

1 **Are urbanization and brood parasitism associated with differences in telomere**
2 **lengths in song sparrows?**

3

4 **Abstract**

5 Urbanization reflects a major form of environmental change impacting wild birds
6 globally. Whereas urban habitats may provide increased availability of water, some food
7 items, and reduced predation levels compared to rural, they can also present novel
8 stressors including increased light at night, ambient noise, and reduced nutrient
9 availability. Urbanization can also alter levels of brood parasitism, with some host
10 species experiencing elevated levels of brood parasitism in urban areas compared to
11 rural areas. Though the demographic and behavioral consequences of urbanization and
12 brood parasitism have received considerable attention, their consequences for cellular-
13 level processes are less understood. Telomeres provide an opportunity to understand
14 the cellular consequences of different environments as they are a well-established
15 metric of biological state that can be associated with residual lifespan, disease risk, and
16 behaviour, and are known to be sensitive to environmental conditions. Here we examine
17 the relationships between urbanization, brood parasitism, and blood telomere lengths in
18 adult and nestling song sparrows (*Melospiza melodia*). Song sparrows are a North
19 American songbird found in both urban and rural habitats that experience high rates of
20 brood parasitism by brown-headed cowbirds (*Molothrus ater*) in the urban, but not the
21 rural, sites in our study system. Among adults and nestlings from non-parasitized nests,
22 we found no differences in relative telomere lengths between urban and rural habitats.
23 However, among urban nestlings, the presence of a brood parasite in the nest was

24 associated with significantly shorter relative telomere lengths compared to when a
25 brood parasite was absent. Our results suggest a novel, indirect, impact of urbanization
26 on nestling songbirds through the physiological impacts of brood parasitism.

27

28 **Introduction**

29 Human-induced rapid environmental change is a threat to biodiversity and is
30 substantially impacting songbirds (Vitousek et al. 1997, Crick 2004, Both et al. 2006).
31 The impacts of one form of anthropogenic change, urbanization, have been especially
32 well-studied (Isaksson 2018, Marzluff 2001). Ecological studies resolving the aspects of
33 urbanization that impact wildlife, including songbirds, have demonstrated that ambient
34 noise, light at night, frequent human disturbance, temperature changes, and shifts in
35 predation intensity and prey availability collectively generate novel ecological pressures
36 in urban habitats (Isaksson 2018, Marzluff 2001). Though urbanization has been linked
37 with local extinctions, many songbird species successfully persist in urban habitats
38 (Blair 1996, Marzluff 2001, McKinney 2002, Both et al. 2006, Bonier et al. 2007,
39 Shochat et al. 2010, Sih et al. 2010, Sol et al. 2013, Wong and Candolin 2015).
40 Whereas some animals can thrive under urban conditions (Blair 1996, McKinney 2006),
41 there is an underlying assumption that inhabiting urban habitats is costly for most
42 individuals (Birnie-Gauvin et al. 2016, Murray et al. 2019). Indeed, some urban-dwelling
43 birds exhibit poor body condition (Capilla-Lasheras et al. 2017, Murray et al. 2019) and
44 reduced reproductive success (Chatelain et al. 2021) compared to rural conspecifics.
45 However, there are also cases where birds inhabiting urban environments exhibit better
46 body condition (Auman et al. 2008, Minias 2016), higher reproductive success (Lane et

47 al. 2023) and increased survival (Møller 2009, Phillips et al. 2018) compared to rural
48 birds of the same species. One approach to resolving the contradictory findings among
49 rural-urban comparisons is to examine cellular and physiological mechanisms driving
50 condition and fitness outcomes and which may also generate variation in these
51 outcomes (Isaksson 2015, Ouyang et al. 2018).

