Multiple carotenoid-based signals are enhanced despite poor body condition in urban male and female Northern Cardinals (*Cardinalis cardinalis*)

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ABSTRACT—If humans aim to sustainably coexist with wildlife, we must understand how our activity impacts the communication systems of urban animal populations. We know much about the effects of anthropogenic noise on bird song, but relatively little about how avian visual signals are affected by urbanization. One way such an effect may occur if urbanization alters the food available to species with color based on carotenoids, which they must obtain from their diet. Over three years, we compared a comprehensive suite of visual signals in male and female Northern Cardinals (*Cardinalis cardinals*) in a rural and an urban population. We predicted that urban birds would have enhanced carotenoid-based signals as they likely have access to more carotenoids from invasive plants, especially honeysuckle (*Lonicera* spp.), that thrive in cities. We used reflectance spectrometry, digital image analyses, and avian visual models to quantify hue, saturation, and brightness of chest (male), underwing (female), and bill (male and female) signals. Compared to rural males, urban males had redder chest feathers in one year and redder

bills in every year. Urban females had more saturated underwing color than rural females in every year. These color differences were sufficient to be distinguished by the avian visual system. Urbanization did not affect female bill color. Interestingly, urban birds had significantly reduced mass-related body condition compared to rural birds. These results show that both male and female urban birds can display enhanced carotenoid-based signals despite being in relatively poor condition. The consequences of this color enhancement are unknown, but it could affect the information content of the signals and the dynamics of the social and mating systems. These results stand in stark contrast to the predominant trend in birds of decreased color in urban areas and highlight the complex and varied potential effects of urbanization on animal communication.

Key words: avian visual model, bill color, *Lonicera*, plumage color, urbanization.

RESUMEN (Spanish)—*Please translate, thank you*

Humans have drastically altered the natural landscape and it is imperative to understand how our activity affects animal populations if we aim to promote sustainable coexistence with wildlife (Seddon et al. 2016, Elmqvist et al. 2019). Beyond the negative impacts of human activities like direct exploitation (e.g., poaching, pet trade) and habitat destruction on wildlife population viability, humans can also more subtly alter evolutionary dynamics (Szulkin et al. 2020). For instance, considerable research demonstrates that humans can disrupt animal communication systems (Shannon et al. 2016, Slabbekoorn et al. 2018). In particular, urbanization has a myriad of impacts on signal production, transmission, and perception (Sepp et al. 2018, Sepp et al. 2020, Heinen-Kay et al. 2021). Urban environments present several

47 challenges that may affect communication systems, including anthropogenic noise and light,
48 heavy metal pollution, habitat fragmentation, and changes in resource availability (Heinen-Kay
49 et al. 2021).

50 In birds, much of this work has focused on anthropogenic disruption of vocal
51 communication, especially in urban areas (Slabbekoorn and den Boer-Visser 2006, Slabbekoorn
52 2013, Derryberry and Luther 2021). Less well understood is how visual signals may also be
53 impacted by human development. Carotenoid-based color patches may be especially affected by
54 urbanization as carotenoids cannot be synthesized de novo by birds and must be obtained from
55 the diet (Hill 1991). One way urbanization can directly impact these signals, and hence the
56 communication system, is if rural and urban areas differ in the access to, amount, or types of
57 resources containing carotenoids. Moreover, carotenoids can undergo extensive physiological
58 modification before being deposited in tissue (Goodwin 1984), leaving room for other more
59 indirect effects of urbanization on color (e.g., via stress or parasites; Møller 2009, Hutton and
60 McGraw 2016). Additionally, rural and urban habitats likely differ in lighting regime, which can
61 also affect carotenoid-based color via selection on conspicuousness (Leveau 2021). Importantly,
62 these mechanisms are not mutually exclusive and might either reduce or enhance color in urban
63 populations. To date, research comparing carotenoid-based signals between rural and urban
64 populations has found reduced ornamentation in urban populations of House Finches
65 (*Haemorhous mexicanus*; Hasegawa et al. 2014, Giraudeau et al. 2015, Giraudeau et al. 2018)
66 and Great Tits (*Parus major*; Grunst et al. 2020). However, urban birds could have more heavily-
67 pigmented, enhanced carotenoid-based color if, for example, they have access to carotenoid-rich
68 invasive plants that thrive in disturbed landscapes (Hobbs 2000, Vilà and Ibáñez 2011). In the
69 midwestern and northeastern United States, invasive honeysuckle (*Lonicera* spp.) has been

implicated in the enhancement of carotenoid-based signals in a variety of bird species (e.g., Northern Cardinal, *Cardinalis cardinalis*; Cedar Waxwing, *Bombycilla cedrorum*; Baltimore Oriole, *Icterus galbula*; Northern Flicker, *Colaptes auratus*; Mulvihill et al. 1992, Hudon et al. 2013, Hudon et al. 2017, Hudon and Mulvihill 2017). *Lonicera* thrives in urban areas (Borgmann and Rodewald 2005, Rodewald 2005, Leston and Rodewald 2006), providing a potential link between urbanization and enhanced color. However, to our knowledge, no study has found enhanced carotenoid-based signals in an urban population of any species. Quantifying the color of rural and urban populations of a species that occurs in both habitats provides a powerful framework to test this idea.

The Northern Cardinal (hereafter "cardinal") is well suited to such a study because it readily occurs in both rural and urban areas, and both males and females exhibit carotenoid-based color patches that are displayed during various territorial, courtship, and pre-copulatory interactions and likely function as signals (Linville et al. 1998, Wolfenbarger 1999a, Wolfenbarger 1999b, Jawor et al. 2003, Jawor et al. 2004, Jawor and Breitwisch 2004). Males have bright red plumage overall and orange-red bills, while females have pink-red underwing patches (hereafter "wing") and orange-red bills. The putative functions of these colors as signals are complex and variable across studies. For example, males with redder chest color had higher quality territories (Wolfenbarger 1999a, Rodewald et al. 2011) and reproductive success (Wolfenbarger 1999a), but females did not prefer redder males (Wolfenbarger 1999b). Males and females mated assortatively based on chest, wing, and bill color (Jawor et al. 2003); and female wing and bill color co-varied with body condition (Jawor et al. 2004). The color of carotenoid-based plumage color in males is related to carotenoid availability in fruits, which they favor during molt in late summer and fall (Linville and Breitwisch 1997); and captive studies confirm

that carotenoid intake affects plumage color (McGraw and Hill 2001). Cardinals have been studied in an urban context where males, but not females, had reduced brightness as urbanization increased across a gradient in Ohio (Jones et al. 2010), which was attributed to the generally negative effects of urban development. In Florida, rural and urban males did not differ in plumage color (Leigh 2012). Unlike plumage, the effects of urbanization on bill color have not been studied in this or any species of which we are aware.

Our goal was to compare multiple carotenoid-based signals (chest, bill, and wing color) in rural and urban males and females while accounting for the sensitivity of the avian visual system, which has not been implemented in this species. We hypothesized that urbanization would affect the color of these signals, specifically that urban birds would have enhanced signals (e.g., redder hue, increased saturation) compared to rural birds, potentially due to increased access to carotenoids. We also explored relationships between mass-related body condition, urbanization, and color.

Methods

Field sites and data collection

We studied cardinals in 2 populations in Central New York that we chose because they differed drastically in the extent of human development (Fig. 1). The rural site, Rice Creek Field Station (43.43°N, 76.55°W; Fig. 1a), was located outside of Oswego (population 17,470), while the urban field site, Barry Park (43.03°N, 76.12°W; Fig. 1b), was located within the city limits of Syracuse (population 142,874). The 2 sites were separated by 57 km. At each site, we captured wild individuals using a territory-based targeted mist net approach between May and July in 2019, 2020, and 2021. We did not re-capture any individuals across years. Although we did not

116 know the precise age of most individuals, we limited our analyses to those we could age as
117 "after-hatching-year" (i.e., at least one-year-old) based on plumage. No captured individuals had
118 yet initiated their prebasic molt.

119 To quantify body condition, we measured mass to the nearest 0.5 g with a spring scale,
120 and tarsus length to the nearest 0.01 mm with digital calipers. Both populations were in the midst
121 of breeding, but we did not have precise information on nest stage for most individuals. For
122 males and females separately, we regressed mass on tarsus length. Males exhibited a significant
123 positive relationship between tarsus length and mass ($F_{1,63} = 18.0$, $P < 0.001$), so we extracted
124 model residuals and used them as an index of body condition. This index described whether an
125 individual was heavier (positive values) or lighter (negative values) than would be predicted by
126 body size. There was no significant relationship between female mass and tarsus length ($F_{1,23} =$
127 0.7, $P = 0.41$), so we used mass as an index of body condition.

