greater in control than experimental nests in both habitats if the observed clutch size maximises the female's fitness payoffs.

In line with the constraint hypothesis, I found that city birds did not replace the removed eggs. Meanwhile, forest birds produced an additional two eggs following egg removal. If urban blue tits were not limited in some way during egg production, then, as observed in the forest and previous studies, females should have laid additional eggs after egg removal to maximise the number of viable offspring produced (Haywood, 1993; Oppliger, 1997; Visser and Lessells, 2001; Mänd *et al.*, 2007). As urban females did not lay replacement eggs, there was no difference in egg volume across the lay sequence between city treatment groups. Conversely, in experimental forest nests, egg volume declined over the lay sequence. As predicted by the constraint hypothesis, this decline in egg size likely reflects the inability of laying females to maintain egg quality when egg production is experimentally upregulated (Haywood, 1993; Oppliger *et al.*, 1997; Visser and Lessells, 2001; Mänd *et al.*, 2007). Nestlings from experimental nests also had a smaller body mass than those in control nests in both habitats two days after hatching. In experimental forest nests, this reduced body mass may reflect

the female's inability to maintain offspring quality in response to increased laying effort. However, experimental nestlings also had a smaller body mass in the city, despite urban females exhibiting no decline in egg volume across the lay sequence. Even at this early-stage body mass is important, with heavier nestlings being more likely to fledge in both habitats.

In both habitats, experimental nests reared fewer offspring than control nests as I did not return the removed eggs before the female initiated incubation. Thus, in the forest, where females laid additional eggs, the increased costs of egg production are confounded with reduced brood-rearing costs. Meanwhile, there was no difference in the number of eggs laid between treatment groups in the city. Thus, any effect of egg removal on nestling growth rates and survival in the city is likely due to the brood reduction. As predicted by the adaptive strategy hypothesis, there were no differences in nestling survival and growth rate between forest treatment groups, but this was not the case in the city. Following the artificial brood reduction in experimental city nests, nestlings were heavier at day 12 and more likely to fledge than their control counterparts. Therefore, urban birds could be producing clutches that are too large to be sustained in the city.

Overall, my results suggest the environmental forces operating on clutch size differ between the city and the forest. Urban conditions are likely imposing constraints on females when laying, preventing increased investment in egg production, such as energy and nutrient limitations (Chamberlain *et al.*, 2009; Mägi *et al.*, 2009; Seress and Liker, 2015). Alternatively, the small clutches of city birds could be an adaptation to maximise the mother's survival if producing large clutches incur a fitness cost (i.e., sacrificing somatic maintenance or future fecundity) (Nager *et al.*, 2001; Ardia *et al.*, 2003; Bowers, 2012). I discuss these alternative explanations of my findings below, including the limitations of this research and future directions to further understand the evolution of clutch size in anthropogenic environments.

4.1 The effects of energy and nutrient limitation on egg production

The energetic costs associated with egg production may be increased in the city, preventing urban birds from replacing eggs after egg removal. As the environment and resources available to urban birds could be of low quality, city females may

differentially allocate energy between activities compared to forest birds. During the pre-laying period, individuals divide energy between nutrient acquisition, thermoregulation, territory defence, mate attraction, and egg production (Drent and Daan, 1980; Perrins and McCleery, 1989; Williams, 2005). Individuals cannot maximise their investment in all tasks, i.e., trade-offs occur between tasks (Stearns, 1989; Zera and Harshman, 2001). For example, the energy costs associated with thermoregulation may limit the energy allocated to egg production (Martin and Wiebe, 2004). In great tits, the ability to replace eggs following egg removal is temperature constrained, with females failing to upregulate egg production in years with lower spring temperatures (Visser and Lessels, 2001). Low temperatures increase the daily energy expenditure of females during egg laying as they expend more energy on thermoregulation (El-Wailly, 1966; Stevenson and Bryant, 2000; Salvante *et al.*, 2007). However, in my study it is unlikely temperature prevented urban birds from replacing eggs. Urban areas are generally 1-3°C warmer than surrounding natural areas due to the heat-island effect (Kim, 1992; Hibbard *et al.*, 2017). Thus, urban birds should spend less energy thermoregulating than forest birds due to the warm city climate. If anything, urban birds should have increased the energy allocated to egg production and laid replacement eggs.

