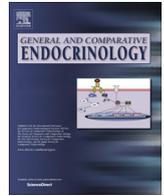


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## Advanced seasonal reproductive development in a male urban bird is reflected in earlier plasma luteinizing hormone rise but not energetic status

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### ABSTRACT

Urban animals inhabit an environment considerably different than do their non-urban conspecifics, and to persist urban animals must adjust to these novel environments. The timing of seasonal reproductive development (i.e., growth of gonads and secondary sex organs) is a fundamental determinant of the breeding period and is frequently advanced in urban bird populations. However, the underlying mechanism(s) by which birds adjust the timing of reproductive development to urban areas remain(s) largely unknown. Here, we compared the timing of vernal reproductive development in free-ranging urban and non-urban male Abert's Towhees, *Melospiza aberti*, in Phoenix, Arizona, USA, and tested the non-mutually exclusive hypotheses that earlier reproductive development is due to improved energetic status and/or earlier increase in endocrine activity of the reproductive system. We found that urban birds initiated testicular development earlier than non-urban birds, but this disparity was not associated with differences in body condition, fat stores, or innate immune performance. These results provide no support for the hypothesis that energetic constraints are responsible for delayed reproductive development of non-urban relative to urban male Abert's Towhees. Urban birds did, however, increase their plasma luteinizing hormone, but not plasma testosterone, earlier than non-urban birds. These findings suggest that adjustment to urban areas by Abert's Towhees involves increases in the endocrine activity of the anterior pituitary gland and/or hypothalamus earlier than non-urban towhees.

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### 1. Introduction

Urbanization profoundly alters ecosystems and produces environments that differ considerably from non-urban areas. Urban areas are characterized by a high proportion of impervious surface (i.e., buildings, roads, etc.), high human density (Nakwa et al., 2008), and elevated levels of noise (Halfwerk and Slabbekoorn, 2013) and artificial light (Gaston et al., 2013). Furthermore, urbanization modifies primary productivity (Buyantuyev and Wu, 2009; Imhoff et al., 2004), food abundance (Cook and Faeth, 2006), and ambient temperature (Imhoff et al., 2010). For urban animal populations to persist, they must adjust to these modified

environmental conditions. As urban spaces are the most rapidly expanding habitat type worldwide (Grimm et al., 2008), the potential impact of urbanization on biodiversity is considerable. There is, therefore, an urgent need to understand the mechanisms responsible for adjustment to these new habitats.

A consistent effect of urbanization on bird populations is an advancement of the timing of seasonal gonadal development (Deviche et al., 2010; reviewed by Deviche and Davies, 2014; Partecke et al., 2005). Although this phenomenon appears to be widespread, a lack of mechanistic studies means that the underlying mechanism(s) remain(s) largely unknown. Most animals have distinct seasonal breeding periods that are synchronized with optimal environmental conditions to maximize fitness (Visser et al., 2006; Williams, 2012). For many vertebrates, including most birds, the transition from the non-breeding to the breeding life history stage is associated with extensive physiological and morphological

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changes, such as increases in the plasma concentrations of reproductive hormones and gonad size, respectively (Murton and Westwood, 1977; Williams, 2012). Reproductive development, in particular growth of the gonads and secondary sex organs, is a fundamental determinant of the breeding period. Hence, it may be advantageous for urban birds to adjust the timing of reproductive development to local environmental conditions.