128 We collected 10 feathers from the same central region of the chest of each captured male
129 for analysis via reflectance spectrometry (rural $n = 43$, urban $n = 22$). In the lab, we mounted the
130 feathers in an overlapping pattern on black construction paper (Strathmore Artagain Coal Black)
131 and measured ultraviolet (UV) and visible light reflectance from 300-700 nm using an Ocean
132 Optics Jaz reflectance spectrometer and a PX2 lamp. The probe was mounted at a 90° angle in a
133 block that held the probe tip 5 mm from the feather surface and excluded ambient light. Between
134 each sample, we calibrated the spectrometer using a WS-1 white standard. We took 3
135 measurements across different regions of the feather sample and averaged the reflectance curves
136 before analysis (Supplemental Fig. S1). To quantify male bill (rural $n = 40$, urban $n = 21$), female
137 bill (rural $n = 16$, urban $n = 9$), and female wing (rural $n = 15$, urban $n = 9$) color, we took
138 photographs in the field using a Canon EOS Rebel T6 digital camera (Supplemental Fig. S2).

139 Variation in sample size across signals was due to some photographs being unusable. We used
140 automatic settings because there were no systematic differences in ISO (t = -0.7, df = 47.3, P =
141 0.5) or aperture (t = -0.8, df = 42.9, P = 0.41) between sites. Each photograph contained color
142 standards and a scale bar (X-Rite ColorChecker Passport) and was taken under diffuse natural
143 sunlight without shadows. The inclusion of color standards in each photo allowed us to compare
144 among images controlling for variation in ambient light. We took 3 photos of each color patch
145 that varied in exposure (one stop under-exposed, normal, and one stop over-exposed) and then
146 selected the best-exposed, sharpest image for analysis.
147
148 **Color analyses**
149 We analyzed reflectance curves of male chest feathers using the program *pavo 2* (Maia et al.
150 2019) in R 3.6.0 (R Core Team 2019). We obtained quantum catch values for each of the 4 avian
151 single cones using the Blue Tit (*Cyanistes caeruleus*) visual model (plus double-cone sensitivity
152 for luminance, hereafter "brightness") and idealized illuminant D65. We then used the quantum
153 catch values to represent each color as a point in tetrahedral color space where the vertices
154 represented stimulation of the u, s, m, and l cones. From the color space, we extracted the angle
155 of the color vector relative to the x-y (theta) and z (phi) plane, which together describe hue.
156 Theta and phi were highly correlated (R = -0.94, P < 0.001), so we only used theta for
157 subsequent analyses. To quantify saturation, we used the distance between the point and the
158 achromatic center relative to the maximum possible distance. Together these measurements
159 described hue, saturation, and brightness of the chest feathers accounting for UV reflectance and
160 the sensitivity of the avian visual system.

We analyzed digital photographs of male and female bills and female wing patches using the Quantitative Color Pattern Analysis (QCPA; van den Berg et al. 2020) framework in the ImageJ 1.53a (Schneider et al. 2012) plugin micaToolbox (Troscianko and Stevens 2015). We first generated a cone catch model accounting for the camera's photosensitivity, the Blue Tit visual model, and idealized illuminant D65. This calibration was achieved by measuring an image of the color standards, which have known reflectance values. The camera was not UV-sensitive, so these analyses were restricted to the visible spectrum (400-700 nm). However, these orange-red color patches reflect predominantly long, visible wavelengths, where most of the variation occurs (Supplemental Fig. S1). Once a cone catch model was built, we converted each image into a normalized multispectral stack standardized by the 3% and 92% reflectance standards and selected regions of interest to measure (Supplemental Fig. S2a–b). We then ran each image through the cone catch model, followed by the QCPA framework that applies spatial acuity and viewing distance correction and then uses the Receptor Noise Limited (RNL) model (Vorobyev and Osorio 1998) to reduce the image into discernable color clusters that can be measured (Supplemental Fig. S2c–d). We used Gaussian acuity correction with 6 cycles per degree and a simulated viewing distance of 0.5 m. We used RNL ranked filtering with the following settings: iterations: 5, radius: 5, falloff: 3. We used RNL clustering with the following settings: visual system Weber fractions: Blue Tit 0.05, luminance Weber fraction: 0.1, color JND threshold: 2, luminance JND threshold: 3, loops: 20, radius multiplier: 2, minimum cluster size: 10. For each color cluster produced by QCPA, we extracted the quantum catch values for the 3 avian single cones (excluding the UV-sensitive cone) and the double cone (i.e., brightness). When an image was split into more than one cluster by QCPA, we computed a weighted average of each quantum catch value accounting for the area of each cluster. Using *pavo 2*, we converted

these values into a color point in triangular color space where the vertices represented stimulation of the s, m, and l cones. From the color space, we extracted the single angle of the color vector (theta), which describes hue. To quantify saturation, we used the distance between the point and the achromatic center. Together these measurements described hue, saturation, and brightness of the male and female bill and female wing patches considering the visible spectrum and the sensitivity of the avian visual system. Note that although both the tetrahedral and triangular color spaces produce angle theta to describe hue, the difference in geometry is such that higher values for male chest correspond to more orange hue, while they correspond to redder hue for male and female bill and female wing.

