Research Article

Daniel J. Becker^{*†}, Elizabeth M. Schultz[†], Jonathan W. Atwell, Ellen D. Ketterson

Urban residency and leukocyte profiles in a traditionally migratory songbird

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Abstract: Many animals are shifting migrations in response to human activities. In particular, human-induced changes to climate and habitat (e.g., urbanization) likely facilitate animals becoming year-round residents. Because migration can be energetically expensive, shifts to sedentary behavior could minimize energetic demands incurred and any immunosuppressive effects. Residency in urban habitats could also provide abundant resources and allow sedentary animals to invest more in immunity. However, urban habitats could also expose sedentary animals to novel stressors that counter such benefits. To examine how recent shifts to residency affects physiology in ways that may shape infectious disease dynamics, we analyzed leukocyte profiles of two dark-eyed junco (*Junco hyemalis*) populations from southern California: the Laguna Mountain population, in which birds breed in high-elevation forests and migrate altitudinally, and the urban University of California San Diego population, which was likely established by overwintering migrants in the 1980s and has since become non-migratory. Over a two-year study of each population's breeding season, we found no difference in the ratios of heterophils to lymphocytes between populations. However, urban residents had more leukocytes than birds from the altitudinal migrant population. A multivariate analysis suggested urban residents had fewer monocytes, but effect sizes were small. These results suggest no differences in energy demands or stressors between urban resident and altitudinal migrant populations during their breeding season. However, urban

residency may confer immunological benefits through anthropogenic resources, which could have important consequences for disease dynamics..

Keywords: white blood cells; HL ratios; ecoimmunology; altitudinal migration; *Junco hyemalis*; urbanization; anthropogenic resources

1 Introduction

Animal migration, the regular movement of individuals between breeding and overwintering sites to track seasonal changes in resources and habitats, is a widespread phenomenon [1]. Such behavior has important ecological implications for nutrient cycling, pollination and propagule dispersal, and species interactions [2, 3]. However, animal migrations are changing globally in response to human activities [4, 5]. Changes in climate and habitat can decrease migratory propensity or drive animals to eliminate migration altogether and form resident populations [6]. Such patterns have been especially well-documented in birds [7, 8]. For example, white storks (*Ciconia ciconia*) have forgone fall migrations to Africa and now subsist on urban landfills in Spain throughout the year [9, 10]. Similarly, urban habitats, by providing supplemental food in the form of bird feeders and possibly milder climates, have facilitated the loss of migration and adoption of sedentary behavior in songbirds such as the Eurasian blackcap (*Sylvia atricapilla*) and European blackbird (*Turdus merula*) [11, 12]. In particular, this colonization of urban habitats in the traditional overwintering range appears to be a widespread phenomenon [13–15].

Because migration can be energetically expensive [16, 17], shifts to sedentary behavior could minimize the energetic demands incurred from long-distance movements. Given that strenuous activities such as migration can force trade-offs with energetic investment in other physiological systems, such as suppressing components of the immune system [18, 19], these

***Corresponding author: Daniel J. Becker**, Department of Biology, Indiana University, Bloomington, IN, E-mail: danbeck@iu.edu
Daniel J. Becker, Jonathan W. Atwell, Ellen D. Ketterson, Department of Biology, Indiana University, Bloomington, IN
Elizabeth M. Schultz, Department of Biology, Wittenberg University, Springfield, OH

Ellen D. Ketterson, Environmental Resilience Institute, Indiana University, Bloomington, IN

[†]Both authors contributed equally

transitions towards residency could in turn improve resistance or tolerance to pathogens and alter infectious disease dynamics [13, 20]. Such effects could be especially amplified from residency in urban habitats, given the potential for greater food abundance from intentional (e.g., bird feeders) and unintentional (e.g., landfills) resources and therefore minimized foraging effort [21, 22]. Environments with more resources could accordingly allow hosts to invest more resources in both immunity and other costly traits (e.g., reproduction) [23, 24]. However, urban habitats could also expose residents to novel stressors through changes in intra-specific competition (e.g., elevated density and crowding) [25, 26], exposure to novel parasites [27–29], artificial light at night and noise pollution [30, 31], and toxicants [32, 33]. Given such opposing effects, understanding how urbanized habitats affect the physiology of recent colonists is necessary to predict the consequences for wildlife health, conservation, and pathogen spillover [34–36]. Such work is especially needed given global increases in urban habitat and loss of migration [13, 37].

To assess how urban residency shapes the physiology of traditionally migratory animals, we focus on two distinct populations of dark-eyed juncos (*Junco hyemalis*),

a songbird species complex found across North America [38]. In southern California, the natural breeding range of juncos (*J. h. thurberi*) is confined to high-elevation forests (e.g., >1500 meters) [39]. However, in the early 1980s, a population of juncos became established in an urban habitat on the coastal campus of the University of California San Diego (UCSD) [40, 41]. Population genetics and stable isotopes of hydrogen suggest this resident population was established by overwintering migrants from multiple mountains, including but not limited to the nearby Laguna Mountains (LM) [42, 43] (Fig. 1). The milder climate and abundant food, water, and nesting resources in the urban environment may have facilitated the adoption of sedentary behavior, in contrast to the altitudinal migrations observed among LM juncos in winter [40, 43, 44]. Indeed, LM juncos still display more migratory restlessness than UCSD juncos [45]. Accordingly, the UCSD population has a breeding season more than twice the length of LM juncos and thus greater fecundity [40].