Alternatively, urban blue tits may not upregulate egg production after egg removal due to being constrained by the poor nutrient quality of anthropogenic food. In small passerines that produce large clutches, the reserves required for egg production exceed what females can store endogenously (Gibb, 1950; Perrins, 1996; Graveland, 1996; Meijer and Drent, 1999). Thus, they must acquire the nutrients for egg production daily from their diet when laying (Ojanen *et al.*, 1988; Saino *et al.*, 2004; Ardia *et al.*, 2006). Breeding females need to source sufficient resources to deposit in the egg during this period, including sulphur-rich proteins (Ramsay and Houston, 1988; Murphy, 1994), antioxidants (Biard *et al.*, 2005; Müller *et al.*, 2012), fatty acids (Sanders, 1988; Andersson *et al.*, 2015), and calcium (Graveland and Drent, 1997; Bañbura *et al.*, 2010; Bañbura *et al.*, 2020). However, in the city, there could be lower availability of these resources when compared to the forest, as invertebrate prey may be less abundant (Senar *et al.*, 2021; Jensen *et al.*, 2022). Thus, urban females may expend more energy on nutrient acquisition than forest birds, limiting the energy they can allocate to egg

production (Anderies *et al.*, 2007; Meyrier *et al.*, 2017). Urban blue tits may compensate for the reduced natural food availability by exploiting abundant and predictable human-provisioned food (Pollock *et al.*, 2017; Isaksson, 2018). However, this food is nutritionally poor, with limited carotenoids, proteins, vitamins, and omega-3 polyunsaturated fatty acids (Isaksson and Andersson, 2007; Stofberg *et al.*, 2019; Sinkovics *et al.*, 2021). Whether calcium is limiting in urban contexts is debated and may be city dependent. Urban parks and gardens may have increased snail abundance due to the prevalence of non-native vegetation, while increased pesticide use and chemical pollution in cities could detrimentally impact snail populations (Bańbura *et al.*, 2010; Bańbura *et al.*, 2020). Overall, following egg removal, urban females may have been unable to source or invest sufficient high-quality resources in the production of additional eggs (Ricklefs, 1974; Nilsson, 1991; Houston *et al.*, 1995; Nager *et al.*, 1997).

Given females deplete endogenous stores of biomolecules by reallocating them to the egg, the mother may face a trade-off between allocating resources for somatic maintenance and egg production, which could underlie the costs of egg production (Nager *et al.*, 2001; Visser and Lessells, 2001; Zera and Harshman, 2001). During laying, females have increased susceptibility to oxidative stress (Wiersma *et al.*, 2004; Metcalfe and Monaghan, 2013), disease (Gustafsson *et al.*, 1994), and parasite infection (Oppliger *et al.*, 1996; Leivesley *et al.*, 2019), with parents investing more in egg production and having fewer resources to allocate to immune function (Oppliger *et al.*, 1997). The effects of nutrient limitation during egg laying on female condition and survival may have been operating differently between habitats. On one hand, the nutrient requirements for the female's somatic maintenance may be fulfilled in the forest. Thus, forest females may invest any additionally acquired resources into upregulating egg production at little cost to their survival. On the other hand, as nutrient limitation may be higher in the city (Bradley and Altizer, 2007; Robb *et al.*, 2008), urban females in poor condition could be selectively investing in somatic maintenance at the expense of egg quantity (Sandell *et al.*, 2007; Isaksson *et al.*, 2015; Toledo *et al.*, 2016; Pick *et al.*, 2016). Thus, after egg removal, urban females may not have sufficient nutrient reserves to form additional eggs, as doing so would reduce survival or future fecundity (Mänd *et al.*, 2007; Bowers, 2012).

4.2 The trade-off between clutch size and egg size

As the resources available to the breeding female are limited, individuals producing larger clutches may compensate by producing smaller eggs (Lack, 1967; van Noordwijk and de Jong, 1986; Enum and Fleming, 2000; Williams, 2001). However, I found that mean egg size did not differ between habitats, despite urban birds producing smaller clutches than their forest counterparts. Not all eggs are equal within the clutch, and the level of investment may be biased across the laying sequence, leading to intra-clutch differences in embryonic development, physiology, and nestling growth (Vinuela, 1997; Bourgault *et al.*, 2007; Mänd *et al.*, 2007; You *et al.*, 2009). In both treatment groups in the urban site, I found that egg volume exhibited no change over the lay sequence: the same holds for control clutches in the forest. Despite this, previous studies find egg size increases over the lay sequence in small passerines, reflecting increased protein content in the last laid eggs (Krist *et al.*, 2004; Mänd *et al.*, 2007), and this may compensate for the detrimental effects of asynchronous hatching (Slagsvold *et al.*, 1984; Hillström, 1999; Rosivall *et al.*, 2005). In experimental forest nests, egg volume declined over the lay sequence. These results are in line with previous studies finding females have a limited ability to maintain egg quality when laying above their usual clutch size (Nager *et al.*, 2000; Williams and Miller, 2003; Williams, 2005; Mänd *et al.*, 2007). Urban birds may be adaptively producing small clutches to maintain an equal investment across the laying sequence. If urban birds laid replacement eggs, this could detrimentally impact egg and offspring quality, which they may be unable to compensate for during nestling rearing due to the limited food availability for offspring provisioning in urban areas (Metcalf and Monaghan, 2001; Mänd *et al.*, 2007; Pollock *et al.*, 2017). Thus, in cities, females may terminate laying earlier than forest birds before they pass their critical physiological threshold where egg size and clutch size are traded off against each other (Hillström, 1999; Rosivall *et al.*, 2005; Mänd *et al.*, 2007). Meanwhile, forest blue tits might be able to upregulate egg production following egg removal, as they can compensate for any detrimental effects of egg size on offspring quality later in the reproductive cycle.