If we discovered a significant difference in color variables between factors, we further explored whether these differences would be recognizable by the signal receiver. We input the quantum catch values into the noise-weighted color distance (dS) calculator in *pavo 2* using the following settings: Weber fraction: 0.05, noise: neural, photoreceptor densities: u = 1, s = 2, m = 2, l = 4. We compared the resulting values to dS = 1, or the perceptual threshold indicating colors that can be distinguished by the avian visual system (Vorobyev and Osorio 1998).

Statistical analyses

Because hue, saturation, and brightness represent different and potentially informative aspects of a color, we analyzed them as separate response variables for each signal. We considered site (rural vs. urban) and year (2019, 2020, 2021) as factors because color could vary depending on habitat characteristics or could change systematically due to environmental differences over time. When checking for collinearity among predictors, we found a significant correlation between site and body condition using a linear model (see Results), and thus did not include

body condition as a factor in these models. We used a model selection approach, with all possible combinations of factors as predictors in a linear model, as well as an intercept-only model. We ranked models by Akaike's Information Criterion corrected for small sample sizes (AICc) and considered the model with the lowest AICc score to be best supported by the data. We considered models with ΔAICc < 2 to have similar fits compared to the top model. Additionally, we used log likelihoods and normalized Akaike weights to compare models. We conducted post-hoc comparisons using Tukey's HSD corrected for multiple comparisons. To determine whether dS values between factors were larger than the avian perceptual threshold, we tested whether the average pairwise dS value of all cross-factor comparisons (e.g., between every rural and urban color) was significantly greater than one using a one-sample *t*-test. Analyses were performed using R 3.6.0 (R Core Team 2019) and the *MuMIn* package (Bartón 2020).

Results

The top model for male chest hue included an interaction between site and year (Supplemental Table S1). There was a significant effect of year ($F_{2,59} = 18.7$, $P < 0.001$) and the interaction between site and year ($F_{2,59} = 5.0$, $P = 0.01$). Male chest hue in 2020 was higher (more orange) than 2019 and 2021 (both $P < 0.001$), and urban hue was lower (redder) than rural hue in 2020 ($P = 0.03$, Fig. 2a). The top model for male chest saturation included year only (Supplemental Table S1). There was a significant effect of year ($F_{2,62} = 8.7$, $P < 0.001$) such that saturation in 2019 was higher than 2020 ($P = 0.003$) and 2021 ($P = 0.01$, Fig. 2b). Similarly, the top model for male chest brightness also included year only (Supplemental Table S1). There was a significant effect of year ($F_{2,62} = 13.4$, $P < 0.001$) such that brightness in 2021 was lower than 2019 and 2020 (both $P < 0.001$, Fig. 2c). In 2020, the significant difference in male chest hue between the

230 rural and urban site resulted in an average pairwise dS value that was significantly greater than
231 the perceptual threshold (t = 6.0, df = 35, P < 0.001, Fig. 4a).
232 The top model for male bill hue included site only (Supplemental Table S2). There was a
233 significant effect of site ($F_{1,59}$ = 12.4, P = 0.001) such that urban hue was higher (redder) than
234 rural hue (Fig. 3a). There were no significant effects of any factors on male bill saturation or
235 brightness, as the top models were indistinguishable from an intercept-only model (Supplemental
236 Table S2). The significant difference in male bill hue between the rural and urban site resulted in
237 an average pairwise dS value that was significantly greater than the perceptual threshold (t =
238 35.5, df = 839, P < 0.001, Fig. 4a). There were no significant effects of any factors on female bill
239 hue, saturation, or brightness (Supplemental Table S3). For female wing saturation, the top
240 model included site only (Supplemental Table S4). There was a significant effect of site ($F_{1,22}$ =
241 4.9, P = 0.04) such that urban saturation was higher than rural saturation (Fig. 3b). There were no
242 significant effects of any factors on female wing hue or brightness (Supplemental Table S4). The
243 significant difference in female wing saturation between the rural and urban site resulted in an
244 average pairwise dS value that was significantly greater than the perceptual threshold (t = 11.1,
245 df = 134, P < 0.001, Fig. 4a).
246 Mass-related body condition of both males and females was affected by site. Male body
247 condition score was higher in rural compared to urban birds ($F_{1,63}$ = 14.9, P < 0.001, Figure 5a),
248 and female mass was higher in rural compared to urban birds ($F_{1,23}$ = 4.5, P = 0.05, Figure 5b).
249

250 **Discussion**

251 The color of multiple carotenoid-based signals was enhanced in males and females compared to
252 rural birds. Specifically, these colors were redder or more saturated in urban birds (Fig. 2–3),