We capitalized on a longitudinal study comparing UCSD and LM juncos across their breeding seasons in 2006 and 2007 [46, 47]. We first used differential leukocyte counts from blood smears to assess whether

Figure 1. (A) Dark-eyed junco distribution in the southwestern United States and northern Mexico; dark grey highlights the wintering range derived from BirdLife International and the International Union for Conservation of Nature [95]. (B) Study sites within the UCSD campus (32°40'N, 117°10'W) and LM range (32°52'N, 116°25'W) are shown alongside land cover from Google Maps. Sites are separated by approximately 70 kilometers.

urban habituation minimizes or amplifies stressors. Stimulation of the hypothalamic–pituitary–adrenal axis can cause acute increases in plasma corticosterone (CORT) [48]; however, baseline levels are rapidly altered by capture [49]. However, elevated CORT stimulates an influx of heterophils from bone marrow to blood while distributing lymphocytes from blood to other tissue, increasing the ratio of heterophils to lymphocytes (i.e., HL ratios) [50]; these hematological shifts can remain unchanged during an acute stressor and only elevate hours to days after treatment [51]. Importantly, HL ratios shift in response to mild and chronic stressors [52]; for example, whereas Eurasian kestrel (*Falco tinnunculus*) nestlings elevated HL ratios and CORT in response to starvation, only HL ratios increased with less-severe stressors such as poor weather [53]. Elevated HL ratios from subtle, chronic stressors can remain elevated for long durations (e.g., a week or more) [53, 54]. We also estimated total white blood cell (WBC) counts to indicate investment in cellular defense. High total WBC counts can suggest more robust defenses or an inflammatory response to infection [22, 55]. Considering how HL ratios and total WBC counts both vary between urban resident and altitudinal migrant populations, alongside leukocyte composition more broadly (e.g., eosinophil counts can decline with stress but increase with parasitism, high monocyte counts can indicate infection [56, 57]), can inform whether hematological patterns indicate stressors, disease, or altered defense [51].

Because UCSD juncos appear to benefit reproductively from urban residency [40], we predicted that HL ratios would be lower in urban residents compared to birds from the altitudinal migrant population [58]. Lower HL ratios could also be expected in urban residents given that attenuated plasma CORT responses have been observed in UCSD juncos [47] and other urban resident birds [59]. If the greater abundance of food, water, and nesting resources in urban habitats allows juncos to invest more resources in both immunity and reproduction, we would also predict UCSD juncos display greater total WBC counts compared to LM birds [23, 24]. Alternatively, the additional stressors of urban residency (e.g., light pollution, crowding, disease) could have a greater influence on physiology than resource abundance or minimized energetics, which could result in urban residents showing higher total WBC counts (indicating infection) alongside elevated HL ratios (indicating infection or other stressors) than LM birds [33, 60].

2 Methods

2.1 Junco sampling

In 2006 and 2007, we used mistnets and Potter traps to sample juncos in the respective breeding seasons of the UCSD population (February–August) and LM population (May–July) [46, 47]. We fit each bird with an aluminum U.S. Fish & Wildlife Service leg band. We determined age class from wing plumage and secondarily from iris color [38]. Sex was determined by plumage coloration, wing chord length, and breeding morphology (i.e., cloacal protuberance or brood patch) [38]. We obtained ~100 μ L of blood by pricking the brachial vein with a sterile 26G needle, followed by collection with heparinized capillary tubes (hematocrit was obtained for all birds sampled in 2006). Blood smears were prepared on glass slides, fixed with methanol, and stained with Wright–Giemsa [61]. All birds were bled shortly after capture ($\bar{x}$ =17.18 minutes, SD =9.27) to minimize effects of capture stress on circulating leukocyte numbers [62]. All sampling protocols were approved by the Indiana University Institutional Animal Care and Use Committee (06-242) and conducted under scientific collecting permits issued by the U.S. Fish and Wildlife Service, the California Department of Fish and Game, and the U.S. Forest Service.

2.2 Leukocyte profiles

Smears were analyzed from a subset of all sampled birds (n =75; Fig. S1) by two individuals who received identical training, including a phase where both observers analyzed a subset of the same slides to validate correspondence in cell identification and enumeration. More samples were analyzed from 2006 (n =51) than 2007 (n =24), although sampling effort was consistent between LM (n =36) and UCSD (n =39). Greater variation in sampling date stems from the longer breeding season of the UCSD population [40]. For differential leukocytes, we recorded the percentage of heterophils, lymphocytes, monocytes, eosinophils, and basophils by counting at least 50 WBCs at 1000X magnification (oil immersion) [61]. By recording total leukocytes detected during this screening and the number of fields scored, we estimated the total WBCs per 10,000 red blood cells based on the approximation of 100 red blood cells per field. This method is common in ecological studies [22, 55, 63] but is less precise than hemocytometer-derived total WBC counts [64]. This semi-quantitative approach also assumes that the total

red blood cell count is constant across birds. To account for this variation, we multiplied the total WBC counts by hematocrit (i.e., adjusted total WBC count) [65]; however, hematocrit was only available for 2006 birds.

We also used blood smears to screen all individuals for the presence of intracellular and extracellular blood parasites [61]. We scanned each blood smear at 200X for the presence of microfilariae and haemosporidian parasites (e.g., *Plasmodium* spp. and *Haemoproteus* spp.).

2.3 Statistical analysis of leukocyte data

Differential WBC data are compositional; changes in the proportion of one leukocyte type correspond to changes in the other four cell types [66]. Because analyses of compositional data deal with logratio transformations [67], we treated zeros as values below the detection limit and replaced them with 50% of the bounds for detection (i.e., 0.5%) [68]. We next calculated HL ratios and $\log_{10}$ -transformed both this outcome and the total WBC counts; these two physiological measures were not strongly associated with one another ($\rho=0.11$, $p=0.33$).