The timing and/or rate of reproductive development have the potential to be modulated by the activity of the hypothalamo–pituitary–gonadal (HPG) axis through changes in hormone secretion, hormone carrier protein concentrations, and hormone receptor densities. The HPG axis begins with the hypothalamus, the site of production of gonadotropin-releasing hormone-I (GnRH-I; Sharp and Ciccone, 2005). Gonadotropin-releasing hormone-I stimulates the release of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland (Kuenzel, 2000). Gonadotropins initiate gonad development, gametogenesis, secretion of the sex steroids (testosterone (T) and estradiol (E<sub>2</sub>) in males and females, respectively), and expression of reproductive behaviors (Deviche et al., 2010; Murton and Westwood, 1977). The activity of the HPG axis is determined by information provided by a suite of environmental cues that can predict future conditions (Dawson, 2008). In seasonally breeding birds, the annual change in day length (photoperiod) is the initial predictive cue used to begin reproductive development. As changes in day length at a given latitude are constant from year to year, birds use a host of supplementary cues, such as ambient temperature (Schaper et al., 2012b) and food availability (Davies and Deviche, 2014; Hahn et al., 2005), to fine-tune development to a given year's conditions. Urbanization potentially modifies some or all of these non-photoc cues. In particular, urbanization potentially creates differences in food availability between urban and non-urban areas. For example, in Phoenix (Arizona, USA), where the present study was conducted, urbanization is associated with an earlier seasonal growth of plants (Buyantuyev and Wu, 2009, 2010), as well as increased arthropod abundance (Cook and Faeth, 2006).

Understanding the mechanism by which food availability influences seasonal reproductive development is complicated by the fact that food may provide both environmental information (i.e., an abundance of food could signal optimal conditions via physiological pathways independent of energetics; O'Brien and Hau, 2005; Watts and Hahn, 2012) as well as influence energetic status (via effects on food consumption; Davies et al., in press; Hahn et al., 2005). Within the window of opportunity for reproductive development governed by day length, a bird's energetic status, defined as the amount of available energy stores, is thought to constrain the timing of reproductive development (Hahn et al., 2005; Meijer and Drent, 1999). Life history theory posits that when resources are limited there is a resource allocation trade-off between reproduction and self-maintenance, in which allocation into reproduction comes at a cost to somatic processes, such as immune function (Stearns, 1989; Zera and Harshman, 2001). If urbanization affects food availability (see above), this may lead to differences in energetic status between urban birds and their non-urban conspecifics, and, in turn, a disparity in reproductive development, energy stores, and investment into somatic processes, such as innate immunity, between urban and non-urban populations.

We compared the timing of reproductive development in urban and non-urban male Abert's Towhees, *Melospiza aberti*, in Phoenix, Arizona and tested the non-mutually exclusive hypotheses that (1) earlier reproductive development is due to improved energetic status and/or (2) earlier increase in endocrine activity of the reproductive system. We predicted that urban towhees would develop their testes and cloacal protuberance (CP, a T-dependent secondary

sexual characteristic) earlier than non-urban conspecifics. Furthermore, we compared endocrine activity of the HPG axis between these populations and predicted that plasma LH and T, hormones essential for reproductive development and male reproductive function (Deviche et al., 2010), would rise earlier in urban than non-urban birds. If urbanization increases food availability, we also predicted that urban male towhees would have greater endogenous fat stores, be in better body condition (i.e., body mass corrected for body size), and have higher innate immune function compared to non-urban birds.

## 2. Methods

### 2.1. Study species

Abert's Towhees, *M. aberti*, are common in riparian woods and marshes of the Sonoran Desert and also throughout the Phoenix Metropolitan area, particularly in urban yards (Rosenberg et al., 1991). They consume a variety of foods including arthropods and seeds, but arthropods are thought to dominate the diet in all seasons (winter: 73%; summer: 96%; Rosenberg et al., 1991). In urban areas, this species will also consume a wide variety of human-provided food (S. Davies, pers. obs.). Abert's Towhees are sedentary, form life-long pair bonds, and hold a permanent territory (1.5–2 ha; Rosenberg et al., 1991). Captive studies indicate that males are photoperiodic and develop their reproductive system in response to increasing day length (S. Davies, unpublished data). Free-ranging towhees can have multiple broods in a given breeding season and active nests have been found from February to September (Tweit and Finch, 1994). Most brood attempts occur during spring and the number of active nests and CP width increase substantially during March and peak in April (Tweit and Finch, 1994). Breeding during the summer is dependent on monsoon rainfall (Tweit and Finch, 1994), suggesting that, in addition to day length, this species modulates its reproductive activity based on the use of supplementary environmental cues.

### 2.2. Study sites

To investigate the effect of urban areas on vernal development of the reproductive system, we compared adult male Abert's Towhees from six urban and four Sonoran Desert localities in Maricopa County, Arizona (Davies et al., 2013; Table 1). Urban localities were distributed throughout the Phoenix metropolitan area: in the cities of Phoenix (320 m above sea level [m.a.s.l.]; latitude: 33°25'N; longitude: 112°04'W), Gilbert (242 m.a.s.l.; latitude: 33°21'N; longitude: 111°44'W), and Tempe (357 m.a.s.l.; latitude: 33°26'N; longitude: 111°56'W), and include the Arizona State University Tempe campus, residential housing, city parks, and riparian areas adjacent to the Salt River. Desert localities were Robbins Butte Wildlife Area (247 m.a.s.l.; latitude: 33°19'N; longitude: 112°38'W), Powers Butte Wildlife Area (242 m.a.s.l.; latitude: 33°18'N; longitude: 112°43'W), and the confluence of the Agua Fria and Gila Rivers (278 m.a.s.l.; latitude: 33°23'N; longitude: 112°21'W). On average, desert study sites were 9 km from the nearest urban area (i.e., Buckeye, AZ) and 61 km from urban study sites. These desert locations border the Gila River and the vegetation is characteristic of the Sonoran Desert, including mesquite (*Prosopis* spp.), palo verde (*Parkinsonia* spp.), saltbush (*Atriplex* spp.), creosote (*Larrea tridentata*), white bursage (*Ambrosia dumosa*), and willows (*Salix* spp.). These areas also contain dense thickets of invasive salt cedar (*Tamarix* spp.) and, in the case of the Robbins Butte and Powers Butte areas, retired agricultural lands.

**Table 1**

The location and the proportion of impervious surface, vegetation, and open water for each of the study sites sampled. We also provide a qualitative description of the predominant land use type for each of the urban sites.

	Site name	Latitude	Longitude	Predominant urban habitat type	Impervious surface	Vegetation	Open water
Urban	Arizona State University Campus	33°25'N	111°56'W	Residential	51.2	29.3	0.3
	Gilbert Riparian Preserve	33°21'N	111°44'W	Urban park	19.9	40.5	5.3
	Indian Bend Wash	33°26'N	111°54'W	Industrial	35.8	17.9	17.3
	Lo Piano Habitat	33°26'N	111°56'W	Urban park	29.5	15.0	15.5
	Rio Salado	33°25'N	112°04'W	Industrial	45.9	8	1
	West Tempe	33°25'N	111°57'W	Residential	50.7	17.5	0.1
Non-urban	Confluence of the Agua Fria and Salt Rivers	33°23'N	112°21'W	–	0.3	31.4	20.4
	Powers Butte	33°18'N	112°43'W	–	0.0	41.2	4.9
	Robbins Butte (east)	33°19'N	112°37'W	–	3.3	37.2	20.0
	Robbins Butte (west)	33°19'N	112°39'W	–	0.0	35.8	11.2

### 204 2.3. Land use and land cover

205 To classify the land use and land cover (LULC) around our study  
 206 sites, we obtained LULC data within a 1 km radius of each study  
 207 site from the Central Arizona-Phoenix Long-Term Ecological  
 208 Research program database (Li et al., 2014). Briefly, this database  
 209 uses high spatial resolution, 4 band aerial photography from the  
 210 National Agricultural Imagery Program (NAIP) and classifies LULC  
 211 to a resolution of 1 m<sup>2</sup> using object-based image analysis (OBIA).  
 212 Each square meter was classified as one of the following 12 LULC  
 213 types: building, road, soil, tree, grass, shrub, cropland, fallow, lake,  
 214 canal, swimming pool, or seasonal river. These types were then  
 215 grouped into categories of 'impervious surface' (i.e., building and  
 216 road), 'vegetation' (i.e., tree, grass, shrub, cropland, and fallow),  
 217 and 'open water' (i.e., lake, canal, swimming pool, and seasonal  
 218 river).

### 219 2.4. Bird capture and blood collection

220 We caught adult male Abert's Towhees using a mist net and  
 221 playback of conspecific song and 'squeal duets,' a vocalization used  
 222 by both males and females presumably to strengthen pair bonds  
 223 (Tweitt and Finch, 1994). The time of capture ranged from 05:45  
 224 AM to 11:30 AM (mean capture time: urban = 07:41 AM;  
 225 desert = 07:46 AM PST). The study was conducted between  
 226 January and May in 2011 and 2012. In total, we sampled 79  
 227 towhees ( $n = 37$  urban and 42 desert), and any given individual  
 228 was sampled only once during the study. We determined sex and  
 229 age based on behavior (singing and territorial aggression only in  
 230 males; Tweitt and Finch, 1994), skull pneumatization, and morphol-  
 231 ogy (developed CP only in adult males, incubation patch only in  
 232 adult females). To quantify plasma LH and T, we collected a blood  
 233 sample (maximum ~300  $\mu$ l) from the right jugular vein using a  
 234 heparinized 0.3 cc syringe with a 29.5 gauge needle. Although  
 235 the duration of song playback has been shown to be unrelated to  
 236 plasma T in these populations of Abert's Towhees (Fokidis et al.,  
 237 2011) and other Sonoran Desert songbirds (Deviche et al., 2006),  
 238 in many vertebrate species, including multiple species of birds,  
 239 the stress of capture and handling causes a rapid decrease in  
 240 plasma levels of this hormone (Deviche et al., 2012b). Therefore,  
 241 we collected samples within 3 min of capture to represent initial  
 242 (i.e., pre-stress) values. Samples were placed on ice until cen-  
 243 trifuged for 10 min at 10,000 rpm later the same day (within 8 h)  
 244 and the plasma was harvested using a Hamilton glass syringe.  
 245 We stored samples at  $-80^{\circ}\text{C}$  until assay. All experimental proce-  
 246 dures were approved by the Arizona State University  
 247 Institutional Animal Care and Use Committee and conducted under  
 248 appropriate scientific collecting permits issued by the Arizona  
 249 Game and Fish Department and the US Fish and Wildlife Service.

### 250 2.5. Morphometrics

251 Following blood collection, we measured body mass ( $\pm 0.5$  g),  
 252 tarsus length ( $\pm 0.1$  mm), and CP width ( $\pm 0.1$  mm). Body mass and  
 253 tarsus length were linearly related (linear regression:  $r^2 = 0.13$ ,  
 254  $P = 0.001$ ), so we used these parameters to calculate body condi-  
 255 tion following the scaled mass index approach (Peig and Green,  
 256 2009). Body condition is generally assumed to reflect the energy  
 257 stores of an individual; however, this assumption may not hold  
 258 in all cases and direct measures of energy stores may be a more  
 259 accurate estimate of energetic status (Wilder et al., in press). To  
 260 that end, we also quantified fat stores by visually inspecting the  
 261 amount of furcular fat and assigned a score of 0–5 (a score of 0 rep-  
 262 resenting no fat, 5 representing bulging fat deposits). Scaled mass  
 263 index and fat score were positively related (Spearman's rank-order  
 264 correlation:  $r^2 = 0.40$ ,  $P < 0.001$ ), suggesting that scaled mass index  
 265 is a measure of the energetic state of an individual (Peig and Green,  
 266 2009). Following an injection of analgesic (Meloxicam; 0.1 mg/kg)  
 267 into the left pectoral muscle, we measured left testis length and  
 268 width ( $\pm 0.5$  mm) via unilateral laparotomy. Briefly, the bird was  
 269 restrained to a working surface and the feathers on the left flank  
 270 were dampened with 70% alcohol to move them out of the surgical  
 271 field. We swabbed the surgical field with betadine and then topi-  
 272 cally applied lidocaine anesthetic (Akorn, