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1 **Anthropogenic noise is associated with telomere length and carotenoid-based coloration in free-**
2 **living nestling songbirds**

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4 **Anthropogenic noise and telomere length**

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15

16 **ABSTRACT**

17 Growing evidence suggests that anthropogenic noise has deleterious effects on the behavior and
18 physiology of free-living animals. These effects may be particularly pronounced early in life, when
19 developmental trajectories are sensitive to stressors, yet studies investigating developmental effects of
20 noise exposure in free-living populations remain scarce. To elucidate the effects of noise exposure during
21 development, we examined whether noise exposure is associated with shorter telomeres, duller
22 carotenoid-based coloration and reduced body mass in nestlings of a common urban bird, the great tit
23 (*Parus major*). We also assessed how the noise environment is related to reproductive success. We
24 obtained long-term measurements of the noise environment, over a ~24-hour period, and characterized
25 both the amplitude (measured by LA_{eq} , LA_{90} , LA_{10} , LA_{max} .) and variance in noise levels, since more
26 stochastic, as well as louder, noise regimes might be more likely to induce stress. In our urban

27 population, noise levels varied substantially, with louder, but less variable, noise characteristic of areas
28 adjacent a highway. Noise levels were also highly repeatable, suggesting that individuals experience
29 consistent differences in noise exposure. The amplitude of noise near nest boxes was associated with
30 shorter telomeres among smaller, but not larger, brood members. In addition, carotenoid chroma and hue
31 were positively associated with variance in average and maximum noise levels, and average reflectance
32 was negatively associated with variance in background noise. Independent of noise, hue was positively
33 related to telomere length. Nestling mass and reproductive success were unaffected by noise exposure.
34 Results indicate that multiple dimensions of the noise environment, or factors associated with the noise
35 environment, could affect the phenotype of developing organisms, that noise exposure, or correlated
36 variables, might have the strongest effects on sensitive groups of individuals, and that carotenoid hue
37 could serve as a signal of early-life telomere length.

38
39 **Capsule:** Loud noise was associated with shorter telomeres among small, but not large, brood members,
40 whereas variance in the noise environment was correlated to carotenoid-based plumage traits.

41
42 **Keywords:** Anthropogenic noise, Developmental stress, Telomeres, Carotenoid-based coloration, Body
43 mass, Size rank, *Parus major*

44 45 **1. Introduction**

46 Anthropogenic environments present organisms with complex mosaics of highly developed and less
47 disturbed habitat, in which novel stressors are experienced (Gaston et al., 2013; Swaddle et al., 2015;
48 Bauerová et al., 2017). Anthropogenic noise is one such stressor that may seriously interfere with animal
49 behaviors, with cascading effects on health status and fitness (Barber et al., 2010; Kight and Swaddle,
50 2011; Swaddle et al., 2015; Shannon et al., 2016; Kleist et al., 2018). In habitats with high levels of
51 anthropogenic noise, sounds and signals no longer propagate in the same way through the environment,
52 with implications for foraging, vigilance, and communication (Leonard and Horn, 2005, 2008; Quinn et

53 al., 2006; Kight and Swaddle, 2011; Narango and Rodewald, 2016). Moreover, innocuous anthropogenic
54 noises may be perceived as stressors, and consistent activation of stress responses may result in chronic
55 stress and pathology (Blickley et al., 2012; Tennessen et al., 2014; Kleist et al., 2018).

56 Exposure to loud noise may affect animals across life history stages (Francis and Barber, 2013), and
57 may have particularly strong effects early in life, when the phenotype remains sensitive to organizational
58 effects (Metcalf and Monaghan, 2001; Monaghan, 2008). A growing number of studies on developing
59 animals document effects of noise exposure on phenotypic traits, including body condition, oxidative
60 status, glucocorticoid and haptoglobin levels, and growth and metabolic rates (Potvin and MacDougall-
61 Shackleton, 2015; Brischoux et al., 2017; Davies et al., 2017; Raap et al., 2017; Injaian et al., 2018a, b).
62 However, the mechanisms underlying phenotypic effects of early-life stress exposure in general, and
63 anthropogenic noise exposure in particular, remain poorly understood.

64 One mechanism that may link later-life fitness effects to early-life stress exposure is telomere loss.
65 Telomeres are conserved repeats of nucleotide sequences that cap chromosomes and protect coding DNA
66 from damage and malfunction (Monaghan and Hausmann, 2006; Hausmann et al., 2012; Monaghan,
67 2014). Telomeres shorten upon cellular division, with rapid rates occurring during development in
68 association with rapid growth (Heidinger et al., 2012). Once reduced beyond a threshold length, telomere
69 shortening triggers cellular senescence. Shorter telomeres have been related to stress exposure, increased
70 biomolecular aging rates, and pathology (Monaghan, 2014 for review). On a related vein, faster
71 shortening of telomeres is correlated with disease and lower survival prospects (Hausmann et al., 2005;
72 Heidinger et al., 2012; Boonekamp et al., 2014; Wilbourn et al., 2018).

73 Early-life noise exposure may also affect other condition-dependent traits, such as plumage
74 pigmentation, with potential later-life effects on mating and reproductive success. Carotenoid-based
75 pigmentation is a common sexually-selected trait in vertebrates, which is responsible for red, yellow and
76 orange coloration in integumentary tissues (McGraw, 2006). Carotenoids must be obtained from the diet,
77 since they cannot be synthesized *de novo* by animals (Isaksson, 2009), and may serve as antioxidants,
78 meaning that depositing carotenoids in the skin or feathers may tradeoff against combating oxidative

79 stress (Alonso-Alvarez et al., 2004, 2008; McGraw, 2006). Thus, increased stress levels associated with
80 noise exposure may reduce the intensity of carotenoid-based coloration. Moreover, if carotenoid-based
81 coloration is sensitive to stressors, such as loud noise, then coloration may serve as a visual signal of
82 associated declines in physiological state, such as telomere loss and reduced body condition. Indeed, the
83 role of carotenoids as antioxidants is debated, but their condition-dependence is well-established, and
84 researchers have proposed that carotenoid-based pigmentation could serve as a bioindicator trait for
85 environmental stress exposure (Hill, 1991, 1995; Eeva et al., 1998; McGraw, 2006).

