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Body Condition and Chronic stress in Urban and Rural Noisy Miners

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Abstract: Cities are potentially stressful environments for birds for numerous reasons, including their high volumes of pedestrian and vehicular traffic. Native birds inhabiting cities tolerate such human disturbance, but may still potentially incur some cost that is reflected in body condition and the level of chronic stress experienced, unless they are inherently relatively insensitive to urban stressors. We compared body mass and condition, three erythrocyte variables and heterophil: lymphocyte ratios (HL) of adult Noisy miners (*Manorina melanocephala*) in urban Melbourne, Australia and its rural hinterland. Urban individuals had a significantly higher HL (mean 0.995) than rural con-specifics (0.719), suggesting that they may have been experiencing higher chronic stress levels. Body condition (mass-size residuals) and haematocrit were similar in urban and rural individuals, but urban individuals were a little heavier (~ 1%) and rural individuals had a 0.6 g dl^{-1} higher whole blood haemoglobin concentration. There were no significant relationships between body condition indices and blood variables of the kind demonstrated in some bird species; their absence in Noisy miners may either reflect a lack of winter fattening or confirm that the occurrence of these relationships is species-specific.

Keywords: Noisy miner, heterophil, lymphocyte, chronic stress, body condition, erythrocyte variables, urban environment.

INTRODUCTION

Proximity to humans and their activities can negatively influence bird assemblages, populations and individuals [1]. Documented effects of such proximity include reduced species richness and altered composition of communities [2, 3] and decreased population densities [4]. Proximity to human disturbance also disrupts vital behaviour [5], stimulates intra-specific aggression [6], reduces fecundity [7] and causes chronic stress and even mortality [8, 9]. These effects apparently occur because birds often respond to humans as potential predators, even when they are harmless, and avoid close proximity to people or become 'distressed' when avoidance is impossible or uneconomical [10] .

Cities not only have high densities of pedestrian and vehicular traffic, but also physical and biotic features that can be problematic for some colonizing birds [11, 12]. They are thus potentially quite stressful environments for members of many bird species. Although the birds that inhabit cities tolerate human proximity and traffic quite effectively [13-15], possibly through habituation or adaptive down-regulation of the hypothalamus-pituitary-adrenal (HPA) stress response, there may still be some physiological cost to successful urban colonization that is reflected in reduced body condition and an elevated level of potentially debilitating chronic stress [11,16] (the 'cost hypothesis'). It has even been proposed that urban populations will be dominated numerically by weak competitors in poor body condition because of resource overmatching (the 'credit card hypothesis') [17]. Alternatively, perhaps the only birds that thrive in cities are precisely those that are inherently bold and relatively insensitive to urban stressors and so do not exhibit these symptoms [18, 19] (the 'reduced sensitivity hypothesis'). Thus the 'cost hypothesis' predicts that urban members of a species will exhibit higher chronic stress levels (higher HL) and possibly poorer body condition than rural conspecifics, whilst the 'reduced sensitivity hypothesis' predicts that urban and rural members of a species will not differ in chronic stress levels and body condition (similar HL and body condition index). However, the few studies addressing this issue have yielded quite mixed results [11, 20-24] (see Table **1**).

In Australia as elsewhere [25], a 'second wave 'of native bird species has recently joined the original avian colonizers of conurbations, often as part of a broader geographic range expansion triggered by anthropogenic habitat alteration [26] . In eastern Australia, the Noisy miner (*Manorina* m*elanocephala,* Latham), a gregarious,70 g, native honeyeater (Meliphagidae) which mainly inhabits open *Eucalyptus* forests, grassy woodlands and roadside vegetation strips in rural areas [27] , has recently successfully colonized many coastal and inland cities and towns. It now occurs at high densities in suburbia, particularly in areas with little understorey and a sparse, patchy *Eucalyptus* canopy [28]. Noisy miners are aggressive towards con- and hetero-specifics [29] and their aggression towards a range of small-bodied bird species is instrumental in structuring the composition of avian assemblages in rural and urban areas [30-32].