4.3 Egg size, nestling mass, and survival

As parents can directly affect their nestling's body mass by maximising investment in egg quality (Parsons, 1970; Sinervo, 1990; Enum *et al.*, 2004; Krist, 2009), one

may predict that an increase in egg size may be advantageous, especially in the city where resources are limited in the nestling-rearing phase, as large eggs should result in chicks with increased nutrient reserves that facilitate rapid growth after hatching (Lack, 1968; Hepp *et al.*, 1987; Williams, 1994; Krist, 2011; Capilla-Lasheras *et al.*, 2021). However, I found no difference in nestling body mass two days after hatching between the urban and forest sites, but experimental nestlings had a smaller body mass than control nestlings in both habitats. In experimental forest nests, where blue tits upregulated egg production, the smaller body mass of nestlings two days after hatching may reflect the reduced investment into the last laid eggs (Nager *et al.*, 2000). However, experimental nestlings were also smaller in the city, despite no difference in the number of eggs laid between city treatment groups. I did not return the removed eggs to the female in experimental nests, with experimental nestlings always coming from beyond egg four in the laying sequence. As the investment in the clutch is biased, even if egg volume did not differ across the lay sequence in the city, the nutrients required for nestling development may be allocated differentially between eggs (Bourgault *et al.*, 2007). Even though the protein content of the eggs may increase over the lay sequence, other nutrients may decrease, such as vitamin E and carotenoids (Hörak *et al.*, 2002; Valcu *et al.*, 2019). Thus, from egg five onwards in the laying sequence, the yolk content may be of lower quality than in eggs one to four, resulting in nestlings with a smaller body mass.

I found that the nestling's body mass two days after hatching predicted nestling survival, with heavier individuals more likely to fledge. Therefore, the level of pre-natal investment may dictate whether a nestling recruits into the population (Williams, 1994; Krist, 2009), especially in the city where nestling survival was lower than in the forest. This pattern is also observed in white-browed sparrow weavers (*Plocepasser mahali*), where variation in egg volume has fitness implications for the offspring, as nestling mass at hatching predicts survival until fledging (Capilla-Lasheras *et al.*, 2021). Nestlings that are heavier at hatching may have higher fitness prospects, with the increased nutrient reserves from the egg improving growth rates, boosting immunocompetence, and allowing them to be more competitive at feeding (Parsons, 1970; Anderson *et al.*, 1997; Blomqvist *et al.*, 1997; Dawson and Clark, 2000). A meta-analysis of 283 studies by Krist (2011) found that juvenile survival was correlated with egg size, and the effects of egg

size on survival persisted into the post-fledging stage. However, the benefits of large eggs may be confined to early in the post-hatching period as increased egg size does not always result in increased growth rates or survival (Brooke, 1978; Svensson and Nilsson, 1995; Hipfner and Gaston, 1999; Krist, 2004; Monteith *et al.*, 2012). To my knowledge, no study has attempted to link how egg size impacts other components of fitness in urban and non-urban bird populations.

4.4 Maladaptive responses to the city environment

In the city, nestling survival was greater in experimental nests, where the parents reared fewer nestlings as I did not replace the removed eggs, than in control nests. Therefore, my results suggest urban females may be making maladaptive decisions when laying as they could maximise offspring survival by producing a smaller clutch. The clutches of urban birds may be too large due to the lack of barriers preventing gene flow from the forest. Clutch size is a heritable trait (e.g., in great tits, $h = 0.48$), often under directional selection to increase (Perrins and Jones, 1974; van Noordwijk *et al.*, 1980; Price and Liou, 1989; Cooke *et al.*, 1990; Branston *et al.*, 2021). Immigration between the city and forest may have homogenised gene pools (Kawecki and Ebert, 2004; Lemoine *et al.*, 2016), preventing the evolution of adaptive clutch size by gene flow if the two populations have a different fitness optimum for clutch size (Dhondt *et al.*, 1990; Postma and van Noordwijk, 2005). Alternatively, cities may function as ecological traps (Isaksson, 2018). Although cities may have more resources and stable conditions during the winter than in the forest, leading birds to preferentially settle here, the poor food quality and exposure to pollution may impair nestling development (Demeyrier *et al.*, 2016; Pollock *et al.*, 2017). Therefore, urban birds may be misinterpreting habitat quality when settling and, subsequently, producing clutches that are too large to be sustained. These maladaptive decisions occur as females are time constrained during laying and quickly assess habitat quality based on limited knowledge of the environment (Donovan and Thompson, 2001; Hollander *et al.*, 2011; Stracey and Robinson, 2012).