86 Past studies demonstrate that early-life stress associated with factors such as sibling competition
87 (Nettle et al., 2013, 2015; Stier et al., 2015), environmental conditions at high elevations (Stier et al.,
88 2016), and nutritional stress (Nettle et al., 2017), can accelerate telomere shortening during development.
89 In addition, great tit (*Parus major*) nestlings in urban areas were found to have shorter telomeres than
90 those in rural areas (Salmón et al., 2016). Finally, three recent studies report negative effects of
91 anthropogenic noise exposure on the telomere length of young birds (Meillère et al., 2015; Dorado-Correa
92 et al., 2018; Injaian et al., 2019).

93 However, the association between early-life noise exposure and the expression of carotenoid-based
94 pigmentation remains unexplored. Moreover, noise environments are multidimensional, and past studies
95 have incompletely captured this complexity since they generally rely on single types of manipulations or
96 short-term measurements of noise amplitude, thus ignoring patterns of temporal variance in noise (Gill et
97 al., 2015). In addition to the amplitude of noise, variance in the noise environment may also significantly
98 affect the phenotypic traits of animals, with ramifications for community dynamics and fitness. More
99 variable noise regimes may be more likely to activate stress responses and result in phenotypic changes
100 that have fitness consequences, whereas organisms might more readily habituate to consistent noise
101 regimes (Blickley et al., 2012; Gill et al., 2015; Injaian et al., 2018b).

102 We explored the hypothesis that exposure to anthropogenic noise negatively affects telomere length,
103 carotenoid-based pigmentation, and body mass in great tit (*Parus major*) nestlings, and also assessed the
104 potential for duller carotenoid-based pigmentation to signal telomere shortening and reduced body mass.

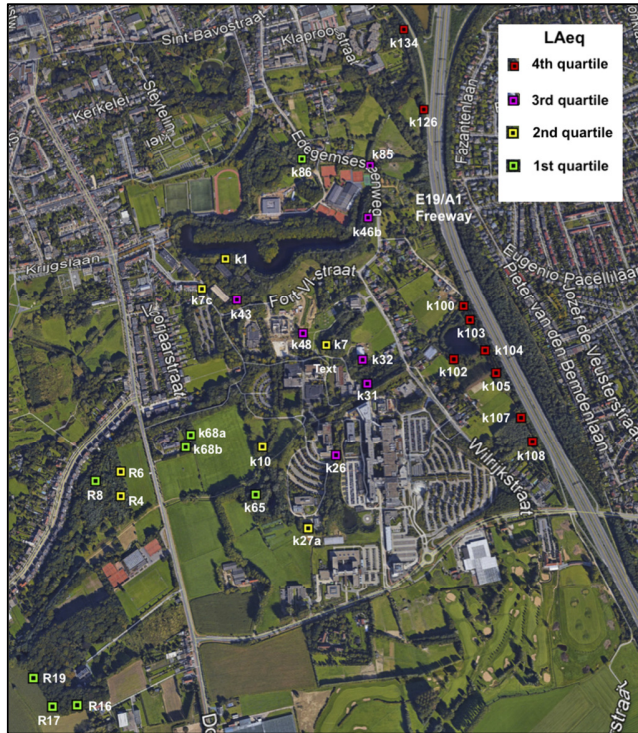
105 We obtained relatively long-term measurements of the noise environment (across an ~24-hour period),
106 comprehensively quantified the noise environment during the nestling period, and assessed whether more
107 variable, or well as louder noise environments, have deleterious effects on nestlings. In addition, we
108 evaluated whether phenotypic associations with the noise environment are modified by a variable that
109 may affect susceptibility to environmental stress, nestling size rank within a brood. Nestling size rank has
110 been shown to affect competitive dynamics (Nettle et al., 2013), and we previously found that nestling
111 size rank was associated with shorter telomere length in nestling great tits, whereas nestling sex and mass
112 were not (Grunst et al., 2019a). Finally, we tested the hypothesis that reproductive success is reduced in
113 loud, or variable, noise environments.

114

115 **2. Methods**

116 **2.1. Study species and site:** We studied the effects of noise exposure in a nest box population of great
117 tits on the University of Antwerp's Campus Drie Eiken (CDE; Wilrijk, Belgium; 51°9'44"N, 4°24'15"E).
118 This population contains ~150 nest boxes and has been studied since 1997 (e.g. Van Duyse et al., 2000,
119 2005; Rivera-Gutierrez et al., 2010, 2012; Raap et al., 2016a, b, 2017; Vermeulen et al., 2016). Great tits
120 are a model species for studies of ecology and evolution, and are successful urban colonizers that feature
121 prevalently in urban ecology studies (Halfwerk et al., 2011; Raap et al., 2016a, b, 2017; Salmón et al.,
122 2016; Biard et al., 2017). Past studies have reported that exposure to anthropogenic noise negatively
123 affects the reproductive success of great tits (Halfwerk et al., 2011) and induces changes in singing
124 behavior (Slabbekoorn and den Boer-Visser, 2006), physiology (Raap et al., 2017), and nestling
125 provisioning (Naguib et al., 2013). Nest boxes in the CDE population are distributed across a gradient of
126 noise exposure (Fig. 1). We selected 31 nest boxes, distributed across this gradient (Fig. 1). We used
127 only first broods, which hatched between April 22 and May 10, 2018 (median: May 1) and had a mean \pm
128 SE brood size of 8.13 ± 0.257 nestlings (range: 6-11). The body mass of nestlings at day 15 averaged
129 16.1 ± 0.104 g (range: 8.8-19.0 g).

