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Abstract

Urbanisation is a threat to natural ecosystems worldwide, as it alters wildlife community composition and foodweb structure. City habitats are shaped and influenced through man-made structures and impervious surfaces, but also by the accumulation of environmental pollutants. Living in an urban habitat is stressful to many animals. Depending on their tolerance and adaptability, we discriminate between urban avoiders, urban adapters and urban exploiters, the latter being species that cope well and even thrive in urban habitats. In avian species that are highly mobile, an individual is not necessarily exposed to urban stressors all day long, but specifically nestlings of altricial birds have no choice but to stay in the nest for their entire development. Such a city-dwelling species with a nestling phase of approximately 30 days is the Eurasian Kestrel (*Falco tinnunculus*), a medium-sized raptor. Kestrels in Vienna provide their nestlings with a diverse diet of vertebrates (mainly rodents and passerines) and invertebrates (such as insects), which differs in composition between inner-city and suburban habitats. Prey items vary in their nutritional value and contents of micronutrients like carotenoids, which might have an impact on nestlings' health. Carotenoids are yellow to red pigments, which are incorporated into integument tissues and thus constitute the colouration of feathers and bare parts. They also have antioxidant and immunostimulatory capacity, and there is a trade-off in allocation between these functions. In this study, we measured carotenoid colouration and other indicators of individual health (Heterophil/Lymphocyte-ratio, body condition and susceptibility to the ectoparasite *Carnus hemapterus*) of 154 nestling Kestrels (n=91 nests) along an urban gradient in the city of Vienna from 2010 to 2015. We found skin yellowness of nestlings from nest-sites in the city-centre to be least pronounced. This result might indicate that inner-city nestlings are strongly affected by novel urban stressors and depleted their stores of dietary carotenoids for health-related functions rather than colouration. In addition, skin yellowness intensified with age and male nestlings were more intensely coloured, as well as those chicks from earlier nests in the breeding season. The immune system of Kestrel nestlings is still developing, thus younger chicks might need more antioxidants to combat environmental stress. This is also supported by the result that younger nestlings had higher Heterophil/Lymphocyte-ratios, indicating higher physiological stress. Competition for food in the nest might be biased by sex or the sexes may have different growth rates, resulting in varying use of dietary nutrients. The other health indicators were not directly linked to the urban gradient, but some were influenced indirectly. Parasite infection intensity was highest in nests laid earlier in the season and in nestlings with less intense skin yellowness. In combination with results from previous studies, our results might indicate that Kestrels in Vienna are indeed in danger of falling into an 'ecological trap' both in terms of productivity, but also individual health.

Keywords: urban ecology, city-dwelling species, integument colouration, H/L-Ratio, leukocyte profile, body condition, individual quality, offspring size - number trade-off, altricial nestlings, Eurasian Kestrel *Falco tinnunculus*, ectoparasites, *Carnus hemapterus*, ecological trap

1. Introduction

Wildlife around the globe faces the dangers of a novel, quickly spreading habitat infiltrating their natural environment: urban areas, i.e. cities and other human settlements. To date, 50% of the human population lives in urban areas (Marzluff 2001, Grimm et al. 2008). Through the drastic changes imposed by cities, such as increased impervious surfaces, noise, light and chemical pollution, introduced alien species and predators, diet alteration, habitat loss and fragmentation, urbanisation acts as a filter for bird communities (McKinney 2002, Blair 2004, Shanahan et al. 2014). This leads to biodiversity loss through both random processes and mal-adaption of some species (Sol et al. 2014). Thus, urbanisation is a novel and strong evolutionary force (Hoffmann and Hercus 2000, Partecke and Gwinner 2007). According to their adaptability to urban landscapes, bird species are categorised in urban avoiders, adapters or exploiters (McKinney 2002, Blair 2004), reflecting their ability to cope with these novel urban stressors. Some species seem to cope well with urban environments and react with rapid evolutionary adaptations to city life (Atwell et al. 2012, Partecke 2014). Other species however show negative responses to anthropogenic stressors in terms of productivity and individual health. Furthermore, urbanisation influences host susceptibility to parasites and infectious diseases and can intensify the detrimental effects of pathogens by weakening the immune system (Bradley and Altizer 2007).

Some individuals might be especially exposed to urban landscapes, namely the altricial nestlings of urban breeding birds. Birds are usually highly mobile, but these nestlings are bound to their nest and dependent on parental care for most of their early development. Thus, they have to endure variation in food availability, exposure to parasites and predators, sibling competition, adverse weather and other environmental stressors, with some buffer by their parents (Blas et al. 2005). Stress leads to higher glucocorticoid levels; prolonged stress might result in lower body condition (Schoech et al. 1997, Kitaysky et al. 1999, Pereyra and Wingfield 2003, Cockrem et al. 2006, Jenni-Eiermann et al. 2008). Yet, increased circulating glucocorticoids can have positive effects on nestlings, as higher levels of these hormones might promote behaviours which can increase individual survival (Kitaysky et al. 2001). Additionally, as apex predators, altricial raptor nestlings are dependent on fluctuating prey populations, e.g. vole cycles and other cascading effects within their food chain (Almasi et al. 2009). For raptors living in urban landscapes, the altered availability of prey species is challenging, but can also be beneficial specifically for raptors that feed on passerines and other small prey or have smaller home ranges (Chace and Walsh 2006).

Raptors are particularly interesting to study in an urban context; as umbrella species their loss can have cascading effects on many other species (Palomino and Carrascal 2007, Lyly et al. 2015, Mueller et al. 2016), which is why they are also used as surrogates for environmental change (Donázar et al. 2016). Yet, raptors depend on large expanses of suitable habitat with stable prey populations and thus might be vulnerable in urban areas (Newton 1979). Additional threats for raptors are direct and indirect poisoning and other persecution (Mannan and Boal 2004; but illegal hunting is generally more pronounced in rural regions Chace and Walsh 2006). In spite of this, the trend of raptors colonising cities is on the rise, with more species moving into urbanised areas, for example Harris's Hawk (*Parabuteo unicinctus*, Dwyer and Mannan 2007), Cooper's Hawk (*Accipiter cooperii*, Rosenfield et al. 2015), Black Sparrowhawk (*Accipiter melanoleucus*, Sumasgutner et al. 2016), Northern Goshawk (*Accipiter gentilis*, Rutz 2008), Crowned Eagle

(*Stephanoaetus coronatus*, McPherson et al. 2016), Booted Eagle (*Hieraaetus pennatus*, Palomino and Carrascal 2007), Mississippi Kite (*Ictinia mississippiensis*, Welch and Boal 2015), Red Kite (*Milvus milvus*, Orros and Fellowes 2015), Barn Owl (*Tyto alba*, Hindmarch and Elliott 2015), Merlin (*Falco columbiarus*, Warkentin and James 1988), Peregrine Falcon (*Falco peregrinus*, Caballero et al. 2016), American Kestrel (*Falco sparverius*, Hogg and Nilon 2015), and the focus of this study, the Eurasian Kestrel (*Falco tinnunculus*), or simply Kestrel. Kestrels breed in many European cities (e.g. Kostrzewa and Kostrzewa 1993, Piattella et al. 1999, Kübler et al. 2005, Riegert et al. 2007, Kal'avský and Pospíšlová 2010, Sumasgutner et al. 2014a), and the diet of urban Kestrels can heavily differ from their rural counterparts (Village 1990). In Vienna, Kestrels breeding in highly urbanised areas increasingly feed on birds, reptiles and insects compared to Kestrels breeding in the outskirts of the city, which feed primarily on voles (Sumasgutner et al. 2013, Kreiderits et al. 2016). However, cities may also pose more subtle physiological risks to raptors, even to those species which appear to do well in urban areas in terms of number of breeding pairs. Examining the mechanisms behind the perceived success of raptors in urban areas can thus help to establish whether these species are falling into ecological traps or whether they are true urban-adapters.

The concept of stress is not easy to define (Romero et al. 2009), but assessing physiological stress of animals via the measurement of circulating glucocorticoids in blood is a widely used method to quantify an individual's stress response (Wikelski and Cooke 2006). These hormone levels can change very quickly and rise especially during handling, making it difficult to measure a baseline concentration (Vleck et al. 2000, Romero and Reed 2005). To avoid these limitations, an alternative method to assess physiological stress was used in this study, namely white blood cell counts from blood smears (Cīrule et al. 2012). The so-called leukocyte profile is closely linked to glucocorticoid levels: high stress hormone levels lead to an increase in number and heterophils, while decreasing the number of lymphocytes (Davis et al. 2008). Therefore, high heterophils to lymphocyte ratios (H/L-ratio) imply high glucocorticoid levels (but see Müller et al. 2011).

One important physiological response to unfavourable environmental conditions is the imbalance of intracellular oxidants and antioxidants, termed oxidative stress. Living in urban environments is a driver of oxidative stress and is responsible for a multitude of negative health effects in humans and wildlife (Chuang et al. 2007, Chávez-Zichinelli et al. 2013, Gillis et al. 2014, Herrera-Dueñas et al. 2014, Isaksson 2015). To counteract the destructive effects of oxidative stress, i.e. damage of DNA, living organisms need to produce or ingest molecules with antioxidant properties. One class of antioxidants are carotenoids (Pérez-Rodríguez 2009), yellow to red pigments which are strictly dietary for vertebrates (Chew 1996, Blount et al. 2003). Raptors feed on a variety of prey species that might differ in their carotenoid contents. Being specialised vole hunters (Village 1990), Kestrels might face a trade-off to meet their dietary needs: voles (*Microtus spp.*) are easy to hunt and have high calorie but low carotenoid content, whereas their alternative prey (small birds or larger insects) is comparably less calorific, but higher in its carotenoid content (Bortolotti et al. 1996, Olson and Owens 1998).

Carotenoids serve many functions in animals, most visibly the colouration of nearly all vertebrate integumentary tissues like skin, eyes, reptilian scales, bird feathers and beaks (McGraw 2006), but also other physiological functions, e.g. antioxidant capacity and immunomodulatory properties (Chew 1996, Møller et al. 2000, Blount et al. 2003, Alonso-Alvarez et al. 2004; but see Isaksson et

al. 2007, Pérez-Rodríguez 2009, Müller et al. 2011). Due to the diverse functions of carotenoids, trade-offs in the allocation of these important micronutrients have been proposed (Olson and Owens 1998). Carotenoid-based colouration of birds' bare skin and beaks has been identified as a dynamic, condition-dependent trait (Faivre et al. 2003) in adult (Casagrande et al. 2006) and nestling (Casagrande et al. 2009) Kestrels. This suggests that the expression of the trait is an honest indicator of individual quality (Lozano 1994, von Schantz et al. 1999). Especially nestlings need carotenoids, since their immune system is not yet fully developed, and hatching asynchrony or insufficient parental care can lead to undernourishment (Hõrak et al. 2001, Fitze et al. 2003, Casagrande et al. 2007). It is known from other raptor species that the intake of carotenoid-rich food sources can improve individual health, including during the nestling phase (Sternalski et al. 2010, 2012a, b).