To address whether there is a physiological cost associated with urban-dwelling in this species, we determined whether urban and rural individuals differed in body condition and chronic physiological stress levels. This was achieved by comparing (a) body mass, (b) body condition determined from mass-size residuals (MSR), which are thought to reflect fuel reserves [33], (c) a differential leuko-

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Table 1. Chronic and Acute Stress Levels of Urban and Ex-urban Conspecifics in Several Bird Species. HL is Heterophil: Lymphocyte Ratio, CORT is Plasma Corticosterone Concentration (ng ml^{-1}), U is Urban and R is Rural

Species	Variable Measured	Result	Reference
Noisy Miner	**HL**	**U>R**	**This Study**
5 passerine species	baseline CORT	generally U=R	11
	post-handling CORT	generally U>R	
Rufous-collared sparrow	HL	U>R	20
	BG	U>R	
Florida scrub-jay	baseline CORT	R>U	21
	post-handling CORT	U>R	49
European blackbird (hand-reared)	baseline CORT	U=R	22
	post-handling CORT	R>U (winter; (spring-males only)	
		U=R (autumn)	
White-crowned sparrow	baseline CORT	U>R (males)	23
		U=R (females)	
Dark-eyed junco (females)	baseline CORT	U=R	24
	post-handling CORT	U>R	

cyte count reflecting chronic stress levels [34] and (d) three erythrocyte variables often considered to reflect nutritional and health status, namely haematocrit (Hct), whole blood haemoglobin concentration (Hb) and mean cell haemoglobin concentration [35, 36] in adult Noisy miners inhabiting urban Melbourne, Australia and the surrounding rural hinterland.

The second aim of the study was to determine if there was a relationship that transcended environment between the measured (or calculated) blood variables and body mass and condition. This is a contentious issue for erythrocyte variables, for which such relationships are often simply assumed [37]. However, the evidence is conflicting [38-41] and we need to examine this question in a broader range of species to establish whether or not such relationships are widespread among birds. An additional problem is that the utility of erythrocyte variables as condition indicators can be complicated by the fact that they are influenced by multiple factors [42], making interpretation of any observed relationship with condition difficult.

METHODS

Study Area and Sites

Data were collected in Melbourne, Australia (37^0 50'S, $145^0$0'E) and the adjacent rural hinterland between 2005 and 2010. The five urban sites in Melbourne's northern and eastern suburbs were about 2.5 to 28 km apart and located in residential gardens and open wooded parkland. The 10 rural sites were about 68 to 200 km southeast and north of the city, 3 to 300 km apart and located in residential gardens and at the farmland/open *Eucalyptus* woodland ecotone. Rural gardens were usually adjacent to agricultural land rather than to multiple houses and other gardens. Noisy miners live in a sedentary colony whose activity space can be up to 40 ha when the group is very large [27]. However, most of our study sites were much further apart than this, so their occupants were clearly members of distinct populations.

The maximal latitudinal difference between urban and rural study sites was 1.3°. Monthly mean maximum and minimum ambient temperatures in Melbourne range from 13.4 – 25.9 and 6.0 -14.6^{0}C, respectively, whilst mean monthly precipitation ranges from 47.6 to 66.2 mm. The highest mean monthly maximum and minimum temperatures in central Victoria, where most of the rural sites were located, were 3.6°C higher and lower, respectively, than those in Melbourne.