52 In addition to effects at the species level, urbanization can impact community
53 structure, with one potentially important effect being an alteration in rates of brood
54 parasitism (Burhans & Thompson 2006, Møller et al. 2016, Zhang et al. 2023). Avian
55 obligate brood parasitism is a life-history strategy in which brood parasites lay their
56 egg(s) in the nest of another species, the host, which then provides parental care for the
57 young (Payne 1977, Rothstein, 1990). Although there are only a handful of known avian
58 brood parasites, consisting of only ~1% of birds globally, (Payne 1977, Davies 2000),
59 many bird species - global estimates suggest more than 14% - can serve as hosts
60 (Kennerley et al. 2002). Cowbirds (genus: *Molothrus*) alone are known to parasitize the
61 nests of over 144 species of songbirds (Friedmann and Kiff 1985). Brood parasitism can
62 negatively impact both the host parents and offspring, though the magnitude of impact
63 varies among species (Payne 1977, Pollock et al. 2021). Urbanization can substantially
64 alter interactions between brood parasites and their hosts, though in some systems
65 urbanization is associated with reduced rates of brood parasitism (Rodewald et al.
66 2013, Buxton et al. 2018), and in others it is associated with increased rates of brood
67 parasitism (Burhans and Thompson 2006, Tewksbury et al. 2006, Rodewald 2009,
68 Padilla and Sutherland 2022). The most common brood parasite in North America is the
69 brown-headed cowbird (*Molothrus ater*, hereafter “cowbirds”; Peer et al. 2013), a native

70 species of songbird that has historically used human-disturbed habitats (i.e., farmlands;
71 Chace et al. 2005) and is now successfully inhabiting urban environments (Rodewald
72 2009, Padilla and Sutherland 2022). Cowbird offspring compete with host offspring for
73 resources provided by host parents (Rothstein 1975, Lichtenstein and Sealy 1998,
74 Kilner et al. 2004, Moskát et al. 2017), often resulting in reduced host nestling hatch
75 rate, growth rate, and survival (Lorenzana and Sealy 1999, Hauber 2003, Hoover 2003,
76 Scharf et al. 2021). Though the negative effects of brood parasitism on host nestlings
77 are well-documented, the physiological mechanisms mediating these effects are less
78 clear (but see Ibáñez-Álamo et al. 2018, Scarf et al. 2021).

79 One approach to understanding the impacts of urbanization on songbirds
80 experiencing multifaceted ecological changes is to examine physiological and molecular
81 responses. These measures have the potential to reveal both pathological effects of
82 living in urban habitats and provide insight into the adaptive mechanisms underpinning
83 responses that facilitate urban living (Isaksson 2015). Previous work has shown
84 associations between urbanization and altered levels of hormones, oxidative stress,
85 hematological parameters, and immune function (Bonier 2012, Isaksson 2015, Isaksson
86 2018, Goodchild et al. 2022, Bonier 2023). Additionally, there is increasing interest in
87 the impacts of urbanization on telomeres - tandem repeats of DNA and a shelterin
88 protein complex at the end of chromosomes (Shay and Wright 2019, Salmón and
89 Burraco 2022). Functionally, telomeres protect the ends of linear chromosomes and are
90 also involved in various cellular-signaling processes, including cellular senescence
91 (Casagrande and Hau 2019, Shay and Wright 2019). Telomeres are relevant to aging,
92 cancer, and disease, and variation in telomere lengths (i.e., the number of tandem

93 repeats) and their dynamics (i.e., within-individual changes in telomere lengths) relate to
94 differences in lifespan within (Wilbourn et al. 2018, Schneider et al. 2022) and between
95 species (Dantzer and Fletcher 2015, Sudyka et al. 2016, Tricola et al. 2018, Wirthlin et
96 al. 2018). Within species, telomeres can also correlate with life-history strategies,
97 behavior, and individual quality (Bateson and Nettle 2018, Young 2018, Angelier et al.
98 2019, Giraudeau et al. 2019, Sudyka 2019, Tobler et al. 2021, Vernasco and Watts
99 2022). Thus, telomeres can provide an integrative measure of somatic state (Monaghan
100 2010, Boonekamp et al. 2013, Bateson et al. 2015). Importantly, telomeres and their
101 dynamics can be impacted by environmental factors (Angelier et al. 2018), including
102 anthropogenic disturbances (Salmón and Burraco 2022). At the time of writing, the
103 relationships between urbanization and telomere lengths has been examined in only
104 two avian species, European blackbirds (*Turdus merula*) and great tits (*Parus major*)
105 (Salmón et al. 2016, Biard et al. 2017, Salmón et al. 2017, Ibáñez-Álamo et al. 2018).
106 Further, the relationship between brood parasitism and host telomere lengths in adults
107 and offspring has not been described.