Living in urban environments, the exposure to anthropogenic stressors might not only lead to immediate health problems, but also to reduced reproductive performance and survival (review in (Isaksson 2010). Urbanisation increases the contact between humans and domestic animals as well as wildlife. The change in density probably also alters the interactions between hosts and pathogens. This can lead to more abundant parasites or vectors and thereby facilitates the rise of pathogens and multiplies the transmission of infectious diseases in urban environments (Ditchkoff et al. 2006, Brearley et al. 2013). Together with increased physiological stress, the severity of diseases might increase, including higher infection prevalence with haemo-, endo-, and ecto-parasites (Giraudeau et al. 2014). The parasitic fly *Carnus hemapterus* is a common ectoparasite of many bird species, including Kestrels (Village 1990, Dawson and Bortolotti 1997, Kal'avský and Pospíšlová 2010). They mainly infest nestlings, with the highest infection rates in younger and last-hatched chicks, i.e. junior siblings (Roulin et al. 2003). Infections with ectoparasites have been connected to reduced expression of carotenoid-based signals, reflecting the strong immune response against parasites (Møller et al. 2000). Urbanisation can also negatively affect body condition of adults (Shochat 2004, Liker et al. 2008) and juveniles (Meillère et al. 2015).

We propose two competing hypotheses regarding (1) integument colouration: (I) dietary antioxidants are supposedly restricted in vole hunters; since Kestrels in the city centre increasingly feed on alternative prey, inner-city nestlings should show more intense carotenoid (i.e. yellow) colouration or, (II) because environmental stress is increased in the city, carotenoids are used for antioxidant defence and inner-city nestlings should show less intense carotenoid colouration.

At the same time breeding performance is known to be lower in inner-city Kestrel pairs and malnutrition was named as one of the potential factors leading to a high nestling mortality (Sumasgutner et al. 2014a, b). Hence, high physiological stress could lead to a measurable drop in health-related traits. On the other hand, already smaller clutches and lower fledged brood size could result in reduced sibling competition for limited food, resulting in an overall higher quality of surviving fledglings (along the offspring size-number trade-off; Gyllenberg et al. 2011). Both of these competing hypotheses predict a significant correlation between the urban gradient and novel urban stressors, measured as (2) body condition, (3) H/L-Ratio and (4) susceptibility to the ectoparasite *Carnus hemapterus*.

2. Material and Methods

Study species

The Eurasian Kestrel (*Falco tinnunculus* LINNÉ, 1758), henceforth simply Kestrel (Figure 1), is one of the most common birds of prey of the palaearctic region. It is widespread throughout its range, occupying a wide range of open natural habitats to human modified landscapes, avoiding only densely forested areas (Village 1990, Glutz von Blotzheim and Bauer 1991, Kostrzewa and Kostrzewa 1993). In Vienna, Kestrels are the most common aerial predators, reaching densities of 89 - 122 breeding pairs/100 km^2 (Sumasgutner et al. 2014b), which is higher than in other European cities or rural areas (Gamauf 1991, Kübler et al. 2005). Kestrels are specialised in hunting voles, but also feed on other small vertebrates and large invertebrates (Sumasgutner et al. 2013, Mebs and Schmidt-Rothmund 2014, Kreiderits et al. 2016). Most of the Kestrels breeding in Vienna are only present during the breeding season, arriving in March and leaving the city in August/September to spend the winter elsewhere, potentially in rural areas or migrating to the Mediterranean area (Schmidt et al. 2014). Some males seem to be resident year round or at least some adults can be observed even during winter time (pers. ob. A. Gamauf, P. Sumasgutner).

Figure 1: Typical Kestrel nest-site on a building in central Vienna. Male (left) and female (right) with 3 nestlings (about 10 days old). The female feeds a juvenile Great Tit (*Parus major*) to her chicks (Photograph by Franz Kerschbaum).

Study area

All data for this study was gathered in the city of Vienna, Austria (48°12’N, 16°22’E; 415 km^2; 150 - 500 m a.s.l.; 1.8 million inhabitants). Since there are large forests (e.g. Donau-Auen National Park and Vienna Woods) and also farm land within the city boundaries, we defined the urban gradient by the percentage of sealed soil and excluded rural areas with <1% sealed soil, thus comprising an urban study area of 243 km^2 (Figure 2). The proportion of impervious surfaces was calculated using ESRI ArcGIS 10 based on coverage by buildings and traffic areas for r = 500 m around the nest (Sumasgutner et al. 2014a, b). Kestrel nesting sites used in this study were found from 19% soil sealing in suburban areas to 97% in the inner city.

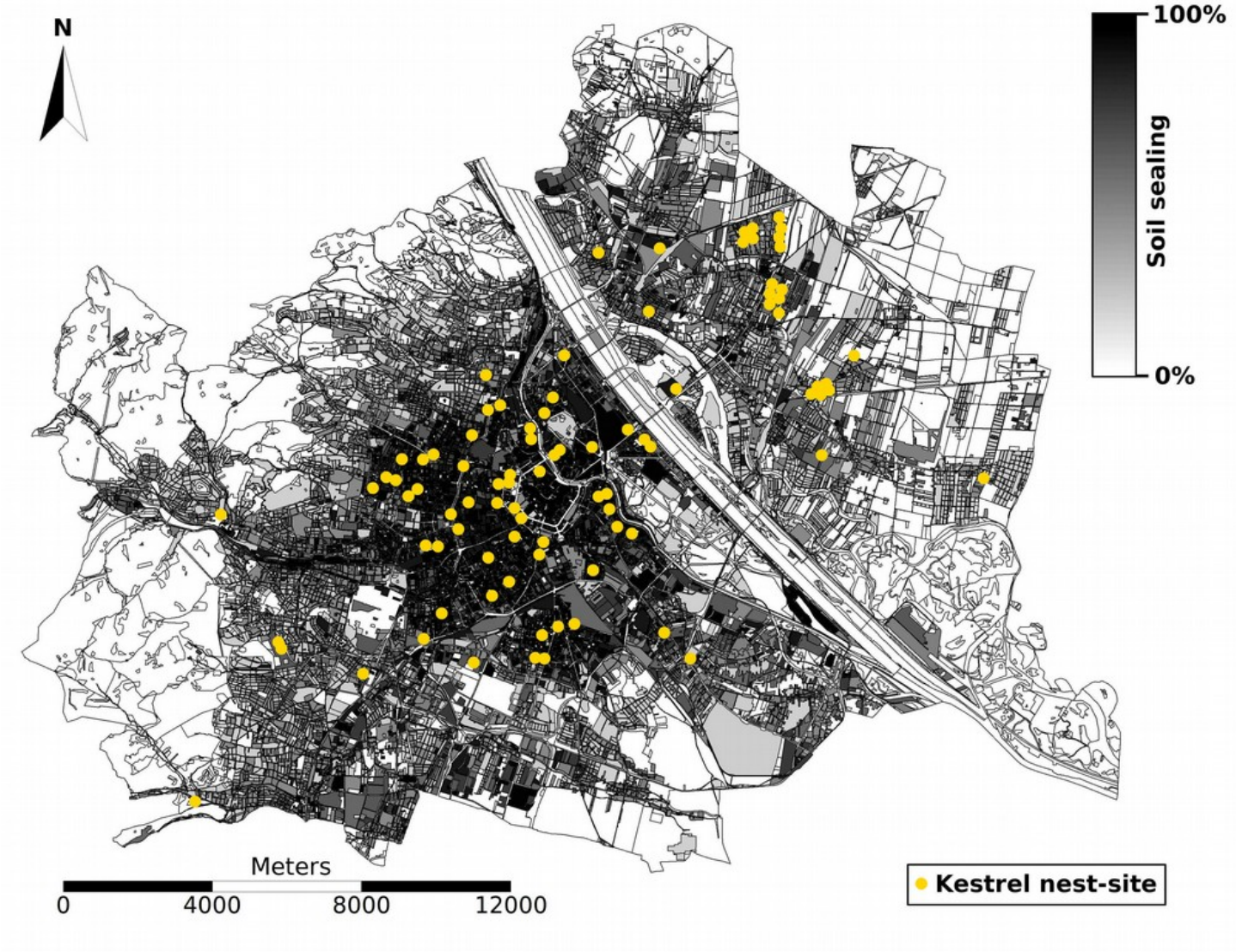


Figure 2: Urban study area (243 km^2) in Vienna, Austria. The urban gradient is displayed from black to light grey (white areas (<1%) are outside the study area), and locations of Kestrel nest-sites used in this study are marked.

Field procedure: morphometric measurement and ectoparasite counts

Kestrel nesting sites in the city of Vienna were located by sight and with the help of the general public from 2010 to 2015 and regularly checked for occupation during the nesting season (Sumasgutner et al. 2013, 2014a, b, Kreiderits et al. 2016). For this study, we used data from 91 Kestrel nest-sites from all six years. When occupied, nests were visited two to three times if accessible through the attic, by tree and façade climbing or with help from the Viennese fire-fighters to assess the number of eggs, number of hatched nestlings and number of fledged chicks. The nestlings were ringed, weighed and measured: length of the fourth primary (numbered from the outermost towards the inside, after Svensson et al. (2009) and length of the first rectrix to the nearest 0.1 mm (with callipers) and wing length to the nearest mm (with a zero-stop ruler; Eck et al. 2011). The laying date was determined either through direct observation or using back estimation based on the age of the nestlings. Each nestling was also screened for ectoparasites. The most significant arthropod ectoparasite in Kestrels is the blood-sucking fly *Carnus hemapterus* (Piechocki 1991), but other Diptera flies, lice (Mallophaga), mites and ticks (Acarina) also live on the nestlings' skin. Parasite infestation decreases with age of the nestlings, and the youngest chick of a clutch usually has most parasites (Roulin et al. 2003, Sumasgutner et al. 2014c). The number of

all ectoparasites was counted in each nestling on the day they were ringed (aged six to 33 days), and parasite load was defined as a score: 0 (no *C. hemapterus* visible), 1 (one to three *C. hemapterus*), 2 (four to nine *C. hemapterus*), 3 (more than 10 *C. hemapterus* on the nestling's skin).

Blood from nestlings was collected by puncturing the brachial vein with a sterilised 21 gauge needle and extraction of the blood through capillary action with 75µl heparinised capillary tubes after desinfection of the skin with alcohol swabs. Blood smears were prepared directly in the field and air-dried before they were fixed with absolute methanol (5 x 1 sec) and stained with Giemsa's stain (using Hemacolor Rapid staining of blood smear set, Merck Germany, standard protocol).

Blood cell count and H/L-ratio

The stained blood smears where examined using a binocular light microscope (Nikon Labophot-2) with oil immersion lenses. To obtain leukocyte profiles (i.e. white cell blood counts), blood smears were screened at 1000x magnification and all lymphocytes, heterophils, eosinophils, basophils and monocytes counted until 100 leukocytes were examined. To calculate the H/L-ratio, the number of heterophils was divided by the number of lymphocytes counted for each blood smear.