As our data included recaptured juncos ($n=10$ individuals), we used generalized linear mixed models (GLMMs) with Gaussian errors and a random effect of band number to analyze HL ratios and total WBC counts. We mainly tested the hypothesis that hematological outcomes varied between the LM and UCSD population; however, we also adjusted for factors such as sex (e.g., leukocyte counts are often female-biased [69]), age (e.g., immune senescence [70]), and time (e.g., leukocytes can vary within seasons and across years [71]). We also considered interactions between population and each of these factors, because the effect of population on physiology could also be modified by individual or temporal variation. Because including all these predictors would dramatically overfit any one GLMM given our sample size ($n=75$), we used a model comparison approach [72]. We considered a base GLMM with population, sex, age, year, and Julian date alongside models that included each interaction between population and these individual and temporal predictors; this produced five *a priori* GLMMs. We used the *lme4* package in R to fit GLMMs with maximum likelihood, and we used *MuMIn* to compare models with Akaike information criterion corrected for small sample sizes (AICc) [73–75]. We considered models within two AICc to be competitive [72]. We then refit GLMMs with restricted maximum likelihood to quantify marginal and conditional R^2 (R^2_m and R^2_c) [76].

Variation in total red blood cell counts could introduce variation into our estimated total WBC count. Because

hematocrit data were only available for juncos sampled in 2006 ($n=51$), we repeated this model comparison approach with the $\log_{10}$ -transformed adjusted total WBC count and removed year as a predictor. To assess whether hematocrit itself varied between LM and UCSD juncos, we logit-transformed this response variable and fit our most parsimonious model.

We lastly used a multivariate approach to analyze leukocyte composition in relation to population. We used a permutational multivariate analysis of variance (PERMANOVA) with the *vegan* package to quantify the proportion of variance explained by population, sex, age, year, and Julian date [77]. The PERMANOVA used Euclidean distances on the isometric logratio (ILR)-transformed relative abundances of all leukocytes [78]. We visualized differences using a robust principal components analysis (PCA) with the *robCompositions* package [79, 80]. Such methods appropriately analyze compositional data (given dependencies among proportions) and handle outliers; however, results can still be vulnerable to inconsistencies in cell counts (e.g., inaccurate smear detection of monocytes [81]). To facilitate interpretation of the PERMANOVA, we also logit-transformed each leukocyte type, fit our parsimonious GLMM, and adjusted the p values.

3 Results

3.1 Analysis of HL ratios

Junco HL ratios varied from 0.02 to 2 ($\bar{x}=0.31$, $SD=0.29$), and a Grubbs test on the $\log_{10}$ -transformed values detected no outliers ($G=2.77$, $U=0.90$, $p=0.34$). The simplest model was the most parsimonious but had poor explanatory power ($w_i=0.41$, Table 1A). After adjusting for sex, age, and time, HL ratios were weakly but not significantly higher in UCSD juncos compared to LM juncos ($\chi^2=2.41$, $p=0.12$, $\beta=0.16$; Fig. 2A). Sex ($\chi^2=0.26$, $p=0.61$) and age ($\chi^2=0.09$, $p=0.76$) showed no associations with HL ratios, whereas HL ratios were weakly higher in 2007 ($\chi^2=2.69$, $p=0.10$) and increased across the breeding season ($\beta=0.004$, $t=1.92$, $p=0.05$; Fig. 2B).

3.2 Analysis of total WBC counts

Juncos at our southern California sites ranged from 0.12 to 1.85 WBCs per field of view, and estimated total WBCs were more variable than HL ratios ($\bar{x}=0.67$, $SD=0.37$). We also detected no outliers ($G=2.74$, $U=0.90$, $p=0.38$). The simplest

Figure 2. Modeled relationship between HL ratios, junco population (A), and time (B). Upper and lower bounds of the shaded regions display 95% confidence intervals from the GLMM, with thick lines showing mean prediction. Points indicate are jittered to reduce overlap (A) and are colored by population. The HL ratio axis is displayed on a $\log_{10}$ -transformed scale.

Table 1. Comparison of GLMMs predicting $\log_{10}$ -transformed junco HL ratios (A), estimated total WBC counts (B), adjusted total WBC counts (C), and hematocrit (logit-transformed) as a function of population, sex, age, year, Julian date, and interactions. Models are ranked by ΔAICc with the number of coefficients (k), Akaike weights (w_i), and marginal and conditional R^2 .

Model structure	k	ΔAICc	w_i	R^2_m	R^2_c
<i>(A) HL ratios</i>					
~ population + sex + age + year + date + (1 band)	6	0.00	0.41	0.07	0.58
~ population * year + sex + age + date + (1 band)	7	1.14	0.23	0.09	0.52
~ population * date + sex + age + year + (1 band)	7	2.48	0.12	0.07	0.57
~ population * sex + age + year + date + (1 band)	7	2.50	0.12	0.07	0.59
~ population * age + sex + year + date + (1 band)	7	2.60	0.11	0.07	0.58
<i>(B) Estimated total WBC count</i>					
~ population + sex + age + year + date + (1 band)	6	0.00	0.38	0.21	0.50
~ population * year + sex + age + date + (1 band)	7	1.11	0.22	0.22	0.49
~ population * sex + age + year + date + (1 band)	7	1.81	0.15	0.21	0.51
~ population * age + sex + year + date + (1 band)	7	1.92	0.14	0.22	0.51
~ population * date + sex + age + year + (1 band)	7	2.48	0.11	0.21	0.50
<i>(C) Adjusted total WBC count</i>					
~ population + age + sex + date + (1 band)	5	0.00	0.38	0.15	0.70
~ population * age + sex + date + (1 band)	6	0.70	0.27	0.17	0.79
~ population * date + sex + age + (1 band)	6	1.51	0.18	0.16	0.72
~ population * sex + age + date + (1 band)	6	1.66	0.17	0.17	0.69
<i>(D) Hematocrit</i>					
~ population + age + sex + date + (1 band)	5	NA	NA	0.24	0.24

model was again the most parsimonious ($w_i=0.38$), but here the fixed effects explained 21% of the hematological variation (Table 1B). Estimated total WBC counts varied by population ($\chi^2=5.73$, $p=0.02$) and sex ($\chi^2=4.96$, $p=0.03$) and were higher in urban residents ($\beta=0.15$, $t=2.40$) and in females ($\beta=0.13$, $t=2.23$; Fig. 3A).

