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LEUKOCYTE PROFILE AND BODY CONDITION OF THE HOUSE FINCH (*HAEMORHOUS MEXICANUS*) IN TWO SITES WITH DIFFERENT LEVELS OF URBANIZATION IN CENTRAL MEXICO

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Abstract · Urban birds are exposed to many potentially detrimental factors including human disturbance, noise, changes in predator communities, exposure to toxins, etc., which may influence their health and reproductive success. Some physiological indices provide immediate measures to evaluate the effects of human-induced disturbances on birds and may offer information on the ability of populations to adapt to these disturbances. In this study we compared leukocyte profile (cellular immunity) and the H/L ratio (heterophils/lymphocytes) in House Finches (*Haemorhous mexicanus*) captured at two sites with different levels of urbanization in central Mexico. A total of 73 individuals were captured, 41 at the highly urbanized site and 32 at the site with lower urbanization. In general, we did not find differences in the H/L ratios, although some leukocyte parameters differed between sites. Birds captured at the site with higher urbanization had higher percentages of lymphocytes and eosinophils, which may be a response to parasitic illness. The proportions of basophiles were higher in individuals captured at the site with lower urbanization, which suggests physiological stress and disease. Even though only two sites were compared in this study, our results suggest an effect of the level of urbanization, and more studies are required that include various sites and that represent different urbanization conditions.

Resumen · Perfil leucocitario y condición corporal del Gorrión Mexicano (*Haemorhous mexicanus*) en dos sitios con diferente nivel de urbanización en el centro de México
Las aves urbanas están expuestas a factores potencialmente perjudiciales incluyendo la perturbación humana, el ruido, cambios en la comunidad de depredadores, el incremento en la depredación, la exposición a toxinas, entre otros, los cuales influyen en su salud y éxito reproductivo. Ciertos índices fisiológicos proveen medidas inmediatas para evaluar los efectos de la perturbación humana en las aves y pueden ofrecer información de las habilidades de las poblaciones para adaptarse a dichas perturbaciones. En este estudio comparamos el perfil leucocitario (inmunidad celular) y la proporción H/L (heterófilos/linfocitos) en el Gorrión Mexicano (*Haemorhous mexicanus*) en dos sitios con diferente nivel de urbanización en el centro de México. Un total de 73 individuos fueron capturados, 41 en el sitio más urbanizado y 32 en el sitio menos urbanizado. En general, no encontramos diferencias en el índice H/L, sin embargo algunos parámetros leucocitarios difirieron entre sitios. Las aves capturadas en el sitio más urbanizado tuvieron mayor porcentaje de linfocitos y eosinófilos, lo cual puede estar indicando una respuesta a enfermedades parasitarias. El porcentaje de basófilos fue mayor en individuos capturados en el sitio menos urbanizado, lo que sugeriría estrés fisiológico o presencia de enfermedades. A pesar de que sólo fueron comparados dos sitios, los resultados sugieren un efecto del nivel de urbanización, pero se requieren trabajos con un mayor número de réplicas o sitios que representen diferentes condiciones de urbanización.

Key words: *Haemorhous mexicanus* · H/L ratio · Leukocyte profile · Immune function · Urbanization level · Mexico

INTRODUCTION

Most environments have been altered by human activities and organisms living in these areas must have the ability to cope with the new conditions in order to survive (Sih et al. 2011). Urbanization has been identified as

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one of the major threats to global biodiversity and the level of urbanization in an area is an important factor that determines which species can colonize, survive and thrive in cities (Chace & Walsh 2006). Ultimately those populations that can use the urban environment or adapt to the challenges will flourish, and those that cannot, will decline or disappear (Shanahan et al. 2014). In particular, urban birds are exposed to many challenges that result from habitat destruction, degradation and an increase in edge effects. For example, birds nesting in cities may experience higher rates of brood parasitism (Chace et al. 2003). Also, the presence of noise (Slabbekoorn & Peet 2003), artificial lighting (Longcore & Rich 2004), direct human disturbance (Fernandez-Juricic et al. 2001) and exposure to toxins (Eens et al. 1999) may alter behavior and survival. These factors might influence the health and reproductive success of urban bird populations (Drake 2003, Liu & Stiling 2006, Fokidis et al. 2008). However, urban areas can also be beneficial for some birds, for example urban centers may provide rich foraging grounds, except at the highest intensities of urbanization (Chace & Walsh 2006). Although natural food availability might be significantly reduced in urban areas due to the loss of native vegetation, many resources are commonly provided by people, for example introduced plants or direct bird feeding (Chace & Walsh 2006, Davies et al. 2009, Thompson et al. 2003; see Shanahan et al. 2014).