Colour measurement and validation study

The colours of the unfeathered skin covering tarsus, cere and eye ring (orbital ring, see Figure 3) were scored by comparing the bird under natural light conditions to a reference colour charts booklet (MICHEL 2000). Scoring colours by visual matching with colour charts has been widely used in studies on avian colouration (Bortolotti et al. 1996, Sternalski et al. 2010, 2012a, b, Blas et al. 2013). The method might be problematic, depending on the subjectivity of the observer and the quality of ambient light (Montgomerie 2006). To estimate the reliability of the chart method used in this study, photospectrometral colour measurement was carried out in the field season of 2015, measuring both tarsus and cere colours of 25 nestling Kestrels. Measurements were taken using a portable JAZ photospectrometer (Ocean Optics, Inc.) with a pulsed xenon lightsource (190 - 1000 nm optical range) and an optical fibre with a selfmade probe pointer, firmly attached to the optical fibre. The handheld probe pointer was cut in a 45° angle and could then be placed firmly on the animals' skin (coincident oblique technique; Andersson and Prager 2006) to avoid distortions of the measurements due to the glossy surface of the skin. Reflectance of each body patch was measured three times, removing the probe pointer in between measurements. The raw data files were read into R (R Development Core Team 2015) and analysed with the package pavo (Maia et al. 2013).

To compare the two different colour measurements, colour charts and spectral reflectance, we extracted three colourimetrics from the reflectance curves within the 400 - 700 nm spectral range: (a) carotenoid chroma (the difference between the reflectance at 700 nm and 450 nm divided by the reflectance at 450 nm, i.e. relative reflectance in the region of highest reflectance in carotenoid-based colours), a measure of carotenoid concentration (Andersson and Prager 2006), (b) carotenoid saturation (after Montgomerie 2006, Blas et al. 2013), the ratio between total brightness (area under the curve) and the reflectance in the region of interest (from 550 nm to 700 nm, corresponding to yellow, orange and red wavelengths, Montgomerie 2006); and (c) spectral saturation of the yellow segment ($S1_y$), calculated as the sum of the reflectance values in the wavelength range 550 - 625 nm (associated with yellow colours) divided by the total reflectance (Montgomerie 2006, Maia et al. 2013).

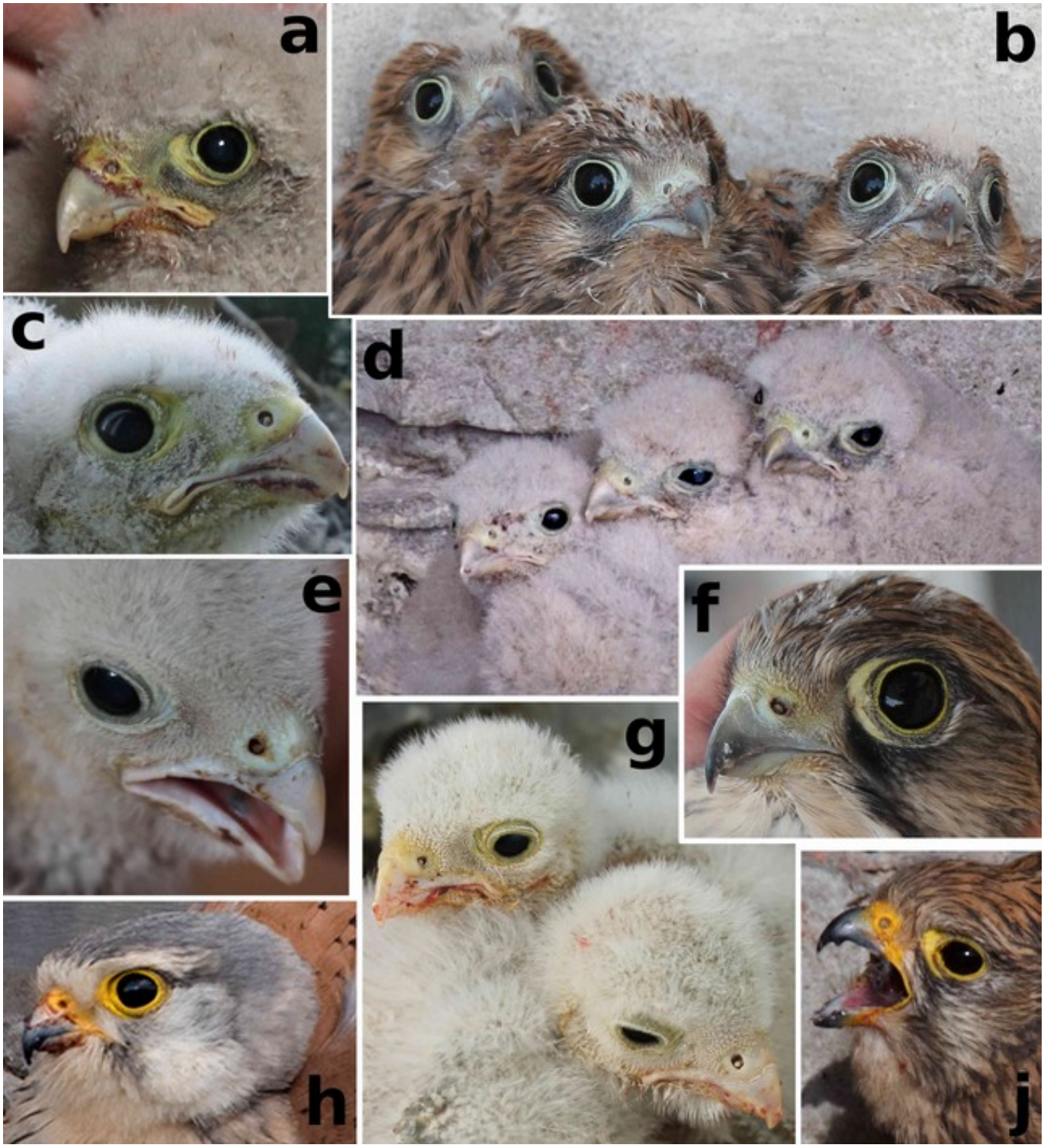


Figure 3: Photographs of Kestrel nestlings (a) - (g), adult male (h) and female (j) from Vienna, showing great variation in colouration of cere and eye ring skin. Colour intensity usually increases from young (d, e, g) to older chicks (a, c) and those almost about to fledge (b, f). Adults have very intensely yellow coloured skin. Photographs courtesy of Bernhard Paces (a, b, f, g), Barbara Kofler (c), Franz Kerschbaum (d, j), Katharina Stefan (e) and Leander Khil (h).

Since there were no colourimetrics available for the colour charts used in this study, I used an image scanner (Canon imageRUNNER Advance C5235i) to extract high resolution digital photographs of the colour charts in standardised lighting conditions. The images of the colour charts were then analysed with GIMP (Kimball et al. 2015). For each colour chart I measured the average red, green

and blue components (RGB colour space). The three components have equal values in white (high values) and black (zero values) colours, respectively. The relative difference of the three colour components determines the hue of the colour. For example, the bright and saturated yellows have high values in the red and green component and very low values in the blue component. I calculated a “yellowness score” for each colour chart by subtracting the ratio of red and green values from the blue value (essentially using the blue value, correcting for red and green; (Villafuerte and Negro 1998, Mougeot et al. 2007, 2009, 2010). I used this RGB-derived colour chart score as a continuous variable in subsequent analyses (Hill 1998, Jawor et al. 2003, Montgomerie 2006), calling it “skin yellowness” hereafter.

Since cere skin yellowness and skin yellowness of the orbital ring were highly correlated (cor = 0.93; $p < 0.001$), but tarsus skin yellowness not as strongly, a Principal Component Analysis (PCA) was run on the three colour measurements. The PCA resulted in two principal components, together explaining 97.3% of the variation in the data, with PC1 consisting mainly of cere colour and eye ring colour and PC2 consisting mainly of tarsus colour (Table A1 in Appendix). PC1 was used as “face skin yellowness” and PC2 as “tarsus skin yellowness” in subsequent analyses. Note that the values of PC1 and PC2 are antipodal: low values of PC1 represent more intense yellow colours and vice versa, low values of PC2 represent dull/whitish shades of yellow.

Skin yellowness was only measured in one nestling of a brood, which was randomly chosen. Between 2010 and 2012, the youngest sibling was excluded (due to its high mortality during the nestling phase), whereas between 2013 and 2015, the chosen nestling could also be the youngest sibling within the brood. This selection resulted in a dataset of n = 154 nestlings from 6 breeding seasons and 91 nest locations.

Sex determination

For genetic sex determination, DNA was extracted from full blood using standard protocol of QIAGEN DNeasy Blood & Tissue kit, and PCR products of the sex specific primers 2718R and 2550 F (Fridolfsson and Ellegren 1999) were visualised on 2% agarose gels. See details in Sumasgutner et al. (2014a).

Age determination and body condition index

Age of the nestlings was determined from plumage development, as the exact age (in days) was seldomly known from direct observation (Kostrzewa and Kostrzewa 1993). Another method, developed by Birdlife Finland (http://netti.nic.fi/~mattisj/petopull_ika_index.html), estimates the age of nestlings only from wing length. These two methods resulted in differing nestling ages, the finish formula making the Viennese nestlings on average 2.36 ± 0.06 (standard error) days younger than the method by Kostrzewa and Kostrzewa (1993), which is based on Kestrel populations in Germany. Since both methods for age determination are based on European Kestrel populations outside our study area, the average of both values was used in statistical analyses.

Nestlings were not all measured at the same age; hence, to derive a nestling’s body condition index we followed the method described by Roulin et al. (2007): First we extracted residuals from a second-order curve of body mass on wing length (with each Kestrel nestling appearing only once in the analysis, n = 556), and then removed variation in these residuals explained by sex (two-way

ANOVA; females are heavier than males). The latter residuals were our body condition index used in statistical analyses.

If not known from direct observation, the laying date of the first egg of the clutch was inferred by subtracting 30 days, the average breeding time of Kestrels (median 29 days, Glutz von Blotzheim and Bauer 1991); about 30 days, Kostrzewa and Kostrzewa 1993), from the hatching day, calculated from the age of the nestling at the time of ringing.To correct for the size difference of nestlings due to hatching asynchrony (Martínez-Padilla and Viñuela 2011, Hardey et al. 2013), we ranked the nestlings within the brood as senior ("1", first-hatched/largest, 1-3 chicks per brood) sibling, junior ("3", last-hatched/smallest) sibling and "2", all the other nestlings in between.