130



131

132

133 **Figure 1.** Distribution of nest boxes (colored squares) used within the Campus Drie Eiken population.

134 The color of squares indicates which quartile the noise level falls into, as measured by average LA_{eq} . The

135 E19/A1 freeway runs along the study site's eastern border, and is associated with the loudest noise

136 environments. The Rekreatief Park (nest boxes numbers beginning with R) is located on the study site's

137 south-western border, most distant from the freeway, and provides a relative refuge from noise. Imagery

138 from Google Earth.

139

140 **2.2. Noise measurements:** With one exception, we took noise measurements on two nest boxes per day,

141 between May 6 and May 20, 2018 using WPK-633 noise measurement kits with CEL633C1 sound level

142 meters (20-140 dB; weight A; NoiseMeters Inc.). To control for seasonal and weather effects on noise

143 levels (Halfwerk et al., 2011), we aimed to take measurements on one nest box in a relatively loud area

144 and one nest box in a relatively quiet area each day. We took noise measurements during the nestling

145 stage, and on or before day 15 (mean \pm SE, range: 11.0 ± 0.225 , 7-15), with the exception of one nest box,

146 for which the measurement occurred on day 16. To assess the repeatability of noise measurements, we
147 repeated measurements of the noise environment on 15 nest boxes after nestlings had fledged, between
148 May 21 and June 4.

149 Noise measurements commenced at or before 1600 (mean \pm SE, range: 1352 ± 6.60 min, 945 to 1600)
150 and ended at or after 930 (1214 ± 7.44 min, 930 to 1545). Measurement duration averaged 22.4 ± 0.072
151 hours (range: 18.5 to 24 hrs). We placed the sound level meter ~3 meters from the nest box, with the
152 microphone erected at nest box level (1.5-2 m off the ground). The meter was calibrated prior to
153 initiation of each measurement. We used a fast response, and synchronized the timing of measurements,
154 such that noise levels were recorded at the hour, and every 15 min thereafter.

155

156 **2.3. Field data collection:** Birds in our population begin breeding late in March. At this time, we
157 commenced checking nest boxes every other day to determine laying, hatching and fledging date. When
158 nestlings were 15 days old (hatch date = day 1), we used brachial veinipuncture to obtain ~80-100 μ l of
159 blood, which was immediately dispensed from microcapillary tubes into glycerol buffer (50 mM Tris-Cl,
160 5 mM MgCl, 0.1 mM EDTA, 40% glycerol). Blood was kept cool on ice until frozen in liquid nitrogen
161 within 4 hours and stored at -80°C . We also collected ~15-20 yellow contour feathers, and weighed (\pm
162 0.1 g) and ringed each nestling. Due to time limitations, we did not measure tarsus length for nestlings in
163 this study, so we were unable to calculate a body condition metric. Feathers were stored in paper
164 envelopes in a dark location. We captured a subset of adults ($N = 38$ birds, 25 nest boxes) during the
165 nestling stage, measured body mass and tarsus length, and calculated body condition as the residuals of a
166 regression predicting mass from tarsus length (Schulte-Hostedde et al., 2005).

167

168 **2.4. Noise data analysis:** From the noise level meter, we extracted several variables that are used in
169 environmental noise monitoring (McKenna et al., 2016). In particular, we used A-weighted (1) LA_{eq} ,
170 which reflects average sound levels, (2) LA_{max} , which reflects maximum sound levels, (3) LA_{90} , which

171 reflects sound levels exceeded 90% of the time, or background noise, and (4) LA₁₀, the sound level
172 exceeded 10% of the time. Variables were calculated for each 15-minute period, and averaged to obtain a
173 mean. In addition, we calculated the variance in each variable, and initially divided data into day and
174 night time periods.

175

176 **2.5. Telomere qPCR:** We extracted DNA using the Macherey-Nagel NucleoSpin® blood kit, and
177 measured concentration and purity using a Nanodrop (260/280 ratio (mean ± SE): 1.847 ± 0.031; 260/230
178 ratio: 2.632 ± 0.277). We diluted samples to ~1 ng/μl prior to qPCR.

179 We used a real-time qPCR assay performed on a LightCycler® 480 System (Roche) to measure the
180 length of telomeres relative to a single copy reference gene, glyceraldehyde-3-phosphate dehydrogenase
181 (GAPDH) (Criscuolo et al., 2009; Atema et al., 2013; see Grunst et al., 2019a for qPCR reaction and
182 thermocycling conditions). Melting curve analysis confirmed that a single product of appropriate length
183 was produced. Amplification efficiency (mean ± s.d) was 93.6 ± 4.45 (r²: 98.1 ± 0.975) and 91.4 ± 5.86
184 (r²: 99.5 ± 0.221) for the telomere and GAPDH reactions, respectively. A “golden standard” reference
185 sample was produced by combining DNA from multiple individuals. This reference sample and a
186 negative control were included on each plate. All samples were run in duplicate.

187 In analyses, we used calibrator-normalized relative telomere length (RTL), which reflects the amount
188 of telomere sequence relative to GAPDH (Pfaffl, 2001; Grunst et al., 2019a). Mean intra-plate variation
189 of C_T values (crossing threshold; number of amplification cycles before products exceed a threshold
190 florescence) was 1.00% and 0.40%, and inter-plate variation was 4.30% and 1.40%, for the telomere and
191 GAPDH reactions, respectively. Mean intra-plate variation for RTL was 12.32%.