108 Here, we examined whether blood telomere lengths differed between song
109 sparrows (*Melospiza melodia*) living in replicate urban and rural study sites, and further,
110 whether brood parasitism is a specific means by which telomeres may be impacted in
111 urban habitats. Song sparrows, a common songbird native to North America, are an
112 excellent model to study physiological responses to urbanization because they
113 successfully breed in both rural and urban habitats. Adult song sparrows in our urban
114 and rural study sites show consistent behavioral differences (Hyman et al. 2004, Evans
115 et al. 2010, Davies and Sewall 2016, Davies et al. 2018, Fossett and Hyman 2021) and

116 urban birds have higher reproductive success (Lane et al. 2023). However, hormone
117 concentrations, oxidative stress, and hematological measures do not reliably differ
118 across habitats (Foltz et al. 2015, Lane et al. 2021, Goodchild et al. 2022), nor is there
119 detectable genetic differentiation between birds at our rural and urban sites (Brewer et
120 al. 2020). Song sparrows are a common host for brown-headed cowbirds (Hauber and
121 Russo 2000), and at our study sites brood parasitism occurs at high rates in urban
122 habitats but is very rare in rural habitats (Lane et al. 2023). Specifically, across 5 years
123 of nest monitoring 44% of urban nests were parasitized (n=84/191) compared to only
124 8% of rural nests (n=10/125) (Lane et al. 2023). Here, we sought to examine the
125 associations among telomere lengths, urbanization, and brood parasitism. To do this,
126 we compared telomere lengths between adult birds in rural and urban habitats. For
127 nestling birds, we evaluated the effects of urbanization apart from brood parasitism by
128 comparing telomere lengths from urban and rural birds belonging to non-parasitized
129 nests. To evaluate the effects of brood parasitism, specifically, we compared telomere
130 lengths from urban nestlings belonging to parasitized or non-parasitized nests.
131 Additionally, this dataset afforded us the opportunity to examine age- and sex-related
132 differences in telomere lengths and age-specific associations between telomere lengths
133 and urbanization.

134

135 **Methods**

136 ***Adult capture***

137 Adult song sparrows were captured from February to October in 2019 – 2022 at
138 6 established urban and rural sites (n = 3 each) in Southwestern Virginia, USA that are

139 at the ends of an urban-rural gradient (Figure S1, Davies et al. 2016, Davies et al.
140 2018). Birds were captured through blanket mist netting, targeting males on their
141 territories using simulated territorial intrusions (Hyman et al. 2004), and capturing
142 females as they returned to or left active nest sites. After capture, a small blood sample
143 was taken via brachial venipuncture with a 26-gauge needle, and blood was collected
144 with heparinized 70 μ L capillary tubes. Whole blood was stored on ice in the field. Red
145 blood cells were then separated from plasma within 6 hours and stored in 100% ethanol
146 at -80°C until being shipped to Washington State University (WSU) on dry ice. At WSU,
147 samples were stored at -20°C until DNA extraction. Across 4 years, 64 urban (21
148 females and 43 males) and 58 rural (23 females and 35 males) adult song sparrows
149 were sampled.

150

151 ***Nest searching and nestling sampling***

152 Song sparrow nests were located and monitored at the same sites and in the
153 same four breeding seasons described above. Nests were found in the morning (0500
154 to 1100) during peak parental behavior, from early March to late July through behavioral
155 observations of adults and systematic searching of known territories and nesting
156 substrates (Martin & Geupel 1993). Complete clutches were inspected for brown-
157 headed cowbirds eggs using previously established methods to distinguish host eggs
158 from those of brown-headed cowbirds (Smith & Arcese 1994). If a nest was found after
159 hatch, visual identification and morphometrics were used to identify cowbird nestlings
160 (Pyle 1997). Blood samples were collected from nestlings at an advanced day of
161 development (day 5-11 post-hatch) and samples were stored using the methods

162 described above. Across 4 years 90 urban nestlings (51 from 28 parasitized nests and
163 39 from 21 non-parasitized nests) and 34 rural nestlings from 14 non-parasitized nests
164 were sampled.

165

166 ***Measurement of relative telomere lengths***

167 The Gentra Puregene Blood Kit (Qiagen) and the modified extraction protocol described
168 in Vernasco et al. (2021) were used to extract DNA from whole blood. This extraction
169 approach results in high molecular weight DNA that is suitable for telomere
170 measurement by real-time quantitative PCR (qPCR; Eastwood et al. 2018, Vernasco et
171 al. 2021). A NanoDrop ND-1000 was used to assess DNA purity and concentration
172 (mean DNA concentration \pm SD = 288 ± 145.5 ng/ μ l, range of DNA concentrations =
173 7.73-778.76 ng/ μ L, mean 260/280 ratio \pm SD = 1.87 ± 0.06 , mean 260/230 ratio \pm SD =
174 2.19 ± 0.22). Extracted DNA was stored at -20°C and relative telomere lengths were
175 quantified using qPCR following Criscuolo et al. (2009) and Eastwood et al. (2018). This
176 qPCR approach measures an individual's relative telomere length by comparing the
177 number of telomere-repeats to the number of copy numbers of a single-copy gene.
178 Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the single-copy
179 gene. Samples were analyzed on a CFX Duet Real-Time PCR System (Bio-Rad) on 96
180 well plates and with 15 μ L reaction volumes. Reactions included 7.5 μ L of
181 SsoAdvanced™ Universal SYBR® Green Supermix, 3.6 μ L of DNase-free water, 0.15
182 μ L of forward and reverse 100nM telomere or single-copy control gene primers (Sigma-
183 Aldrich), and 3.6 μ L of template DNA (4.5 ng of DNA per reaction). Separate plates
184 were used for telomere and GAPDH primers. A “golden sample” was made up by

185 combining DNA from 3 different adult song sparrows that were not a part of the current
186 study and included on each plate for use as the inter-plate control. Telomere primer
187 sequences were Tel1b 5' - CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT
188 TGG GTT - 3' and Tel2b 5' - GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT
189 TAC CCT - 3' (Criscuolo et al. 2009). The GAPDH primers were developed from the
190 song sparrow reference genome (NCBI GCA_022749695.1) using the primer design
191 tool in Geneious v10.2.3 (Kearse et al. 2012; Forward: 5' - TCA TCC CTC CTT AGG
192 CGT GA - 3', Reverse: 5' - GCC TTC TCC ATG GTG GTG AA - 3'). Melt curve
193 analyses identified a single peak in the qPCR products of both primer sets. Reaction
194 conditions for telomere primers were as follows: 95°C for 10 min, followed by 40 cycles
195 of 1 min at 95°C, and 1 min at 58°C. GAPDH reaction conditions were identical except
196 for the annealing and extension temperature was set to 60°C. Samples were randomly
197 assigned across seven plates. Three randomly selected samples were also included on
198 all seven plates to estimate inter-assay repeatability. Two no template controls were
199 included in each plate and all samples, standards, and controls were run in duplicate.
200 Cycle quantification (Cq) values and individual well qPCR efficiencies for samples were
201 calculated using LINREGPCR version 11 (Ruijter et al. 2009; mean \pm SD: Cq-GAPDH =
202 23.75 ± 0.51 , Cq-telomere = 8.86 ± 0.44 , Efficiency-GAPDH = 1.95 ± 0.02 , Efficiency-
203 telomere = 2.07 ± 0.05). Samples were excluded if duplicates Cq values differed by
204 more than > 0.5 (Eastwood et al. 2018), though no samples required exclusion using
205 this criterion. Relative telomere lengths were calculated following equation one in Pfaffl
206 (2001) and values from the sample replicates were then averaged. Technical
207 repeatability and 95% confidence intervals (CIs) were calculated using the rptR package

208 within R Statistical Software (Stoffel et al. 2017). Duplicate measurements of relative
209 telomere length values were used to estimate technical repeatability and samples
210 measured on more than one plate were used to estimate inter-plate repeatability
211 (Kärkkäinen et al. 2021). Technical repeatability was estimated to be 0.97 (95% CIs
212 [0.955, 0.973], $p < 0.001$) and inter-plate repeatability was estimated to be 0.85 (95%
213 CIs [0.82, 0.88], $p < 0.001$).

214

215 ***Statistical analysis***

216 Statistical analyses were conducted using R Statistical Software (R Core Team
217 2022 v. 4.2.2). All telomere data were analyzed using linear mixed-effects models
218 (LMM). All LMM's were fit using the package "lme4" (Bates 2010, Bates et al. 2014). We
219 tested the fixed effects in the LMMs using the lmerTest (Kuznetsova et al. 2017)
220 package, which estimates degrees of freedom (*df*) with the Satterthwaite approximation.
221 Backward stepwise variable selection was performed such that only fixed effects or
222 interactions between variables with a p-value of 0.2 or lower were included in the final
223 model (Wang et al. 2007) except for the variables of interest derived from our
224 hypotheses, which were always retained. All final models were examined using the
225 *check_model()* function within the "performance" package to check the assumptions of
226 LMMs (Lüdtke et al. 2021). In all models, relative telomere length (rTL) was the
227 response variable and qPCR plate number was included as a random effect to control
228 for variance among plates. All models also included year as a random effect to control
229 for non-independence of rTLs collected within years.

230 The effects of age on rTL were analyzed using a model with habitat type and age
231 (adult/nestling) as categorical fixed effects with an interaction indicated. In this model,
232 the unique nest ID was included as a random effect to control for non-independence
233 among nestlings from the same nest. As the interaction between habitat type and age
234 was not significant ($p\text{-value} = > 0.2$) in this model, the interaction was not included in the
235 final model.

236 The effect of habitat type on adult rTL was examined using an initial model that
237 included habitat type and genetic sex as categorical fixed effects with an interaction
238 indicated. In this model, the interaction between habitat type and sex was > 0.2 and the
239 interaction was dropped from the final model.

240 Two separate models were used to examine the effects of urbanization and
241 brood parasitism on nesting rTL, using subsets of the data, to isolate the effects of each
242 factor on nestling rTL. To examine the effect of habitat type on nestling rTL, only data
243 from nests that hadn't been parasitized by brown-headed cowbirds were included in a
244 model with habitat type as a fixed effect. A model that included brood parasitism as a
245 fixed effect was used to examine the effect of brood parasitism on nestling rTL using
246 data from parasitized and non-parasitized, urban nests. Nests from rural sites, which
247 experience very low levels of brood parasitism, were excluded. The two nestling models
248 included nest ID as a random effect and the initial models also included brood size and
249 nestling age as fixed-effects covariates to control for potentially influential sources of
250 variation in rTL. In the model examining the relationship between habitat type and
251 nestling rTL, habitat type, nestling age, and brood size were all included as fixed effects
252 in the final model. In the final model examining the effect of brood parasitism on nestling

253 rTL, only brood parasitism was retained as a fixed effect. See supplemental materials
254 for a full summary of models and results.