Statistical analysis

Each of the five health parameters used in this study (body condition index, H/L-ratio, ectoparasite infection, face skin yellowness and tarsus skin yellowness) was used as a response variable in generalised linear mixed models (GLMM) with the urban gradient (% cover of urban space) as an explanatory variable. To explore additional factors influencing individual health, age, sex, rank of the nestlings and the egg-laying date were added as additional co-variates to the model (after accounting for potential correlation of fixed effects). The GLMMs for body condition index, face skin yellowness and tarsus skin yellowness followed a Gaussian error structure. Ectoparasite infection was analysed with a Poisson, and H/L-ratio with a Gamma error structure. The year (breeding season) and nest ID (location where the nestlings were sampled) were included as random terms in each model, in order to account for pseudoreplication due to the lack of independence of several individuals produced within years or the same nest between different years (Hurlbert 1984). Apart from sex and year (factor variables), and rank (ordinal), all continuous variables were standardised in order to scale their effect size (Schielzeth 2010). All analyses were conducted in R (R Development Core Team 2015), using Rstudio (RStudio Team 2015) and the lme4 (Bates et al. 2015), car (Fox and Weisberg 2011), MASS (Venables and Ripley 2002), MuMIn (Bartoń 2016), effects (Fox 2003), lsmeans (Lenth 2016), lattice and RVAideMemoire (Hervé 2016) packages. Pseudo-R^2 for GLMMs was calculated after (Nakagawa and Schielzeth 2013), using the function "r.squaredGLMM" implemented in the package MuMIn, for the GLMMs with Gaussian and Poisson error distributions. Since it is not (yet) possible to calculate pseudo-R^2 using the above method for models with Gamma error distribution (S. Nakagawa, personal communication), I instead calculated the likelihood-ratio based pseudo-R^2 (Nagelkerke-modification) implemented in the function "r.squaredLR" in the MuMIn package.

Ethical note

The study was performed under license from the Environmental Protection Bureau of Vienna (MA22/1263/2010/3), the Ministry for Science and Research BM.W_F (BM.WF – 66.006/0021-II/3b/2013) and the ethics committee of the University of Veterinary Medicine, Vienna (BGBI.Nr.501/1989i.d.g.F.). All data were acquired strictly following current Austrian and EU law as well as the Weatherall Report and the guidelines for treatment of animals in behavioural research and teaching (ASAB 2015).

3. Results

Colour validation study

Photospectrometral colour measurement was successfully carried out on 25 Kestrel nestlings from 25 different nest sites in the breeding season of 2015. Correlations of Kestrel nestlings' skin yellowness from visual classification using reference colour charts (RGB) and photospectrometral colour measurements were strong when considering only the birds' tarsi (n = 21): S1y (Pearson's product-moment correlation: r = -0.81, p < 0.001), carotenoid chroma (r = 0.73, p < 0.001) and carotenoid saturation (r = -0.72, p < 0.001). The correlations are less strong but still significant in the birds' ceres (n = 25): S1y (r = -0.63, p < 0.001), carotenoid chroma (r = 0.41, p = 0.043) and carotenoid saturation (r = -0.43, p = 0.033) and when combining the whole dataset (n = 46) : S1y (r = -0.69, p < 0.001; Figure 4), carotenoid chroma (r = 0.48, p < 0.001) and carotenoid saturation (r = -0.32, p = 0.029).

Four abberant outlier tarsus spectra (all measured on the same day) had to be excluded in the analysis, which altered the correlation of RGB with S1y (z = -2.43, p = 0.015); however, this did not change the correlations significantly, either of RGB with carotenoid chroma (z = 0.82, p = 0.41) or of RGB with carotenoid saturation (z = 1.07, p = 0.29).

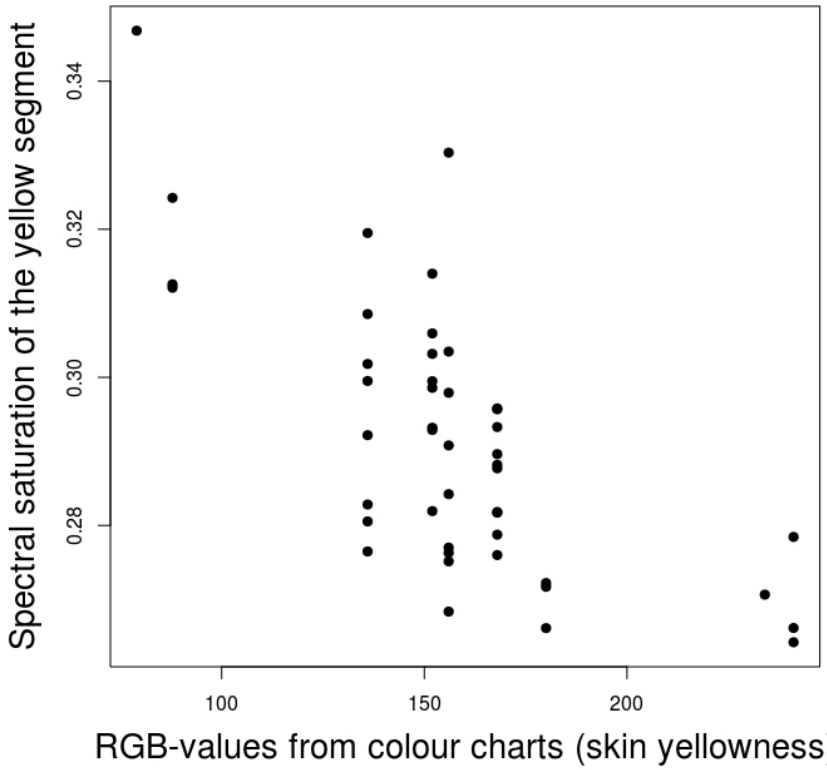


Figure 4: Scatterplot of colour chart classifications (RBG-values) and photospectrometral colour measurements (S1y) of 21 kestrel nestlings' tarsi and 25 ceres (r = -0.69, p<0.001).

Impact of urbanisation on health parameters

Kestrel nesting sites were distributed across the city within a wide range of degree of urbanisation (19.4% to 96.8%, Figure 5). We found no significant relationship between the degree of urbanisation around Kestrels' nesting sites and the body condition of the nestlings (GLMM 'urban gradient' single term: $\chi^2_{(153)} = 0.20$, p = 0.65, $R^2 = 4.3$; Figure 6a, Table 1). Ectoparasite *Carnus hemapterus* was found on 41 of the 154 nestlings (26.6% infection prevalence), the infection intensity ranging from one to 50 individuals of *C. hemapterus* per Kestrel nestling (mean = 1.4 ± 0.09 SE). Infection intensity with *C. hemapterus* was not found to be related to the urban gradient directly (GLMM 'urban gradient' single term: $\chi^2_{(153)} = 0.80$, p = 0.37, $R^2 = 1.51$; Figure 6b).

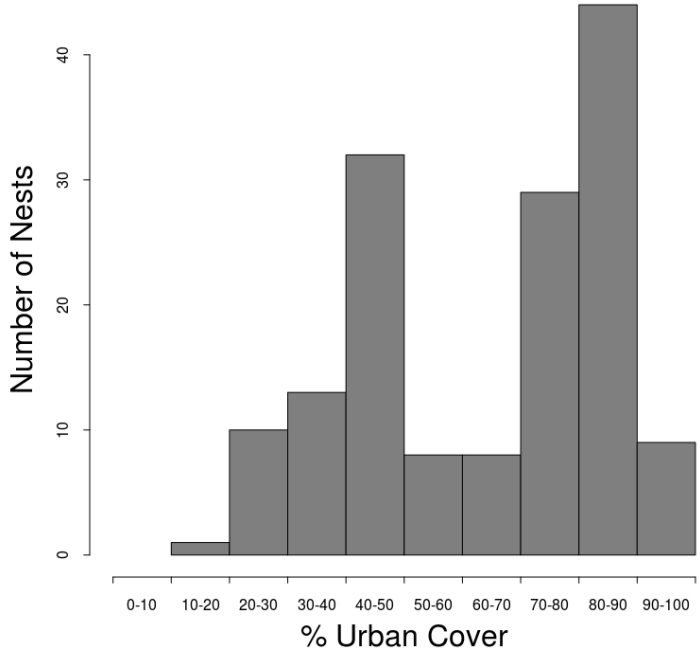


Figure 5: Frequency plot showing the number of nest sites (N = 154) along the urban gradient in 10% slots.

Blood samples were taken from all 154 individuals and the Heterophile/Lymphocyte-Ratio (H/L-Ratio) could be calculated from 75 of those individuals. H/L-Ratio ranged between 0.22 and 2.5 (mean = 0.97 ± 0.54 SD), but was not significantly influenced by the degree of urbanisation around the nest (GLMM 'urban gradient' single term: $\chi^2_{(74)} = 0.28$, p = 0.59, $R^2 = 24.5$; Figure 6c).

Table 1: Results of GLMMs exploring direct effects of the urban gradient as single explanatory variable on five health parameters. Significant p-values in bold.

Health Parameter	Error structure	Est.	SE	Chi²	p	R² for GLMM	R²LR	AICc
Body Condition Index (N = 154)	Gaussian	0.04	0.08	0.20	0.655	4.30	0.20	447.26
Ectoparasite infection intensity (N = 154)	Poisson	-0.13	0.14	0.80	0.370	1.51	2.95	252.31
H/L-Ratio (N = 75)	Gamma	-0.05	0.10	0.28	0.596	*NA*	24.47	96.39
Tarsus skin yellowness (N = 154)	Gaussian	-0.08	0.08	0.89	0.346	9.27	4.34	443.17
Face skin yellowness (N = 154)	Gaussian	0.18	0.08	5.03	**0.025**	9.24	4.25	445.21

The measurement of skin yellowness (RGB-values from colour chart classification) was available for all 154 kestrel nestlings in the dataset. Tarsus skin yellowness was not found to be related to the urban gradient (GLMM 'urban gradient' single term: $\chi^2_{(153)} = 0.89$, $p = 0.35$, $R^2 = 9.27$, Figure 6d), but face skin yellowness was less pronounced towards areas with a higher degree of urbanisation (GLMM 'urban gradient' single term: $\chi^2_{(153)} = 5.03$, $p = 0.025$, $R^2 = 9.24$, figure on single term not shown, but see Figure 6e and Table 2).

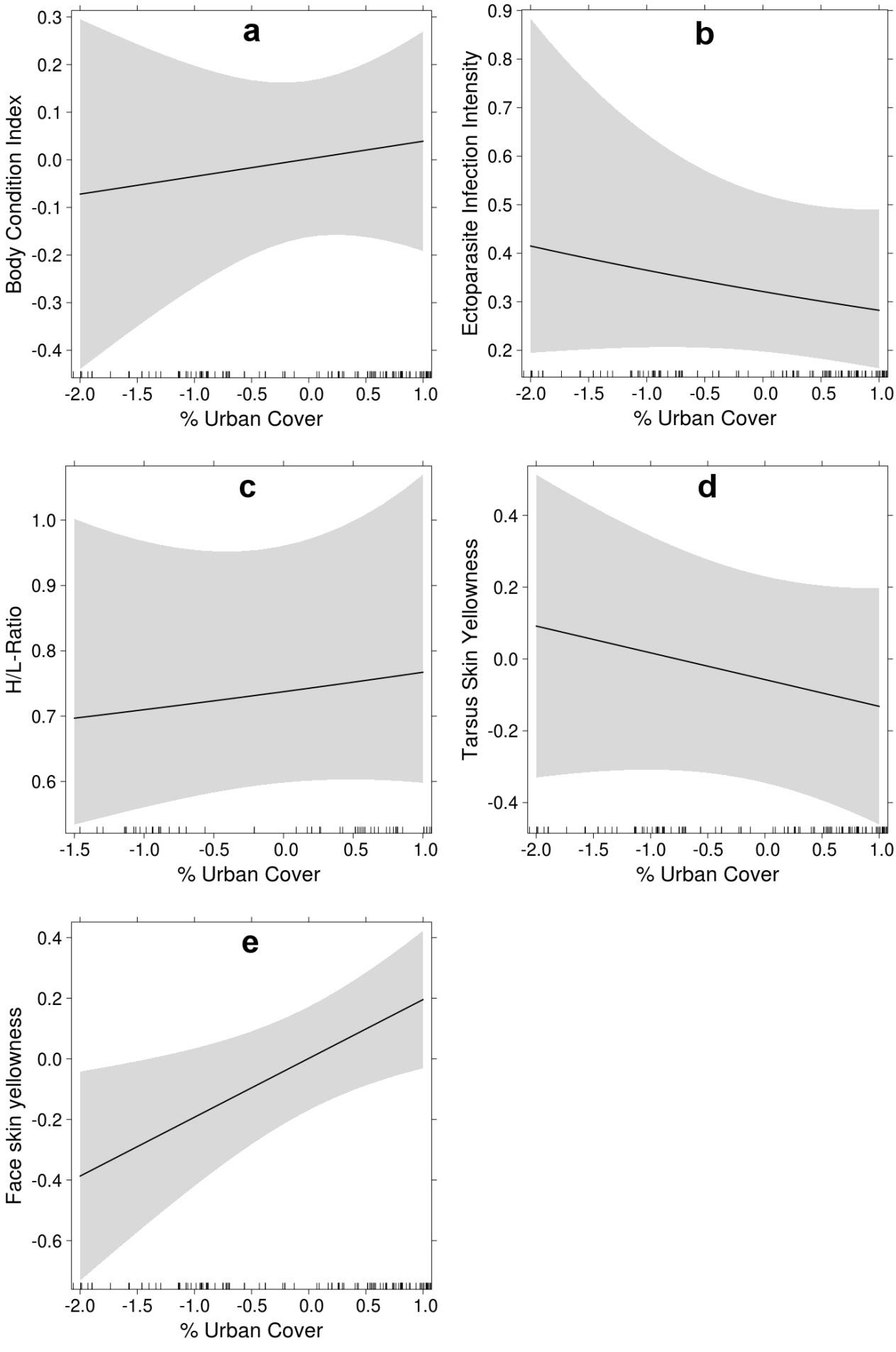
Explanation of health parameters by including additional explanatory parameters to the urban gradient in GLMMs yielded the best (most parsimonious, ranked by Akaike's Information Criterion, AIC) models. Body Condition Index, however, could not be explained by any combination of the explanatory variables used. The combination of nestling age, tarsus and face skin yellowness explained 57% (Table 2) of the variation in the Heterophil/Lymphocyte-Ratio.

Table 2 : Results of best GLMMs seeking to explain the health parameters (H/L-Ratio, ectoparasite infection intensity, tarsus skin yellowness, face skin yellowness) fitted with more explanatory variables than the urban gradient alone. Significant effects in bold.

Health Parameter	Est.	SE	Chi²	Df	p	Sign.	R² for GLMM	R²LR	AICc
H/L-Ratio (N = 75)							NA	56.83	75.35
(Intercept)	1.63	0.21	62.06	1	**<0.001**	***			
Age	0.39	0.09	20.16	1	**<0.001**	***			
Tarsus skin yellowness	-0.19	0.06	8.32	1	**0.004**	**			
Face skin yellowness	0.14	0.05	8.84	1	**0.003**	**			
Ectoparasite infection intensity (N=154)							12.86	10.96	243.99
(Intercept)	-1.25	0.26	22.96	1	**<0.001**	***			
Face skin yellowness	0.39	0.15	6.62	1	**0.010**	*			
Laying date	-0.37	0.16	5.80	1	**0.016**	*			
Tarsus skin yellowness (N = 154)							12.57	10.39	438.11
(Intercept)	-0.10	0.14	0.47	1	0.491				
Age	0.19	0.08	5.66	1	**0.017**	*			
Rank [a]			6.30	2	**0.043**	*			
Rank (middle)	0.10	0.19							
Rank (senior)	-0.28	0.14							
Face skin yellowness (N = 154)							22.80	19.94	426.03
(Intercept)	-0.20	0.12	2.74	1	0.098	.			
Age	-0.28	0.08	12.25	1	**<0.001**	***			
Urban gradient	0.19	0.08	6.56	1	**0.010**	*			
Laying date	0.14	0.08	3.10	1	0.078	.			
Sex [b]			5.15	1	**0.023**	*			
Sex (female)	0.35	0.15							

[A]: Rank (junior) was the reference category

[b]: Sex (female) was the reference category


a
Body Condition Index
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
% Urban Cover
b
Ectoparasite Infection Intensity
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
% Urban Cover
c
H/L-Ratio
1.0
0.9
0.8
0.7
0.6
-1.5
-1.0
-0.5
0.0
0.5
1.0
% Urban Cover
d
Tarsus Skin Yellowness
0.4
0.2
0.0
-0.2
-0.4
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
% Urban Cover
e
Face skin yellowness
0.4
0.2
0.0
-0.2
-0.4
-0.6
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
% Urban Cover

H/L-ratio decreases with increasing age of the nestlings (GLMM ‘age’ term: $\chi^2_{(72)} = 20.16$, $p < 0.001$; Figure A1a in Appendix), and is higher in nestlings with more intense skin yellowness of both tarsus (GLMM ‘tarsus skin yellowness’ term: $\chi^2_{(72)} = 8.32$, $p = 0.004$; Figure A1b) and face (GLMM ‘face skin yellowness’ term: $\chi^2_{(72)} = 8.84$, $p = 0.003$; Figure A1c). Ectoparasite infection intensity is higher in nestlings with less intense face skin yellowness (GLMM ‘face skin yellowness’ term: $\chi^2_{(152)} = 6.62$, $p = 0.01$; Figure A2a) and also in nestlings hatching earlier in the breeding season (GLMM ‘egg-laying date’ term: $\chi^2_{(152)} = 5.80$, $p = 0.016$; Figure A2b). The model with face skin yellowness and laying date explained only 13% of the variation in Ectoparasite infection intensity (Table 2). Tarsus skin yellowness intensity increases with age (GLMM ‘age’ term: $\chi^2_{(150)} = 5.66$, $p = 0.017$; Figure A3a) and depends on the rank of the chick in the brood, with middle siblings being more intensely yellow coloured than senior siblings (GLMM ‘rank’ term: $\chi^2_{(150)} = 6.30$, $p = 0.043$; Figure A3b). Post-hoc pairwise comparisons revealed a marginally significant difference only between senior (rank 1) and middle (rank 2) siblings (least-squares means: t-ratio = -2.41, $p = 0.045$). The best model explained 13% of the variation in tarsus skin yellowness (Table 2). Face skin yellowness intensity increases with age (GLMM ‘age’ term: $\chi^2_{(149)} = 12.25$, $p < 0.001$; Figure A4a), decreases with the increasing degree of urbanisation (GLMM ‘urban gradient’ term: $\chi^2_{(149)} = 6.56$, $p = 0.01$; Figure 6e) and differs between sexes (GLMM ‘sex’ term: $\chi^2_{(149)} = 5.15$, $p = 0.023$; Figure A4b), with males being more intensely coloured than females (post-hoc pairwise comparison, least-squares means: t-ratio = -2.20, $p = 0.029$). Additionally, nestlings from earlier broods show slightly more intense face yellowness (GLMM ‘egg-laying date’ term: $\chi^2_{(149)} = 3.10$, $p = 0.078$, Figure A4c). The best model explained 23% of variation in face skin yellowness (Table 2).

4. Discussion

In this study, we confirmed the method of scoring skin colouration of Kestrel nestlings with colour charts by comparison with measurements of spectral reflectance. Along the urban gradient, we found skin yellowness of nestlings from nest-sites in the city-centre to be least pronounced, which might indicate that those nestlings are strongly affected by novel urban stressors and indeed allocate these micronutrients to antioxidant defence. In addition, skin yellowness intensified with age and male nestlings and chicks from earlier nests were more intensely coloured. The other health indicators used in this study, i.e. body condition, H/L-ratio and parasite infection intensity were not directly linked to the urban gradient (Figure 7), but had some indirect effects.

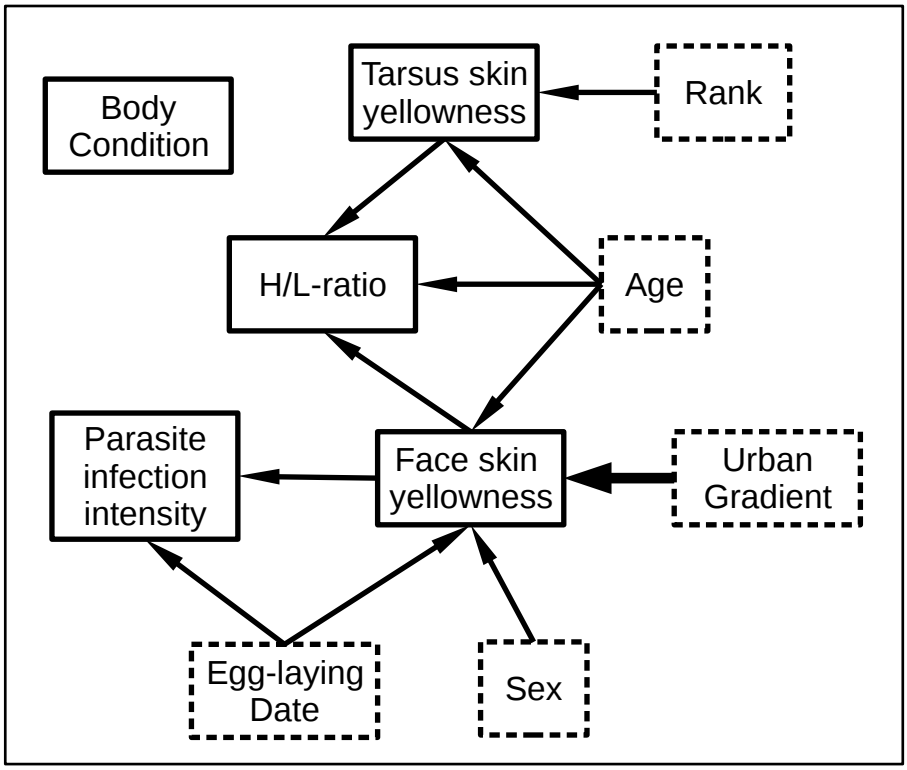


Figure 7: Schematic diagram showing interrelations of health-related variables used in this study. Arrows depict connections between variables and point from explanatories to response variables resulting from GLMMs. Variables in solid-lined boxes have been used as responses and explanatories in GLMMs, variables in dashed boxes only as explanatories. The arrow stemming from the urban gradient is highlighted to display it's importance in the analysis.

