

Sex-specific consequences of life in the city

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The vast majority of species are excluded from human-dominated landscapes, but some species persist and appear to thrive, despite facing challenges they would not encounter in less disturbed habitat. Corticosteroid (cort) secretion may play a significant role in enabling an animal to cope with urban breeding habitat because it helps mediate physiological and behavioral responses to environmental challenges. Here we present tests of 3 hypotheses relating to cort secretion in urban animals: 1) environmental challenges vary between urban and rural habitat, as reflected in differing levels of cort secretion in animals breeding in these habitat types, 2) there are fitness correlates associated with variation in cort levels within populations, and 3) parasite load is one of the mechanistic links between variation in cort levels and reproductive success. Male white-crowned sparrows (*Zonotrichia leucophrys*) in urban habitat had significantly higher baseline corticosterone levels than males in rural habitat, whereas female *Z. leucophrys* hormone levels did not differ between the 2 habitat types. To assess the fitness correlates of variation in hormone levels, we monitored seasonal reproductive success in one urban population and found that baseline corticosterone levels were predictive of female, but not male, reproductive success: females with higher corticosterone fledged fewer offspring. Data do not suggest a direct relationship between corticosterone levels and fitness and instead indicate an indirect relationship between hormone secretion, territory quality, body condition, and reproductive success. Females with blood parasites had lower heterophil:lymphocyte ratios, lower body condition scores, fewer mates, and fledged fewer offspring than females without parasites. In contrast, parasites did not negatively affect reproductive success in males but were associated with reduced body condition. Corticosterone levels did not differ between birds with and without parasites. In tests of all 3 of our hypotheses, we found sex-specific effects, highlighting the importance of considering sex in investigation of physiological responses to disturbance. *Key words:* avian, blood parasites, corticosterone, reproductive success, urbanization, white-crowned sparrow. [*Behav Ecol* 18:121–129 (2007)]

A dubious landmark in modern history has recently been passed: most of the global human population now lives in urban areas (Crane and Kinzig 2005). Increasing urbanization is predicted to continue, particularly in less developed regions. As urban populations increase, the area occupied by urban centers will expand at an even greater rate (O'Meara 1999). This expansion will have numerous and significant impacts on ecosystems (Mills et al. 1989; Sauvajot et al. 1998; Marzluff 2001; Milesi et al. 2003).

As urban areas expand, most species decline, nonetheless a few persist (Douglas 1983). Urban environments present organisms with novel challenges, including increased human activity (Fernández-Juricic et al. 2001), noise (Slabbekoorn and Peet 2003), and toxin levels (Eens et al. 1999). Although urban habitat may offer benefits such as increased food resources (Contesse et al. 2004) and sometimes decreased predation (Gering and Blair 1999), urban organisms are still presented with a suite of novel challenges that they would not encounter in rural areas.

Despite the ubiquity of urbanization, the effects of the urban environment on organisms and their physiology are poorly understood. Corticosteroids (corts) are a group of hormones that mediate behavioral and physiological reactions to conditions that challenge homeostasis (Sapolsky et al. 2000; Kruk et al. 2004; Landys et al. 2004; Romero 2004). Thus cort levels reflect an integration of the internal and external challenges placed on an organism at the time of sampling. As conditions worsen, and challenges increase, cort secretion increases as part of the animal's effort to maintain homeostasis.

If urban environments create novel challenges, cort might play an important role in mediating the behavioral and physiological responses of organisms to those challenges. Cort secretion is flexible, varying among individuals (Koolhaas et al. 1999) and seasonally within populations (Astheimer et al. 1995). Although cort is widely accepted to be critical to survival, chronically elevated levels of cort can have serious negative effects (Sapolsky et al. 2000; Padgett and Glaser 2003; Korte et al. 2005). The point at which the costs of cort secretion begin to outweigh the benefits is unknown. Few studies have documented fitness (i.e., survival or reproductive success) correlates of variation in cort levels, although potential fitness consequences of variation in cort levels are routinely discussed (for review, see Silverin 1998; Korte et al. 2005). Studies exploring this important relationship have found varying, context-dependent relationships between cort levels and fitness components (Romero and Wikelski 2001; Clinchy et al. 2004; Mullner et al. 2004; Brown et al. 2005; Good et al. 2005; Meylan and Clobert 2005).

Blood-borne hematozoa parasites (*Haemaphysalis* spp.) can have significant effects on fitness (Merino et al. 2000; MacDougall-Shackleton et al. 2002; Marzal et al. 2005) and have been found to be associated with increased cort levels (Oppliger et al. 1998), potentially providing a mechanistic link between cort and reproductive success. If these parasites place an increased energetic demand on a bird, parasitized birds should have elevated cort levels and reduced reproductive success. Alternatively, increases in cort levels might increase susceptibility to parasites by suppressing the immune system (Oppliger et al. 1998). Regardless of the causality in the relationship between parasites and cort levels, energy allocated to an immune response to parasites might lead to reduced reproductive effort, providing a link between cort levels and fitness correlates.

We explored the relationship between cort secretion, habitat type, individual condition, and a measure of fitness

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Received 20 February 2006; revised 12 August 2006; accepted 1 September 2006.

(number of offspring fledged) in an urban-adapted bird to test the hypotheses that 1) environmental challenges vary between urban and rural populations, as reflected in differing levels of cort secretion; 2) variation in cort levels are associated with fitness correlates within populations; and 3) parasite load is one of the mechanistic links between variation in cort levels and reproductive success.