192

193 **2.6. Measuring carotenoid-based coloration:** We measured carotenoid reflectance spectra using an
194 Ocean Optics USB4000 spectrophotometer with a deuterium-halogen light source (DH-2000-BAL; Ocean
195 Optics; the Netherlands) and Spectra Suite software (Grunst et al., 2019b for details). Carotenoid

196 pigmentation displays reflectance peaks for both ultraviolet and visible light (Andersson and Prager,
197 2006). To describe reflectance spectra, we calculated: (1) carotenoid chroma, which reflects saturation in
198 yellow wavelengths, and carotenoid content (Saks et al., 2003; Andersson and Prager, 2006; Shawkey et
199 al., 2006; Peters et al., 2007; Grunst et al., 2014a, b, c; Biard et al., 2017), (2) UV chroma, which reflects
200 UV saturation, and is affected by carotenoid content and structural properties of feathers (Andersson and
201 Prager, 2006; Bleiweiss, 2004, 2005), (3) average reflectance, a measurement of brightness, and (4) the
202 slope of the reflectance curve between 450 and 520 nm, a measurement of hue. We analyzed variation in
203 coloration variables separately, because they can convey distinct information (Galván, 2010; Jacot et al.,
204 2010), and to remain consistent with our recent work on adult great tits (Grunst et al., 2019b).

205

206 **2.7. Statistical analyses:** We completed statistical analyses in R 3.4.1 (R Core Team, 2017). To
207 characterize variation in noise exposure, we compared noise levels between nest boxes situated in three
208 locations: (1) near the Highway, (2) on Campus and (3) in the Rekreatief, using linear mixed effects
209 models (LMMs; R package lme4; Bates et al., 2015), with nest box location as a fixed effect and nest box
210 as a random effect (noise measurements were repeated at 15 boxes). We also looked at differences
211 between the noise variables during the day and night. In addition, to characterize how noise variables are
212 affected by roadways, we constructed LMMs for each noise variable with proximity to the Highway and
213 smaller road as fixed effects and nest box as a random effect. In this analysis, we used the log-
214 transformed distance to the Highway and a smaller road, since road-associated pollution, including noise,
215 decreases more rapidly at first with distance (Guarnaccia et al., 2011; Yazdi et al., 2015). Furthermore,
216 we used LMMs to test whether noise levels varied depending on whether the noise recording included
217 the weekend (fixed effect) with nest box location as an additional fixed effect and nest box as a random
218 effect. We assessed repeatability in the noise environment using the repeated measurements at 15 nest
219 boxes and R package rptR (Stoffel et al., 2017).

220 To reduce the complexity of our dataset for further analyses, we performed a principal components
221 analysis on the noise variables using function “principal” in R package psych (Revelle, 2018). We used a
222 varimax rotation to maximize loadings and facilitate interpretation of principal components (PCs).

223 To investigate whether RTL (square-root transformed), body mass (cubed) or the four carotenoid
224 coloration variables (carotenoid and UV chroma, squared) were associated with the noise environment,
225 we used LMMs with the 3 noise PCs with eigenvalues greater than 1 (see Results) as fixed effects and
226 nest box as a random effect. We used qPCR assay number as an additional random effect when
227 predicting RTL. We also included the two-way interactions between the noise PCs and size rank (largest
228 nestling = rank 1, according to day 15 mass), and entered hatching date and brood size as covariates. We
229 did not include nestling sex, because we previously found no relationship between sex and telomere
230 dynamics (Grunst et al., 2019a), size rank and sex are correlated, and not all nestlings were sexed,
231 meaning that including sex would have reduced our sample size. The carotenoid coloration variables are
232 to some extent correlated. Thus, to avoid collinearity in models predicting RTL and mass, we added each
233 coloration variable to final models separately and applied a Holm-Bonferroni correction. See Tables S8-
234 S12 for full models.

235 We used general linear models (GLMs) with a Poisson distribution to assess whether clutch or brood
236 size were influenced by noise exposure, and GLMs with a binomial error structure and logit link function
237 to assess whether fledging or hatching success were affected by noise. When predicting fledging success,
238 we entered number of fledglings as the independent variable and brood size as the binomial denominator.
239 When predicting hatching success, we entered number of eggs that hatched as the independent variable
240 and clutch size as the binomial denominator. In all models, we used the 3 PCs as fixed effect predictor
241 variables, and included hatching date as a covariate.

242 We also repeated the models described above while replacing the noise PCs as fixed effects with the
243 log-transformed distance from the Highway and a smaller road. Although noise levels are correlated to
244 proximity to roads, this analysis grants insight into whether noise can better predict biotic effects than

245 distance to roads alone, or whether other variables associated with roads, such as chemical pollution, may
246 be having effects that are not entirely captured by noise levels.

247 Finally, to assess the contingency that non-random distribution of adults may affect relationships
248 between noise levels and nestling phenotypes, we constructed a linear model predicting adult body
249 condition from the noise PCs, with age (second year versus older), sex and capture date as covariates and
250 nest box as a random affect. In a parallel model, we replaced the 3 noise PCs with nest box location. We
251 only used adults that were captured at nest boxes while breeding. In addition, we assessed the
252 contingency that unmeasured local effects may have affected results by repeating models while including
253 box location as an additional random effect. However, results did not change substantially.

254 We centered predictor variables using the scale function in R, and calculated degrees of freedom using
255 Satterthwaite approximations (R package lmerTest; Kuznetsova et al., 2017). We reduced models via
256 backwards step-wise elimination, by first removing non-significant interactions ($\alpha = 0.05$). Global
257 models (provided in the Supplementary Material) supported similar conclusions as reduced models.

258

259 **Ethical statement:** The ethical committee of University of Antwerp (ID number: 2017-90) granted
260 approval for procedures, and we took all measures possible to reduce stress. The Belgian Royal Institute
261 for Natural Sciences (Koninklijk Belgisch Instituut voor Natuurwetenschappen) provided banding
262 licenses for authors and technical personnel.

263

264 **3. Results**

265 **3.1. Variation and repeatability in noise:** LA_{eq} , LA_{10} , LA_{90} , and LA_{max} were higher at Highway nest
266 boxes relative to at Campus or Rekreatief nest boxes (Fig 2.; Table S1, S2). Noise variables at Campus
267 nest boxes were higher than in the Rekreatief, with the exception of LA_{90} (Fig 2.; Table S2). See Tables
268 S3 and S4 for a comparison of noise levels during the day (615 to 2045) and night (2100 to 600).

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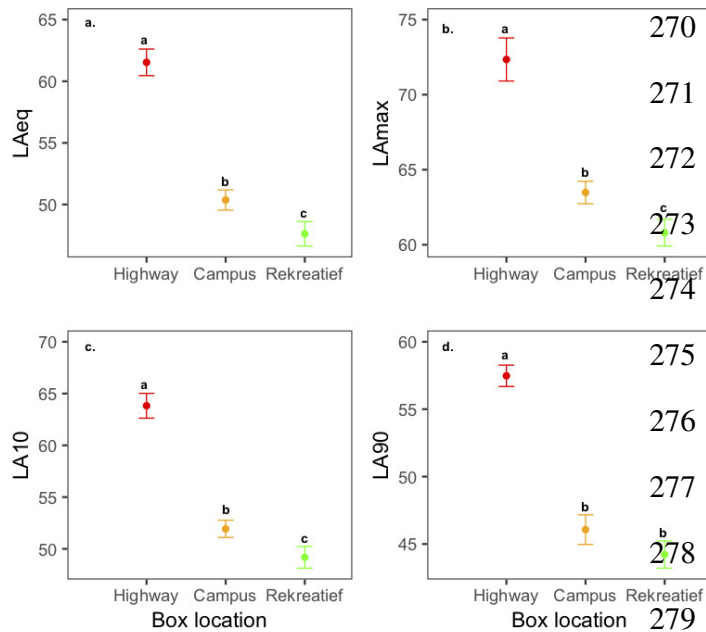


Figure 2. Mean (a) LA_{eq} , (b) LA_{max} , (c) LA_{10} and (d) LA_{90} at nest boxes near the Highway, on Campus, and in the Rekreatief. Bars denote standard error. Different letters above bars indicate statistically significant differences. N = 31 nest boxes (Highway: 9, Campus: 16, Rekreatief: 6)

280 Variance in LA_{eq} , LA_{10} , and LA_{max} were higher on Campus relative to near the Highway (Fig 3.;

281 Tables S1, S2). Variance in LA_{max} was higher at Rekreatief nest boxes than at Highway nest boxes (Fig

282 3.). On the other hand, variance in LA_{90} was higher at nest boxes near the Highway than at Campus or

283 Rekreatief nest boxes (Fig 3.). Variance in LA_{eq} and LA_{10} did not differ between nest boxes near the

284 Highway and in the Rekreatief (Fig 3.). There was no difference in variance at nest boxes on Campus and

285 in the Rekreatief for any noise variable (Fig 3.; Table S1, S2). With the exception of LA_{90} , differences in

286 the variance in noise variables were most pronounced during the day (Tables S3, S4). The only marginal

287 effect of whether the noise recording included the weekend was that variance in LA_{max} tended to be lower

288 on weekends ($\beta = -12.1 \pm 6.56$, $t_{33} = -1.84$, $p = 0.075$, N = 31 nest boxes, 46 observations).

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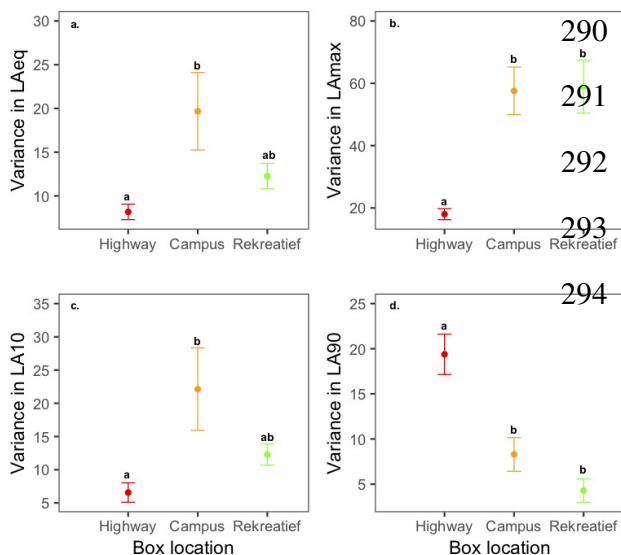


Figure 3. Variance in (a) LA_{eq} , (b) LA_{max} , (c) LA_{10} and (d) LA_{90} at nest boxes located near the Highway, on the Campus, and in the Rekreatief. Bars denote standard error. Different letters above bars indicate statistically significant

295 differences. N = 31 nest boxes (Highway: 9, Campus: 16, Rekreatief: 6)

296

297

298 Day time noise levels were ~4 dB higher than at night (Fig. S1; Tables S3, S4). Conversely, variance
299 in noise was higher at night on Campus and especially near the Highway, where variance in LA_{eq} , LA_{10}
300 and LA_{90} was ~10 times higher during the night than during the day (Fig. S1; Table S3).

301 LA_{eq} , LA_{10} , LA_{90} , and LA_{max} were negatively associated with distance from the Highway and road, but
302 with the exception of LA_{max} , the effect of the road was not significant when included in the same model
303 with distance to the Highway. In contrast, distance to the Highway and to a road had opposite effects on
304 variance in LA_{eq} , LA_{10} , and LA_{max} , with distance from the Highway having a positive effect, but distance
305 from a road a negative effect (non-significant for LA_{max}). Both distance from the Highway and a road had
306 a negative effect on variance in LA_{90} (Table S5).

307 Repeatability was >0.90 for mean values of noise variables, and lower, but still highly significant, for
308 variance (Table 1; Table S6 for the day and night time periods).

309

310

311

312

313

314 **Table 1.** Repeatability estimates for noise variables ($p < 0.001$ in all cases).

Noise variable	R ± SE	95% CI
LA_{eq}	0.955 ± 0.022	0.901, 0.983
LA_{10}	0.946 ± 0.025	0.885, 0.979
LA_{90}	0.929 ± 0.035	0.839, 0.972
LA_{max}	0.967 ± 0.016	0.926, 0.987
LA_{eq} var	0.587 ± 0.168	0.152, 0.823

LA ₁₀ var	0.686 ± 0.137	0.362, 0.872
LA ₉₀ var	0.732 ± 0.123	0.403, 0.895
LA _{max} var	0.736 ± 0.114	0.445, 0.889

315

316 **3.2. Principal components analysis:** The PCA of the noise variables extracted 3 axes of variation with
 317 eigenvalues greater than 1 that explained 92% of the variance. PC1 (49% of variance) was most strongly
 318 related to the mean amplitude of noise variables, with LA_{eq}, LA₁₀, LA₉₀, and LA_{max} positively loaded on
 319 this axis. PC2 (31% of variance) was positively related to variance in LA_{eq}, variance in LA₁₀, and
 320 variance in LA_{max}. PC3 (13% of variance) was positively related to variance in LA₉₀ (Table S7).

321

322 **3.3 Telomere length:** There was a negative interaction between size rank and PC1 in predicting RTL.
 323 Nestlings that were smaller than their brood mates had shorter telomeres when exposed to loud noise,
 324 whereas noise was not associated with the RTL of larger brood members (Table 2; Fig 4.; Table S8 for
 325 global model). There was a positive relationship between carotenoid hue and RTL (Table 2). PC2, PC3,
 326 brood size, hatching date, carotenoid and UV chroma, and average reflectance were unrelated to RTL ($p >$
 327 0.10; Table S8). Distance to the Highway and road had no effect on RTL, or on any variable examined
 328 below ($p > 0.40$; Table S14-S19).

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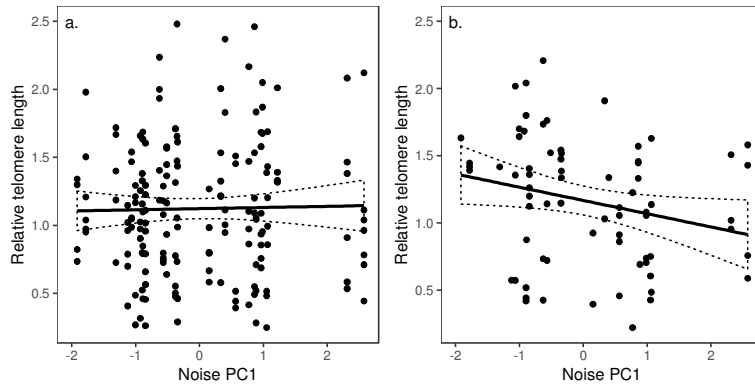
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332 **Table 2.** Linear mixed effect model predicting RTL from the noise PCs and covariates. Significant p-
 333 values appear in bold. SR = size rank.

Fixed effects	$\beta \pm SE$	Df	T	P > t	Random effects	Variance	SD	N
Intercept	1.03 ± 0.05	7.31	18.01	<0.001	Nest	0.003	0.050	31
PC1	-0.003 ± 0.01	25.6	-0.24	0.81	Assay number	0.025	0.157	8

SR	0.009 ± 0.01	223	0.82	0.41	Residual	0.030	0.174	243
Hue	0.030 ± 0.01	227	2.76	0.006				
PC1 × SR	-0.020 ± 0.01	217	-2.22	0.027				

334



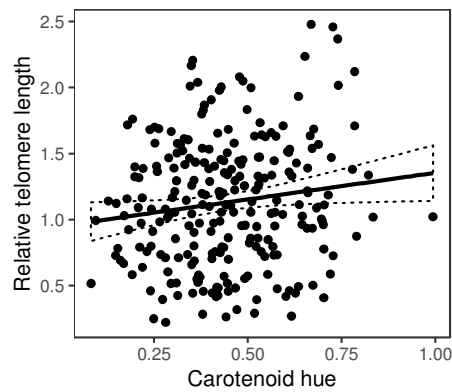
335

336 **Figure 4.** The relationship between noise PC1 and RTL within (a) size rank 1-5 (N = 173) and (b) size

337 rank 6-9 (N = 71). Shaded regions show 95% confidence intervals.

338

339



340

341 **Figure 5.** The relationship between carotenoid hue and RTL (N = 243). Shaded regions represent 95%

342 confidence intervals.

343

344 **3.4. Body mass:** The noise environment was unrelated to body mass ($p > 0.20$; Table S9). Size rank was

345 negatively associated with body mass ($\beta = -632 \pm 18.3$, $t_{217} = -34.5$, $p < 0.001$; $N = 249$). Hatching date,
 346 and brood size, and carotenoid-based coloration variables were unrelated to body mass, and all two-
 347 interactions tested were non-significant ($P > 0.10$; Table S9).

348

349 **3.5. Carotenoid-based coloration:** Carotenoid chroma and hue were positively related to PC2 (Table 3a,
 350 b; Fig. S2), and hue was also higher in nestlings that hatched later. Carotenoid chroma and hue were
 351 unrelated to PC1 and PC3, or any other covariate or interaction ($p > 0.10$; Table S10, S11). UV chroma
 352 was negatively associated with hatching date (Table 3c), but was not related to the noise environment, or
 353 any other covariate ($p > 0.10$; Table S12). Average reflectance was negatively associated with PC3 and
 354 was higher in nestlings of lower size rank and nestlings that hatched later (Table 3d). Average reflectance
 355 was unrelated to PC1, PC2, or any other covariate or interaction ($p > 0.10$; Table S13).

356

357

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359

360 **Table 3.** Linear mixed effect model predicting carotenoid-based coloration variables. Significant p-
 361 values ($\alpha=0.05$) appear in bold. $N = 248$ nestlings, 31 nest boxes. HD =hatching date, SR = size rank.

(a) Carotenoid chroma							
Fixed effects	$\beta \pm SE$	Df	T	P > t 	Random effects	Variance	SD
Intercept	0.21 ± 0.01	28.5	36.6	<0.001	Nest box	<0.001	0.007
PC2	0.02 ± 0.01	41.5	2.93	0.005	Residual	0.007	0.084
(b) Carotenoid hue							
Intercept	0.45 ± 0.01	28.3	35.5	<0.001	Nest box	0.002	0.042
PC2	0.04 ± 0.01	35.5	2.15	0.038	Residual	0.023	0.152

HD 0.04 ± 0.01 24.6 2.86 **0.008**

(c) UV Chroma

Intercept	0.16 ± 0.01	28.2	22.3	<0.001	Nest box	0.001	0.038
HD	-0.03 ± 0.01	27.6	-3.61	0.001	Residual	0.002	0.047

(d) Average reflectance

Intercept	42.3 ± 0.64	29.3	65.8	<0.001	Nest box	2.25	1.50
PCN3	-1.63 ± 0.62	30.0	-2.62	0.014	Residual	82.4	9.08
SR	-1.16 ± 0.58	241	-1.99	0.047			
HD	2.69 ± 0.65	27.2	4.15	<0.001			

362

363 **3.6. Reproductive success and adult condition:** Hatching and fledging success averaged 94.7 ± 0.017

364 % and 99.2 ± 0.006 %, respectively. All nestlings with RTL measured fledged. The noise environment

365 was unrelated to clutch size, brood size, hatching success or fledging success ($p > 0.30$, for PC1-3).

366 Adult body condition was unrelated to noise levels or nest box location ($p > 0.07$; Table S20, S21).

367

368 **4. Discussion**

369 Our study suggests that variation in the noise environment within the urban matrix may affect telomere

370 loss and acquisition of carotenoid-based plumage coloration in great tit nestlings. With respect to

371 telomere dynamics, we found shorter RTL in loud noise environments among small brood members.

372 With respect to carotenoid-based coloration, carotenoid chroma and hue were higher in noise

373 environments characterized by higher variance in average and maximum noise levels, and average

374 reflectance was lower in noise environments with higher variance in background noise. In addition, there

375 was a positive relationship between carotenoid hue and RTL, suggesting that this aspect of carotenoid-

376 based coloration may signal early-life telomere length, and potentially associated fitness correlates. On

377 the other hand, we found no relationship between the noise environment and nestling body mass or

378 reproductive success. We proceed to discuss the implications of these results.

379 First, our study provides insight into variation in noise within an urban area, which may translate into
380 effects on free-ranging animals. Significant, repeatable differences in noise levels existed between nest
381 boxes close to the Highway, on Campus, and in the Rekreatief. Thus, refuges from noise exist, and
382 individuals experience marked differences in noise exposure. The Highway area was characterized by
383 high amplitude noise, but this noise was also consistent, and potentially easier to habituate to than more
384 stochastic noise. Similarly, although noise decreased at night, which could reduce interference with sleep,
385 variance in noise actually increased near the Highway and on Campus, which could increase disruptive
386 effects. Noise decreased when moving away from both the Highway and smaller roads. However,
387 proximity to the Highway decreased, whereas proximity to a road increased, variance in noise. Thus,
388 different types of roadways have both similar and distinct effects on the noise environment.

389 With respect to biotic effects, our study offers support for a deleterious effect of noise exposure on
390 RTL, but also suggests that loud noise environments do not affect all individuals equivalently. Rather,
391 exposure to noise was negatively correlated with RTL only among smaller brood members. Among
392 larger brood members, RTL was not associated with noise levels. Thus, negative phenotypic
393 ramifications of noise exposure may only be manifest in competitively disadvantaged groups. Large
394 brood members could be preferentially fed and might more effectively compete for lucrative positions in
395 the nest (Cotton et al., 1999; Nettle et al., 2013), and thus might be relatively buffered against negative
396 effects of noise. In addition, the adrenocortical stress response could be upregulated in smaller brood
397 members to support increased begging activity, potentially translating into increased sensitivity to
398 external stimuli such as noise (Nunez de la Mora et al., 1996; López-Jiménez et al., 2015). Given
399 individual-specific effects of noise on organisms, studies that do not differentiate between more and less
400 vulnerable groups might conclude that there is no precedence for noise mitigation policies.