Colour validation study

We showed that scoring integument colouration with colour charts is a reliable method for measuring skin yellowness, i.e. carotenoid-based colouration in Kestrel nestlings. This finding confirms the use of colour charts in scoring colouration as a frequently used method in animal research (Bortolotti et al. 1996, McDonald 2003, Eraud et al. 2007, Sternalski et al. 2010, 2012a, b, Blas et al. 2013). Compared to the usually accurate and precise spectral measurement of colours with a photospectrometer, the use of colour charts has several advantages: colour charts are less

expensive, easier to use, easier to analyse (given the choice of appropriate colour charts), easier to transport in the field, do not rely on electricity and are applied faster. This reduces the handling time of the animal tremendously and hence reduces human induced stress (Vleck et al. 2000, Romero and Reed 2005). On the other hand, colour chart "measurements" rely on the objectivity of the observer, ambient light and the limitations of human colour vision, i.e. the inability to perceive UV radiation, and thus can be complex to interpret (Endler 1990, Andersson and Prager 2006, Montgomerie 2006). The choice of an appropriate colour chart is crucial, with even-spaced colour intervals or pre-defined values in a tristimulus colour space (e.g. Munsell colour system; see Montgomerie 2006) to be preferred. Since spectral reflectance is "the most objective and context-independent measure of (…) color variation" (Andersson and Prager 2006 p. 42), it is inevitable to validate any scoring method using photospectrometry at least on a subsample, but not many studies have done so (but see Blas et al. (2013) for an exemplary study). In our study, the correlations of three different colourimetrics extracted from photospectrometral colour measurements with a colour score derived from colour charts were strong. Thus we confirmed the adequacy of these measurements, and henceforth use them as our colouration value.

Habitat or diet dependence of carotenoid integument colouration

Carotenoid-based colouration of birds' bare parts like the bill or the skin is a dynamic, condition-dependent signal, and supposedly an honest signal that reflects an individual's health status (Faivre et al. 2003, Casagrande et al. 2009). These findings are challenged, though, since recent research in a raptor has found that correlation between circulating carotenoids and skin colouration can be limited (Garcia-Heras unpublished manuscript). Past research has shown that Kestrels nesting in Vienna's (and other European cities'; e.g. Piattella et al. 1999, Riegert and Fuchs 2004, Kübler et al. 2005) inner-city habitats feed their chicks on a higher proportion of carotenoid-rich prey (i.e. birds, lizards and insects; Olson and Owens 1998, Bortolotti et al. 2000, Costantini et al. 2005) than their suburban conspecifics (Sumasgutner et al. 2013, 2014a, b, Kreiderits et al. 2016). Following these findings and the differences in brood sizes and nestling mortality along the urban gradient, we hypothesised that either (I) the intensity of integument carotenoid colouration is highest in nestlings from more urbanised areas or (II) the opposite, since breeding in highly urbanised areas is connected with strong stressors and subsequent depletion of carotenoids for e.g. antioxidant defence. In concordance with hypothesis (II), we found nestlings from the city centre to have least intense yellow colouration. Several non-exclusive reasons may explain this finding: (a) urban stressors, e.g. chemical pollution, noise and light pollution and heavy metals may result in oxidative stress, hence city Kestrels need to use all dietary antioxidants, such as carotenoids, to reduce oxidative damage. Furthermore, inflammations and infections with parasites may be more frequent in urbanised areas (see for example Delgado-V. and French 2012), although we did not find variation in ectoparasite infection along the urban gradient in this study), which activates the immune system. This immune system activation requires more circulating carotenoids, which are thus consumed for physiological needs and cannot be used for integument pigmentation anymore (Eraud et al. 2007, Pérez-Rodríguez 2009). (b) Carotenoid content of urban prey species such as great tits (*Parus major*) is not very high after all (Isaksson et al. 2005), since those urban passerines mainly forage on low-quality food resources themselves (i.e. caterpillars with low carotenoid contents, Isaksson and Andersson 2007, due to low carotenoid levels in inner-city trees, Isaksson 2009). This entire urban food chain reflects limited availability of dietary antioxidants and strong environmental stress. (c) It may also be possible that this finding is in part caused by a sampling

effect. Often, nestlings from inner-city nest-sites were sampled at a younger age than those in the periphery due to field work constraints: limited visibility of the brood niche calls for more frequent nest visits, and, since the cavities are approached mainly from behind, there is an increased risk of nestlings jumping out of the nest onto the street, which is why we tend to handle them earlier than for example broods in window boxes that can be aged easily without disturbing and that are overall used to human activities and less prone to induced-fledging.

If explanation (a) would hold true and urban stressors indeed create such a massive stress for Kestrel nestlings that all carotenoids are used for antioxidant defence, we would expect a direct link to the physiological stress measurement taken in this study, i.e. the ratio of heterophils to lymphocytes (H/L-ratio). In fact, our results opposed each other, since H/L-ratio was higher in more intensely yellow coloured nestlings. High values of H/L-ratio are generally thought to indicate higher glucocorticoid levels and therefore reflect higher stress levels (Davis et al. 2008, Cīrule et al. 2012), but this has been challenged by other research where no such connection was found (Müller et al. 2011). Since urbanisation changes whole ecosystems and the species networks (Marzluff 2001), effects of increased oxidative stress will act on all trophic levels from primary producers to apex consumers. Explanation (b) supports this relation of overall low carotenoid contents of biota in urban areas, just as evidence from plants (Joshi and Swami 2009) and passerines (Eeva et al. 1998, Møller et al. 2010, Giraudeau et al. 2015a, b) does.

The potential link between carotenoid-colouration and the urban gradient described above was found in the facial integuments (i.e. cere and orbital ring) of Kestrel chicks, but not for their tarsal skin. Cere and orbital ring colour scores in our Kestrel nestlings were often identical (62% of all 454 measurements) and usually very similar. Most often, tarsus colour scores differed from the two facial colouration scores, in that the legs were usually more intensely yellow whereas facial colouration also ranged between greenish-yellow and bluish-yellow colours (see Figure 3). The intensity of yellow integument colouration (both facial and tarsial skin yellowness) of Kestrel nestlings increased with age in our study, as known from other raptor nestlings, including Kestrels (Casagrande et al. 2007, Sternalski et al. 2010). This positive age-dependence of carotenoid-based colouration may mirror the condition of the nestlings which usually increases with age and the maturation of the immune system. It is also possible, though, that older chicks are more intensely yellow because carotenoids had more time to accumulate in the integuments or the absorption and deposition processes are more mature in older nestlings (Casagrande et al. 2007). The observed effect of nestling rank on tarsus skin yellowness was likely an artefact of unequal sample sizes in our data, with only 17 junior individuals measured (compared to 55 senior and 82 middle ranked chicks).

In our study, male nestlings were more intensely coloured (face skin yellowness) than females, which is in line with a study on marsh harrier (*Circus aerugionsus*) nestlings (Sternalski et al. 2012a), but not with other Kestrel nestlings (Casagrande et al. 2007). Possibly males and females have different carotenoid allocation strategies, resulting in differential maturation of traits important for sexual selection like intense male skin yellowness. This trait may already be present in nestlings but does not acquire its signalling function until adulthood (Sternalski et al. 2012a). Another possible explanation is that sexes follow different growth strategies in that females, the larger sex in size-dimorphic raptors (Glutz von Blotzheim and Bauer 1991, Mebs and Schmidt-Rothmund 2014), invest more in growth and require more carotenoids as antioxidants against growth-related free

radical production (Surai and Speake 1998). This explanation would lead to less intense skin yellowness in females, as well as the possibility that female nestlings are larger and stronger and could therefore, to some extent, control the distribution of prey items among siblings. Possibly, they thus consume larger prey which is a poorer source of carotenoids (mammals) and leave the smaller prey (birds and insects, with higher carotenoid contents) to their brothers (Sternalski et al. 2012b).

The best model explaining face skin yellowness included the egg-laying date, although it was not significant. Nestlings from clutches laid earlier in the breeding season tended to have more intense face skin yellowness. Early breeding, especially in migratory species such as Kestrels in Vienna (Schmidt et al. 2014), is generally linked to superior individual quality, i.e. healthier individuals migrate to the breeding grounds earlier than their conspecifics and occupy better habitats (Kokko 1999, Sergio et al. 2007). Early breeding in Viennese urban Kestrels has been associated with city-centre-breeders, which in turn was connected with smaller clutches, lower hatching success and overall lower breeding success (support for the "ecological trap" hypothesis; Sumasgutner et al. 2014a). The results from this study suggest that early breeders might not be food-limited at the time they feed their chicks. For early breeders, especially in the city centre, competition for scarce food may not yet be as drastic as a few weeks later, when the density of breeding pairs is higher and the nearest-neighbour-distance smaller (Sumasgutner et al. 2014a). Our results further suggest that nestlings from early broods experience less stress than nestlings in later broods, or that parents of higher individual quality provide overall more food or prey that contains a higher level of dietary antioxidants. Our results may indicate that chicks from early clutches are at least not carotenoid-limited.

The hard times of urban living: reactions to novel urban stressors

Kestrels and other birds living in human-made landscapes are exposed to novel stressors associated with the urban environment (Marzluff 2001). Many animals react to these stressors with physiological impairment. In Vienna, Sumasgutner et al. (2014a) suggested the lack of available main prey (diurnal rodents) to result in food scarcity and starvation of nestlings. Provisioning of suitable prey might be further decreased due to high competition in the city centre, i.e. high breeding density and low nearest-neighbour-distances. Since Viennese Kestrels have lower breeding success in the city centre (Sumasgutner et al. 2014a, b), we predicted two competing effects: I) urban life imposes high physiological stress on Kestrel nestlings leading to a measurable drop in health-related traits; and II) given that less chicks fledge per nest in the inner-city, sibling competition for limited food could be lower, resulting in an overall higher quality of surviving fledglings (offspring size - number trade-off). We could not show a direct effect of the urban gradient on either body condition, H/L-ratio or infection intensity with *C. hemapterus*, the most abundant ectoparasite of Kestrel nestlings. In fact, we could not identify any variable that was directly correlated with body condition as found in other studies. For instance, body condition decreased with increasing urbanisation in house sparrows (*Passer domesticus*; (Shochat 2004, Liker et al. 2008). We expected environmental stress in the urban habitat to be reflected in the H/L-ratio. Yet, likewise to body condition, we found no direct effect of the urban gradient on H/L-ratio, but a somewhat indirect effect through face skin yellowness that was linked to the urban gradient (Figure 7). H/L-ratio rose with increasing face skin yellowness. This suggests that the most intensely carotenoid-coloured nestlings also have the highest physiological stress levels. At the same time more intensely yellow coloured individuals were found in more suburban nesting sites. This is very

interesting in regard to previous findings (Sumasgutner et al. 2014a), where breeding in the city centre was related to higher chick mortality during the nestling phase. It might well be that lower nestling competition in smaller broods is linked to this finding, due to reduced food stress in more urban environments due to the high mortality of weak chicks leaving more resources for the remaining smaller brood. Additionally, H/L-ratio was negatively correlated to nestling age, implying that older chicks are less stressed than younger chicks. The immune system is still developing during the nestling stage and hence younger chicks heavily invest in innate immunity, which increases H/L-ratio (Dehnhard et al. 2011, Banbura et al. 2013). Thus, our result is not surprising given that chicks hatch asynchronously, and if nestling competition should be the main driver for individual stress and individual colouration, younger chicks should be stronger negatively affected than their older siblings. This connection was also found in e.g. Montagu's Harrier (*Circus pygargus*, Sternalski et al. 2012a) and Black Kite (*Milvus migrans*, López-Jiménez et al. 2016) but not in Kestrel nestlings (Müller et al. 2010). The interpretation of H/L-ratio as a stress measure is not undebated. The positive correlation of circulating glucocorticoid hormone levels in blood with H/L-ratio (Davis et al. 2008) was not found in Kestrel nestlings and was therefore previously questioned (Müller et al. 2011). The correlation was found in laboratory studies on Chickens (*Gallus gallus* f. dom., Gross and Siegel 1983), but only in stable, low-stress environments, and not in stressed conditions on free-living birds (Vleck et al. 2000, Clinchy et al. 2004, Müller et al. 2011). H/L-ratio did correlate, though, with several environmental stressors in numerous animal studies (Vleck et al. 2000, Suorsa et al. 2003, 2004, Lobato et al. 2005, Davis et al. 2008), including Kestrel nestlings (Müller et al. 2011), with all correlations pointing in the expected direction: worse conditions produced higher H/L-ratios. Apparently, circulating glucocorticoid levels and H/L-ratio display different stressors and thus the choice of the appropriate measure must comply with the research question. Since H/L-ratio reflects the number of different types of leucocytes and therefore the body's defences in general, it also changes with inflammations and disease, not only environmental stressors. Müller et al. (2011) found that H/L-ratio correlated positively with many subtle, not immediately life-threatening stressors (contrary to circulating glucocorticoids) and also rose in response to immediately dangerous stressors of nestling Kestrels. They concluded that H/L-ratio is not always a good indicator of severe stress (review of the poultry literature in Maxwell 1993), but rather of stress over a longer period of time, which was also the interest of this study.