Some physiological indices provide immediate measures of the effects of human-induced disturbances on birds and, potentially, may offer information on the ability of populations to adapt to these disturbances (Lucas et al. 2006). For example, the cellular component of the immune system can be assessed using the relative abundance of leukocytes (Roitt et al. 2001), and its study can provide useful information on the health status of wild animals (Davis 2005, Salvante 2006). Specifically, the relation heterophils/lymphocytes (H/L ratio) has been used as a reliable stress indicator in birds. When a bird is stressed, an increase in the number of heterophils and a decrease in the number of lymphocytes are usually observed (Gross & Siegel 1983, Maxwell 1993, Ots et al. 1998). This ratio is positively related to the magnitude of the stressor and to the circulating levels of glucocorticoids, and there is evidence that the H/L ratio in birds is influenced by diseases and infections (Davis et al. 2008). Poultry researchers were the first to use the H/L ratio (Gross & Siegel 1983) and laid the basis for using leukocyte parameters to infer physiological stress in other avian species (Davis et al. 2008). Lately, ornithologists have used this information and have quantified H/L ratios in wild birds across different habitats (reviewed by Davis et al. 2008). However, available information on the range of variation of this ratio is scarce for wild populations, especially for passerines (Norte et al. 2009a).

It is known that different levels of urbanization influence the immune function of individuals (Ruiz et al. 2002, Fokidis et al. 2008). For instance, exposure to city-associated challenges may negatively impact immune function, probably through an increase in circulating glucocorticoids that are released in response to stress (Partecke et al. 2006, Bonier et al. 2007) or through exposure to environmental toxins (Eeva et al. 2005). For example, Ruiz *et al.* (2002) found that urban individuals of the Rufous-collared Sparrow (*Zonotrichia capensis*) have lower values of body weight and lymphocytes, as well as higher levels of heterophils than rural ones. However, the response of the immune function varies depending on age, sex, foraging habits, and habitat (Allander & Bennett 1994, Dávila & Campo 2005, Sol et al. 2003, Perez et al. 2009). For example, in urban forest individuals of Great Tits (*Parus major*) the H/L ratio increased with age for females and males (Norte et al. 2009).

In this paper, we compare aspects of the immune function (using a leukocyte profile that included the percentages of lymphocytes, monocytes, heterophils, eosinophils, and basophils, as well as H/L ratios) of the House Finch (*Haemorhous mexicanus*), in two areas with different levels of urbanization in central Mexico. We also analyze how it varies according to sex (males and females) and age (juveniles and adults). We expected that the leukocyte parameters, H/L ratios, and body mass would differ between levels of urbanization, and between different sexes and ages. We also expected that all individuals, including females, males, juveniles, and adults, would be more stressed and have a poorer state of health at the site with a higher level of urbanization, according to the different kinds of pressure that urbanization has on the immune function.

METHODS

The House Finch is a common and widely distributed species throughout Mexico and the United States, and can be found in many natural habitats, especially in arid and semi-arid regions, but it also inhabits and thrives in urban and other disturbed areas (Bartholomew & Cade 1956, Peterson & Chalif 1994, Howell & Webb 1995, Dunn & Garrett 1997, Carbó-Ramírez & Zuria 2011). It is a common resident bird in our study site, and it usually benefits from human settlements where food, shelter and nesting sites are abundant (Nocedal 2011). Although the House Finch has been widely studied, there is still very little information on its leukocyte profiles and their relation to urbanization, particularly for Mexico.

Study area. The study was conducted in the city of Pachuca, Hidalgo, Mexico (Figure 1). It is located between 19°50’ and 20°10’N, and between 98°41’ and 98°57’W, at 2400–2800 m. a.s.l. The metropolitan area covers approximately 100 km^2 and it has a human population of around 500,000 inhabitants (INEGI 2011). About 75% of the city area consists of housing developments (Fleming 1999), and there are no permanent water bodies (Carbó-Ramírez & Zuria

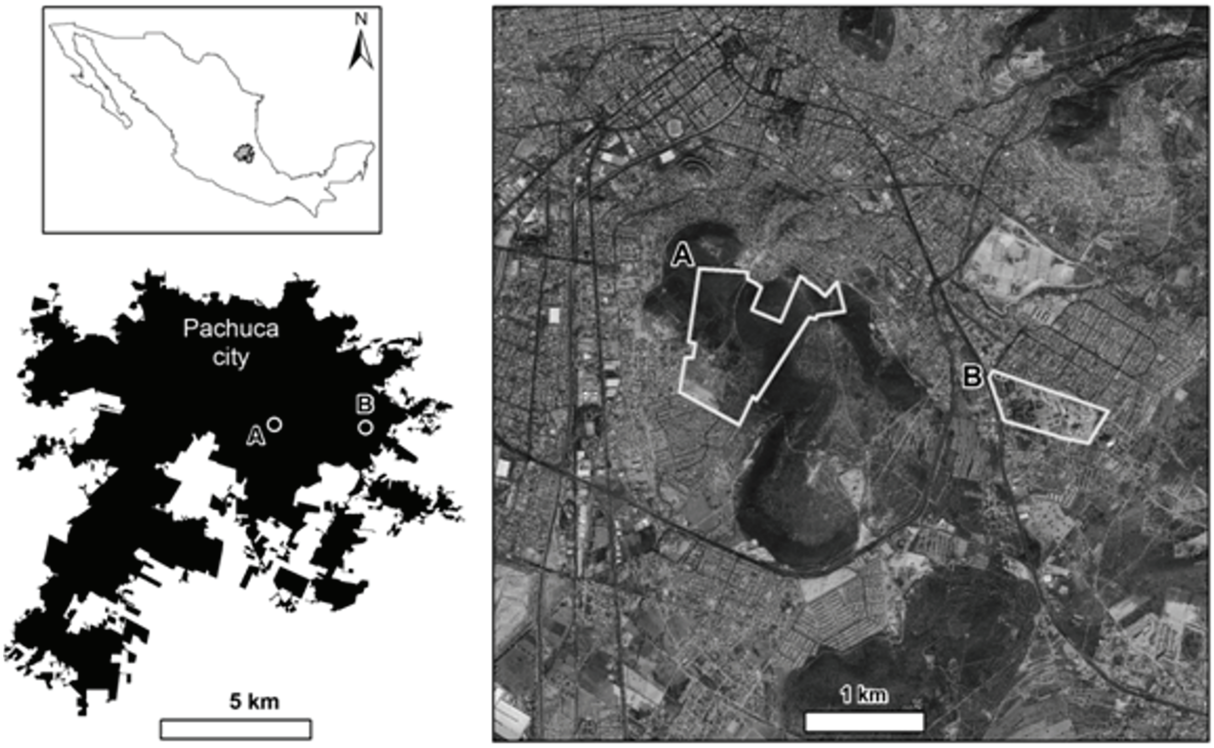

Figure 1. Study sites in the city of Pachuca, state of Hidalgo, Mexico. A) The site with lower urbanization (Parque Ecológico Cubitos) and B) the site with higher urbanization (University campus, Ciudad Universitaria, Universidad Autónoma del Estado de Hidalgo).

2011). The mean annual temperature is 15°C and the mean annual precipitation is 367.6 mm; in summer, the mean temperatures reach 15.5°C (9.5–25.4°C) and the wettest months are July and September; in winter, the mean temperature is 12°C (4.2–24.5°C) and the driest month is December with 6.6 mm of rain (INEGI 2005, Pavón & Meza-Sánchez 2009). The native vegetation consists of small patches of arid tropical scrub or scrub forest (see Carbó-Ramírez & Zuria 2011 for a detailed description).

Within the city, we selected two sites with different levels of urbanization. 1) The site with high urbanization level was the main campus of Universidad Autónoma del Estado de Hidalgo, located in the eastern part of the city (20°05’39”N, 98°42’33”W), with an area of 31 ha (Figure 1); and 2) the site with low urbanization level was located inside a nature reserve (Parque Ecológico Cubitos), located in the southeastern part of the city (20°06’33’’N, 98°44’60’’W), with an area of 92 ha (Figure 1).