White-crowned sparrows (*Zonotrichia leucophrys*) persist, breed, and are even spreading in urban environments. These versatile birds breed in diverse habitat types from Arctic tundra to coastal chaparral, and large breeding populations can be found both in remote wilderness and in cities (Chilton et al. 1995). We measured baseline levels of corticosterone (the primary avian cort) in 60 *Z. leucophrys* during the same breeding stage in 3 pairs of populations in both urban and nearby rural habitat from the central coast of California to northern Washington, USA. We predicted that urban and rural birds would differ in their levels of cort secretion, reflecting variance in challenges confronted by birds in these populations. To address the second and third hypotheses, we monitored reproductive success in 25 focal pairs of *Z. leucophrys* within one urban population to quantify the fitness correlates of among-individual variation in cort secretion and presence of avian blood parasites.

METHODS

Hormone comparisons between birds in urban and rural habitat

We sampled 60 breeding *Z. leucophrys* (30 urban and 30 rural birds) in 2003 and 2004 from 3 urban populations, each of which was paired with 3 rural populations (Table 1). Both populations in each habitat pair were sampled in the same year, and are no more than 150 km apart, at similar elevations and latitudes, and experience similar temperatures and rainfall during the breeding season. To control for variation in cort secretion associated with life history stage (Romero et al. 1998), we sampled all birds during the incubation stage of breeding. We identified breeding pairs and determined their stage of breeding through behavioral observations and through location of nests. To control for diel variation in cort levels, we captured all birds in mist nets within 3 h of dawn and collected an initial blood sample into heparinized micro-capillary tubes, via alar vein puncture within 3 min of when birds became entangled in nets. This sample was used for measurement of the bird's circulating baseline hormone level. We held birds for an additional 30 min and collected 2 more blood samples to measure capture stress-induced hormone levels (data not presented here).

Table 1
Summary of sampling of *Z. leucophrys* at 3 urban and 3 rural sites

Region	Urban site	Rural site	Distance (km) ^a	N (urban) ^b	N (rural) ^b	Baseline corticosterone (males) ^c	Baseline corticosterone (females) ^c
Central coast of California	Monterey	Garrapata State Park	14	3, 4	3, 4	U: 24.9 ± 3.0 R: 4.1 ± 0.8	U: 14.1 ± 2.0 R: 10.0 ± 0.8
Northern coast of California	San Francisco	Point Reyes National Seashore	55	4, 5	6, 4	U: 19.1 ± 2.0 R: 14.4 ± 1.6	U: 10.3 ± 1.6 R: 17.2 ± 2.2
Coastal Washington	Seattle	Westhaven State Park	150	9, 5	10, 3	U: 15.8 ± 0.9 R: 9.8 ± 0.4	U: 11.2 ± 1.3 R: 21.0 ± 6.1

^a Distance between urban and rural sites in kilometers.

^b Number of individuals sampled: males, females.

^c Baseline corticosterone levels (ng/ml) mean ± standard error, urban (U) above rural (R).

All birds were color-banded and released after sampling. We then confirmed our assessment of the bird's life history stage through behavioral observations, using color bands to identify individuals and confirm that sampled females were in fact incubating eggs. Because only the female incubates in this species (Chilton et al. 1995), behavioral observations of the male interacting with the female and defending the area around her nest were used to confirm that he was the social mate.

Z. leucophrys typically nest on the ground or in shrubs in open habitat (Chilton et al. 1995). In urban sites, we found nests in landscaped areas around shopping centers, parking lots, university campuses, small urban parks, and public walkways. In rural sites, birds nest in open scrub, such as the poison oak (*Toxicodendron diversilobum*) and lupine (*Lupinus* spp.) found along the coast of central and northern California. We sampled birds randomly within designated sites, while avoiding the interiors of large urban parks, as these may be refugia from urban conditions. All urban bird territories were in areas with high levels of human activity, containing human-made structures, not because we preferentially sampled these areas but because breeding birds were found in such areas. All rural sites were on public land and had no human-made structures within the birds' territories. Our sampling included 2 of the 5 recognized *Z. leucophrys* subspecies: *Z. l. nuttalli*, sampled in California, and *Z. l. pugetensis*, sampled in Washington. These 2 subspecies breed along the west coast of North America, from southern California to southern British Columbia. These subspecies are the most notably urban of the 5 (Dawson 1909; Jewett 1953), though this may simply be a by-product of the level of disturbance they encounter in their breeding ranges.

For our comparison of baseline cort levels across 3 urban and 3 rural populations, we compared baseline cort measures using a general linear model (GLM) analysis, with habitat type (urban or rural), sex, and region (central California, northern California, or Washington), as well as all possible interaction effects as factors. We simplified the model by individually removing nonsignificant factors until we found the best-fit model (maximum R-squared value for the whole model). The best-fit model included habitat type and 3 interaction effects, habitat by sex, habitat by region, and sex by region, as factors. We followed up the GLM analysis with post hoc Tukey tests to compare mean cort levels across habitat types for both males and females.