We found no direct effect of urbanisation on the ectoparasite infection intensity with the blood-sucking fly *C. hemapterus*. The infection intensity was higher in nestlings with paler face skin yellowness. Since face skin yellowness also decreased with the degree of urbanisation, the combination of these results would lead to the conclusion that nestlings from inner-city nest sites tend to have more ectoparasites. Parasite infection intensity was also highest in nestlings from early nests, decreasing over the course of the breeding season. A seasonal trend of infection with ectoparasites is known from raptor studies, but usually parasite numbers increased during the season (Roulin et al. 2007), although Sumasgutner et al. (2014c) found a seasonal decline of parasite infection intensity in Kestrel nestlings, limited to nest boxes that were left un-cleaned and consecutively used for breeding. In Vienna, we basically have the same situation, since Kestrels mainly breed in roof openings with pellets and other prey remains from earlier breeding attempts, where *C. hemapterus* can overwinter. The results from this study are further support for the hypothesis that Kestrels breeding in Vienna choose nest-sites due to their attractiveness and prey remains from past successful breeding attempts as public information, but are unable to assess the

true quality of the surrounding hunting territory (Sumasgutner et al. 2014a, b). Together with the findings on the health status provided in this current study, Kestrels breeding in Vienna's city centre could indeed be in danger of falling into an 'ecological trap'.

5. Zusammenfassung

Durch Urbanisierung werden weltweit immer mehr natürliche Ökosysteme bedroht. Dadurch werden Tier- und Pflanzengesellschaften gefährdet sowie Nahrungsnetze verändert. Städtische Habitate werden sowohl durch versiegelte Flächen und menschengemachte Strukturen, als auch durch die Anreicherung von Umweltschadstoffen geformt und beeinflusst. Viele Tiere, die in der Stadt leben, werden von diesen Faktoren beeinflusst und beeinträchtigt. Anhand ihrer Toleranz und Anpassungsfähigkeit unterscheidet man zwischen Arten, die die Stadt vermeiden, sich an diese anpassen oder diese ausnutzen. Letztere werden durch den Lebensraum Stadt sogar gefördert. Vogelarten sind mobil und können daher räumlich und zeitlich urbanen Stressfaktoren ausweichen. Die Küken des Turmfalken (*Falco tinnunculus*), ein mittelgroßer Greifvogel und Stadtbrüter, sind jedoch Nesthocker und verweilen etwa 30 Tage lang am Nistplatz. Diese Küken sind daher urbanen Stressfaktoren unvermeidlich ausgesetzt. Die Ernährung von Turmfalkenküken in Wien ist vielseitig und besteht aus Vertebraten (hauptsächlich Nagern und Singvögeln) und Invertebraten (etwa Insekten). Die Nahrungszusammensetzung unterscheidet sich zwischen der Innenstadt und den Außenbezirken. Die Beutetiere weisen unterschiedliche Nährwerte und Konzentrationen von Nährstoffen wie Carotinoiden auf, was sich auf die Gesundheit der Küken auswirken kann. Carotinoide sind gelb-rote Pigmente, die unter anderem in Geweben wie Haut und Federn eingelagert werden und daher deren Farbe maßgeblich beeinflussen. Sie fungieren zudem als Antioxidantien und können das Immunsystem stimulieren. Abhängig von ihrer Nahrung können Carotinoide für Turmfalken eine limitierende Resource sein, was zu einem Konflikt in deren Verteilung für diese verschiedene Funktionen führen kann. Um die Auswirkungen des städtischen Lebensraumes auf Turmfalken zu untersuchen, wurden die carotinoid-basierte Färbung der Haut und weitere Indikatoren der individuellen Gesundheit (Heterophile/Lymphozyten-Verhältnis, körperliche Verfassung und der Befall mit dem Ektoparasiten *Carnus hemapterus*) von 154 Turmfalkenküken (aus 91 Nestern) in den Jahren 2010 bis 2015 entlang eines Urbanitätsgradienten in Wien gemessen. Die innerstädtischen Küken waren am schwächsten gelb gefärbt. Eine mögliche Erklärung ist, dass diese Küken von städtischen Stressfaktoren am stärksten beeinflusst werden und daher die zur Verfügung stehenden Carotinoide nicht für die Färbung der Haut, sondern für gesundheitsrelevante Funktionen genutzt werden. Außerdem wurde festgestellt, dass Männchen generell stärker gelb gefärbt sind, die Gelbfärbung mit dem Alter der Küken zunimmt und Küken, die im Vergleich früher in der Brutsaison geschlüpft sind, eine stärkere Gelbfärbung aufweisen. Da sich das Immunsystem von Turmfalkenküken noch in der Entwicklung befindet, brauchen jüngere Küken wahrscheinlich mehr Antioxidantien um dem Umweltstress entgegen zu wirken. Zudem hatten jüngere Küken eine höheres Heterophile/Lymphocyten-Verhältnis, was unsere Vermutung auf erhöhten Stress erhärtet. Eines der Geschlechter könnte im Wettstreit um Nahrung zwischen den Küken eines Geleges im Vorteil sein; oder die Geschlechter haben unterschiedliche Wachstumsgeschwindigkeiten, die zu einem Unterschied in der benötigten Menge an Nährstoffen führen könnte. Es konnte keine direkte Abhängigkeit von den anderen Gesundheitsindikatoren zum Urbanitätsgradienten gezeigt werden. Jedoch wurden manche indirekt davon beeinflusst. Der Befall mit Ektoparasiten war am stärksten bei Küken aus Gelegen, die früher in der Brutsaison gelegt wurden und bei Küken mit weniger ausgeprägter Gelbfärbung der Haut. In Kombination mit Erkenntnissen aus früheren Untersuchungen weisen die Ergebnisse dieser Studie darauf hin, dass Turmfalken in Wien in eine „ökologische Falle“ tappen könnten, da zwar viele Nistmöglichkeiten zum Brüten einladen, aber sowohl Produktivität als auch individuelle Gesundheit beeinträchtigt sind.

6. References

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7. Appendix

Table A1: Results of a Principal Components Analysis (PCA) on the colour chart measurements of 3 bodyparts of kestrel nestlings: tarsus, cere and orbital ring. PCs 1 and 2 were used as "face skin yellowness" and "tarsus skin yellowness", respectively, in further analyses.

Rotation:	**PC1**	**PC2**	**PC3**
Tarsus RGB	0.31	-0.95	0.07
Cere RGB	0.66	0.27	0.71
Orbital ring RGB	0.69	0.17	-0.70
Importance of components:			
Standard deviation	69.99	32.67	12.82
Proportion of Variance	0.80	0.17	0.03
Cumulative Proportion	0.80	0.97	1.00

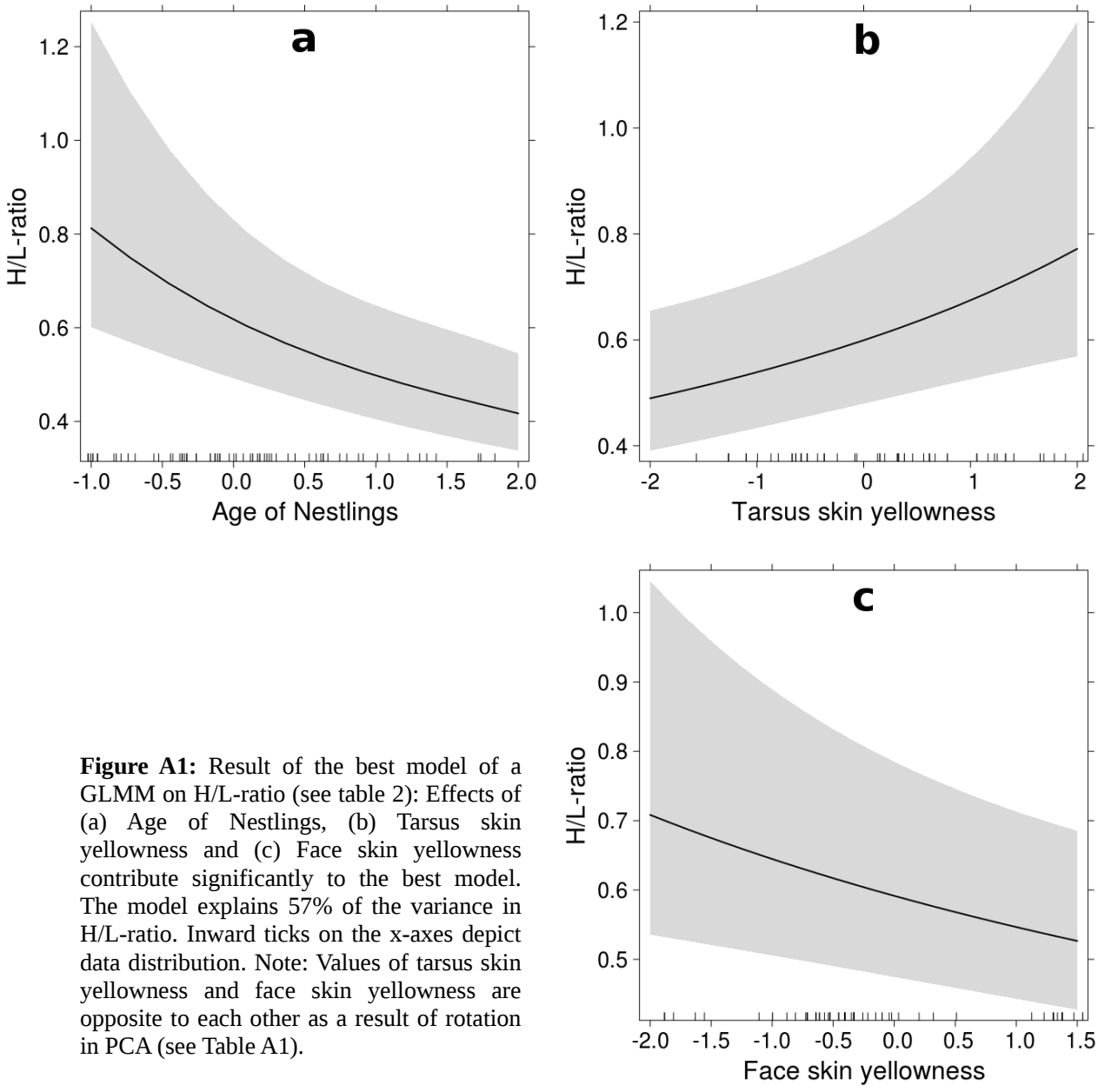

Figure A1: Result of the best model of a GLMM on H/L-ratio (see table 2): Effects of (a) Age of Nestlings, (b) Tarsus skin yellowness and (c) Face skin yellowness contribute significantly to the best model. The model explains 57% of the variance in H/L-ratio. Inward ticks on the x-axes depict data distribution. Note: Values of tarsus skin yellowness and face skin yellowness are opposite to each other as a result of rotation in PCA (see Table A1).