253 despite these individuals being in relatively poor mass-related body condition compared to rural
254 birds (Fig. 5). Furthermore, although subtle to the human eye, these color differences were large
255 enough to be distinguished by the avian visual system (Fig. 4). These findings suggest that urban
256 living may reduce mass-related body condition, but that urban birds have enough access to
257 carotenoids that they are nonetheless able to produce what are typically thought of as costly
258 honest signals. In rural habitats, carotenoids may be a much more limiting resource, such that
259 rural birds have reduced ornamentation overall, but color may be a more honest signal of
260 condition. Preliminary analyses have found weak or no relationships between body condition and
261 ornament color within either population, but we are hesitant to over-interpret these results due to
262 low sample sizes. Our findings are in line with previous work on cardinals that found a
263 dampened relationship between body condition, plumage color, and reproductive success as
264 urbanization increased (Jones et al. 2010, Rodewald et al. 2011). Whether cardinals attend to the
265 color variation documented here, whether that variation is linked to reproductive success, and
266 whether the urban color enhancement disrupts the typical signaling dynamics are unknown in our
267 populations. However, our study stands apart in that we found a distinct enhancement of multiple
268 carotenoid-based signals in urban birds of both sexes, apparently not associated with increased
269 mass-related body condition. This is the first such result we are aware of, as the overwhelming
270 trend in the literature is for reduced ornamentation in urban areas, even among melanin-based or
271 structural colors (Sepp et al. 2018, Sepp et al. 2020, Heinen-Kay et al. 2021).
272 A closer inspection of the chest color results supports the idea that increased and/or
273 consistent access to carotenoids in urban areas could be what promoted the color enhancement.
274 In 2020 when we found a significant difference in chest hue between rural and urban males,
275 overall hue was more orange than in other years, but this difference was greater in the rural

habitat, which drove the rural-urban difference (Fig. 2A). This pattern suggests that carotenoid availability may fluctuate more widely over time in the rural habitat, but that urban birds have a more stable carotenoid supply that allows them to maintain redder plumage. Leigh (2012) made a similar interpretation based on urban male cardinals in Florida exhibiting less among-individual variation in chest brightness than rural males. We suggest that the relative abundance of exotic fruiting shrubs, especially *Lonicera* spp., in urban areas (Borgmann and Rodewald 2005, Borgmann and Rodewald 2005, Leston and Rodewald 2006) is responsible for the pattern we observed. Although we have not quantified plant diversity at our sites, anecdotally, *Lonicera* is much more established at the urban site compared to a more diverse vegetation community at the rural site (DTB, 2019, pers. observ.). We did not choose these sites a priori based on this difference. Furthermore, an increase in sampling sites would help determine if the observed color variation is due to *Lonicera* abundance specifically or other potential differences between rural and urban sites. Additionally, work is ongoing to quantify carotenoids in blood plasma and feathers to determine more conclusively if urban birds ingest and/or deposit more carotenoids than rural birds.

Whether or not carotenoids are more available to urban birds, it is important to note that many other unmeasured factors may influence whether they are acquired and deposited in tissue. For example, even if carotenoid-based color is a condition-dependent trait in cardinals, our mass-related measures of body condition offer only a small glimpse into what might be physiologically relevant to true condition. Rural and urban birds may differ in other more meaningful measures of condition such as immune function or stress. Wright and Fokidis (2016) found that urban male cardinals had lower circulating corticosterone and a lower stress response than rural males. Urban habitats may be less stressful for cardinals and/or they may modulate their stress response

299 such that they can more efficiently metabolize and deposit carotenoids in their tissues regardless
300 of availability (Giraudeau et al. 2015). Related to this idea, in a suite of Western Palearctic
301 species, Møller (2009) found that urban birds had a stronger immune response than rural birds,
302 which might free up carotenoids to be deposited in tissue. Additionally, if the lighting conditions
303 and/or visual background differs between sites, urban and rural birds may experience disruptive
304 selection to maximize the conspicuousness of their visual signals (Dalrymple et al. 2018, Leveau
305 2019). In summary, it is difficult with the present data to determine the mechanism of the
306 observed color variation, and there are several, non-mutually exclusive possibilities.