Data Collection

Data collection occurred in all seasons of the year, but was biased (77% of data) towards autumn and winter (March-August), when Noisy miner breeding is much less frequent [27]. Sampling was spread over the five-year period similarly in urban and rural regions. Noisy miners were caught in mist nets and funnel traps between 0700 and 1900 and uniquely banded. A 70 μl blood sample was taken from each bird within a few minutes of capture by piercing the brachial vein with a 27 gauge syringe needle. Whole blood haemoglobin concentration (± 0.1 g dl^{-1}) was measured immediately with either a Hemocue Classic or Hemocue 201™ haemoglobin photometer, which yield a value for bird blood that is within ~15% of that obtained by cyanmethaemoglobin spectrophotometry [43]. Haematocrit (± 1%) was determined by centrifugation for 3 min at 12000g of blood that had been transported to the laboratory on ice. Mean cell haemoglobin concentration (MCHC) (g l^{-1}) was calculated from Hb and

Hct [44]. Blood smears were made immediately by the pull-wedge method, air-dried and stained with Wright's stain [44]. The first 50 leukocytes encountered on each slide viewed under a microscope at 40× magnification were counted and the ratio of heterophils to lymphocytes (HL) was calculated. The ratio was averaged for the three smears made per bird. Haematocrit and Hb influence blood oxygen carrying capacity and vary in response to several factors, including some diseases, parasite infections, short- and long-term stress and nutritional status (C. Johnstone *et al*. personal communication). The heterophil:lymphocyte ratio varies in response to a wide variety of stressors, including some diseases, long-distance migration, parasite infection and nutritional status [34].

Each sampled bird was weighed (± 1g) and the following morphological characters were measured with a butt-ended ruler or digital callipers: length of head +bill, bill depth and tarsus (all ± 0.1 mm) and wing length (flattened chord) (± 1mm). Wing length was omitted from analyses if the primaries were being moulted. Moult was scored simply as present or absent.

Approval to conduct the project was obtained from the Monash University Biological Sciences Animal Experimentation Committee, the Victorian Department of Sustainability and Environment and the Australian Bird and Bat Banding Scheme.

Data Analysis

Statistical analysis was conducted with *Systat* v. 13 (Systat Software Inc) and R (R Development Core Team, 2011). Three indices commonly used in studies of avian body condition (body condition indices, BCI) were calculated for each bird by the method described by Schulte-Hostedde *et al.* [33]. For BCI 1, principal components analyses (PCA) of all the morphometric data gathered for urban and rural birds, respectively, were used to derive estimates of structural size (Principle Component 1, PC1) [33]. Tarsus length cubed and wing chord were used as the structural size metrics for the other two BCIs. Mass-size residuals, the residuals of ordinary least squares linear regressions of body mass on the structural size metric (PC1, tarsus length^3 and wing chord) were used as an estimate of body fat content. It has been shown for several bird species that this method of estimation is accurate [45].

Separate two-factor (REGION - urban and rural; SEASON - autumn/ winter and spring/ summer) analyses of variance (ANOVA) were conducted for body mass, each blood variable and each BCI to determine whether urban and rural Noisy Miner populations differed with respect to these variables. The spread of sampling over five years and 15 sites made the study more robust and representative; however, the data were too unevenly spread to incorporate sampling YEAR and SITE in these analyses, because most samples were obtained in three years and a few rural sites provided a very small percentage of the data. Data were tested to determine whether they met the assumptions of linear regression and ANOVA and transformation was used where appropriate.

To determine whether there were significant relationships among morphometric variables, among blood variables and between blood variables and BCIs, Pearson correlation coefficients (*r*) were calculated for the urban and rural samples combined. Separate linear mixed models (LMM) were also run on the combined samples to explore the relationships between blood variables and BCIs, with REGION and SEASON (as above) being included as random effects. Data are reported as mean ± standard error (se).

RESULTS

Chronic Stress and Body Condition in Urban and Rural Environments

There were significant urban/rural differences in mean body mass (urban 0.6 g> rural), Hb (rural 0.6 g dl^{-1}> urban), MCHC (urban 0.2 g l^{-1}> rural) and HL (urban 0.276 > rural), but not in Hct or any BCI (Table **2**). A similar percentage of Noisy miners was moulting in the urban and rural environments (33.3 versus 35.6%; n = 54 and 45, respectively; $\chi^2_{(1)}$ = 0.054, P >0.05).