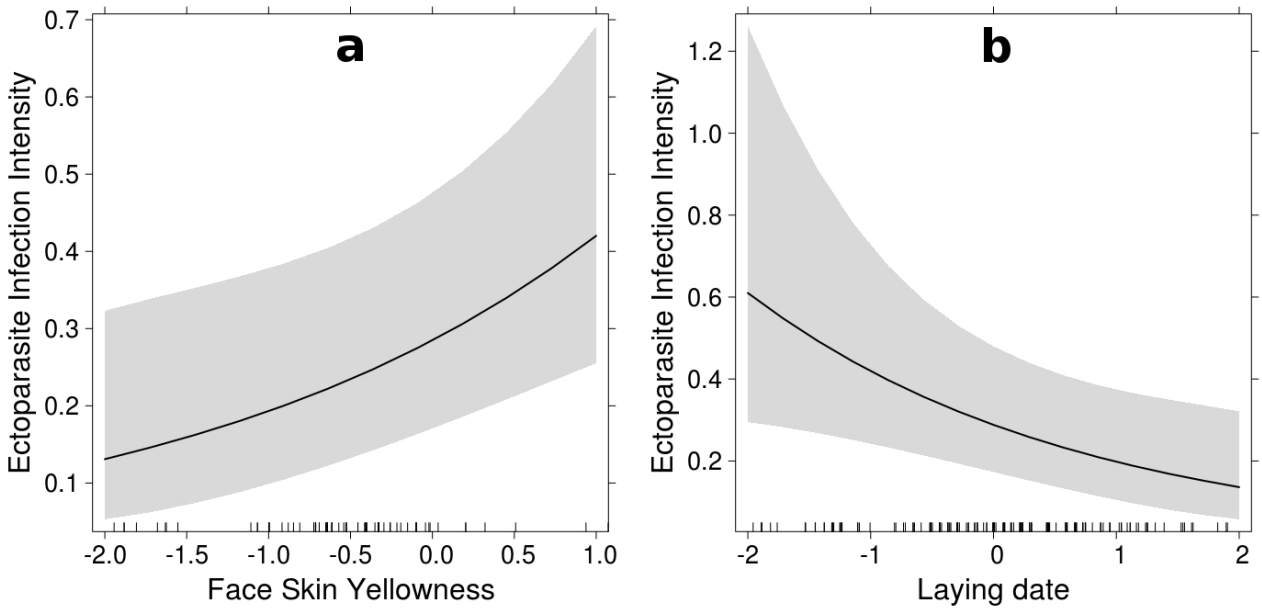

Figure A2: Result of the best model of a GLMM on Ectoparasite infection intensity. Effects of (a) Face skin yellowness and (b) the egg-laying date contribute significantly to the best model (see Table 2). The model explains 13% of the variance in ectoparasite infection intensity. Inward ticks on the x-axis depict data distribution.

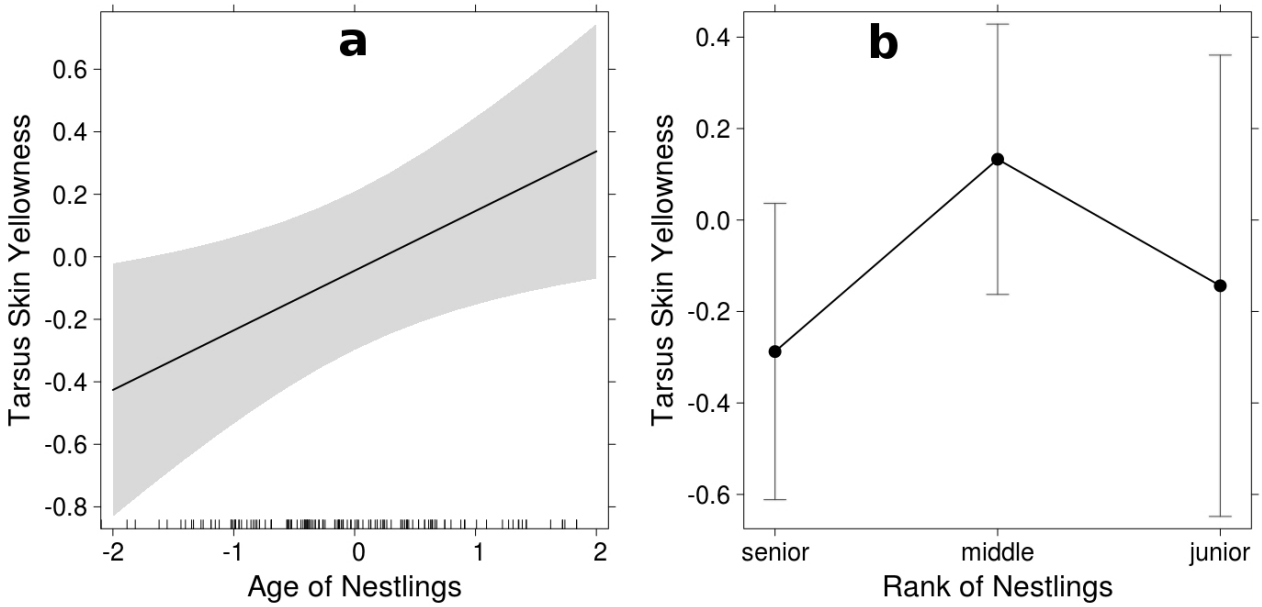

Figure A3: Result of the best model of a GLMM on Tarsus skin yellowness. Effects of (a) age of nestlings and (b) rank of nestlings contribute significantly to the best model (see Table 2). The model explains 13% of the variance in tarsus skin yellowness. Inward ticks on the x-axes depict data distribution.

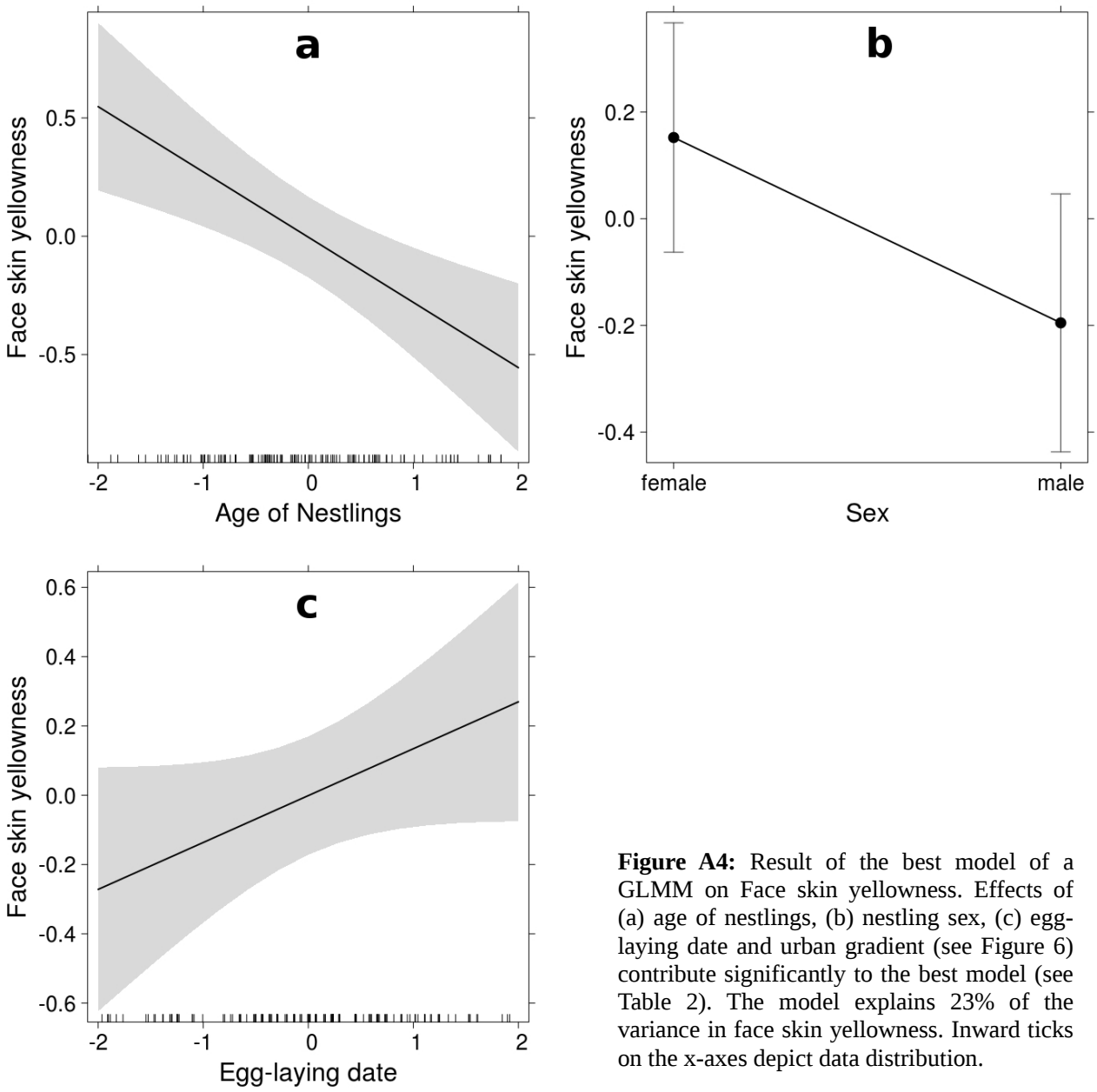

Figure A4: Result of the best model of a GLMM on Face skin yellowness. Effects of (a) age of nestlings, (b) nestling sex, (c) egg-laying date and urban gradient (see Figure 6) contribute significantly to the best model (see Table 2). The model explains 23% of the variance in face skin yellowness. Inward ticks on the x-axes depict data distribution.