307 It is unclear why urban birds were in worse mass-related body condition than rural birds.
308 Potential explanations include limited access to protein-rich insect prey, benefits of reduced mass
309 for predator evasion, and adaptive changes in nestling development. In cardinals, previous
310 studies have found little to no effect of urbanization on similar body condition metrics
311 (Rodewald and Shustack 2008, Jones et al. 2010) or even that urban birds were in better
312 condition than rural birds (Wright and Fokidis 2016). However, the potential impacts of
313 urbanization on body condition may be site-specific, and there is evidence in other species of a
314 negative effect (Jiménez-Peñuela et al. 2019). Additionally, urban birds may not need to
315 accumulate energy reserves in the form of body mass in urban areas where food availability is
316 more predictable and nutritional stress is not as severe as in rural areas (Salleh Hudin et al.
317 2016). Regardless of the cause, we found that despite this reduction in mass-related body
318 condition, urban birds were still able to produce enhanced color signals compared to rural birds.

319 We found an enhancement of both feather and bill color in urban birds. The timing of
320 production of these two signal types has different implications for the relationship with
321 carotenoid availability. Cardinals molt once per year after the breeding season, so chest and wing

322 color should mainly be influenced by carotenoid deposition at that time only. However, it is
323 possible that the color variation is due to differences between rural and urban birds in the extent
324 to which feather color wears over time (e.g., due to differences in sun exposure). In contrast to
325 plumage, carotenoid-based bill color can vary much more dynamically and over shorter time
326 periods (Rosen and Tarvin 2006, Ardia et al. 2010). If the rural-urban color difference in bill
327 color is driven by carotenoid availability, these results may suggest that urban birds have greater
328 access to carotenoids not only during molt, but potentially throughout the breeding season (when
329 these birds were captured and photographed). Since *Lonicera* fruits are mainly available in late
330 summer and fall, carotenoids producing bill color may come from different sources (e.g., insect
331 prey), although fruits can ripen as early as June (DTB, 2020, pers. observ.). However, the extent
332 to which cardinal bill color changes over time is unknown, and color measured during the
333 summer breeding season may well have resulted from carotenoids ingested and sequestered
334 previously. Importantly, the factors influencing color (e.g., carotenoid availability, body
335 condition, stress) may vary across the signals measured in this study.
336
337 **Conclusion**
338 Urbanization typically dulls or reduces the extent of color in birds (Sepp et al. 2018, Leveau
339 2019, Heinen-Kay et al. 2021). Here we show instead that both male and female urban cardinals
340 had significantly enhanced color of multiple carotenoid-based signals compared to rural birds.
341 We suggest this enhancement is potentially due to the widespread and stable availability of
342 carotenoids in exotic plants that thrive in disturbed areas, although there are other possible
343 explanations, and these mechanisms deserve additional investigation. If carotenoids are a
344 limiting resource in rural but not urban habitats, the reliability of these signals as honest

indicators of condition may deteriorate (Jones et al. 2010, Rodewald et al. 2011). In support of this idea, we also found that urban birds were in worse mass-related body condition compared to rural birds, suggesting a decoupling of condition and color. Together, these results underscore the complex and subtle impacts of urbanization on wildlife. Not only can urbanization negatively affect populations by destroying and fragmenting habitat, it may also disrupt communication systems and potentially alter the evolutionary dynamics of signals.

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Figure 1. A map of the rural (Rice Creek Field Station, a) and urban (Barry Park, b) field sites.