Table 2. Mean ± se [n] Values for Blood Variables and Condition Indices in Urban and Rural Noisy Miners. Full Names of Blood Variables given in Methods. Significant (<0.05) P-values in Bold. Mean Values for Body Condition Indices given in Scientific Notation for Simplicity

Variable	Regional Mean ± s.e Urban	Source of Variation Rural	Region	F_{df} ratio and P-value Season
Hb (g dl^{-1})	17.4 ± 0.3 [53]	18.0 ± 0.3 [49]	$F_{1, 98}$ 5.094 **P 0.026**	$F_{1, 98}$ 5.553 **P 0.02**
Hct (%)	49.3 ± 0.6 [53]	50.8 ± 0.8 [50]	$F_{1, 99}$ 0.003 P 0.957	$F_{1, 99}$ 1.759 P 0.188
MCHC (g l^{-1})	35.5 ± 0.6 [52]	35.3 ± 0.4 [49]	$F_{1, 97}$ 4.042 **P 0.047**	$F_{1, 97}$ 1.768 P 0.187
H/L	0.995 ± 0.066[53]	0.719 ± 0.050 [49]	$F_{1, 99}$ 8.554 **P 0.004**	$F_{1, 99}$ 0.667 P 0.2
BCI 1	-2.1e-14± 0.702 [53]	-9.3e-18± 0.713 [48]	$F_{1, 97}$ 0.085 P 0.771	$F_{1, 97}$ 0.049 P 0.825
BCI tarsus^3	-1.9e-14 0.808 [53]	-7.7e-15 ± 0.901 [50]	$F_{1, 99}$ 1.097 P 0.298	$F_{1, 99}$ 0.01 P 0.919
BCI wing chord	-3.7e-14 ± 0.779 [53]	-4.5e-14 ± 0.689 [48]	$F_{1, 97}$ 2.14 P 0.147	$F_{1, 97}$ 0.826 P 0.366
Mass (g)	71.7 ± 0.6 [54]	70.6 ± 0.9 [50]	$F_{1, 100}$ 4.164 **P 0.044**	$F_{1, 100}$ 0.29 P 0.591

The only significant seasonal difference in a BCI or a measured blood variable was in Hb (means: spring/summer 16.8 ± 0.7, n = 12, versus autumn/winter 17.8 ± 0.2 g dl^{-1}, n = 90) (Table **2**), but the spring/summer sample was very small.

Relationship between Body Condition and Blood Variables

There were significant correlations between all pairs of morphometrics (r = 0.35-0.671, n = 81-103, all P<0.01). In the PCAs of all morphometrics for both regions, head-bill length had the highest loadings of any morphometric on PC1 (urban 0.804, rural 0.911), whilst all loadings were positive on PC1. PC1 (eigenvalues both > 2) explained 50% and 63% of the variance in the morphometrics in the urban and rural regions, respectively (Table **3**). There were significant correlations between Hb and Hct (r 0.473, n= 100), Hb and MCHC (r 0.519, n= 101) and Hct and MCHC (r -0.509, n= 101) (all P<0.001). However, Pearson correlation coefficients between blood variables and BCIs ranged from 0.006 to 0.113 (n= 97-102) and were not significant (P>0.05). The LMMs likewise revealed no significant relationships between blood variables and BCIs (including mass) (Table **4**), and no model explained more than 25% of the variance in any blood variable. REGION (urban *versus* rural) explained 12-15% of the variance in HL in three models, but not more than 7% for any of the erythrocyte models. SEASON (autumn-winter/ spring-summer) accounted for little of the variance in the HL or erythrocyte models (0-9%), except in the BCI $tarsus^3$ × Hct (23%) and BCI wing length × Hb (25%) models.

DISCUSSION

Urban-rural Comparison

The statistically significant urban/rural disparity in HL (0.276) was modest compared with that (1.27) in the Rufous-collared sparrow (*Zonotrichia capensis*, Müller) [20]. Currently there are too few intra-specific comparisons of HL in free-living birds inhabiting contrasting environments to satisfactorily evaluate the biological significance of the observed modest difference in Noisy miners. However, the higher HL of urban Noisy miners was not associated with poorer body condition (MSR); urban individuals were actually slightly (about 1g) heavier than rural con-specifics, on average. Again, this contrasts with the finding that less chronically-stressed rural Rufous-collared sparrows were 19% heavier than urban con-specifics [20]. It is also not in accord with the 'credit card hypothesis' [17], which predicts that weak competitors in poor body condition will survive and dominate urban populations because of resource overmatching.