When we adjusted total WBC counts by hematocrit, the fixed effects from the simplest model ($w_i=0.38$) explained 15% of hematological variation (Table 1C). However, the adjusted total WBC counts were no longer associated with population ($\chi^2=2.29$, $p=0.13$), although the effect direction was similar ($\beta=0.09$; Fig. 3B) and the prior sex difference remained ($\beta=0.12$, $p=0.04$). When analyzing

hematocrit itself, values were dependent upon population ($\chi^2=11.97$, $p<0.001$). Junco hematocrit was significantly lower in UCSD than in LM ($\beta=-0.16$; Fig. S2).

3.3 Compositional leukocyte profiles

Juncos from both populations were dominated by lymphocytes and heterophils (Table 2). The first two PCs explained 88% of variance in leukocyte composition, with the former indicating negative eosinophil loadings and the latter indicating negative monocyte loadings (Table S1).

The PERMANOVA showed that leukocyte composition

Figure 3. Modeled relationships between estimated total WBC counts, junco population, and sex (A), alongside results after adjusting for hematocrit (B, 2006 only). Upper and lower bounds of the shaded regions display 95% confidence intervals from the GLMMs, with thick lines showing mean prediction. Points are jittered to reduce overlap and are colored by population.

Table 2. Means and standard deviations (SD) for each leukocyte type per junco population. The χ^2 statistics and p values (corrected for multiple comparisons) are from a GLMM fit to the logit-transformed relative abundances of each cell after adjusting for sex, age, year, and Julian date.

Leukocyte	LM (n=36)		UCSD (n=39)		GLMM	
	Mean	SD	Mean	SD	χ^2	p
Heterophil	0.175	0.110	0.199	0.103	3.61	0.14
Lymphocyte	0.704	0.149	0.717	0.112	0.16	0.86
Monocyte	0.075	0.057	0.043	0.030	14.03	0.001
Eosinophil	0.040	0.030	0.035	0.026	0.02	0.88
Basophil	0.005	<0.001	0.005	<0.001	1.40	0.39

differed only by population ($F_{1,68}=5.36$, $p=0.001$, $R^2=0.07$; Table S2). Our post-hoc analysis showed this difference was driven by UCSD juncos having relatively fewer monocytes than LM juncos (Fig. 4, Table 2).

3.4 Blood parasites

Across our 75 individual juncos, we detected only *Haemoproteus* spp. in blood smears. This parasite was present in only two individuals, one from each population (LM: 1/36; UCSD: 1/39). We note that microscopy is not sensitive enough to conclude all other birds were uninfected [82].

4 Discussion

As many animals are decreasing their migratory propensity or are abandoning migration in response to urbanization [11–15], understanding how urban residency is associated with physiology is necessary to predict consequences for wildlife health, conservation, and pathogen spillover [34–36]. By focusing on the recent colonization of the UCSD campus by migratory juncos and comparing UCSD birds to juncos from a nearby altitudinal migrant population (LM), we show that urban residents only weakly differ in their HL ratios. However, a multivariate analysis suggested that monocyte counts may be lower in urban residents and that UCSD birds may have higher estimated total WBC counts. Urban residents and altitudinal migrants thus do not differ in hematological proxies of stress, and the observed hematological differences could instead indicate urban variation in either parasite exposure or immunological investment.

One of our predictions was that urban resident juncos could have lower HL ratios than juncos from the LM population, as the adoption of sedentary behavior could reduce energy expenditures associated with seasonal altitudinal migrations [16, 17]. The abundant food, water, and nesting resources in the UCSD environment could amplify this effect through minimizing additional energy demands associated with foraging and nest building [13, 22]. However, HL ratios varied only weakly between populations and tended to be slightly higher in the UCSD population. This pattern agrees with prior work in which female juncos from these populations also did not differ in baseline plasma CORT [47]. Because HL ratios can detect the long-term impact of more mild stressors [52, 53], our results suggest migrants that become resident are not more stressed and have similar energetic demands,

Figure 4. Multivariate effects of population on junco leukocyte composition (H=heterophils, L=lymphocytes, M=monocytes, E=eosinophils, B=basophils). The biplot shows the first two back-transformed centered logratio (CLR) coordinates from a robust PCA of ILR-transformed leukocyte proportions. Points are colored by population with 95% data ellipses.

at least during their breeding season. One parsimonious explanation for this pattern could be that the relatively short altitudinal migrations of LM juncos do not carry sufficient energetic costs to alter HL ratios. Comparisons of leukocyte profiles between UCSD juncos and mountain populations across their broad range in California (e.g., [41, 42]) would be informative, because overwintering migrants in UCSD are likely from breeding populations across a wide latitudinal gradient [43]. We also sampled urban residents and juncos from the altitudinal migrant population during their breeding season, when animals are also faced with other reproductive stressors. Following the need for more studies across the annual cycle more broadly [83], sampling urban residents alongside migrants closer to the migration itself (e.g., prior to breeding in the high-elevation forest habitats and recently after arrival in the UCSD habitat) would help quantify the physiological costs of bird migration.

Although we observed no difference in HL ratios between LM and UCSD juncos, urban residents had higher estimated total WBC counts. We caution that the relationship between population and total WBC counts was weaker when we accounted for hematocrit; however, hematocrit data were only available from one year, and thus it is unclear whether this weaker association stems from temporal or hematocrit variation. Hematocrit was higher in LM juncos likely from increased metabolic

requirements of altitudinal migration or high elevation [84, 85]. As total leukocyte counts show inconsistent relationships with stressors [51], higher estimated total WBC counts of urban residents instead suggests stronger constitutive immunity or a greater likelihood of acute infections [51]. The former could arise if urban residents, through having greater energy reserves from more access to food, water, and nesting resources, could maintain a higher leukocyte reserve in preparation for fighting infections [22]. Alternatively, increased risk of parasite exposure in urban sedentary populations offers an alternative explanation. Shifts from migratory to resident behavior have been associated with greater parasite exposure owing to the loss of ecological mechanisms that reduce transmission [86, 87]. In the junco system, a resident subspecies in the eastern United States (*J. h. carolinensis*) maintains higher prevalence of haemosporidians than migrants (*J. h. hyemalis*) during seasonal sympatry [88]. However, our microscopy screening for haemosporidians was inconclusive; only *Haemoproteus* was detected at low prevalence and was not sufficiently sensitive to detect low-intensity infections [82]. We instead used a multivariate analysis of leukocyte composition to provide additional insights [51]. Our PCA loaded strongly by monocytes, for which elevated relative counts were higher in LM juncos (Table 2) and could indicate high bacterial infection risks and inflammation [51, 61]. However, we note that population alone only explained a relatively small fraction of variance in leukocyte composition. We note that avian pox and ectoparasites are more prevalent in UCSD juncos [89]. Yet additional data on parasite prevalence or even diversity, such as that offered by advances in metagenomic approaches [90, 91], would be necessary to assess whether such immunological variation between populations stems from parasite risk or urban resources.

Higher estimated total leukocyte counts of urban residents could signal the reduced energetic demands of non-migratory individuals and improved resources in urban habitats [22]. However, other metrics of constitutive immunity, such as microbicidal or haptoglobin activity, could better test how urban residency influences host defense [24]. Such work warrants further investigation, because potentially stronger immunity of urban residents could carry important implications for infectious disease dynamics. Prior mathematical models have examined the consequences of urban residency when migrants and residents are sympatric in the breeding season [92]. Further consideration of how susceptibility varies between urban residents and migrants during the breeding season, either in sympatry or allopatry, may further alter disease dynamics. For example, immunological benefits in

urban residents could reduce or prevent transmission by homogenizing resource allocation to resistance [93, 94]. However, if stronger immunity of urban residents translates into greater tolerance of infection, adoption of sedentary behavior could increase prevalence and make urban residents a source for infecting vectors [92].

As an increasing number of traditionally migratory species reduce migratory propensity or fully adopt sedentary behaviors in urbanized habitats, understanding whether such novel environments represent a net stressor or benefit to hosts is important to ultimately predict how infectious diseases and the health of migratory populations will respond to environmental change [12, 13, 15, 92]. By using leukocyte profiles to assess physiological differences between recently established urban residents and a nearby altitudinal migrant population, we show that these two populations do not differ in their HL ratios but may differ in their estimated total WBC counts. Future work studying a broader range of urban resident and mountain populations would help capture a wider gradient of migratory distances, and sampling such populations more broadly across their annual cycles (including but not limited to closer to the timing of migration) would help quantify the physiological consequences of long-distance movements. Further assessments of parasite diversity and immunology will also be necessary to disentangle if urban residency is associated with greater exposure or stronger defense. Incorporating potential immunological effects of urban habitats into new modeling frameworks could have significant effects on infectious disease dynamics not only within residents but also for seasonally sympatric migrants.