4.5 Limitations and future investigations

My study has limitations that could influence the interpretation of the results. First, most females were not individually identified. I was, therefore, unable to assess how parental quality and age affected the ability of females to upregulate

egg production. Low-quality or young individuals that naturally produce smaller clutches may be pushed into the city if free territories in the forest are no longer available (Anderies *et al.*, 2007; Lepczyk *et al.*, 2017; Isaksson, 2018). Indeed, laying following egg removal may be more common in the forest if females are more experienced at breeding (Högstedt, 1980; Oppliger, 1997; Winder *et al.*, 2022). Therefore, in the future, there is a need to determine if the parental quality or the age structure of the city and forest populations differ and if that further explains differences in clutch size between urban and forest habitats. Second, adding the super controls to the number of eggs produced analysis may have artificially increased within group variance. However, there was no visible difference in the homogeneity of variance plots when super controls were included compared to when they were excluded. Therefore, this suggests that the variance between groups did not change greatly when the super controls were added to the analysis. Ideally, super controls should have also been added in the city, however, all urban nests were used in the experiment, with there being no spare to function as super controls. Third, my study consists of only one urban and one forest population, with no quantitative assessment of the urban environment. Comparative work across cities is needed to see if my results generalise across study populations. Species may also exhibit different responses to egg removal if the factor limiting egg production varies inter-specifically. Information on the costs of egg production should be obtained from urban and natural populations of different species filling various niches. Fourth, the number of fledglings is only one component of fitness. Ideally, I should have measured the number of offspring recruited or the parent's lifetime reproductive success. Similarly, I was also unable to assess the parent's survival. Indeed, the fitness costs of egg production may be paid by the mother rather than by the offspring (Visser and Lessells, 2001). Fifth, future work should compare the costs of each stage of the reproductive cycle (egg laying, incubation, and nestling rearing) between urban and forest birds, and assess the consequences of these costs, as there may be interactive effects between reproductive phases that operate differently between the city and the forest (Monaghan and Nager, 1997). Finally, a common garden or transplant experiment would be valuable to further consolidate my findings and confirm that the small clutches of urban birds reflect a constraint.

5. Conclusion

My study provides experimental support for blue tits being more constrained during egg production in urban than forest habitats. Urban birds may experience greater energetic or nutrient constraints than forest birds that either immediately restricts egg formation or exacerbates the trade-off between somatic maintenance and egg production in urban breeding females. Additionally, urban birds may produce small clutches as an increase in clutch size would be traded off against egg size, the detrimental effects of which city birds cannot compensate for during the nestling rearing stage. My results also suggest urban birds produce clutches too large to be sustained in the city. Egg removal in urban experimental nests resulted in a brood reduction, and the nestlings in the reduced broods had higher survival prospects than those in control nests. Thus, the clutch sizes of urban birds may be maladaptive, either due to gene flow between the forest and the city preventing the evolution of clutch size or through birds misjudging the quality of the urban environment as they are time constrained when laying. Overall, my results emphasise a need to incorporate the environmental constraints and fitness costs associated with egg production when attempting to explain variation in reproductive investment and success for birds breeding in anthropogenically modified landscapes.

Ethics Statement

All work involving nest disturbance, egg removal, and cross-fostering was covered by the license 207317 issued by NatureScot to DMD. Permission for bird ringing was granted by the British Trust for Ornithology, with licenses to DMD (permit number: C6822), CJB, and PC-L.