255

256 **Results**

257 There was no difference in rTL between adult urban and rural song sparrows of
258 either sex (Habitat: $\beta_{\text{Urban}} = 0.01 \pm 0.03$, $t_{116.33} = -0.42$, $p = 0.67$; Sex: $\beta_{\text{Male}} = 0.0004 \pm$
259 0.03 , $t_{116.33} = 0.01$, $p = 0.67$; Figure 1). However, there was a significant difference
260 between adult and nestling rTL across both habitat types, with nestlings having longer
261 rTLs compared to adults (Age: $\beta_{\text{Nestling}} = 0.09 \pm 0.03$, $t_{168.21} = 2.78$, $p = 0.006$; Figure 2).

262 We failed to detect a significant difference between urban and rural nestling rTL
263 (Habitat: $\beta_{\text{Urban}} = 0.11 \pm 0.06$, $t_{18.89} = 1.71$, $p = 0.10$; Figure 3). However, older nestlings
264 had significantly longer rTLs (Nestling age: $\beta = 0.05 \pm 0.02$, $t_{29.92} = 2.50$, $p = 0.02$) and
265 rTL was positively associated with brood size (though only in this model; Brood size: $\beta =$
266 0.07 ± 0.03 , $t_{24.76} = 2.08$, $p = 0.048$). Finally, among urban nestlings, brood parasitism
267 had a significant, negative effect on rTLs, with nestlings from parasitized nests having
268 shorter telomeres compared to those from non-parasitized nests on average (Brood
269 parasitism: $\beta_{\text{Parasitized}} = -0.14 \pm 0.06$, $t_{28.06} = -2.25$, $p = 0.03$; Figure 4).

270

271 **Discussion**

272 Although urban habitats are often presumed to present challenges for birds, here
273 we found no evidence for an association between urbanization and rTLs - an indicator
274 of somatic state - in adults or in nestlings when we controlled for brood parasitism.
275 Among adult song sparrows, rTLs did not differ between rural and urban habitats (Fig.

276 1). Further, even during early development when animals are expected to be particularly
277 vulnerable to adverse conditions (Nowicki et al. 2002, Monaghan 2008, Chaby 2016),
278 nestlings from non-parasitized nests in urban habitats had similar rTLs to their non-
279 parasitized rural counterparts (Fig. 3). However, urban habitats may impact nestling
280 rTLs indirectly as we found that among urban nestlings, those from parasitized nests
281 had shorter rTLs compared to those from non-parasitized nests (Fig. 4). This could
282 reflect an indirect effect of urban living in several species because brood parasitism can
283 be more prevalent in urban habitats for many host species (Burhans and Thompson
284 2006, Tewksbury et al. 2006, Rodewald 2009, Padilla and Sutherland 2022, Lane et al.
285 2023). Among adult birds, we found no sex differences in rTLs, which is consistent with
286 previous studies (Barrett & Richardson 2011, Remot et al. 2020). We did find that rTLs
287 varied with age in two ways. First, nestlings had longer telomeres compared to adults, a
288 pattern common, though not ubiquitous, among birds and many other vertebrates
289 (Tricola et al. 2018, Remot et al. 2022). Second, rTLs increased with nestling age
290 among birds 5-11 days post-hatch. A lengthening of telomeres early in life, before a
291 later decline, has been reported in other vertebrates (Ujvari & Madsen 2009, Anchin et
292 al 2011, Ujvari et al. 2017), though this is not a common pattern (Remot et al. 2022).
293 Viewed together, these results provide new evidence describing the extent to which the
294 multi-faceted environmental changes associated with urbanization are associated with
295 variation in telomere lengths in a songbird that persists in both urban and rural habitats.

