RESEARCH ARTICLE

Urbanization enhances multiple carotenoid-based signals despite poor body condition in male and female Northern Cardinals

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ACKNOWLEDGEMENTS

Susan DeVries, Sylvia Halkin, Jodie Jawor, Gavin Leighton, and Daniel Shustack provided helpful comments on early drafts of this manuscript. Comments from anonymous reviewers greatly improved the manuscript. Shyla Luna helped collect data in the field. We are grateful to Syracuse University and the Onondaga County Department of Water Environment Protection for access to Barry Park, and to Alan Harris for logistical support at Rice Creek Field Station. Lauren Silvernail provided childcare while fieldwork was being conducted.

Funding statement: This research was supported by Rice Creek Associates and the Scholarly and Creative Activities Committee at SUNY Oswego.

Ethics statement: This research was approved by the SUNY Oswego Institutional Animal Care and Use Committee. D.B. had appropriate federal, state, and local permits.

Author contributions: D.B. conceived the idea, developed methods, collected data, analyzed data, and wrote the paper. H.B., K.D., B.G., M.M., and D.R. collected data and substantially edited the paper.

Data availability: Analyses in this article can be reproduced using the data provided by Baldassarre et al. (2022).

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. 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.

Keywords: avian visual model, bill color, carotenoids, *Lonicera*, Northern Cardinal, plumage color, signals, urbanization

LAY SUMMARY

- Urbanization typically dulls bird color, but carotenoid-based signals may be enhanced if urban birds have increased access to carotenoids from invasive plants.
- Urban Northern Cardinals of both sexes had enhanced signal color compared to rural birds, but were in worse body condition.
- Urban birds likely had greater access to carotenoids from invasive plants, allowing them to produce enhanced color despite poor condition.
- Urbanization can disrupt animal communication and possibly alter evolutionary dynamics.

INTRODUCTION

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, 2020; Heinen-Kay et al. 2021). Urban environments present several challenges that may affect communication

systems, including anthropogenic noise and light, heavy metal pollution, habitat fragmentation, and changes in resource availability.

In birds, much of this work has focused on anthropogenic disruption of vocal communication, especially in urban areas (Slabbekoorn and den Boer-Visser 2006, Slabbekoorn 2013, Derryberry and Luther 2021). Less well understood is how visual signals may also be impacted by human development. Carotenoid-based color patches may be especially affected by urbanization as carotenoids cannot be synthesized de novo by birds and must be obtained from the diet (Hill 1991). One way urbanization can directly impact these signals, and hence the communication system, is if rural and urban areas differ in the access to, amount, or types of resources containing carotenoids. Moreover, carotenoids can undergo extensive physiological modification before being deposited in tissue (Goodwin 1984), leaving room for other more indirect effects of urbanization on color (e.g., via stress or parasites). To date, research comparing carotenoid-based signals between rural and urban populations has found reduced ornamentation in urban populations of House Finches (*Haemorhous mexicanus*; Hasegawa et al. 2014, Giraudeau et al. 2015, 2018) and Great Tits (*Parus major*; Grunst et al. 2020). However, urban birds could have enhanced carotenoid-based color if, for example, they have access to carotenoid-rich invasive plants that thrive in disturbed landscapes (Hobbs 2000, Vilà and Ibáñez 2011). In the 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, 2017; Hudon and Mulvihill 2017). *Lonicera* thrives in urban areas (Rodewald 2005, Borgmann and Rodewald 2005, Leston and Rodewald 2006), providing a potential link

116 between urbanization and enhanced color. However, to our knowledge, no study has found
117 enhanced carotenoid-based signals in any species because of urbanization. Quantifying the color
118 of rural and urban populations of the same species provides a powerful framework to test this
119 idea.

120 The Northern Cardinal (hereafter "cardinal") is well suited to such a study because it
121 readily occurs in both rural and urban areas, and both males and females exhibit carotenoid-
122 based color patches that are displayed during various territorial, courtship, and pre-copulatory
123 interactions and likely function as signals (Linville et al. 1998, Wolfenbarger 1999a, b; Jawor et
124 al. 2003, 2004; Jawor and Breitwisch 2004). Males have bright red plumage overall and orange-
125 red bills, while females have pink-red underwing patches (hereafter "wing") and orange-red bills.
126 The putative functions of these colors as signals are complex and variable across studies. For
127 example, males with redder chest color had higher quality territories (Wolfenbarger 1999b,
128 Rodewald et al. 2011) and reproductive success (Wolfenbarger 1999b), but females did not
129 prefer redder males (Wolfenbarger 1999a). Males and females mated assortatively based on
130 chest, wing, and bill color (Jawor et al. 2003); and female wing and bill color co-varied with
131 body condition (Jawor et al. 2004). The color of carotenoid-based plumage color in males is
132 related to carotenoid availability in fruits, which they favor during molt in late summer and fall
133 (Linville and Breitwisch 1997); and captive studies confirm that carotenoid intake affects
134 plumage color (McGraw and Hill 2001). Cardinals have been studied in an urban context where
135 males, but not females, had reduced brightness as urbanization increased across a gradient in
136 Ohio (Jones et al. 2010), which was attributed to the generally negative effects of urban
137 development. It is not obvious how carotenoid access would affect brightness per se, whereas the
138 link between increased pigmentation and more saturated, redder hue is clear. Unlike plumage,

139 the effects of urbanization on bill color have not been studied in any species of which we are
140 aware.