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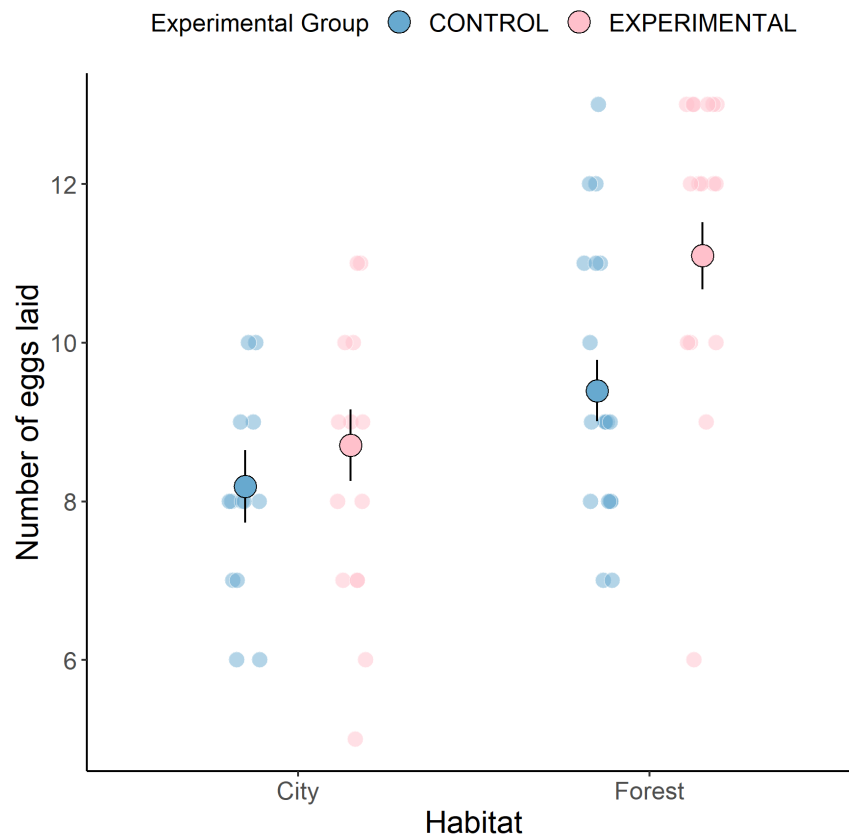
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Appendix

Appendix 1. a) Likelihood-ratio tests (LRT) for the following predictors explaining the number of eggs produced: the treatment group × habitat interaction and first egg date. Significant P-values are highlighted in bold. **b)** Global model coefficients from the linear mixed model of the effects of the following predictors on the number of eggs produced: habitat × treatment group interaction and first egg date. The standard errors and 95% confidence intervals are provided for each model coefficient. N = 60 clutches.

Global model: Number of eggs produced				
a) Likelihood-ratio test results				
<i>Predictors</i>	χ^2	<i>df</i>	<i>P-value</i>	
Habitat	14.366	1	<0.001	
Treatment group	7.723	1	0.005	
1 st egg laying date ¹	10.808	1	0.001	
1 st egg laying date ²	0.126	1	0.723	
Treatment group × Habitat	2.069	1	0.150	
b) Model coefficients				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Control group in the urban habitat)	8.188	0.458	7.271	9.105
Treatment group: <i>Experimental</i>	0.517	0.632	-0.749	1.783
Habitat: <i>Forest</i>	1.207	0.603	-0.0004	2.414
1 st egg laying date ¹	-0.147	0.047	-0.242	-0.052
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i>	1.186	0.854	-0.524	2.897



Appendix 2. The effect of egg removal on the number of eggs laid by the females in the city and the forest. Large dots represent global model predictions \pm standard error. Small dots are the raw data points. N = 61 clutches.

Appendix 3. a) Likelihood-ratio tests (LRT) for the following predictors explaining the number of eggs produced in the city: the treatment group and first egg date. Significant P-values are highlighted in bold. **b)** Model coefficients from the linear mixed model of the effects of the following predictors on the number of eggs produced: the treatment group and first egg date. The standard errors and 95% confidence intervals are provided for each model coefficient. N = 27 clutches.

Number of eggs produced in the city				
a) Likelihood-ratio test results				
<i>Predictors</i>	χ^2	<i>df</i>	<i>P-value</i>	
Treatment group	0.701	1	0.403	
1 st egg laying date ¹	3.108	1	0.078	
1 st egg laying date ²	0.960	1	0.327	
b) Model coefficients				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Control group)	7.941	0.426	7.061	8.821
Treatment group: <i>Experimental</i>	0.472	0.594	-0.754	1.697
1 st egg laying date ¹	-0.105	0.062	-0.232	0.022

Appendix 4. a) Likelihood-ratio tests (LRT) for the following predictors explaining the number of eggs produced in the forest: the treatment group and first egg date. Significant P-values are highlighted in bold. b) Model coefficients from the linear mixed model of the effects of the following predictors on the number of eggs produced in the forest: the treatment group and first egg date. The standard errors and 95% confidence intervals are provided for each model coefficient. N = 34 clutches.

Number of eggs produced in the forest				
a) Likelihood-ratio test results				
<i>Predictors</i>	χ^2	<i>df</i>	<i>P-value</i>	
Treatment group	7.267	1	0.007	
1 st egg laying date ¹	7.110	1	0.008	
1 st egg laying date ²	0.335	1	0.563	
b) Minimal model coefficients				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Control group)	9.643	0.408	8.812	10.476
Treatment group: <i>Experimental</i>	1.632	0.600	0.407	2.856
1 st egg laying date ¹	-0.192	0.062	-0.338	-0.046

Appendix 5. a) Likelihood-ratio tests (LRT) for the following predictors explaining the number of eggs produced in the forest: the treatment group (control or super control) and first egg date. Significant P-values are highlighted in bold. b) Model coefficients from the linear mixed model of the effects of the following predictors on the number of eggs produced in the forest: the treatment group, and first egg date. The standard errors and 95% confidence intervals are provided for each model coefficient. N = 53 clutches.

Number of eggs produced in the forest				
a) Likelihood-ratio test results				
<i>Predictors</i>	χ^2	<i>df</i>	<i>P-value</i>	
Treatment group	2.409	1	0.121	
1 st egg laying date ¹	19.920	1	<0.001	
1 st egg laying date ²	0.664	1	0.415	
b) Minimal model coefficients				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Control group)	9.214	0.318	8.569	9.860
Treatment group: <i>Super control</i>	-0.681	0.450	-1.595	0.234
1 st egg laying date ¹	-0.167	0.034	-0.236	-0.098

Appendix 6. a) Likelihood-ratio tests (LRT) of following predictors explaining egg volume: the treatment group \times habitat interaction, number of eggs laid, and egg laying date. Nest box ID was included as a random effect. Significant P-values are highlighted in bold. **b)** Model coefficients from the minimal linear mixed model of the effects of the following predictors on egg volume: habitat \times treatment group interaction, the number of eggs laid, and egg laying date. The standard errors and 95% confidence intervals (calculated by running 500 parametric bootstrap simulations of the model) are provided for each model coefficient. N = 496 eggs.

Global model: Egg volume (mm ³)				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Habitat	-	-	-	
Treatment group	-	-	-	
Number of eggs laid	2.693	1	0.260	
Egg laying date¹	11.413	1	0.003	
Egg laying date ²	0.219	1	0.640	
Habitat \times Treatment group	2.154	1	0.341	
b) Model coefficients				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Control group in the urban habitat)	1370.059	30.849	1304.913	1429.423
Treatment group: <i>Experimental</i>	23.239	37.895	-55.942	98.099
Habitat: <i>Forest</i>	-40.598	37.434	-112.778	41.654
Number of eggs laid	-7.283	7.113	-20.762	5.811
Egg laying date¹	-4.137	1.315	-6.701	-1.594
Habitat \times Treatment group	-35.031	49.680	-138.450	55.525
Random effects				
Groups	Variance	Standard deviation		
Nest box ID	7984	89.350		
Residual	6423	80.140		

Appendix 7. a) Likelihood-ratio tests (LRT) for the following predictors explaining the total volume of egg produced by a female: the treatment group × habitat interaction and first egg date. Significant P-values are highlighted in bold. **b)** Global model coefficients from the global linear model of the effects of the following predictors on the total volume of egg material produced: the habitat × treatment group interaction and first egg date. The standard errors and 95% confidence intervals are provided for each model coefficient N = 61 clutches.

Global model: Total volume of egg (mm ³) produced by a female				
a) Likelihood-ratio test results				
<i>Predictors</i>	<i>x²</i>	<i>df</i>	<i>P-value</i>	
Habitat	-	-	-	
Treatment group	-	-	-	
1st egg laying date¹	11.763	1	0.001	
1 st egg laying date ²	0.392	1	0.531	
Treatment group × Habitat	1.149	1	0.284	
b) Model coefficients from the global model				
<i>Fixed Effects</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>95% CI (Lower Upper)</i>	
Intercept (Control group in the urban habitat)	11299.690	665.440	9964.990	12634.389
Treatment group: <i>Experimental</i>	678.780	921.490	-401.718	3099.423
Habitat: <i>Forest</i>	1348.85	872.780	-1169.499	2527.049
1st egg laying date	-235.18	68.130	-371.826	-98.534
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i>	1265.330	1228.550	-1198.824	3729.490

Appendix 8. a) Likelihood-ratio tests (LRT) for the minimal model of the following predictors explaining the total volume of egg produced by a female in the city: treatment group, habitat, and first egg date. Significant P-values are highlighted in bold. **b)** Model coefficients from the minimal linear model of the effects of the following predictors on total volume of egg material produced in the city: treatment group, and first egg date. N = 61 clutches.

Total volume of egg (mm ³) produced by a female in the city				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Treatment group	0.578	1	0.447	
1 st egg laying date ¹	5.352	1	0.021	
1 st egg laying date ²	0.077	1	0.078	
b) Model coefficients				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Control group in the urban habitat)	10889.770	646.201	9549.624	12086.462
Treatment group: <i>Experimental</i>	645.830	900.303	-1221.292	2512.942
1 st egg laying date ¹	-210.932	92.056	-401.827	-20.041

Appendix 9. a) Likelihood-ratio tests (LRT) for the minimal model of the following predictors explaining the total volume of egg produced by a female in the forest: treatment group, habitat, and first egg date. Significant P-values are highlighted in bold. **b)** Model coefficients from the minimal linear model of the effects of the following predictors on total volume of egg material produced in the forest: treatment group, and first egg date. N = 61 clutches.