Fitness correlates of variation in cort levels

During April through August of 2005, we tracked the reproductive success of 25 focal pairs of *Z. leucophrys* in urban habitat in Seattle, Washington. Using the methods described above, we collected a blood sample for measurement of

baseline and stress-induced cort levels from each bird. We also used approximately 5 μ l of the initial blood sample for a blood smear, which we used for quantification of several hematological parameters (see below). We measured each bird's body mass (± 0.1 g, Pesola scale), tarsus and wing length (± 0.1 mm, caliper), and furcular fat score (scale 0–5, for details, see Wingfield and Farmer 1978) by inspecting visible subcutaneous fat stores in the furculum (interclavicular depression). Because birds store most of their fat subcutaneously, fat score is a reliable indicator of total body fat content, particularly if interobserver variation is controlled (Kremetz and Pendleton 1990), thus all fat scores were made by F.B. We sampled all birds during the incubation stage, in most cases during incubation of the first clutch. In 4 cases, the first clutch was depredated before birds could be sampled, and these individuals were sampled during subsequent nesting attempts.

The subspecies of *Z. leucophrys* found in Seattle have multiple broods in one breeding season, so we located and monitored every nest for the 25 focal pairs to arrive at one cumulative measure of seasonal reproductive success for each bird. We are confident that we found all successful nests on the study site because birds were monitored a minimum of once every 3 days throughout the season. Behavioral observations easily confirmed the breeding stage for each pair because behavior is stereotypical during the various stages. For example, during incubation, the female is off the nest for no more than 20 min at a time; during the nestling stage, the female and male can be observed carrying food to the nest; and during the fledgling stage, both adults continue to carry food for at least 1 week, but bring it to fledglings out of the nest (Bonier F, personal observations). The birds' territories are contiguous, so that missing an extrapair breeding of one of the focal males on the study site would be unlikely. We confirmed termination of breeding through failure to locate the pair on their territory or anywhere on the study site (using conspecific song playback to survey for birds), observation of presence of molting (this species loses all of its tail feathers at once during the postbreeding molt, which is conspicuous), and/or observation of flocking behavior.

Although *Z. leucophrys* are socially monogamous, extrapair fertilizations (EPFs) have been documented (Sherman and Morton 1988; MacDougall-Shackleton et al. 2002). To determine genetic paternity, we collected a small blood sample from each nestling that survived to day 6 of the nestling stage (see below). We monitored nests through regular observations and recorded the number of offspring that successfully fledged for each focal pair. We defined fledging success as observation of an empty nest on day 7 or later of the nestling stage, in combination with observations of the focal pair carrying food, which is evidence they are feeding dependent young. Following such observations, we recorded all nestlings in the nest prior to fledging as having successfully fledged.

Because EPFs have been documented in *Z. leucophrys*, we genotyped all adults and fledglings at 5 polymorphic microsatellite loci to assign genetic paternity. These loci have been previously described for use in this species (MacDougall-Shackleton and MacDougall-Shackleton 2001; MacDougall-Shackleton et al. 2002). We extracted DNA using the Qiagen DNeasy Tissue Kit, following the manufacturer's instructions for extraction from nucleated blood cells. We amplified fragments at the 5 loci (Table 2) using 10 μ l polymerase chain reactions (PCR) containing 50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl₂, 0.2 mM deoxynucleoside triphosphate, 0.5 μ M of each primer, 0.25 units of polymerase, and 25 ng of template. The following thermal conditions were used for all loci: 94 °C for 3 min followed by 10 cycles of 94 °C for 30 s, 62 °C for 30 s, decreasing by 1 °C each cycle, and 72 °C for 45 s; followed by 30 cycles of 94 °C for 30 s, annealing temperature (Table 2)

for 30 s, and 72 °C for 45 s; and a final extension step of 72 °C for 10 min. We separated all PCR products on an Applied Biosystems 3100 capillary electrophoresis system and assigned genotypes using Applied Biosystem's GeneScan version 3.7.

We used the software program Cervus (Marshall et al. 1998) to assign paternity for 70 offspring that we recorded as having successfully fledged. We found the analysis to be robust to changes in the starting parameters. The mean number of alleles per locus was 8, with an expected heterozygosity of 0.66. All loci were in Hardy-Weinberg equilibrium and were in linkage equilibrium. Mean null allele frequency per locus was estimated to be < 0.01 . With the 5 loci we used, we had a cumulative exclusionary power of 85% for the first and 97% for the second most likely paternal candidates. Eight of the offspring could not be assigned with 95% confidence (the level we used for strict assignment), but all 70 offspring could be assigned with 85% confidence, with no genotype mismatches between offspring and their known (maternal) and candidate (paternal) parents at either of the confidence levels. We repeated subsequent statistical analyses on reproductive success twice, once at each confidence level and found no difference in results; therefore, the data we present here include all offspring. From these analyses, we quantified each individual's reproductive success and number of different pairings resulting in fledged offspring (referred to as number of mates).

Female reproductive success was determined without genetic methods, as all nestlings in a given female's nest are known to be her biological offspring. Conspecific brood parasitism has never been documented in this species (Chilton et al. 1995). Furthermore, in our observations of the complete laying sequence in 25 nests, we never found more than one *Z. leucophrys* egg added to the clutch on a given day (which would be evidence of conspecific brood parasitism).

To explore the relationship between reproductive success and variation in parental baseline cort levels, we conducted 2 Poisson regressions, 1 for males and 1 for females, of total number of offspring fledged on baseline cort measures. Poisson regression was used because count data, such as number of offspring fledged, violate assumptions of linear regression (Crawley 2002). Because we suspected that a bird's condition might affect its reproductive strategy, we further analyzed the relationship between baseline cort levels and number of within-pair (those offspring produced with the bird's social mate) and extrapair (those offspring produced with another mate) offspring for males and females using 2 Poisson regressions.