We characterized each of the sites according to its urbanization level, calculated as the percentage of urban cover (impervious surfaces: buildings, parking lots and roads) and vegetated areas (e.g., parks, gardens, agricultural fields, natural vegetation) within a 500 m width buffer delineated around the border of each site, using a high-resolution satellite image (IKONOS-2, 1-m resolution) and ArcView (ver. 3.2, ESRI). We also measured temperature and relative humidity with a multiple environmental meter (Sper Scientific LTD), and noise levels (expressed in A-weighted decibels, dBA) using a digital sound level meter (Extech 407732). These measurements were carried out each visit during the morning and under ideal meteorological conditions (no wind and no rain). We recorded instantaneous noise levels every 10 s for 1 min.

Bird captures. Birds were captured weekly from June 2009 to September 2010 at both sites. Mist nets were opened just before sunrise (06:30 h) and closed 5 hours later, with a total mist net effort of 2999 net-hours. Immediately after being captured, we took approx. 75 µL of blood from the brachial vein of each bird. The vein was punctured with a 26-gauge needle and blood was collected directly into micro-hematocrit capillary tubes (Abbot & Oring 1997). Each bird was banded with a numerated aluminum ring and a colored plastic ring, and we obtained the following data: sex, age, weight, and wing chord length. Age and sex were determined from plumage characteristics (Hill 1993). Juveniles refer to first year birds. We collected blood samples from 15 adults (8 males and 7 females) and 26 juveniles (9 males and 17 females) at the site with higher urbanization, and from 7 adults (6 males and 1 female) and 25 juveniles (11 males and 14 females) at the site with lower urbanization.

A blood smear was later prepared at the laboratory. Blood smears were air-dried and then stained with Wright-Giemsa stain. We did not use methanol to fix blood smears since it has been shown that methanol can affect basophile identification (Maxwell

Table 1. Habitat variables measured in the two study sites: University campus and nature reserve from November 2009 to March 2010 in Pachuca, Hidalgo, Mexico. We present averages ± standard error, range (in parentheses) and sample size (N).

Variable	Higher Urbanization (university campus)	Lower Urbanization (nature reserve)	Statistic t/U	*P*
Noise level (dBA)	50.8 ± 0.26	44.0 ± 0.22		
	(37.2–70.3)	(31.1–65.9)	U = 37952.0	< 0.001
	N = 433	N = 540		
Temperature (°C)	20.2 ± 0.51	17.8 ± 0.47		
	(11.7–28.4)	(7.9–27.7)	t = 3.44	< 0.001
	N = 66	N = 78		
Relative humidity (%)	32.2 ± 1.63	49.9 ± 1.65		
	(7.1–71.4)	(12.2–82.4)	t = -4.58	< 0.001
	N = 66	N = 78		

1993, Dubiec et al. 2005, Vinkler et al. 2010). Blood smears were then examined under a microscope at a magnification of 100x. We examined 100 leukocytes on each slide (Davis et al. 2004) and we report percent values for each cell type. We used the leukocyte profile as an indicator of cellular immunity function because it is relatively inexpensive and easier than measuring direct glucocorticoid levels (Davis et al. 2008). We identified the different cell types using standard avian guidelines (Lucas & Jamroz 1961, Campbell 1994, Clark et al. 2009). The H/L ratio was then calculated as the number of heterophils divided by the number of lymphocytes (Tejeda et al. 1997).

Data analysis. The two sites were considered independent because the distance between them was greater than 1 km, and this distance is larger than the maximum expected home range size of House Finches (Manley & Schlesinger 2000). Also, birds were re-captured or re-sighted in the site where they were originally banded and we never re-captured or re-sighted individuals of the university campus at the nature reserve or vice versa. We never sampled the same individual more than once.

The environmental variables and noise levels were compared statistically between the two sites with t-tests (Sokal & Rohlf 1995). When the assumption of normality was violated (based on a Kolmogorov-Smirnov test), we used Mann-Whitney *U* tests (Table 1). The leukocyte parameters, H/L ratios, and body mass variables were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test). We normalized percentages of lymphocytes, monocytes, heterophils, and H/L ratios using an arcsin square-root transformation (Zar 1999). We used non-parametric tests for basophils, eosinophils, and body mass, which were not normally distributed even after transformation.