If we assume that the difference in HL in the Noisy miner *was* biologically meaningful, it suggested that urban individuals were exhibiting higher chronic stress levels, which is more in accord with the 'cost' than the 'reduced sensitivity' hypothesis, although the cost admittedly appeared relatively small. We have no information on the population densities of, and food resources available to, Noisy miners in the study sites. Urban environments often provide more abundant and novel food resources [46, 47] which can result in higher avian population densities and more intense competition than rural environments [14, 48], potentially adding to the stress of city-living. In contrast, some authors have argued that for species that do not exhibit greater stress levels in cities, the greater predictability of the more abundant, stabilized urban food resources has reduced the need for adaptive stress responses [11, 21]. Haemoglobin concentration, sometimes considered an avian condition indicator [38], was significantly higher in rural than urban Noisy miners, although again the mean disparity was fairly small (0.6 g dl^{-1}). Mean cell haemoglobin concentration exhibited a similar small disparity. Haematocrit, however, which is also proposed as such an indicator but is probably less reliable [42], did not differ between urban and rural populations despite the ambient temperature disparity.

The apparently higher chronic stress level in urban than rural Noisy miners agreed with findings for Rufous-collared sparrows [20] and male White-crowned sparrows (*Z. leucophrys*, Forster) [23], but not with those for female White-crowned sparrows [23] or members of seven other passerine species [11, 21, 22, 24] (Table **4**). However, the caveat needs to be added that baseline plasma corticosterone (CORT) concentrations were used to assess chronic stress in most of these species and both low and high values of this variable can reflect high levels of chronic stress [11]. Acute stress responses (based on post-handling CORT), which are beneficial in allowing birds to cope with environmental challenges, were lower in urban than rural Dark-eyed juncos

Table 3. Principle Components Analysis of all Noisy Miner Morphometrics

Morphometrics	Loadings on Principle Component 1	
	Urban	Rural
Head-bill	0.804	0.911
Bill depth	0.768	0.652
Wing chord	0.732	0.867
Tarsus length	0.494	0.703
Eigenvalue	2.017	2.502
% explained variance	50.4	62.6

Table 4. Linear Mixed Models of Relationship between Body Condition and Blood Variables. Full Names of Blood Variables Given in Methods

Blood Variable	(df) F ratio	P-value	Percentage Variation Explained			Model r^2
			Region	Season	Residual	
BCI 1 models:						
Hct	(1, 95) 0.22	0.644	0.67	22.97	76.37	0.082
Hb	(1, 94) 0.99	.322	2.59	0.0	97.41	0.034
MCHC	(1, 93) 0.93	0.336	1.37	2.08	96.55	0.034
HL	(1, 95) 0.02	0.882	11.72	7.59	80.69	0.118
BCI tarsus[3] models:						
Hct	(1, 97) 0.06	0.807	5.98	3.78	90.25	0.074
Hb	(1, 96) 0.42	0.52	0.52	9.31	90.17	0.042
MCHC	(1, 95) 0.10	0.748	10.66	0.0	89.34	0.07
HL	(1, 95) 0.22	0.644	0.67	22.97	76.37	0.082
BCI wing length models:						
Hct	(1, 95) 0.16	0.691	6.66	5.55	87.78	0.08
Hb	(1, 94) 0.65	0.421	0.1	24.52	75.38	0.073
MCHC	(1, 93) 0.69	0.409	4.66	2.24	93.1	0.055
HL	(1, 95) 0.04	0.837	13.79	0.0	86.21	0.106
Body mass models:						
Hct	(1, 98) 0.36	0.548	6.13	3.72	90.15	0.077
Hb	(1, 97) 0.13	0.723	2.23	8.95	88.82	0.054
MCHC	(1, 96) 0.06	0.814	3.82	0.0	96.18	0.04
HL	(1, 98) 0.0	0.956	14.5	0.0	85.5	0.11