Hematological parameters and blood parasites

For 45 of the focal birds from the second component of the field study, we quantified several hematological parameters and recorded the presence or absence of blood parasites (*Haemaphysalis* spp.), which might affect the birds' condition. We used approximately 5 μ l of the initial blood sample to prepare a blood smear on a microscope slide, which was allowed to air dry. Within 1 week of collection, we fixed slides with methanol and stained them using a Wright-Giemsa stain. For each slide, a single observer (K.S.S.) inspected white blood cells (WBCs) under oil-immersion objective using 1000 \times magnification. The observer was unaware of the origin of the slides or the sex or hormone levels of the sampled birds. We screened slides by viewing successive fields perpendicular to the direction of the smear until we reached a total of 100 WBCs. We identified WBCs as lymphocytes, monocytes, eosinophils, heterophils, and basophils (Campbell 1995). We did not include thrombocytes in the analysis following standard protocol for avian clinical pathology (Campbell 1995). We randomly

Table 2
Summary of microsatellite loci used in paternity assignment

Locus	Number of alleles	Primer sequence (forward/reverse)	Annealing temperature	Original reference
Escμ1	9	TTCTCTTGGTCTATGGAAGGTG GCTTGAAGACAGTCACCAGG	56 °C	Hanotte et al. (1994)
Escμ4	6	TTCCCTCACAAATTTCCGAC TATGTGCTGAAGTGAACCATCC	60 °C	Hanotte et al. (1994)
Gf01	11	TAGCATTCTATGTAGTGTATTTTAA TTTATTTATGTTTCATATAAACTGCATG	56 °C	Petren (1998)
Gf06	5	GCTATTGAGCTAACTAAATAAACT CACAAATAGTAATTAAGGAAGTACC	56 °C	Petren (1998)
Mme1	9	AGGAAAAGGGAGGGAGAGGGTG GGGAGTGCAGAATGTGCAAATG	56 °C	Jeffery et al. (2001)

rescored 5 slides to check for precision (average difference of 3.04 WBCs). We noted any parasitized red blood cells (RBCs) (primarily hemosporidian gametocytes) in the peripheral blood. This method is not sensitive enough to conclude that birds without visual evidence of parasites were entirely parasite free (Fallon et al. 2003), but does detect intensity of infection, which is potentially a more important parameter for impact on the bird's reproductive success (Merino et al. 2000).

We found significant sex differences in many parameters and thus analyzed data for males and females separately. We predicted that presence of blood parasites would negatively affect body condition and reproductive success and would induce an immune response reflected in a decreased heterophil:lymphocyte (H:L) ratio as lymphocytes proliferate in response to parasites. To test these predictions, we used 1-tailed *t*-tests (or 1-tailed Mann–Whitney rank sum tests for data that failed tests of normality and equal variance) to compare baseline cort levels, H:L ratios, 2 measures of body condition (see below), number of mates, and number of offspring for birds with and without blood parasites. To assess body condition, we used principal components analysis of tarsus length, wing length, body mass, and fat score. The first principal component (PC1) was most affected by morphometric measures, whereas the second principal component (PC2) primarily reflects variation in fat score (Table 3). In combination, the 2 principal components captured 78% of the variance in these measures. We compared PC1 and PC2 for birds with and without parasites.

Hormone assay

We centrifuged all blood samples within 6 h of collection to separate plasma, which was then drawn out of microcapillary tubes using a Hamilton glass syringe and stored in microtubes at -20°C until assay. We also stored RBCs at -20°C for genetic analyses. We quantified plasma levels of total corticosterone in each sample in duplicate through radioimmunoassay, follow-

ing extraction with doubly distilled dichloromethane (for details, see Wingfield et al. 1992). All samples from a given urban/rural pair were assayed simultaneously. Interassay variation was 8.5%, and intra-assay variation was 5.8%.

RESULTS

Hormone comparisons between birds in urban and rural habitat

The best-fit GLM model explained 29% of the variance in baseline cort levels. Birds responded to urban habitat differently depending on the region of sampling, as reflected by a significant interaction effect of habitat type (urban or rural) with region ($F = 4.28$, degrees of freedom [df] = 2,53, $P = 0.02$). Response to urban habitat was also sex-dependent, as reflected in a significant interaction of habitat type by sex ($F = 11.99$, df = 1,55, $P < 0.01$). None of the other factors had a significant effect. Post hoc tests revealed that urban male birds had significantly higher baseline cort levels than rural males ($P < 0.05$), but females did not differ (Figure 1). When data for males and females were pooled, none of the birds' cort levels differed either by region or by habitat type. Thus, our hypothesis that urban birds would have different cort levels than rural birds was supported for males but not females.

Fitness correlates of variation in cort levels

The extrapair paternity rate in our population was 54%, which is comparable with the rate found in other populations of this species (MacDougall-Shackleton et al. 2002). Of 25 males sampled, 5 sired no offspring that reached fledging age. Of the remaining 20 males, 17 sired at least one extrapair offspring. Three females failed to produce any fledglings. Of the 22 remaining females, 20 produced at least one extrapair offspring.

Male baseline cort levels were not correlated with number of offspring fledged (Figure 2A). Further, male baseline cort levels did not correlate with number of within-pair or extrapair offspring (Figure 2B,C), although the pattern for within-pair offspring approached significance ($z = -1.85$, df = 22, $P = 0.06$). In contrast, females with higher baseline cort levels fledged fewer offspring than females with low cort levels (Figure 3A, $z = -2.33$, df = 24, $P = 0.02$). When reproductive success was divided into within-pair and extrapair offspring, females with higher baseline cort levels fledged fewer within-pair offspring (Figure 3B, $z = -2.61$, df = 24, $P < 0.01$) but did not differ from females with low cort in number of extrapair offspring (Figure 3C). Our hypothesis that variation in cort levels would correlate with a measure of fitness was also found to be sex-dependent, being upheld in females but not males.

Table 3
Summary of principal component loadings for 4 morphometric measures used to assess body condition in 23 female and 22 male *Z. leucophrys*

Metric	PC1	PC2
Tarsus length	0.86	-0.08
Wing length	0.80	0.43
Body mass	0.72	-0.33
Fat score	-0.05	0.94

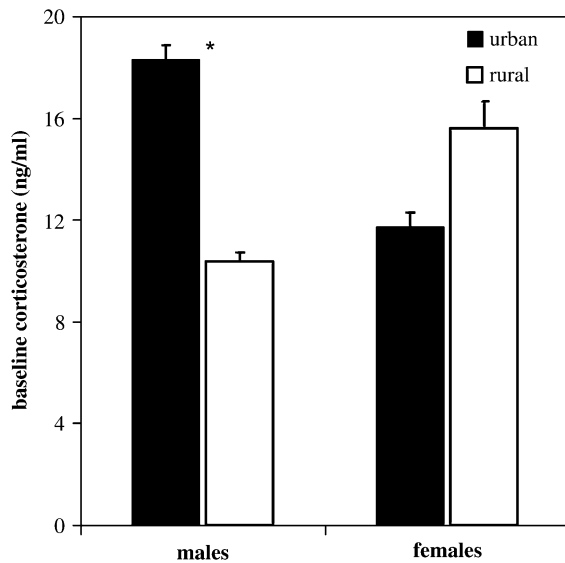


Figure 1
 Comparison of baseline corticosterone levels (mean + standard error) in male and female *Z. leucophrys* breeding in urban (black bars) and rural (white bars) habitat. Urban male *Z. leucophrys* ($N = 16$) had significantly higher baseline cort levels than rural conspecifics ($N = 19$). There were no differences between urban ($N = 14$) and rural ($N = 11$) female birds' hormone levels. Asterisk (*) denotes a significance at the $P < 0.05$ level.

Hematological parameters and blood parasites

Six of 23 females and 7 of 22 males sampled had *Haemaphysalis* infection. Baseline cort levels of females with and without parasites did not differ (Figure 4A). As predicted in birds that have increased lymphocyte production in response to an immune challenge, females with blood parasites had lower H:L ratios than females without parasites (Table 4, Figure 4B, $t = -2.73$, $df = 21$, $P < 0.01$). Females with parasites were in poorer body condition than females without parasites, as reflected by their significantly lower PC2 scores (Figure 4D, $t = -1.68$, $df = 17$, $P = 0.04$). Females' PC1 scores did not differ (Figure 4C). Parasites also were associated with significant fitness costs: females with parasites had fewer mates (Figure 4E, $t = -1.82$, $df = 17$, $P = 0.04$) and fledged fewer offspring (Figure 4F, $t = -2.33$, $df = 17$, $P = 0.01$) than female *Z. leucophrys* without parasites.

Males with parasites were in poorer condition than those without parasites (Figure 4C, PC1: $t = -1.60$, $df = 21$, $P = 0.03$; Figure 4D, PC2: $t = -1.60$, $df = 21$, $P = 0.02$) but did not differ in cort levels, H:L ratios, or reproductive success (Figure 4A,B,E,F). Our hypothesis that parasites may be one of the mechanistic links between fitness and cort secretion was not supported, as birds with and without *Haemaphysalis* parasites did not differ in their cort levels. Nonetheless, parasites were associated with a significant fitness cost in female, but not male, *Z. leucophrys*.

DISCUSSION

Hormone comparisons between birds in urban and rural habitat

Urban male *Z. leucophrys* had higher cort levels than rural males, but females' cort levels did not differ (Table 1, Figure 1). This repeated pattern, found across 3 cities and 2 subspecies, reveals that, at least in the sites we sampled, urban chal-

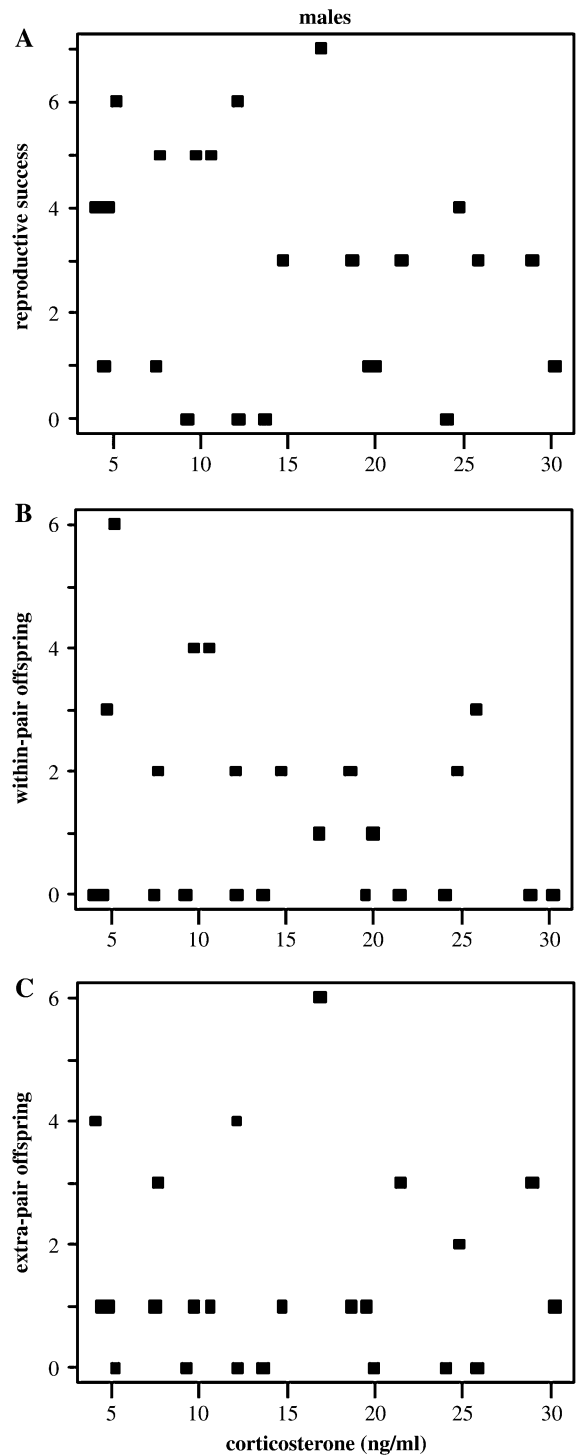


Figure 2
 Poisson regression of cumulative seasonal reproductive success (number of offspring fledged during one breeding season) on male *Z. leucophrys* baseline corticosterone levels. There was no relationship between male reproductive success and baseline corticosterone for total number of offspring fledged (A), number of within-pair offspring (B, those sired with the social mate), or number of extra-pair offspring (C, those sired with another female in the population).

lenges differ from rural challenges and that experience of, or response to, these challenges is sex-specific. Although the difference in cort levels in male birds was consistent across 3 comparisons, the magnitude of the response varied by

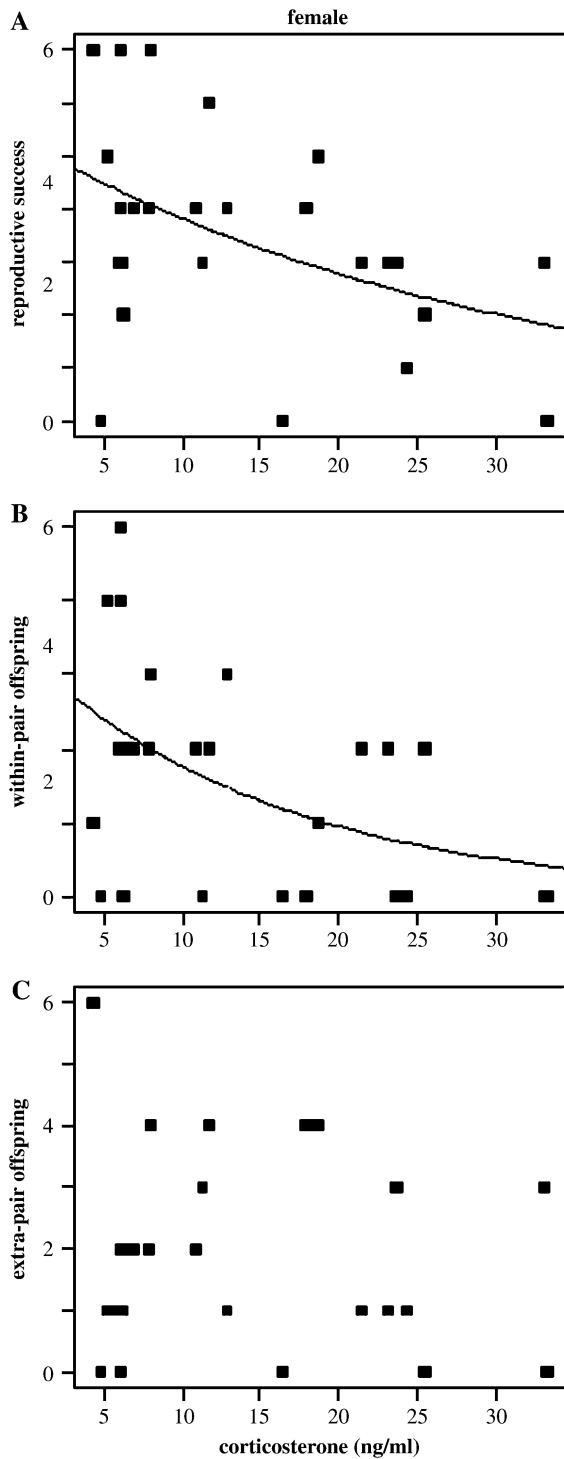


Figure 3
Poisson regression of cumulative seasonal reproductive success (number of offspring fledged during one breeding season) on female *Z. leucophrys* baseline corticosterone levels. Females with high baseline corticosterone levels fledged fewer offspring (A) and had fewer within-pair offspring (B, those sired by the social mate) than females with low hormone levels. There was no relationship between a female's baseline corticosterone level and number of extra-pair offspring (C).

region. This result suggests that population-level differences in physiology, potentially attributable to subspecies differences, and/or differences in environmental factors in each site, such as food availability, population density, and level of hu-

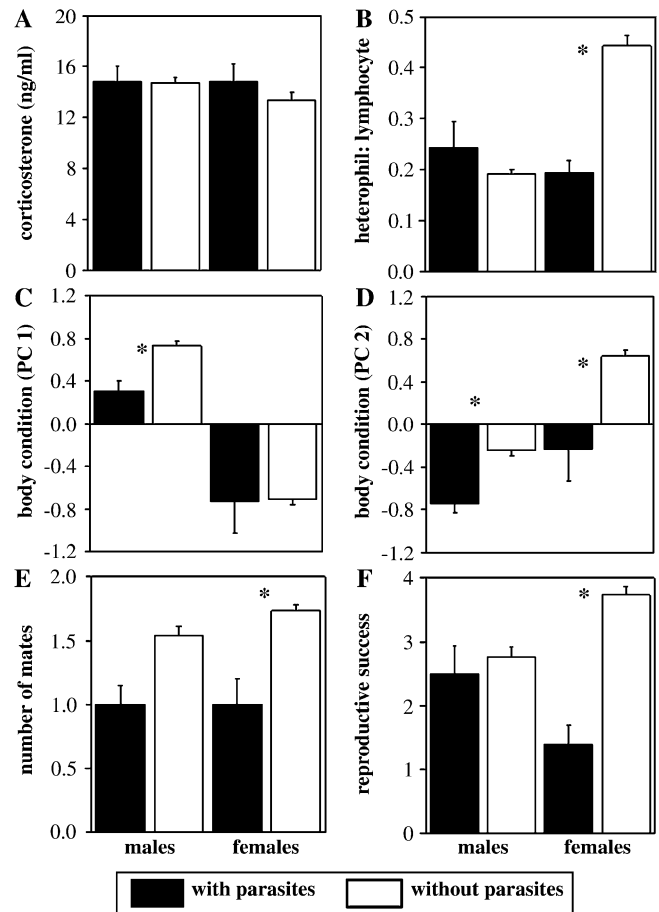


Figure 4
Comparison of several parameters in male and female *Z. leucophrys* with (black bars) and without (white bars) *Haemaphysalis* parasites. There were no differences for either males or females in baseline corticosterone levels (A). Females with parasites had lower H:L ratios (B), lower PC2 scores (D, a measure of body condition), fewer mates (E), and fewer offspring (F) than females without parasites. Males with and without blood parasites differed only in their PC1 (C, a measure of body size and mass) and PC2 scores (D). All data are presented as means + standard error. Asterisks (*) indicate comparisons with $P < 0.05$.

man activity, might also contribute to the birds' physiological response to their environments. Consideration of sex-specific physiological responses might be critical for our understanding of how animals will respond to disturbance and should be considered in future studies. Without an understanding of the fitness consequences of variation in cort levels, the significance of these, or any, findings of variation in cort levels, is difficult to discern.

Fitness correlates of variation in cort levels

We found that cort levels were predictive of female, but not male, reproductive success (Figures 2 and 3). The close link between an animal's condition and cort secretion provides a likely explanation of the costs associated with high cort levels in female *Z. leucophrys*. Cort levels increase with increased physiological demands, including decreased resource availability, history of illness or infection, increased predation risk, and poor territory quality (McEwen and Wingfield 2003; Clinchy et al. 2004). Elevated cort can lead to suppression

Table 4
Summary of WBC counts

Sex	Eosinophils	Basophils	Heterophils	Lymphocytes	Monocytes
Male	<i>0.04 ± 0.00</i>	<i>0.01 ± 0.00</i>	<i>0.13 ± 0.02</i>	<i>0.63 ± 0.02</i>	<i>0.20 ± 0.01</i>
Female	<i>0.04 ± 0.00</i>	<i>0.00 ± 0.00</i>	<i>0.13 ± 0.00</i>	<i>0.66 ± 0.01</i>	<i>0.17 ± 0.00</i>
	0.07 ± 0.00	0.01 ± 0.00	0.20 ± 0.01	0.53 ± 0.01	0.19 ± 0.00

Data presented are mean frequencies of each WBC type (\pm standard error) per 100 cells scored for *Z. leucophrys* with (numbers above, in italics, $N = 6$ female, 7 males) and without (numbers below, $N = 17$ females, 15 males) *Haemaphysalis* infection.

of the reproductive and immune systems (Sapolsky et al. 2000; Padgett and Glaser 2003; Retana-Marquez et al. 2003) and may result in reallocation of resources away from reproduction. All of these effects may be more pronounced in breeding females than in males. Thus, selection may favor resistance to increases in cort levels in females more than males, consistent with our findings of a sex-specific difference in response to urban breeding habitat. Resistance to increased cort levels has been observed in other vertebrates and is hypothesized to minimize fitness costs when the value of current reproductive efforts is high (Wingfield and Sapolsky 2003).

Our finding of a fitness cost associated with increased cort in females may elucidate our previous finding of a lack of a population-level difference between females breeding in urban and rural habitat. Females with increased cort levels in an urban setting appear to be selected against. Habitat selection might be influenced by this selective pressure: females that can resist cort increases in urban settings might preferentially settle there. From our data, we cannot differentiate between nonrandom habitat selection and adaptive responses to selective pressures found in urban settings (i.e., natural selection has shaped urban females to be less physiologically responsive to urban stressors). Both of these possibilities are intriguing and warrant further study. Populations that have recently colonized cities may offer an avenue for investigating this further (cf., Diamond 1986; Yeh and Price 2004).

Urban male *Z. leucophrys* appear to incur reduced fitness costs of increased cort levels, relative to females. Although cort levels did not correlate with offspring fledging success for male breeding birds, we cannot conclude that moderately elevated cort is detrimental in urban *Z. leucophrys*. First, male *Z. leucophrys* in our focal population are the primary parental caregiver during the postfledging stage, when their mate is engaged with initiation of subsequent nesting attempts. Male condition may have a more direct impact on reproductive success at this stage. Potential benefits of increased cort include rapid response to and recovery from challenges, redirection of energy during perturbations, modification of behavior toward coping with an acute challenge, and increased ability to form associations and learn during challenges (Koolhaas et al. 1999; Sapolsky et al. 2000; Pravosudov 2003; Akirav et al. 2004; Romero 2004; Korte et al. 2005). Because of these and other benefits, moderately elevated cort levels may be favored through stabilizing selection in urban male *Z. leucophrys*. Nonlinear fitness relationships are difficult to detect in nature (Kingsolver et al. 2001), but evidence for stabilizing selection on cort levels has been found in a large-scale study of colonially nesting cliff swallows (Brown et al. 2005). Alternatively, poor-quality male birds may tend to be more stress responsive and could predominate in urban environments due to exclusion from higher quality habitat. In this case, high cort levels in urban birds may simply reflect a poor quality of urban habitat.

Hematological parameters and blood parasites

Female *Z. leucophrys* with blood parasites were in poorer body condition, had fewer mates, and fledged fewer offspring than females without parasites, but their cort levels did not differ (Figure 4). Whether the relationship between parasites and reproductive success is a direct or indirect relationship is difficult to discern. An animal's condition may affect allocation of resources for reproduction, as energy must be expended to meet energetic demands. Our findings suggest that infection with blood parasites stimulates the female bird's immune system, as evidenced by decreased H:L ratios, and potentially reduces resources available for reproduction. The reduced body condition of females with parasites further supports this possibility.

An alternative explanation of the relationship between parasites and reproductive success in females is that declining condition results in reduced allocation to reproduction and to the immune system, thereby increasing susceptibility to infections (Oppliger et al. 1998). These 2 explanations are not mutually exclusive, and could in fact act synergistically, making it difficult to identify causality in the relationship we observed. However, if the link between parasites and fitness is condition-dependent, we would expect to find a relationship with cort levels, which increase with declining condition. Additionally, Merino et al. (2000) demonstrated that experimental reduction of *Haemaphysalis* infection in free-ranging blue tits (*Parus caeruleus*) resulted in increased reproductive success suggesting that a causal relationship exists between parasites and fitness.

In contrast to other work in white-crowned sparrows (MacDougall-Shackleton et al. 2002), we found no fitness costs associated with blood parasites in male *Z. leucophrys*. MacDougall-Shackleton et al. (2002) used a different metric to measure reproductive success than we used in this study (number of sired offspring—including all nestlings and unhatched eggs—instead of number of sired fledglings), which could explain the difference in our findings. Further, their study was conducted in a subspecies that is only found breeding at high elevation. In their system, where the breeding season is shorter and resource availability potentially more limited or variable, male parental care might be more important for reproductive success (Badyaev and Ghalambor 2001), favoring females that are choosier in mate selection.

Because of their lack of a relationship with cort levels, parasites do not appear to provide the mechanistic link between cort levels and reproductive success. The ability to mount a physiological response to stressors, as reflected in stress-induced cort levels, might be predicted to provide a link between parasites and reproductive success; but in work conducted in parallel with this study, we found no difference in total or maximum cort levels released in response to capture stress that would support this link (Bonier F, unpublished data).

CONCLUSIONS

Our hypothesis that urban and rural birds would have differing cort levels was supported; urban male *Z. leucophrys* had elevated baseline cort levels as compared with rural conspecifics across 3 paired comparisons, but female birds' hormone levels did not differ (Figure 1). Further, baseline cort levels were negatively correlated with reproductive success in female, but not male, birds (Figure 2 and 3), partially supporting our hypothesis that variation in cort levels is associated with fitness costs. Finally, female birds with blood parasites had fewer mates and fewer offspring than females without parasites, but male reproductive success did not differ (Figure 4). Although there is a significant cost of parasites in female birds, parasites do not appear to provide a mechanistic link between cort levels and fitness, as cort levels did not differ between birds with and without parasites. Variation in physiological and behavioral traits has been found in other urban species (e.g., Ruiz et al. 2002; Slabbekoorn and Peet 2003; Partecke et al. 2004; Schoech et al. 2004; Yeh and Price 2004), but, to our knowledge, our report is the first showing elevated cort levels in an urban organism and one of the first reports of a sex-specific relationship between a fitness component, level of blood parasites, and variation in cort levels (Meylan and Clobert 2005). The repeated pattern of significant sex-specific effects in tests of all 3 of our hypotheses highlights the importance of considering sex when investigating physiological responses to disturbance, particularly in breeding animals.

We thank Lynn Erckmann for logistical support; Ellen Boyd and Tristan Marshall for assistance with analyses; and Z. Morgan Benowitz-Fredericks, Andrew F.G. Bourke, H.D. Toby Bradshaw, Ray Huey, Patrick Kelley, Doug Levey, Ignacio Moore, Trevor Price, Josh Tewksbury, Brian Walker, and 2 anonymous reviewers for helpful comments on early versions of the manuscript. The American Ornithologists' Union, National Science Foundation (IBN 0317141 grant to J.C.W.), Sigma Xi, and a Washington Research Foundation Professorship in Basic Biological Sciences to (H.D. Bradshaw Jr) supported this work. All genetic analyses were conducted in the Comparative Genomics Center in the Biology Department at the University of Washington funded in part by Major Research Instrumentation grant no. 2002236 from the Murdock Foundation. All work was approved by the University of Washington's Institutional Animal Care and Use Committee.

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