401 We also found a positive association between carotenoid hue and RTL. Thus, hue may serve as a
402 signal of early-life RTL, and fitness correlates. In great tits, hue has been previously found to relate
403 positively to nutritional condition (Senar et al., 2003) and survival (Hörak et al., 2001), and negatively to

404 parasite infection (Hörak et al., 2001) and exposure to metal pollution (Dauwe and Eens, 2008; Geens et
405 al., 2009), but our result demonstrating a positive relationship between hue and RTL grants new insight
406 into the information content of carotenoid-based coloration in this species. However, to clarify and
407 further test the mechanistic link between carotenoid hue and telomere length, additional measurements
408 would be needed, such as circulating carotenoid and oxidative stress levels. The noise environment was
409 not negatively related to hue, suggesting that the relationship between RTL and hue is not mediated by
410 noise. In contrast to our study, a previous study on great tit nestlings found no relationship between
411 carotenoid-based coloration and telomere length (Biard et al., 2017), but this study only used carotenoid
412 chroma and reflectance as coloration metrics, and consequently did not consider the relationship between
413 hue and telomere length.

414 We found some evidence that more variable noise environments may suppress coloration. In
415 particular, average reflectance was negatively related to PC3, which was highly associated with variance
416 in background noise. Since background noise represents the noise level exceeded 90% of the time, we
417 find it unlikely that this effect is mediated by stochastic noise events that could activate the stress
418 response. However, high variance in background noise could pose communication challenges, including
419 for begging nestlings, and affect the accuracy with which parents assess the hunger state of offspring.

420 We also found unpredicted positive relationships between carotenoid chroma and hue and variance in
421 average and maximum noise levels (PC2). We hypothesized that variance in noise would be negatively
422 associated with RTL and carotenoid coloration, since more stochastic noise could be more likely to
423 activate stress responses (Blickley et al., 2012; Gill et al., 2015; Injaian et al., 2018b). However, noise
424 environments with higher variance could also provide more intervals of relative quiet, which could
425 facilitate communication and reduce stress. Positive relationships between carotenoid chroma and hue
426 and PC2 could also reflect a positive correlation between PC2 and habitat quality, with PC2 and habitat
427 quality both increasing farther from the Highway.

428 We found no relationship between the noise environment and nestling mass, suggesting that noise does
429 not have large deleterious effects on growth and energetic state, and paralleling results of some past

430 studies (Meillère et al., 2015; Nedelec et al., 2017; Raap et al., 2017). Other studies have found negative
431 effects of noise on size or mass (Injaian et al., 2018a, b; Kleist et al., 2018). However, our study suggests
432 that even in lieu of effects on mass, effects on other phenotypic traits may be manifest.

433 The noise environment was not associated with reproductive success. Hatching and fledging success
434 were high in the year of our study, and conditions were milder than average. Thus, deleterious effects of
435 noise on reproductive success could emerge given more stressful environmental conditions. Furthermore,
436 there could be delayed effects of noise exposure on adult fitness, perhaps via reduced later-life survival of
437 offspring. We also had limited statistical power to test effects on reproductive success, since these
438 metrics are measured at the level of the nest, rather than nestling. In contrast to our results, Halfwerk et
439 al. (2011) found that female great tits laid smaller clutches in noisier areas.

440 We did not find any relationship between distance to roadways and any dependent variable examined,
441 suggesting that noise may better predict biotic effects than distance to roadways. This may be true
442 because pollution levels (both noise and chemical) may be affected by factors other than distance to a
443 roadway, such as traffic volume, type and speed (Guarnaccia et al., 2011; De Coensel et al., 2016).

444 Our study is correlational and thus has limitations. We cannot conclusively determine whether effects
445 observed result from noise exposure. Air and light pollution may be higher near potent noise sources.
446 Furthermore, adults may non-randomly segregate between loud and quiet areas, such that differences in
447 adult quality could influence effects observed. Here, we did not find evidence that adult condition was
448 associated with noise. However, we recently found that carotenoid-based coloration, which is often
449 associated with condition, was lower in adults close to the road (Grunst et al., 2019b), and past work has
450 also reported that great tits and tree swallows (*Tachycineta bicolor*) may preferentially settle in quieter
451 areas (Halfwerk et al., 2016; Injaian et al., 2018c). Telomere length has been shown to be heritable,
452 although estimates are highly variable. Thus, if parents in loud environments are of poorer quality and
453 have shorter telomeres, this could result in shorter nestling telomere length (Dugdale et al., 2018). This
454 explanation seems unlikely since the association between noise and telomere length was only present
455 among smaller brood members. Nevertheless, an experimental approach would be necessary to determine

456 that noise is the agent underlying correlations between the noise environment, RTL and carotenoid-based
457 coloration and not some correlated factor.

458 In conclusion, our results suggest that both loud and more variable noise environments could affect
459 phenotypic traits of developing nestlings. We also found that loud noise environments do not affect all
460 great tit nestlings equivalently. Rather, smaller brood members displayed decreased RTL in loud
461 environments, whereas no impact was apparent among larger brood members. Thus, differentiating
462 between classes of individuals may be necessary to identifying negative physiological effects of noise
463 exposure, and motivating mitigation policies. In addition, carotenoid hue positively correlated with early-
464 life RTL, suggesting that carotenoid-based coloration may have the capacity to signal RTL. Nestling
465 mass and reproductive success were unassociated with noise levels, despite associations with RTL and
466 carotenoid-based coloration. Our study provides precedence for future research to more fully characterize
467 the effects of multiple dimensions of the noise environment on behavior, physiology and fitness.

468

469 **Data availability:** Data will be available in the Dryad Digital Repository.

470

471 **Declaration of competing interests:** The authors declare that there are no conflicts of interest.

472

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477

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