Total volume of egg (mm ³) produced by a female in the forest				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Treatment group	5.310	1	0.021	
1 st egg laying date ¹	6.591	1	0.010	
1 st egg laying date ²	0.348	1	0.348	
b) Model coefficients				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Control group in the forest habitat)	12988.300	577.710	11808.527	14168.036
Treatment group: <i>Experimental</i>	1910.702	834.908	205.667	3615.673
1 st egg laying date ¹	-261.220	101.400	-468.418	-54.064

Appendix 10. a) Likelihood-ratio tests (LRT) for the following predictors explaining egg volume: the treatment group \times location \times laying order group interaction, number of eggs laid, and egg laying date. Nest box ID was included as a random effect. Significant P-values are highlighted in bold. b) Global model coefficients and confidence intervals from the global linear mixed model of the effects of the following predictors on egg volume: treatment group \times location \times laying order interaction, the number of eggs laid, and first egg date. The standard errors and 95% confidence intervals (calculated by running 500 parametric bootstrap simulations of the model) are provided for each model coefficient. N = 460 eggs.

Global model: Egg volume (mm ³)				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Habitat	-	-	-	
Treatment group	-	-	-	
Lay order group	-	-	-	
Number of eggs laid	1.029	1	0.310	
Egg laying date¹	4.141	1	0.042	
Egg laying date ²	2.016	1	0.156	
Habitat \times lay order group \times Treatment group	3.167	1	0.075	
b) Model coefficients from the global model				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Eggs 1-3 from the control group in the urban habitat)	1348.098	36.083	1280.64	1424.988
Treatment group: <i>Experimental</i>	28.962	44.323	-48.836	116.681
Habitat: <i>Forest</i>	-23.336	43.859	-109.579	56.816
Number of eggs laid	-7.625	7.476	-21.733	6.835
Egg laying date¹	-4.067	1.990	-7.786	-0.031
Lay order group: <i>Eggs 4-9</i>	30.397	24.764	-15.271	78.493
Habitat: <i>Forest</i> \times Lay order group: <i>Eggs 4-9</i>	-26.349	28.844	-78.016	34.258
Lay order group: <i>Eggs 4-9</i> \times Treatment group: <i>Experimental</i>	0.026	29.652	-65.887	56.563
Habitat: <i>Forest</i> \times Treatment group: <i>Experimental</i>	28.315	59.024	-83.971	139.262
Habitat: <i>Forest</i> \times Lay order group: <i>Eggs 4-9</i> \times Treatment group: <i>Experimental</i>	-70.311	39.322	-146.609	19.115
Random effects				
Groups	Variance	Standard deviation		
Nest box ID	8495	92.170		
Residual	6140	78.360		

Appendix 11. a) Likelihood-ratio tests (LRT) for the following predictors explaining nestling body mass two days after hatching: the treatment group × habitat interaction, number of siblings in the nest, time of day, and hatch date. Clutch ID was included as a random effect. Significant P-values are highlighted in bold. **b)** Global model coefficients from the linear mixed model of the effects of the following predictors on nestling mass two days after hatching: treatment group × habitat interaction, number of siblings in the nest, time of day, and hatch date. The standard errors and 95% confidence intervals (calculated by running 500 parametric bootstrap simulations of the model) are provided for each model coefficient. N = 372 nestlings.

Global model: Nestling mass (g) two days after hatching				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Habitat	-	-	-	
Treatment group	-	-	-	
Hatch date ¹	0.189	1	0.664	
Hatch date ²	0.219	1	0.640	
Number of siblings	0.725	1	0.395	
Time of day	1.757	1	0.185	
Treatment group × Habitat	0.132	1	0.717	
b) Model coefficients from the global model				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Control group in the urban habitat in the morning)	1.928	0.100	1.720	2.084
Treatment group: <i>Experimental</i>	-0.294	0.167	-0.608	0.047
Habitat: <i>Forest</i>	0.123	0.141	-0.118	0.361
Number of siblings	-0.024	0.028	-0.077	0.008
Hatch date ¹	0.006	0.014	-0.022	0.030
Time of day: <i>Afternoon</i>	0.141	0.104	-0.039	0.310
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i>	0.071	0.196	-0.295	0.414
Random effects				
Groups	Variance	Standard deviation		
Clutch ID	0.097	0.312		
Residual	0.156	0.395		

Appendix 12. a) Likelihood-ratio tests (LRT) for the following predictors explaining nestling body mass: treatment group × habitat × age interaction, number of siblings in the nest, time of day, and hatch date. Clutch ID was included as a random effect. Significant P-values are highlighted in bold. **b)** Model coefficients from the linear mixed model of the effects of the following predictors on nestling body mass: treatment group × habitat × age interaction, number of siblings in the nest, time of day, and hatch date. N = 354 nestlings.