To analyze the effects of categorical factors on leukocyte parameters, H/L ratio, and body mass, we performed GLM analyses with the lymphocytes, monocytes, heterophils, and H/L ratio as dependent variables, and site, sex, and age as independent factors. Interaction terms were also included in the models. For eosinophils, basophils, and body mass, we conducted two-way PERMANOVA (non-parametric MANOVA based on the Bray-Curtis measure; see Anderson 2001 and Jakubas et al. 2015) with fixed factors (sex and age) and their interaction as independent factor. We performed PERMANOVA analyses using PAST 2.17c software (Hammer et al. 2001) and all other analyses were performed using STATISTICA 7 (StatSoft, Inc., Tulsa, OK, USA).

RESULTS

Study site characterization. The two sites were different in terms of urbanization based on values of vegetation cover, species composition and percent cover of impervious surface. At the site with higher urbanization, 64% of the 500-m width buffer was covered by impervious surfaces, while the rest was covered by vegetation, mostly exotic trees, shrubs, and herbaceous plants including *Juniperus* spp. (Cupressaceae), *Ligustrum japonicum* (Oleaceae), *Eucalyptus* spp. (Eucalypteae), *Pinus* spp. (Pinaceae), *Schinus molle* (Anacardiaceae), *Thuja* spp. (Cupressoideae), *Casuarina equisetifolia* (Casuarinaceae), *Cupressus* spp. (Cupressaceae), *Nicotiana glauca* (Solanaceae), *Bromus catharticus* (Poaceae), *Eruca sativa* and *Lepidium virginicum* (Brassicaceae), *Malva parviflora* (Malvaceae) and *Taraxacum officinale* (Asteraceae), and some natives, mainly *Opuntia* spp. (Cactaceae) and *Yucca filifera* (Asparagaceae). At the low urbanization site only 12% of the area was covered by impervious surfaces, and vegetation cover included mainly scrub forest with native plants like *Agave lechuguilla* (Agavaceae), *Hechtia podantha* (Bromeliaceae), *Coryphantha* spp. and *Stenocactus* spp. (Cactaceae), *Senecio praecox* (Asteraceae), *Yucca filifera*, *Opuntia streptacantha*, *O. spinulifera*, *O. robusta*, and some introduced trees (e.g., *Schinus molle*).

The temperature and relative humidity were significantly different between sites (Table 1). The temperature was higher at the site with higher urbanization, while relative humidity was higher in the site with low urbanization (Table 1). Noise levels