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References

- [1] Dingle H., Migration: the biology of life on the move, Oxford University Press, USA, 2014
- [2] Bauer S., Hoyer B.J., Migratory animals couple biodiversity and ecosystem functioning worldwide, *Science*, 2014, 344, 1242552
- [3] Bagstad K.J., Semmens D.J., Diffendorfer J.E., Mattsson B.J., Dubovsky J., Thogmartin W.E., *et al.*, Ecosystem service flows from a migratory species: Spatial subsidies of the northern pintail, *Ambio*, 2019, 48, 61–73
- [4] Wilcove D.S., Wikelski M., Going, going, gone: is animal migration disappearing, *PLoS Biol.*, 2008, 6, e188
- [5] Tucker M.A., Böhning-Gaese K., Fagan W.F., Fryxell J.M., Moorter B.V., Alberts S.C., *et al.*, Moving in the Anthropocene: Global reductions in terrestrial mammalian movements, *Science*, 2018, 359, 466–469
- [6] Shaw A.K., Drivers of animal migration and implications in changing environments, *Evol. Ecol.*, 2016, 30, 991–1007
- [7] Bairlein F., Migratory birds under threat, *Science*, 2016, 354, 547–548
- [8] Fiedler W., Recent changes in migratory behaviour of birds: a compilation of field observations and ringing data, In: *Avian Migration*, Springer, 2003, 21–38
- [9] Gilbert N.I., Correia R.A., Silva J.P., Pacheco C., Catry I., Atkinson P.W., *et al.*, Are white storks addicted to junk food? Impacts of landfill use on the movement and behaviour of resident white storks (*Ciconia ciconia*) from a partially migratory population, *Mov. Ecol.*, 2016, 4, 1
- [10] Tortosa F.S., Manez M., Barcel M., Wintering white storks (*Ciconia ciconia*) in South West Spain in the years 1991 and 1992, *Vogelwarte*, 1995, 38, 41–45
- [11] Partecke J., Gwinner E., Increased sedentariness in European Blackbirds following urbanization: a consequence of local adaptation?, *Ecology*, 2007, 88, 882–890
- [12] Plummer K.E., Siriwardena G.M., Conway G.J., Risely K., Toms M.P., Is supplementary feeding in gardens a driver of evolutionary change in a migratory bird species?, *Glob. Change Biol.*, 2015, 21, 4353–4363
- [13] Satterfield D.A., Marra P.P., Sillett T.S., Altizer S.M., Responses of migratory species and their pathogens to supplemental feeding, *Philos. Trans. R. Soc. B Biol. Sci.*, 2018
- [14] Greig E.I., Wood E.M., Bonter D.N., Winter range expansion of a hummingbird is associated with urbanization and supplementary feeding, *Proc R Soc B*, 2017, 284, 20170256
- [15] Kessler M.K., Becker D.J., Peel A.J., Justice N.V., Lunn T., Crowley D.E., *et al.*, Changing resource landscapes and spillover of henipaviruses, *Ann. N. Y. Acad. Sci.*, 2018, 1429, 78–99
- [16] Wikelski M., Tarlow E.M., Raim A., Diehl R.H., Larkin R.P., Visser G.H., Costs of migration in free-flying songbirds, *Nature*, 2003, 423, 704
- [17] McWilliams S.R., Guglielmo C., Pierce B., Klaassen M., Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective, *J. Avian Biol.*, 2004, 35, 377–393
- [18] Owen J.C., Moore F.R., Swainson's thrushes in migratory disposition exhibit reduced immune function, *J. Ethol.*, 2008, 26, 383–388
- [19] Eikenaar C., Hegemann A., Migratory common blackbirds have lower innate immune function during autumn migration than resident conspecifics, *Biol. Lett.*, 2016, 12, 20160078
- [20] Fritzsche McKay A., Hoyer B.J., Are Migratory Animals Superspreaders of Infection?, *Integr. Comp. Biol.*, 2016, 56, 260–267
- [21] Oro D., Genovart M., Tavecchia G., Fowler M.S., Martínez-Abraín A., Ecological and evolutionary implications of food subsidies from humans, *Ecol. Lett.*, 2013, 16, 1501–1514
- [22] French S.S., Fokidis H.B., Moore M.C., Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban–rural gradient, *J. Comp. Physiol. B*, 2008, 178, 997–1005
- [23] Van Noordwijk A.J., de Jong G., Acquisition and allocation of resources: their influence on variation in life history tactics, *Am. Nat.*, 1986, 128, 137–142
- [24] Strandin T., Babayan S.A., Forbes K.M., Reviewing the effects of food provisioning on wildlife immunity, *Phil Trans R Soc B*, 2018, 373, 20170088
- [25] Rodewald A.D., Shustack D.P., Consumer resource matching in urbanizing landscapes: are synanthropic species over-matching?, *Ecology*, 2008, 89, 515–521
- [26] Murray M.H., Becker D.J., Hall R.J., Hernandez S.M., Wildlife health and supplemental feeding: A review and management recommendations, *Biol. Conserv.*, 2016, 204, 163–174
- [27] Fokidis H.B., Greiner E.C., Deviche P., Interspecific variation in avian blood parasites and haematology associated with urbanization in a desert habitat, *J. Avian Biol.*, 2008, 39, 300–310
- [28] Bradley C.A., Altizer S., Urbanization and the ecology of wildlife diseases, *Trends Ecol. Evol.*, 2007, 22, 95–102
- [29] Evans K.L., Gaston K.J., Sharp S.P., McGowan A., Simeoni M., Hatchwell B.J., Effects of urbanisation on disease prevalence and age structure in blackbird *Turdus merula* populations, *Oikos*, 2009, 118, 774–782
- [30] Davies S., Haddad N., Ouyang J.Q., Stressful city sounds: glucocorticoid responses to experimental traffic noise are environmentally dependent, *Biol. Lett.*, 2017, 13, 20170276
- [31] Ouyang J.Q., de Jong M., Hau M., Visser M.E., van Grunsven R.H., Spoelstra K., Stressful colours: corticosterone concentrations in a free-living songbird vary with the spectral composition of experimental illumination, *Biol. Lett.*, 2015, 11, 20150517
- [32] Series L.E.K., Lea A.J., Epeldegui M., Armenta T.C., Moriarty J., VandeWoude S., *et al.*, Urbanization and anticoagulant poisons promote immune dysfunction in bobcats, *Proc R Soc B*, 2018, 285, 20172533
- [33] Murray M.H., Sánchez C.A., Becker D.J., Byers K.A., Worsley-Tonks K., Craft M.E., City sicker? A meta-analysis of wildlife health and urbanization, *Front. Ecol. Environ.*, 2019
- [34] Hassell J.M., Begon M., Ward M.J., Fèvre E.M., Urbanization and disease emergence: Dynamics at the wildlife–livestock–human interface, *Trends Ecol. Evol.*, 2017, 32, 55–67

- [35] Becker D.J., Streicker D.G., Altizer S., Linking anthropogenic resources to wildlife–pathogen dynamics: a review and meta-analysis, *Ecol. Lett.*, 2015, 18, 483–495
- [36] Ouyang J.Q., Isaksson C., Schmidt C., Hutton P., Bonier F., Dominoni D., A New Framework for Urban Ecology: An Integration of Proximate and Ultimate Responses to Anthropogenic Change, *Integr. Comp. Biol.*, 2018, 58, 915–928
- [37] Grimm N.B., Faeth S.H., Golubiewski N.E., Redman C.L., Wu J., Bai X., *et al.*, Global change and the ecology of cities, *Science*, 2008, 319, 756–760
- [38] Nolan V., Ketterson E.D., Cristol D.A., Rogers C.M., Clotfelter E.D., Titus R.C., *et al.*, Dark-eyed junco (*Junco hyemalis*), American Ornithologists' Union, 2002
- [39] Unitt P., Klovstad A.E., San Diego County Bird Atlas, San Diego Natural History Museum, 2004
- [40] Yeh P.J., Price T.D., Adaptive phenotypic plasticity and the successful colonization of a novel environment, *Am. Nat.*, 2004, 164, 531–542
- [41] Yeh P.J., Rapid Evolution of a Sexually Selected Trait Following Population Establishment in a Novel Habitat, *Evolution*, 2004, 58, 166–174
- [42] Rasner C.A., Yeh P., Eggert L.S., Hunt K.E., Woodruff D.S., Price T.D., Genetic and morphological evolution following a founder event in the dark-eyed junco, *Junco hyemalis thurberi*, *Mol. Ecol.*, 2004, 13, 671–681
- [43] Fudickar A.M., Greives T.J., Abolins-Abols M., Atwell J.W., Meddle S.L., Friis G., *et al.*, Mechanisms Associated with an Advance in the Timing of Seasonal Reproduction in an Urban Songbird, *Front. Ecol. Evol.*, 2017, 5
- [44] Yeh P.J., Hauber M.E., Price T.D., Alternative nesting behaviours following colonisation of a novel environment by a passerine bird, *Oikos*, 2007, 116, 1473–1480
- [45] Peterson M.P., Abolins-Abols M., Atwell J.W., Rice R.J., Milá B., Ketterson E.D., Variation in candidate genes CLOCK and ADCYAP1 does not consistently predict differences in migratory behavior in the songbird genus Junco, *F1000Research*, 2013, 2
- [46] Atwell J.W., Cardoso G.C., Whittaker D.J., Price T.D., Ketterson E.D., Hormonal, behavioral, and life-history traits exhibit correlated shifts in relation to population establishment in a novel environment, *Am. Nat.*, 2014, 184, E147–E160
- [47] Atwell J.W., Cardoso G.C., Whittaker D.J., Campbell-Nelson S., Robertson K.W., Ketterson E.D., Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated evolutionary adaptation, *Behav. Ecol.*, 2012, 23, 960–969
- [48] Sapolsky R.M., Romero L.M., Munck A.U., How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions, *Endocr. Rev.*, 2000, 21, 55–89
- [49] Romero L.M., Reed J.M., Collecting baseline corticosterone samples in the field: is under 3 min good enough?, *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.*, 2005, 140, 73–79
- [50] Gross W.B., Siegel H.S., Evaluation of the Heterophil/Lymphocyte Ratio as a Measure of Stress in Chickens, *Avian Dis.*, 1983, 27, 972–979
- [51] Davis A.K., Maney D.L., Maerz J.C., The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists, *Funct. Ecol.*, 2008, 22, 760–772
- [52] Davis A.K., Maney D.L., The use of glucocorticoid hormones or leukocyte profiles to measure stress in vertebrates: What's the difference?, *Methods Ecol. Evol.*, 2018, 9, 1556–1568
- [53] Müller C., Jenni-Eiermann S., Jenni L., Heterophils/Lymphocytes-ratio and circulating corticosterone do not indicate the same stress imposed on Eurasian kestrel nestlings, *Funct. Ecol.*, 2011, 25, 566–576
- [54] Goessling J.M., Kennedy H., Mendonça M.T., Wilson A.E., A meta-analysis of plasma corticosterone and heterophil: lymphocyte ratios—is there conservation of physiological stress responses over time?, *Funct. Ecol.*, 2015, 29, 1189–1196
- [55] Ots I., Murumägi A., Hõrak P., Haematological health state indices of reproducing Great Tits: methodology and sources of natural variation, *Funct. Ecol.*, 1998, 12, 700–707
- [56] Čirule D., Krama T., Vrublevska J., Rantala M.J., Krams I., A rapid effect of handling on counts of white blood cells in a wintering passerine bird: a more practical measure of stress?, *J. Ornithol.*, 2012, 153, 161–166
- [57] Davis A.K., Cook K.C., Altizer S., Leukocyte Profiles in Wild House Finches with and without Mycoplasmal Conjunctivitis, a Recently Emerged Bacterial Disease, *EcoHealth*, 2004, 1, 362–373
- [58] Bonier F., Hormones in the city: endocrine ecology of urban birds, *Horm. Behav.*, 2012, 61, 763–772
- [59] Partecke J., Schwabl I., Gwinner E., Stress and the city: urbanization and its effects on the stress physiology in European blackbirds, *Ecology*, 2006, 87, 1945–1952
- [60] Ruiz G., Rosenmann M., Novoa F.F., Sabat P., Hematological parameters and stress index in rufous-collared sparrows dwelling in urban environments, *The Condor*, 2002, 104, 162–166
- [61] Campbell T.W., Avian hematology and cytology., Iowa State University Press, 1995
- [62] Davis A.K., Effect of handling time and repeated sampling on avian white blood cell counts, *J. Field Ornithol.*, 2005, 76, 334–338
- [63] Cornelius E.A., Davis A.K., Altizer S.A., How important are hemoparasites to migratory songbirds? Evaluating physiological measures and infection status in three neotropical migrants during stopover, *Physiol. Biochem. Zool.*, 2014, 87, 719–728
- [64] Walberg J., White blood cell counting techniques in birds, *Semin. Avian Exot. Pet Med.*, 2001, 10, 72–76
- [65] Schultz E.M., Hahn T.P., Klasing K.C., Photoperiod but not food restriction modulates innate immunity in an opportunistic breeder, *Loxia curvirostra*, *J. Exp. Biol.*, 2017, 220, 722–730
- [66] Sandmeier F.C., Leonard K.L., Tracy C.R., Drake K.K., Esque T.E., Nussear K., *et al.*, Tools to understand seasonality in health: quantification of microbe loads and analyses of compositional ecoimmunological data reveal complex patterns in tortoise populations, *Can. J. Zool.*, 2019
- [67] Aitchison J., The statistical analysis of compositional data, *J. R. Stat. Soc. Ser. B Methodol.*, 1982, 44, 139–160
- [68] Martín-Fernández J.A., Barceló-Vidal C., Pawłowsky-Glahn V., Dealing with zeros and missing values in compositional data sets using nonparametric imputation, *Math. Geol.*, 2003, 35, 253–278
- [69] Kelly C.D., Stoeckl A.M., Nunn C., Smyth K.N., Prokop Z.M., Sexual dimorphism in immunity across animals: a meta-analysis, *Ecol. Lett.*, 2018, 21, 1885–1894