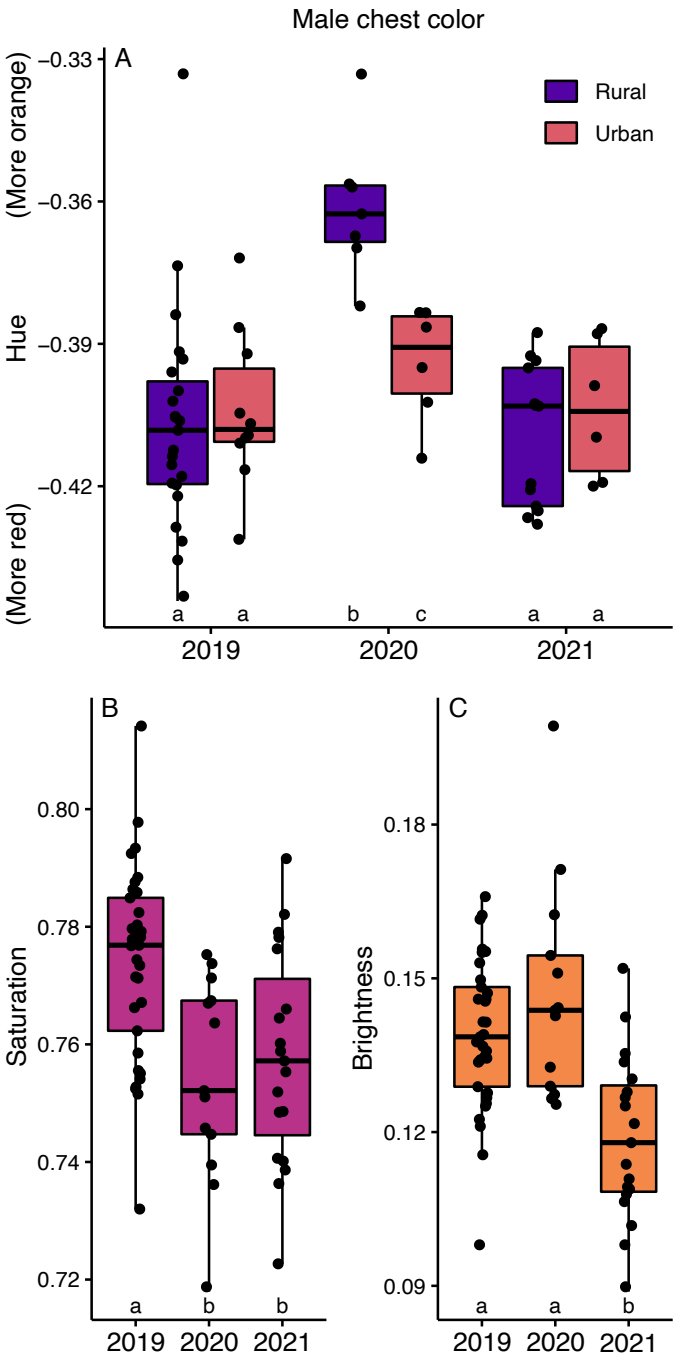


Figure 2. Variation in male chest hue (a), saturation (b), and brightness (c) as a function of urbanization and/or year. There was no significant effect of site on saturation or brightness, so only the effect of year is shown. Lowercase letters indicate which factors are (different letter) or are not (same letter) significantly different from each other.

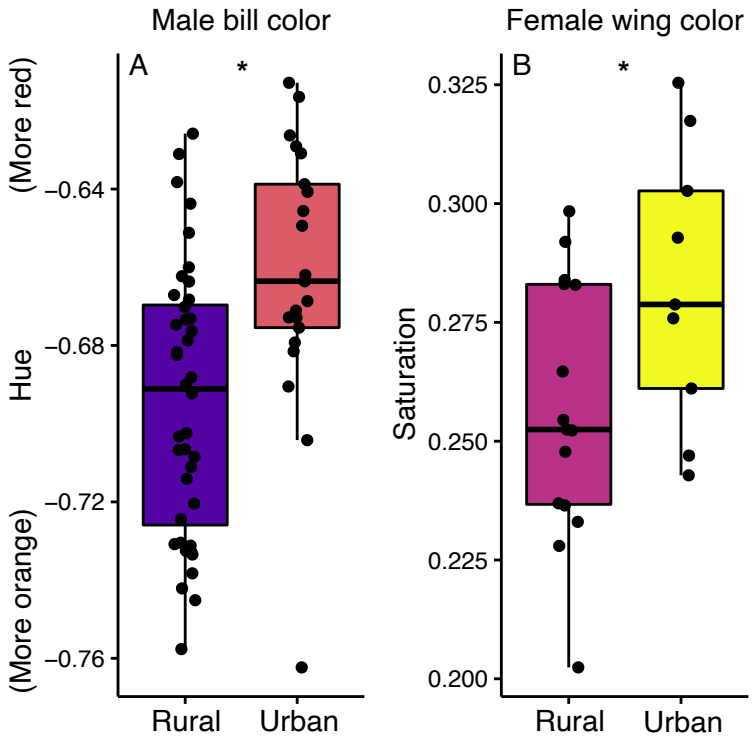


Figure 3. Variation in male bill hue (a) and female wing saturation (b) as a function of urbanization. Asterisks separate factors that are significantly different.

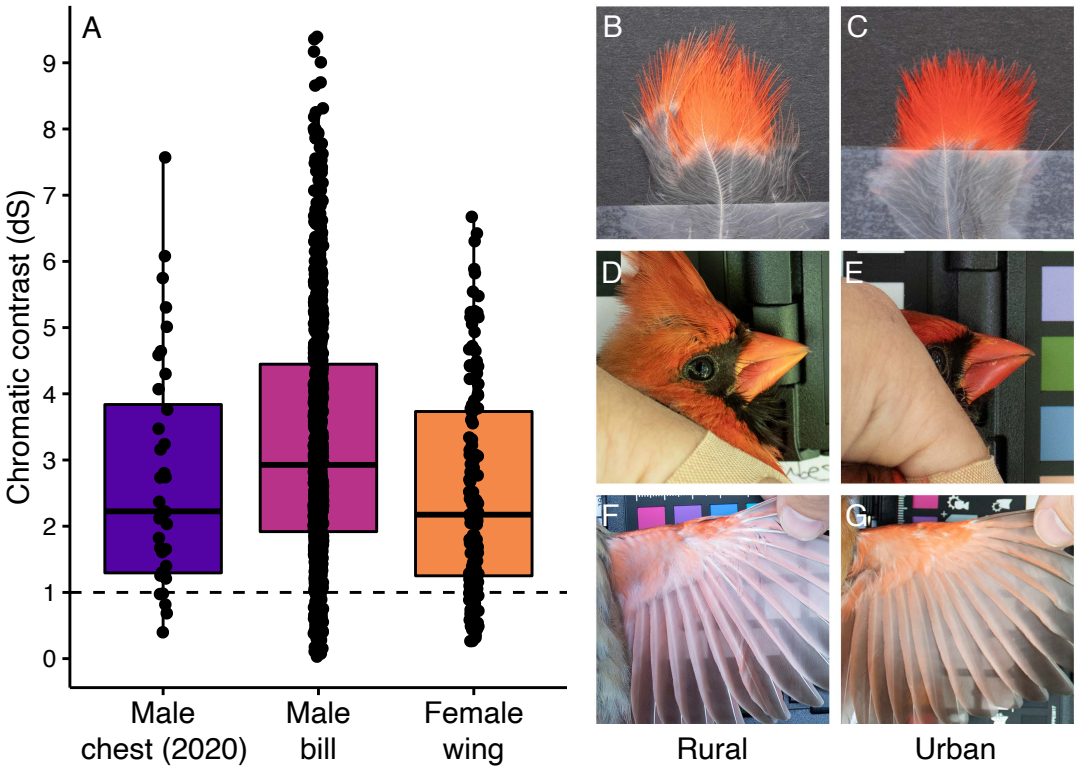
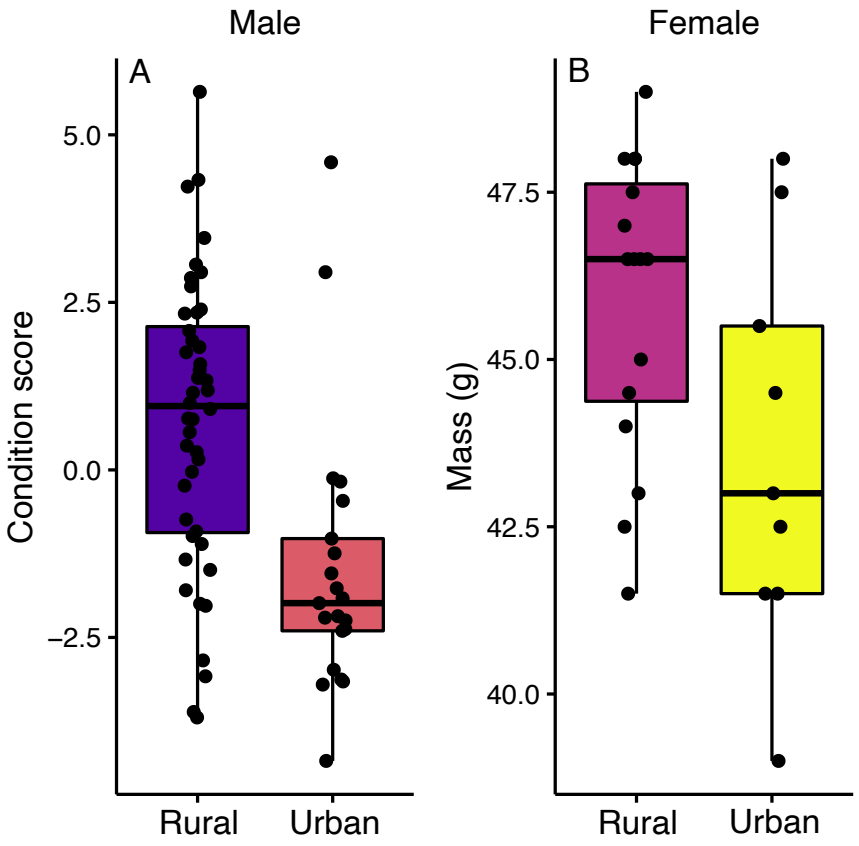


Figure 4: Pairwise chromatic contrast between rural and urban male chest (2020 only), male bill, and female wing (a). The dashed line indicates dS = 1, or the perceptual threshold above which colors can be distinguished by the avian visual system. Photos exemplifying the average rural and urban male chest (b, c), male bill (d, e), and female wing (f, g) signals.



551

552 **Figure 5.** Variation in male body condition score (a) and female mass (b) as a function of

553 urbanization.