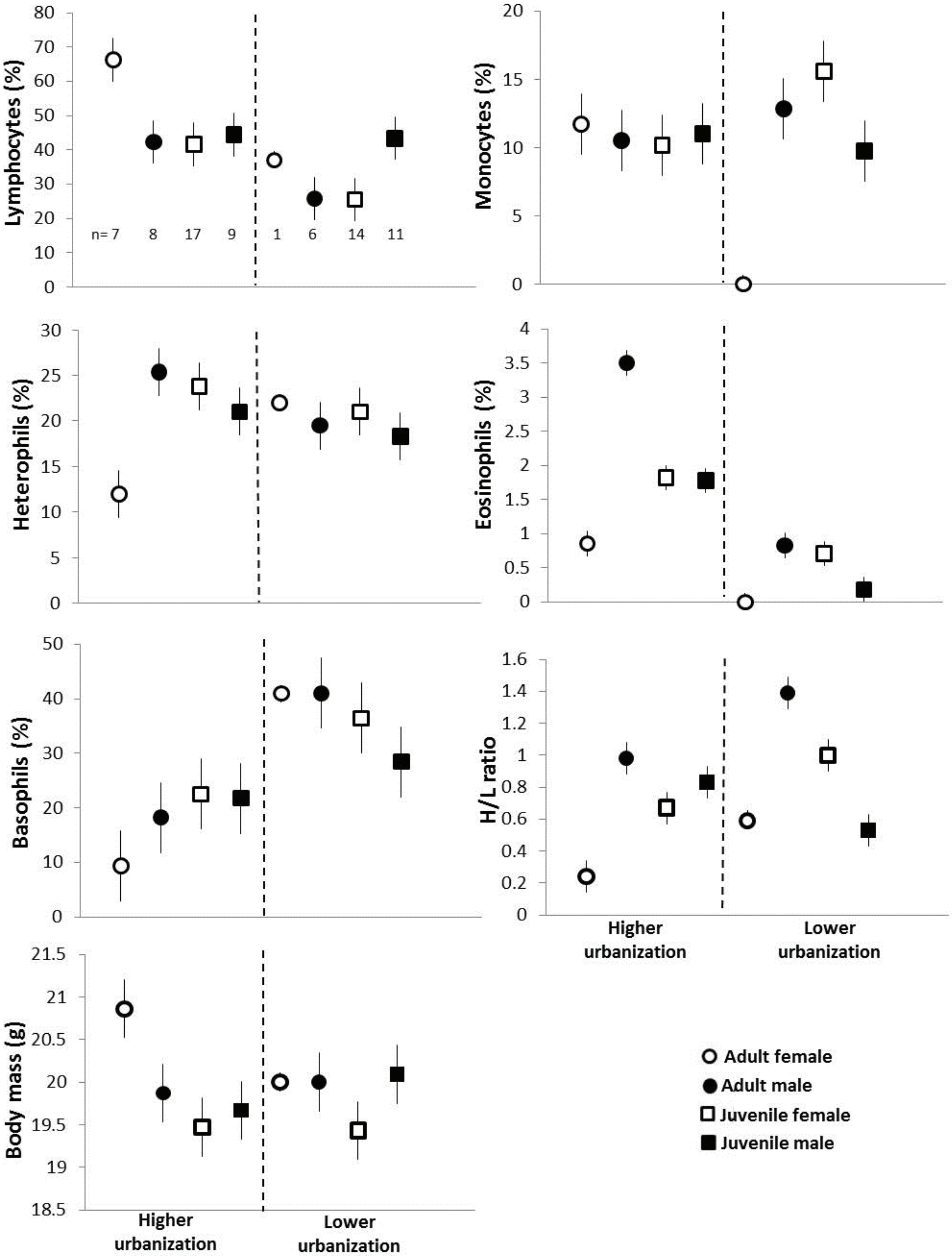

Figure 2. Mean (and SE) values of leukocyte parameters, H/L ratios and body mass for adults and juveniles, females and males of the House Finch (*Haemorhous mexicanus*) in Pachuca, Hidalgo, Mexico.

were significantly higher at the high urbanization site (Table 1).

Leukocyte profile and H/L ratio. Means and standard errors of leukocyte parameters, H/L ratios, and body mass are presented in Figure 2. The level of urbanization significantly affected lymphocytes which were higher at the site with higher urbanization (Table 2, Figure 2). Lymphocytes were also affected by an interaction between sex and age (Table 2). In general,

Table 2. General Linear Models assessing the effects of urbanization (site), sex and age on leukocyte parameters and H/L ratios for the House Finch (*Haemorhous mexicanus*) in Pachuca, Hidalgo, Mexico.

	df	Lymphocytes		Monocytes		Heterophils		H/L	
		F	P	*F*	P	*F*	P	*F*	P
Intercept	1	369.61	**< 0.001**	100.26	**< 0.001**	286.40	**< 0.001**	163.96	**< 0.001**
Site	1	5.84	**0.02**	1.11	0.29	0.005	0.94	1.28	0.26
Sex	1	0.41	0.52	1.03	0.31	0.20	0.66	1.14	0.29
Age	1	0.45	0.50	2.17	0.14	0.02	0.87	0.005	0.94
Site x Sex	1	1.08	0.30	1.68	0.20	0.85	0.36	0.68	0.41
Site x Age	1	1.21	0.27	2.49	0.12	0.50	0.48	0.89	0.35
Sex x Age	1	4.98	**0.03**	2.97	0.09	0.90	0.35	3.69	0.06
Site x Sex x Age	1	0.03	0.87	3.50	0.07	1.40	0.24	0.04	0.84
Error	65								

higher levels of lymphocytes were recorded at the site with higher urbanization, but these differences were larger for females (Figure 2). Monocytes, heterophils, and H/L ratios did not show statistically significant differences between sites, age classes or sexes (Table 2, Figure 2). Eosinophils were significantly higher at the more urbanized site (PERMANOVA, $F_{1,69}$ = 5.99, P = 0.01, Figure 2), and no significant effects were found for sex (P = 0.52), age (P = 0.14), and site x sex (P = 0.99), site x age (P = 0.65), and sex x age interactions (P = 0.28), respectively (Figure 2). The percentage of basophils was significantly higher at the site with lower urbanization (PERMANOVA, $F_{1,69}$ = 5.21, P = 0.005, Figure 2), and we found no significant influence of sex (P = 0.58), age (P = 0.70), and the site x sex (P = 0.96), site x age (P = 0.66), and sex x age interactions (P = 0.40), respectively (Figure 2). Body mass was influenced by the site x age interaction (PERMANOVA, $F_{1,69}$ = -5.37, P = 0.04); adults were heavier at the site with higher urbanization, while juveniles were heavier at the site with lower urbanization (Figure 2). We found no significant influence of sex (P = 0.53), site x sex (P = 0.20), and sex x age interactions (P = 0.43), respectively, on body mass (Figure 2).

DISCUSSION

Our results showed that some leukocyte parameters of House Finches differed between sites. Individuals captured at the site with higher urbanization had higher percentages of lymphocytes and eosinophils than individuals from the site with lower urbanization, while the percentages of basophiles were higher in individuals at the site with lower urbanization. Monocytes, heterophils, and H/L ratios were not affected by site, age or sex. The significant differences between sites may result from variations in habitat quality, food availability and climatic factors, but could also be due to variation in growth and development, which can in turn affect the immune system (Norris & Evans 2000, Dehnhard et al. 2011a, Jakubas et al 2015). Although our sites of study are relative close, the environmental variables and landscape composition are significantly different. For example, the site with higher urbanization had higher temperatures and lower humidity values than the site with lower urbanization, which could affect foraging success or predation risk.

Lymphocytes are responsible for pathogen-specific immune responses (Krams et al. 2012) and higher numbers can be found during parasitic infections (Ots & Hõrak 1998, Bonier et al. 2007) as well as when birds face other immunological challenges (Eeva et al. 2005). Eosinophils are leukocytes responsible for combating multicellular parasites and many viral infections, and higher eosinophil proportions usually indicate the presence of infection (Dein 1986) and gastrointestinal parasites, respectively (Maxwell & Burns 1985, Work et al. 2004, Pollock et al. 2005). Given that both lymphocytes and eosinophils were higher at the site with higher urbanization, it could mean that birds there may be facing a parasitic, bacterial or viral illness.

On the other hand, a decline in the proportion of eosinophils is thought to be caused by glucocorticoid-induced stress, and in general it is believed that changes in the proportions of heterophils, lymphocytes, and eosinophils are necessary to ensure that the different types of cells are directed to where they are needed during the stress response (see Cīrule et al. 2012). Our results show that all individuals at the site with lower urbanization, independently of age or sex, had lower percentages of eosinophils, and therefore might be responding to stress. Temperatures at this site are significantly lower than at the university campus, and at lower temperatures birds need to increase food intake as energy consumption increases and as more nutrients are needed (Davis et al. 1973, Chatelain et al. 2013). Also, at the site with lower urbanization predation pressure may be higher than at the university campus, since predator community includes native predators as well as cats and dogs (Tirado Aviles 2009).

Higher levels of basophils were observed in individuals from the site with lower urbanization. Higher

Table 3. Leukocyte parameters for passerine birds in the American continent (Davis, 2009). Leukocyte types (%): L = lymphocytes; M = monocytes; H = heterophils; E = eosinophils; B = basophils; H/L = heterophil/lymphocyte ratios. A = adults, J = juveniles, *high urbanization site; **low urbanization site.

Species	Sample size	L	M	H	E	B	H/L	Reference
Zonotrichia capensis	75	71.4	2.9	20.2	2.3	3.1	0.28	Ruiz et al. 2002
Haemorhous mexicanus	65	79.0	2.0	5.0	7.0	8.0	0.06	Davis et al. 2004
Melospiza melodia	9	83.3	0.0	11.6	4.0	0.7	0.14	Davis et al. 2004
Turdus migratorius	8	68.5	0.9	20.7	7.4	2.4	0.3	Ricklefs & Sheldon 2007
Turdus grayi	27	69.9	11.4	10.5	6.8	1.2	0.15	Ricklefs & Sheldon 2007
Catharus guttatus	28	69.1	0.3	11.6	9.3	9.7	0.17	Davis et al. 2008
Cardinalis cardinalis	37	84.9	0.8	11.2	1.8	1.5	0.13	Maney et al. 2008
Haemorhous mexicanus	A = 15*	53.5	11.1	19.1	2.3	14.1	0.6	This study
	J = 26*	42.6	10.5	22.8	1.8	22.3	0.7	
	A = 7**	27.4	11.0	19.9	0.7	41.0	1.3	
	J = 25**	33.4	13.0	19.8	0.5	32.9	0.8	