Global model: Nestling mass (g)				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Habitat in nest of rearing	-	-	-	
Treatment group in nest of rearing	-	-	-	
Age	-	-	-	
Habitat in nest of hatching	0.071	1	0.789	
Treatment group in nest of hatching	0.895	1	0.344	
Hatch date ¹	3.075	1	0.080	
Hatch date ²	0.005	1	0.943	
Number of siblings	0.286	1	0.593	
Time of day	20.035	1	<0.001	
Treatment group in nest of rearing × Habitat in nest of rearing × Age	18.895	1	<0.001	
b) Model coefficients from the global model				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Control group two days after hatching, reared in the urban habitat and weighed in the morning)	4.660	0.255	4.117	5.114
Habitat in the nest of hatching: <i>Forest</i>	0.055	0.205	-0.346	0.402
Treatment group in the nest of hatching: <i>Experimental</i>	-0.283	0.298	-0.830	0.189
Habitat in the nest of rearing: <i>Forest</i>	1.079	0.326	0.442	0.157
Treatment group in the nest of rearing: <i>Experimental</i>	0.728	0.457	-0.153	1.486
Age: day 12	2.642	0.169	2.302	2.930
Number of siblings	-0.034	0.063	-0.160	0.071
Hatch date ¹	-0.060	0.034	-0.124	0.005
Time of day: <i>Afternoon</i>	0.346	0.076	0.216	0.480
Treatment group in nest of rearing: <i>Experimental</i> × Habitat in nest of rearing: <i>Forest</i>	-0.5	0.461	-1.404	0.187
Treatment group in nest of rearing: <i>Experimental</i> × Age: <i>day 12</i>	1.408	0.185	0.966	1.793
Habitat in nest of rearing: <i>Forest</i> × Age: <i>day 12</i>	2.022	0.178	1.654	2.340
Treatment group in nest of rearing: <i>Experimental</i> × Age: <i>day 12</i> × Habitat in nest of rearing: <i>Forest</i>	-1.179	0.264	-1.658	-0.766
Random effects				
Groups	Variance	Standard deviation		
Clutch ID of hatching	0.275	0.525		
Clutch ID of rearing	0.441	0.664		
Ring Number	0.267	0.517		
Residual	0.383	0.619		

Appendix 13. a) Likelihood-ratio tests (LRT) for the following predictors explaining the nestlings fate: the nestling body mass 2 days after hatching × treatment group × habitat interaction, number of siblings, time of day, and hatch date. N= 364. Clutch ID of rearing and hatching were included as random effects. Significant P-values are highlighted in bold. **b)** Global model coefficients from the binomial generalised linear mixed model of the effects of the following predictors on the nestling's fate: the interaction between nestling body mass 2 days after hatching × the treatment group × habitat, number of siblings, time of day, and hatch date. The standard errors and 95% confidence intervals are provided for each model coefficient. N= 364 nestlings.

Global model: Fate (Dead or Fledged)				
a) Likelihood-ratio test results				
Predictors	χ^2	df	P-value	
Habitat	-	-	-	
Treatment group	-	-	-	
Mass (g) at day 2	-	-	-	
Number of siblings	2.142	1	0.143	
Hatch date ¹	4.295	1	0.038	
Hatch date ²	2.296	1	0.130	
Time of day	3.545	1	0.060	
Mass at day 2 × treatment group × Habitat	0.256	1	0.611	
b) Model coefficients				
Fixed Effects	Estimate	Standard Error	95% CI (Lower Upper)	
Intercept (Control group in the urban habitat)	-4.123	0.573	-9.538	1.291
Mass (g) at day 2	1.087	0.993	-0.859	3.032
Treatment group: <i>Experimental</i>	3.003	3.960	-4.759	10.764
Habitat: <i>Forest</i>	5.555	4.016	-2.317	13.426
Hatch date ¹	-0.531	0.271	-1.062	-0.001
Number of siblings	0.691	0.464	-0.219	1.600
Time of day: <i>Afternoon</i>	-3.424	1.841	-7.032	0.184
Mass × Habitat: <i>Forest</i>	1.355	1.523	-1.630	4.341
Mass × Treatment group: <i>Experimental</i>	0.691	1.475	-2.201	3.582
Treatment group: <i>Experimental</i> × Habitat: <i>Forest</i>	-3.829	6.093	-15.772	8.112
Mass at day 2 × Habitat: <i>Forest</i> × Treatment group: <i>Experimental</i>	1.515	3.038	-4.439	7.469
Random effects				
Groups	Variance	Standard deviation		
Clutch ID of hatching	5.469	2.339		
Clutch ID of rearing	21.318	4.617		