141 Our goal was to compare multiple carotenoid-based signals (chest, bill, and wing color)
142 in rural and urban males and females while accounting for the sensitivity of the avian visual
143 system. We hypothesized that urbanization would affect the color of these signals, specifically
144 that urban birds would have enhanced signals (e.g., redder hue, increased saturation) compared
145 to rural birds due to increased access to carotenoids. We also explored relationships between
146 mass-related body condition, urbanization, and color.

147

148 **METHODS**

149 **Field Sites and Data Collection**

150 We studied cardinals in two populations in Central New York that differed drastically in the
151 extent of human development (Figure 1). The rural site, Rice Creek Field Station (43.43°N,
152 76.55°W; Figure 1A), was located outside of Oswego (population 17,470), while the urban field
153 site, Barry Park (43.03°N, 76.12°W; Figure 1B), was located within the city limits of Syracuse
154 (population 142,874). The two sites were separated by 57 km. At each site, we captured wild
155 individuals using a territory-based targeted mist net approach between May and August in 2019,
156 2020, and 2021. We did not re-capture any individuals across years. Although we did not know
157 the precise age of most individuals, we limited our analyses to those we could age as "after-
158 hatching-year" (i.e. 1+ years old) based on plumage. To avoid comparing color among
159 individuals with worn and new feathers, we excluded individuals that had initiated their prebasic
160 molt.

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

We collected 10 feathers from the chest of each captured male for analysis via reflectance spectrometry (rural $n = 43$, urban $n = 22$). In the lab, we mounted the feathers in an overlapping pattern on black construction paper (Strathmore Artagain Coal Black) and measured ultraviolet (UV) and visible light reflectance from 300-700 nm using an Ocean Optics Jaz reflectance spectrometer and a PX2 lamp. The probe was mounted at a 90° angle in a block that held the probe tip 5 mm from the feather surface and excluded ambient light. Between each sample, we calibrated the spectrometer using a WS-1 white standard. We took three measurements across different regions of the feather sample and averaged the reflectance curves before analysis (Supplementary Material Figure S1). To quantify male bill (rural $n = 40$, urban $n = 21$), female bill (rural $n = 16$, urban $n = 9$), and female wing (rural $n = 15$, urban $n = 9$) color, we took photographs in the field using a Canon EOS Rebel T6 digital camera (Supplementary Material Figure S2). Variation in sample size across signals was due to some photographs being unusable. We used automatic settings because there were no systematic differences in ISO ($t = -0.7$, df =

47.3, $P = 0.5$) or aperture ($t = -0.8$, df = 42.9, $P = 0.41$) between sites. Each photograph contained color standards and a scale bar (X-Rite ColorChecker Passport) and was taken under diffuse natural sunlight without shadows. The inclusion of color standards in each photo allowed us to compare among images controlling for variation in ambient light. We took three photos of each color patch that varied in exposure (one stop under-exposed, normal, and one stop over-exposed) and then selected the best-exposed, sharpest image for analysis.

Color Analyses

We analyzed reflectance curves of male chest feathers using the program *pavo 2* (Maia et al. 2019) in R 3.6.0 (R Core Team 2019). We obtained quantum catch values for each of the four avian single cones using the Blue Tit visual model (plus double-cone sensitivity for luminance, hereafter "brightness") and idealized illuminant D65. We then used the quantum catch values to represent each color as a point in tetrahedral color space where the vertices represented stimulation of the u, s, m, and l cones. From the color space, we extracted the angle of the color vector relative to the x-y (theta) and z (phi) plane, which together describe hue. Theta and phi were highly correlated (chest $r = -0.94$, $P < 0.001$), so we only used theta for subsequent analyses. To quantify saturation, we used the distance between the point and the achromatic center relative to the maximum possible distance. Together these measurements described hue, saturation, and brightness of the chest feathers accounting for UV reflectance and 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