levels of these cells in birds have been associated with physiological stress and forced molting (Fudge 2000, Latimer & Bienzle 2010), however its function is not fully understood (Campbell & Ellis 2013). Latimer & Bienzle (2010) detected basophilia (a concentration of basophils that it is higher than the upper limit of an appropriate reference range, Clark et al. 2009) in birds with respiratory disease, bacterial infection, acute inflammation, and internal and external parasitism with or without eosinophilia. Individuals at the site with lower urbanization are probably experiencing increased levels of physiological stress and disease, since basophil percentages were almost five times higher than individuals from other studies (Table 3) and two times higher than individuals from the site with higher urbanization.

Levels of lymphocytes and basophiles, as well as values of the H/L ratio, found in adult birds in this study are different from what has been reported in previous studies on House Finches and other passerines (Table 3). In particular, we found lower lymphocyte and eosinophil percentages, and higher percentages of the other parameters (monocytes, heterophils, basophils, and H/L ratio) than those reported by Davis et al. (2004) for House Finches (Table 3). Differences in fixation methods may explain some of these discrepancies, for example methanol fixation decreases the visibility of granules in basophils and thus compromises their detection (Robertson & Maxwell 1990). Unlike Davis et al. (2004), in this study methanol fixation was not used, so we may have achieved better detection rates. In general, leukocyte levels can vary according to species, age, sex, habitat condition and methodology employed for sample preparation. Also, lymphocytes and heterophils have been considered cells with a higher variability (e.g., interspecific or linked to age and sex) and fluctuation (e.g., due to stress, season, or health changes) in counts (Vinkler et al. 2010), while the variability among other leukocyte types is considered rare (Davis 2005, Norte et al. 2009a, Norte et al. 2009b, Krams et al. 2012). Nevertheless, certain illnesses induce quantitative changes in the number of basophils, eosinophils, and monocytes that are comparable to the changes observed in lymphocytes and heterophils (e.g., Fudge 1989, Campbell & Ellis 2013).

Although heterophils and H/L ratios were not affected by site, sex, or age, those are parameters related to stress and our results may suggest that all the individuals at both sites are subject to stressful conditions, because they have higher levels compared previous studies, including values for *Haemorhous mexicanus* (Table 3), and because there are anthropogenic and predator pressure at both sites. On the other hand, lymphocytes and body mass were influenced by age, being lower in juveniles than adults in both sites. These differences could be due to variation in experience and social status, molting patterns, and more efficient foraging in adults (Woodrey & Moore 1997, Quillfeldt et al. 2008). Also, juveniles may rely mostly on their innate immune system, which provides initial protection against pathogens, and it is mainly dependent on monocytes, heterophils, eosinophils, and basophils (Roitt et al. 2001), while the adults also have acquired immunity, this is more specific and it is based in lymphocytes (Roitt et al. 2001, Davis et al. 2008, Campbell & Ellis 2013).

Our study provides data of leukocyte profiles and H/L ratios for the House Finch and, for the first time, leukocyte percentages for juveniles, adults, females and males. The results showed that individuals between sites have differences in their cellular immunity, suggesting a link between habitat use and physiology (Krams et al. 2010). Urban areas have been characterized as seasonally buffered with respect to temporal availability of resources, and this has been suggested to provide an increased opportunity for breeding (Schoech & Bowman 2001, Partecke et al. 2005). Urban birds that are better capable of exploiting food resources may allocate energy to immunity without compromising, for example, reproductive effort (Fokidis et al. 2008). Understanding how species differentially use anthropogenic resources may offer insight into the patterns of body condition and immune function capacity (Fokidis et al. 2008). Our work also highlights the importance of studying how habitat changes at the local level can affect cellular immunity. It is still necessary to focus on comparative studies in order to understand how biological and ecological factors affect immune function in wild birds, especially in urban birds and at broader scales. Studies that analyze changes in immune function at sites with different levels of urbanization (i.e., urbanization gradient) and that have site replicates are particularly needed. Although our study is spatially and temporarily limited, it adds to the understanding on how the immune system responds to changes in habitat potentially helping individuals to cope with environmental conditions, particularly those in urban areas.

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