(*Junco hyemalis*, Linnaeus) [24] and European blackbirds (*Turdus merula*, Linnaeus) (in some seasons) [22], but higher in urban than rural Florida scrub-jays (*Aphelocoma coerulescens*, Bosc) [49] and members of several other passerine species [11] (Table **4**). It would be interesting to make the same determination for Noisy miners. Given the high HL of urban individuals, we might predict that their acute stress response would be attenuated. However, this species apparently has a 'bold phenotype' [50], which is usually associated with a low acute CORT response [51], so differences in acute stress response between urban and rural individuals may not be very marked.

Urban-colonizing bird species thus appear to vary markedly in both their short- (acute) and long-term (chronic) responses to the stressors assumed to characterise city life, suggesting that there may be more than one physiological coping strategy involved in urban colonization by birds. However, many more comparisons are needed before we can detect whether there are discernible majority trends in such strategies [11, 24]. Our investigation, like those discussed above, was limited to comparison of birds in one city with rural con-specifics and ideally needs to be extended to encompass multiple cities and their rural surroundings. Data collection spread over 5 years gave a more representative picture than a one-year 'snapshot', but it would also be valuable to collect sufficient data to examine how urban-rural disparities in chronic stress vary from year to year.

Relationship between Body Condition and Blood Variables

There were no significant relationships between any BCI (including mass) and any blood variable for the combined sample of urban and rural Noisy miners. Haematocrit, Hb and MCHC have been shown to be associated with body condition (MSR or mass) in adults or nestlings of several altricial bird species [40, 43, 52-54]. These associations are not surprising, because these variables are known to be influenced by nutrition, health, parasite infections, toxins and energetic factors [52, 55-59]. Despite this, other studies have also failed to demonstrate any such associations [39-41, 60-63]. Moreover, these erythrocyte variables are affected by multiple factors [42,64] and can also reflect different aspects of body condition at high and low values [65, 66], which makes it difficult to convincingly interpret any relationship with body condition which is established [67]. However, the lack of significant relationships between the BCIs and HL is more puzzling. HL is a reliable chronic stress indicator in birds [34] and significant, usually negative, relationships with MSR or mass would be predicted and have been observed in several other species e.g. [20, 58, 66].

Conceivably the lack of significant relationships between blood variables and body condition in Noisy miners resulted from the three commonly-used BCIs and mass not accurately reflecting the birds' stored fat reserves [68], although they have been shown to do so in several other small bird species [45]. In any case, an indirect relationship might be predicted theoretically, because of the known relationship of these blood variables with health status [42, 58, 65]. It is also possible that the lack of relationships reflected a lack of fat reserves in adult Noisy miners during autumn and winter, our main sampling season. Winter is comparatively mild at low altitude in the relatively low latitudes of temperate south-eastern Australia and overwintering adaptations of resident birds are less pronounced than at high latitudes in the northern hemisphere [69]. Thus Superb fairy-wrens (*Malurus cyaneus*, Ellis), which are only one seventh of the mass of Noisy miners, showed no fuel reserve storage or metabolic or haematological adjustments in winter near Melbourne of the kind recorded for small resident birds overwintering at high latitudes in the north temperate zone [69-71].

It seems increasingly likely that the occurrence of blood variable × body condition relationships is species- or environment-specific among birds [37]. If so, it is necessary to validate rather than assume such a relationship for any species in which body condition is being investigated for the first time.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

LIST OF ABBREVIATIONS

HL	=	Heterophil: lymphocyte ratio
Hct	=	Haematocrit
Hb	=	Whole blood haemoglobin concentration
BCI	=	Body condition index
MSR	=	Mass-size residuals
MCHC	=	Mean cell haemoglobin concentration

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