296 Anthropogenic disturbances (e.g., chemical pollution and noise), many of which
297 are associated with urbanization, generally show negative correlations with telomere
298 lengths in a variety of species (Salmón and Burraco 2022). Yet, prior intraspecific

399 comparisons of telomeric traits in urban and non-urban bird populations report mixed
300 results. In European blackbirds, both yearling and older birds from urban environments
301 have shorter telomeres compared to forest-dwelling conspecifics (Ibáñez-Álamo et al.
302 2018). In contrast, studies on one population of great tits show that urban habitats are
303 associated with shorter nestling telomeres and longer adult telomeres, a pattern
304 mediated by the selective disappearance of fledglings with short telomeres (Salmón et
305 al. 2016, Salmón et al. 2017). However, a study on another population of urban and
306 rural nestling great tits found no differences in telomere lengths, despite phenotypic
307 differences (Biard et al. 2017). Thus, among adults, urban habitats may be associated
308 with shorter telomere lengths, longer telomere lengths, or no differences. Among
309 younger birds, telomere lengths are either shorter or do not differ between urban and
310 rural habitats. These differences in findings may not be surprising given that both the
311 effects of urbanization and telomere dynamics often differ with species' life histories and
312 can also vary geographically (Callaghan et al. 2019, Burraco et al. 2021, Salmón and
313 Burraco 2022, Neate-Clegg et al. 2023). Future studies comparing urban and rural
314 populations should therefore prioritize (1) working with replicate urban and rural sites
315 across broader spatial scales to test for parallel differences in telomere lengths (e.g.,
316 Campbell-Staton et al. 2020) and (2) examining the contributions of interspecific life
317 history variation.

318 Our finding that adult urban and rural birds did not differ in rTLs is consistent with
319 prior studies on song sparrows showing that adult birds in urban and rural areas have
320 similar body condition and physiological and cellular markers of health (Fokidis et al.
321 2009, Bókony et al. 2012, Ibáñez-Álamo et al. 2012, Ibáñez-Álamo et al. 2020,

322 Goodchild et al., 2022). Collectively, these studies suggest that adult birds at our urban
323 study sites are not negatively impacted by urban environmental conditions. It is
324 important to recognize that our urban sites reflect habitat typical of suburban areas, not
325 highly urbanized city centers, which could present more substantial challenges to birds.
326 Nonetheless, previous studies on urban animals have shown that despite the presence
327 of stimuli shown to be stressors (e.g., artificial light at night, ambient noise), urban
328 habitats can also present benefits such as improved access to food and water, respite
329 from nest predation (Fischer et al., 2012, Seress and Liker, 2015), and maintained
330 green space for foraging and breeding territories (McKinney 2002, McKinney 2008,
331 Chamberlain et al. 2009, Reynolds et al. 2019). Our study provides further evidence
332 that for some species, the benefits of some urban habitats may outweigh the potential
333 costs.

334 Although we did not find any differences between urban and rural nestling rTLs,
335 we did find that nestlings from parasitized nests within urban habitats had shorter rTLs
336 compared to those from non-parasitized nests. Our results are consistent with prior
337 studies demonstrating the negative effects of brood parasites on host nestling condition,
338 physiology, and survival (Lorenzana and Sealy 1999, Hoover 2003, Scharf et al. 2021),
339 including in this study system (Lane et al. 2023), though in some cases such negative
340 effects are not observed (Vernasco et al. 2018, Jones et al. 2023). The observed
341 relationship between brood parasitism and telomere lengths we report here could arise
342 because of direct impacts of brood parasites on the growth and physiology of host
343 young. Alternatively, this relationship could arise indirectly if parental telomere lengths
344 are heritable, as has been shown in other vertebrates (Chik et al 2022) and are

345 associated with vulnerability to brood parasitism such that parents with shorter
346 telomeres are more likely to be parasitized. Further work will be necessary to distinguish
347 between these potential underlying causes. By demonstrating an association between
348 brood parasitism and telomere lengths, our results suggest a previously unrecognized
349 correlation between brood parasitism and a measure of somatic state. Disentangling the
350 causal nature of this relationship, whether it persists into adulthood, and whether it
351 impacts fitness will require further study. Whereas many studies have highlighted how
352 urbanization can impact animals through the direct effects of abiotic factors in the urban
353 environment, this study also highlights the potential for the effects of urbanization to
354 occur through indirect processes such as shifts in community dynamics.

355

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