207 first generated a cone catch model accounting for the camera's photosensitivity, the Blue Tit
208 visual model, and idealized illuminant D65. This calibration was achieved by measuring an
209 image of the color standards, which have known reflectance values. The camera was not UV-
210 sensitive, so these analyses were restricted to the visible spectrum (400-700 nm). However, these
211 orange-red color patches reflect predominantly long, visible wavelengths, where most of the
212 variation occurs (Supplementary Material Figure S1). Once a cone catch model was built, we
213 converted each image into a normalized multispectral stack standardized by the 3% and 92%
214 reflectance standards and selected regions of interest to measure (Supplementary Material Figure
215 S2A, B). We then ran each image through the cone catch model, followed by the QCPA
216 framework that applies spatial acuity and viewing distance correction and then uses the Receptor
217 Noise Limited (RNL) model (Vorobyev and Osorio 1998) to reduce the image into discernable
218 color clusters that can be measured (Supplementary Material Figure S2C, D). We used Gaussian
219 acuity correction with six cycles per degree and a simulated viewing distance of 0.5 m. We used
220 RNL ranked filtering with the following settings: iterations: 5, radius: 5, falloff: 3. We used RNL
221 clustering with the following settings: visual system Weber fractions: Blue Tit 0.05, luminance
222 Weber fraction: 0.1, color JND threshold: 2, luminance JND threshold: 3, loops: 20, radius
223 multiplier: 2, minimum cluster size: 10. For each color cluster produced by QCPA, we extracted
224 the quantum catch values for the three avian single cones (excluding the UV-sensitive cone) and
225 the double cone (i.e. brightness). When an image was split into more than one cluster by QCPA,
226 we computed a weighted average of each quantum catch value accounting for the area of each
227 cluster. Using *pavo 2*, we converted these values into a color point in triangular color space
228 where the vertices represented stimulation of the s, m, and l cones. From the color space, we
229 extracted the single angle of the color vector (theta), which describes hue. To quantify saturation,

230 we used the distance between the point and the achromatic center. Together these measurements
231 described hue, saturation, and brightness of the male and female bill and female wing patches
232 considering the visible spectrum and the sensitivity of the avian visual system. Note that
233 although both the tetrahedral and triangular color spaces produce angle theta to describe hue, the
234 difference in geometry is such that higher values for male chest correspond to more orange hue,
235 while they correspond to redder hue for male and female bill and female wing.

236 If we discovered a significant difference in color variables between factors, we further
237 explored whether these differences would be recognizable by the signal receiver. We input the
238 quantum catch values into the noise-weighted color distance (dS) calculator in *pavo 2* using the
239 following settings: Weber fraction: 0.05, noise: neural, photoreceptor densities: u = 1, s = 2, m =
240 2, l = 4. We compared the resulting values to dS = 1, or the perceptual threshold indicating colors
241 that can be distinguished by the avian visual system (Vorobyev and Osorio 1998).

242

243 **Statistical Analyses**

244 Because hue, saturation, and brightness represent different and potentially informative aspects of
245 a color, we analyzed them as separate response variables for each signal. We considered site
246 (rural vs. urban) and year (2019, 2020, 2021) as factors because color could vary depending on
247 habitat characteristics or could change systematically due to environmental differences over
248 time. When checking for collinearity among predictors, we found a significant correlation
249 between site and body condition using a linear model (see Results), and thus did not include
250 body condition as a factor in these models. We used a model selection approach, with all
251 possible combinations of factors as predictors in a linear model, as well as an intercept-only
252 model. We ranked models by Akaike's Information Criterion corrected for small sample sizes

253 (AICc) and considered the model with the lowest AICc score to be best supported by the data.
254 We considered models with ΔAICc < 2 to have similar fits compared to the top model.
255 Additionally, we used log likelihoods and normalized Akaike weights to compare models. We
256 conducted post-hoc comparisons using Tukey's HSD corrected for multiple comparisons. To
257 determine whether dS values between factors were larger than the avian perceptual threshold, we
258 tested whether the average pairwise dS value of all cross-factor comparisons (e.g., between every
259 rural and urban color) was significantly greater than one using a one-sample t-test. Analyses
260 were performed using R 3.6.0 (R Core Team 2019) and the *MuMIn* package (Bartón 2020).
261

262 **RESULTS**

263 The top model for male chest hue included an interaction between site and year (Supplementary
264 Material Table S1). There was a significant effect of year (F = 18.7, df = 2 and 59, P < 0.001)
265 and the interaction between site and year (F = 5.0, df = 2 and 59, P = 0.01). Male chest hue in
266 2020 was higher (more orange) than 2019 and 2021 (both P < 0.001), and urban hue was lower
267 (redder) than rural hue in 2020 (P = 0.03, Figure 2A). The top model for male chest saturation
268 included year only (Supplementary Material Table S1). There was a significant effect of year (F
269 = 8.7, df = 2 and 62, P < 0.001) such that saturation in 2019 was higher than 2020 (P = 0.003)
270 and 2021 (P = 0.01, Figure 2B). Similarly, the top model for male chest brightness also included
271 year only (Supplementary Material Table S1). There was a significant effect of year (F = 13.4, df
272 = 2 and 62, P < 0.001) such that brightness in 2021 was lower than 2019 and 2020 (both P <
273 0.001, Figure 2C). In 2020, the significant difference in male chest hue between the rural and
274 urban site resulted in an average pairwise dS value that was significantly greater than the
275 perceptual threshold (t = 6.0, df = 35, P < 0.001, Figure 4A).