- [70] Nussey D.H., Watt K., Pilkington J.G., Zamoyska R., McNeilly T.N., Age-related variation in immunity in a wild mammal population, *Aging Cell*, 2012, 11, 178–180
- [71] Hegemann A., Matson K.D., Both C., Tieleman B.I., Immune function in a free-living bird varies over the annual cycle, but seasonal patterns differ between years, *Oecologia*, 2012, 170, 605–618
- [72] Burnham K.P., Anderson D.R., Model selection and multimodel inference: a practical information-theoretic approach, Springer Science & Business Media, 2002
- [73] R Core Team, R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria., 2013
- [74] Bates D., Mächler M., Bolker B., Walker S., Fitting Linear Mixed-Effects Models Using lme4, *J. Stat. Softw.*, 2015, 67, 1–48
- [75] Barton K., MuMIn: Multi-model inference. R package version 1.0.0, Vienna Austria R Found. Stat. Comput. See [HttpCRAN R-Proj. Org](http://CRAN.R-project.org/package=MuMIn) package MuMIn, 2011
- [76] Nakagawa S., Schielzeth H., A general and simple method for obtaining R² from generalized linear mixed-effects models, *Methods Ecol. Evol.*, 2013, 4, 133–142
- [77] Dixon P., VEGAN, a package of R functions for community ecology, *J. Veg. Sci.*, 2003, 14, 927–930
- [78] Egozcue J.J., Pawłowsky-Glahn V., Mateu-Figueras G., Barcelo-Vidal C., Isometric logratio transformations for compositional data analysis, *Math. Geol.*, 2003, 35, 279–300
- [79] Templ M., Hron K., Filzmoser P., robCompositions: an R-package for robust statistical analysis of compositional data, 2011
- [80] Filzmoser P., Hron K., Outlier detection for compositional data using robust methods, *Math. Geosci.*, 2008, 40, 233–248
- [81] Bílková B., Bainová Z., Janda J., Zita L., Vinkler M., Different breeds, different blood: Cytometric analysis of whole blood cellular composition in chicken breeds, *Vet. Immunol. Immunopathol.*, 2017, 188, 71–77
- [82] Fallon S.M., Ricklefs R.E., Swanson B.L., Bermingham E., Detecting avian malaria: an improved polymerase chain reaction diagnostic, *J. Parasitol.*, 2003, 1044–1047
- [83] Marra P.P., Cohen E.B., Loss S.R., Rutter J.E., Tonra C.M., A call for full annual cycle research in animal ecology, *Biol. Lett.*, 2015, 11, 20150552
- [84] Krause J.S., Németh Z., Pérez J.H., Chmura H.E., Ramenofsky M., Wingfield J.C., Annual Hematocrit Profiles in Two Subspecies of White-Crowned Sparrow: A Migrant and a Resident Comparison, *Physiol. Biochem. Zool.*, 2016, 89, 51–60
- [85] Fair J., Whitaker S., Pearson B., Sources of variation in haematocrit in birds, *Ibis*, 2007, 149, 535–552
- [86] Satterfield D.A., Maerz J.C., Altizer S., Loss of migratory behaviour increases infection risk for a butterfly host, *Proc. R. Soc. B Biol. Sci.*, 2015, 282, 20141734
- [87] Hall R.J., Altizer S., Bartel R.A., Greater migratory propensity in hosts lowers pathogen transmission and impacts, *J. Anim. Ecol.*, 2014, 83, 1068–1077
- [88] Slowinski S.P., Fudickar A.M., Hughes A.M., Mettler R.D., Gorbatenko O.V., Spellman G.M., *et al.*, Sedentary songbirds maintain higher prevalence of haemosporidian parasite infections than migratory conspecifics during seasonal sympatry, *PLOS ONE*, 2018, 13, e0201563
- [89] Atwell J.W., Responses of associated hormonal, behavioral, and morphological traits following a songbird's colonization of a novel environment, Indiana University, 2011
- [90] Razzauti M., Galan M., Bernard M., Maman S., Klopp C., Charbonnel N., *et al.*, A Comparison between Transcriptome Sequencing and 16S Metagenomics for Detection of Bacterial Pathogens in Wildlife, *PLoS Negl Trop Dis*, 2015, 9, e0003929
- [91] Bergner L.M., Orton R.J., Filipe A. da S., Shaw A.E., Becker D.J., Tello C., *et al.*, Using noninvasive metagenomics to characterize viral communities from wildlife, *Mol. Ecol. Resour.*, 2019, 19, 128–143
- [92] Brown L.M., Hall R.J., Consequences of resource supplementation for disease risk in a partially migratory population, *Phil Trans R Soc B*, 2018, 373, 20170095
- [93] Becker D.J., Hall R.J., Too much of a good thing: resource provisioning alters infectious disease dynamics in wildlife, *Biol. Lett.*, 2014, 10, 20140309
- [94] Hall R.J., Modeling the effects of resource-driven immune defense on parasite transmission in heterogeneous host populations, *Integr. Comp. Biol.*, 2019
- [95] BirdLife International, *Junco hyemalis*, IUCN Red List Threat. Species, 2016