The top model for male bill hue included site only (Supplementary Material Table S2). There was a significant effect of site ($F = 12.4$, df = 1 and 59, $P = 0.001$) such that urban hue was higher (redder) than rural hue (Figure 3A). There were no significant effects of any factors on male bill saturation or brightness, as the top models were indistinguishable from an intercept-only model (Supplementary Material Table S2). The significant difference in male bill hue between the rural and urban site resulted in an average pairwise dS value that was significantly greater than the perceptual threshold ($t = 35.5$, df = 839, $P < 0.001$, Figure 4A). There were no significant effects of any factors on female bill hue, saturation, or brightness (Supplementary Material Table S3). For female wing saturation, the top model included site only (Supplementary Material Table S4). There was a significant effect of site ($F = 4.9$, df = 1 and 22, $P = 0.04$) such that urban saturation was higher than rural saturation (Figure 3B). There were no significant effects of any factors on female wing hue or brightness (Supplementary Material Table S4). The significant difference in female wing saturation between the rural and urban site resulted in an average pairwise dS value that was significantly greater than the perceptual threshold ($t = 11.1$, df = 134, $P < 0.001$, Figure 4A).

Mass-related body condition of both males and females was affected by site. Male body condition score was higher in rural compared to urban birds ($F = 14.9$, df = 1 and 63, $P < 0.001$, Figure 5A), and female mass was higher in rural compared to urban birds ($F = 4.5$, df = 1 and 23, $P = 0.05$, Figure 5B).

DISCUSSION

Urbanization enhanced the color of multiple carotenoid-based signals in both males and females. Specifically, these colors were redder or more saturated in urban birds (Figures 2, 3), despite

these individuals being in relatively poor mass-related body condition compared to rural birds (Figure 5). Furthermore, these color differences – although subtle to the human eye – were large enough to be distinguished by the avian visual system (Figure 4). These findings suggest that urban living may reduce mass-related body condition, but that urban birds have enough access to carotenoids that they are nonetheless able to produce what are typically thought of as costly honest signals. In rural habitats, carotenoids may be a much more limiting resource, such that rural birds have reduced ornamentation overall, but color may be a more honest signal of condition. Preliminary analyses have found weak or no relationships between body condition and ornament color within either population, but we are hesitant to over-interpret these results due to low sample sizes. Our findings are in line with previous work on cardinals that found a dampened relationship between body condition, plumage color, and reproductive success as urbanization increased (Jones et al. 2010, Rodewald et al. 2011). Whether cardinals attend to the color variation documented here, whether that variation is linked to reproductive success, and whether the urban color enhancement disrupts the typical signaling dynamics are unknown in our populations. However, our study stands apart in that we found a distinct enhancement of multiple carotenoid-based signals in urban birds of both sexes, apparently not associated with increased mass-related body condition. This is the first such result we are aware of, as the overwhelming trend in the literature is for reduced ornamentation in urban areas, even among melanin-based or structural colors (Sepp et al. 2018, 2020; Heinen-Kay et al. 2021).

A closer inspection of the chest color results supports the idea that increased and/or consistent access to carotenoids in urban areas could be what promoted the color enhancement. In 2020 when we found a significant difference in chest hue between rural and urban males, overall hue was more orange than in other years, but this difference was greater in the rural

322 habitat, which drove the rural-urban difference (Figure 2A). This pattern suggests that carotenoid
323 availability may fluctuate more widely over time in the rural habitat, but that urban birds have a
324 more stable carotenoid supply that allows them to maintain redder plumage. We suggest that the
325 relative abundance of exotic fruiting shrubs, especially *Lonicera* spp., in urban areas (Borgmann
326 and Rodewald 2005; Leston and Rodewald 2006) is responsible for this pattern. Although we
327 have not quantified plant diversity at our sites, anecdotally, *Lonicera* is much more established at
328 the urban site compared to a more diverse vegetation community at the rural site. Furthermore,
329 work is ongoing to quantify carotenoids in blood plasma and feathers to determine more
330 conclusively if urban birds ingest and/or deposit more carotenoids than rural birds.

331 Even if carotenoids are more available to urban birds, it is important to note that many
332 other unmeasured factors may influence whether they are acquired and deposited in tissue. For
333 example, even if carotenoid-based color is a condition-dependent trait in cardinals, our mass-
334 related measures of body condition offer only a small glimpse into what might be physiologically
335 relevant to true condition. Rural and urban birds may differ in other more meaningful measures
336 of condition such as immune function or stress. Wright and Fokidis (2016) found that urban male
337 cardinals had lower circulating corticosterone and a lower stress response than rural males.
338 Urban habitats may be less stressful for cardinals and/or they may modulate their stress response
339 such that they can more efficiently metabolize and deposit carotenoids in their tissues regardless
340 of availability (Giraudeau et al. 2015). Additionally, if the lighting conditions and/or visual
341 background differs between sites, urban and rural birds may experience disruptive selection to
342 maximize the conspicuousness of their visual signals (Dalrymple et al. 2018, Leveau 2019).

343 It is unclear why urban birds were in worse mass-related body condition than rural birds.
344 Potential explanations include limited access to protein-rich insect prey, benefits of reduced mass

345 for predator evasion, and adaptive changes in nestling development. In cardinals, previous
346 studies have found little to no effect of urbanization on similar body condition metrics
347 (Rodewald and Shustack 2008, Jones et al. 2010) or even that urban birds were in better
348 condition than rural birds (Wright and Fokidis 2016). However, the potential impacts of
349 urbanization on body condition may be site-specific, and there is evidence in other species of a
350 negative effect (Jiménez-Peñuela et al. 2019). Additionally, urban birds may not need to
351 accumulate energy reserves in the form of body mass in urban areas where food availability is
352 more predictable and nutritional stress is not as severe as in rural areas (Salleh Hudin et al.
353 2016). Regardless of the cause, we found that despite this reduction in mass-related body
354 condition, urban birds were still able to produce enhanced color signals compared to rural birds.
355 We found an enhancement of both feather and bill color in urban birds. The timing of
356 production of these two signal types has different implications for the relationship with
357 carotenoid availability. Cardinals molt once per year after the breeding season, so chest and wing
358 color would be influenced by carotenoid intake at that time only. In contrast, carotenoid-based
359 bill color can vary much more dynamically and over shorter time periods (Rosen and Tarvin
360 2006, Ardia et al. 2010). If the rural-urban color difference in bill color is driven by carotenoid
361 availability, these results suggest that urban birds have greater access to carotenoids not only
362 during molt, but potentially throughout the breeding season (when these birds were captured and
363 photographed). Since *Lonicera* fruits are mainly available in late summer and fall, carotenoids
364 producing bill color may come from different sources (e.g., insect prey), although fruits can ripen
365 as early as June (personal observation). However, the extent to which cardinal bill color changes
366 over time is unknown, and color measured during the summer breeding season may well have
367 resulted from carotenoids ingested and sequestered previously. Importantly, the factors

influencing color (e.g., carotenoid availability, body condition, stress) may vary across the signals measured in this study.

CONCLUSION

Urbanization typically dulls or reduces the extent of color in birds (Sepp et al. 2018, Leveau 2019, Heinen-Kay et al. 2021). Here we show instead that both male and female urban cardinals had significantly enhanced color of multiple carotenoid-based signals compared to rural birds. We suggest this enhancement is potentially due to the widespread and stable availability of carotenoids in exotic plants that thrive in disturbed areas, although there are other possible explanations, and this relationship deserves additional investigation. If carotenoids are a 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.

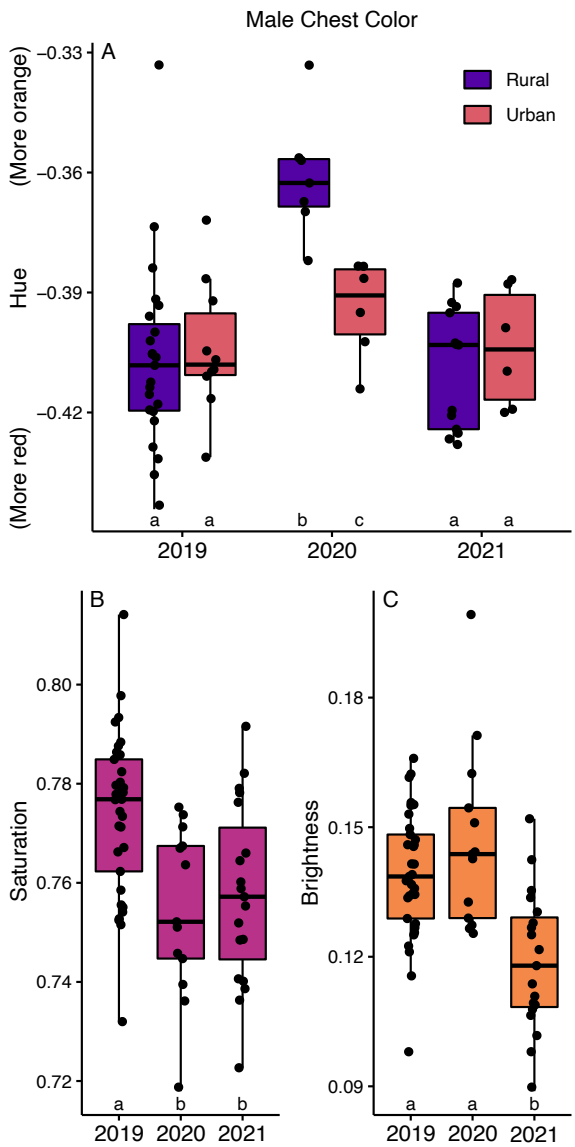


Figure 2. Variation in male chest hue (**A**), saturation (**B**), and brightness (**C**) as a function of urbanization and/or year. There was no significant effect of site on saturation or brightness, so only the effect of year is shown. Lowercase letters indicate which factors are (different letter)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

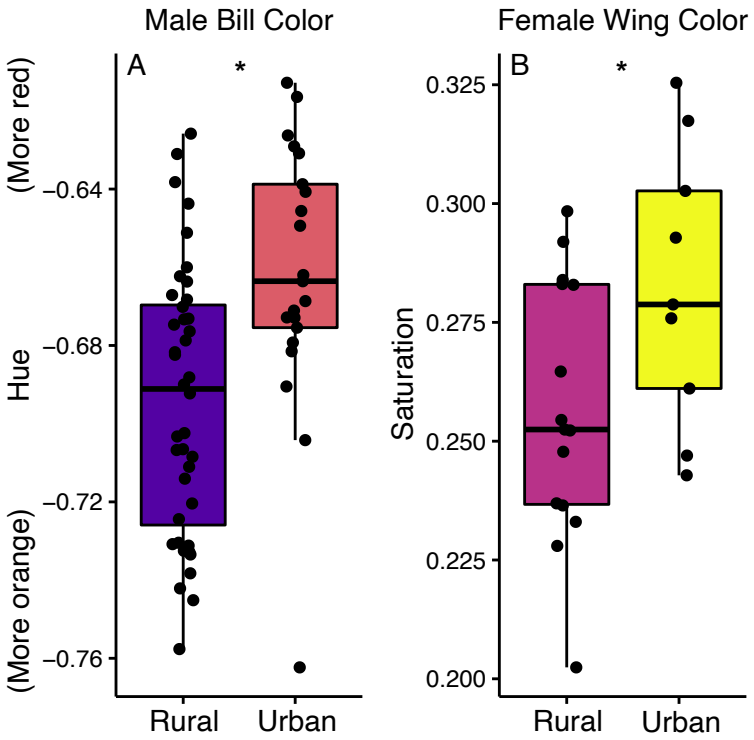


Figure 3. Variation in male bill hue (**A**) and female wing saturation (**B**) as a function of urbanization. Asterisks separate factors that are significantly different.

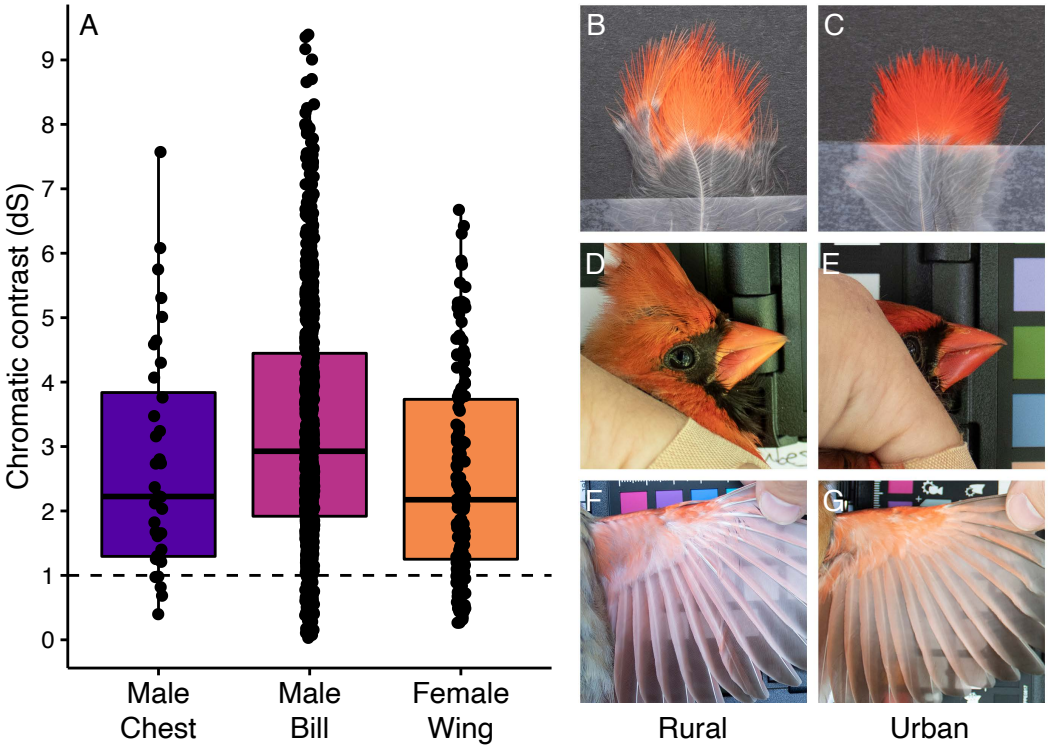
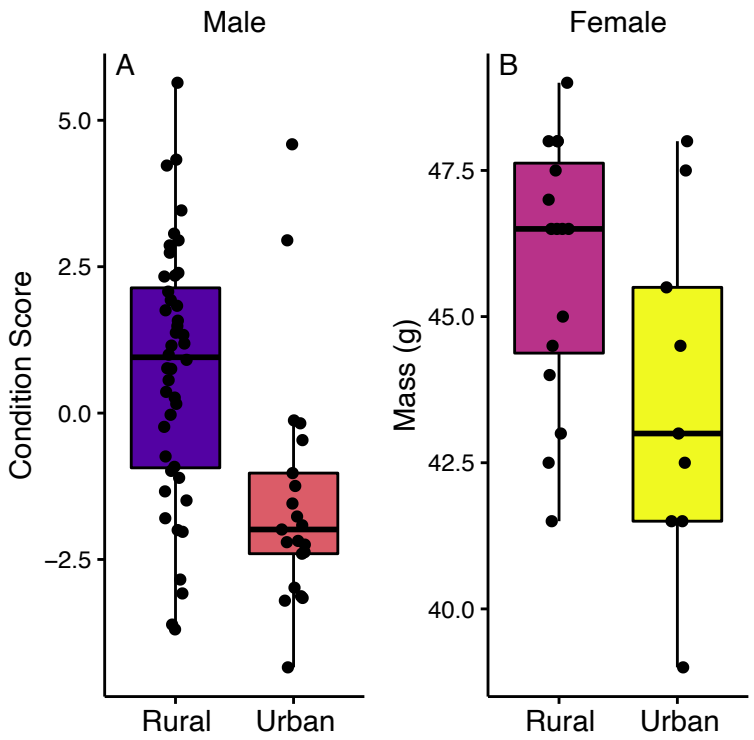


Figure 4: Pairwise chromatic contrast between rural and urban male chest (2020 only), male bill, and female wing (**A**). The dashed line indicates dS = 1, or the perceptual threshold above which colors can be distinguished by the avian visual system. Photos exemplifying the average rural and urban male chest (**B**, **C**), male bill (**D**, **E**), and female wing (**F**, **G**) signals.



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576 **Figure 5.** Variation in male body condition score (**A**) and female mass (**B**) as a function of

577 urbanization.

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SUPPLEMENTARY MATERIAL

Table S1. Summary of model selection results examining male chest hue, saturation, and brightness (n = 65). Only models with ΔAICc < 5 are shown.

Response	Factors	k	logLik	ΔAICc	Weight
hue	site*year	7	170.22	0	0.86
hue	year	4	164.76	3.63	0.14
saturation	year	4	173.58	0	0.66
saturation	site+year	5	173.58	2.35	0.21
saturation	site*year	7	175.62	3.23	0.13
brightness	year	4	175.87	0	0.58
brightness	site*year	7	178.50	2.05	0.21
brightness	site+year	5	176.01	2.07	0.21

Table S2. Summary of model selection results examining male bill hue, saturation, and brightness (n = 61). Only models with ΔAICc < 5 are shown.

Response	Factors	k	logLik	ΔAICc	Weight
hue	site	3	120.41	0	0.50
hue	site+year	5	122.53	0.43	0.40
hue	site*year	7	123.61	3.29	0.10
saturation	site	3	115.65	0	0.29
saturation	intercept	2	114.51	0.07	0.28
saturation	site+year	5	117.69	0.58	0.22
saturation	year	4	116.48	0.63	0.21
brightness	intercept	2	116.29	0	0.39
brightness	year	4	118.24	0.62	0.29
brightness	site	3	116.68	1.44	0.19
brightness	site+year	5	118.62	2.24	0.13

Table S3. Summary of model selection results examining female bill hue, saturation, and brightness (n = 25). Only models with ΔAICc < 5 are shown.

Response	Factors	k	logLik	ΔAICc	Weight
hue	intercept	2	47.09	0	0.50
hue	year	4	49.10	1.43	0.25
hue	site	3	47.35	2.07	0.18
hue	site+year	5	49.41	3.98	0.07
saturation	year	4	50.13	0	0.43
saturation	intercept	2	47.29	0.23	0.38
saturation	site	3	47.30	2.81	0.10
saturation	site+year	5	50.15	3.12	0.09
brightness	year	4	46.56	0	0.45
brightness	intercept	2	43.60	0.47	0.36
brightness	site	3	43.60	3.07	0.10
brightness	site+year	5	46.56	3.15	0.09

Table S4. Summary of model selection results examining female wing hue, saturation, and brightness (n = 24). Only models with ΔAICc < 5 are shown.

Response	Factors	k	logLik	ΔAICc	Weight
hue	intercept	2	42.30	0	0.51
hue	site	3	42.93	1.37	0.26
hue	year	4	43.97	2.21	0.17
hue	site+year	5	44.57	4.22	0.06
saturation	site	3	52.73	0	0.75
saturation	intercept	2	50.34	2.15	0.25
brightness	year	4	27.03	0	0.33
brightness	intercept	2	24.21	0.11	0.31
brightness	site+year	5	28.10	1.09	0.19
brightness	site	3	24.94	1.27	0.17

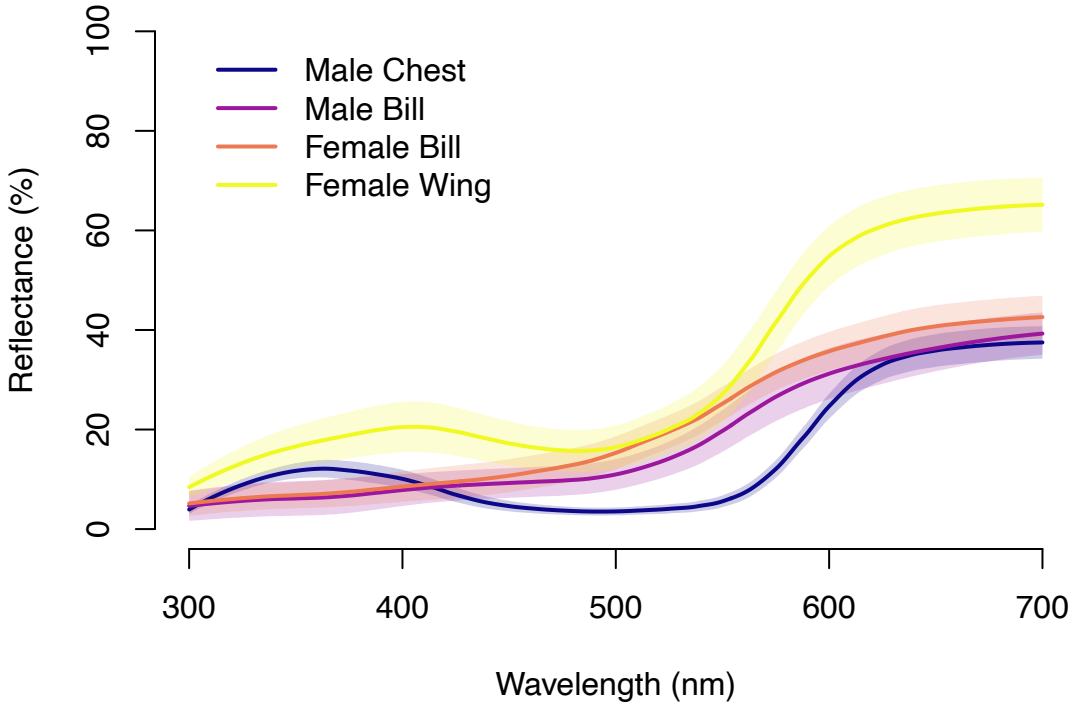

Figure S1. Examples of reflectance curves for the signals measured in the study. The dark line represents average reflectance and the shaded area represents one standard deviation. The male chest curve was generated from ten random males measured in the study. The male bill, female bill, and female wing curves were generated from ten measurements of museum skins. All signals reflect predominantly long wavelengths and have minimal ultraviolet (UV) reflectance.

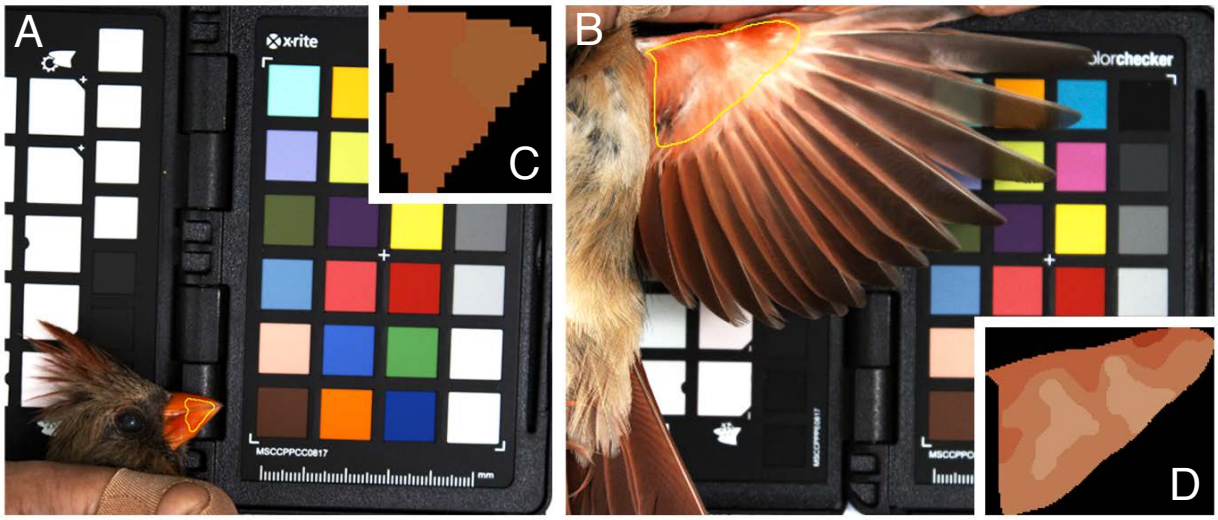

Figure S2. Examples representing the analysis of male and female bill (**A**) and female wing (**B**) photos. After selecting regions of interest, we used the QCPA framework to break the color patch up into discernable clusters (**C**, **D**) that could be measured for hue, saturation, and brightness.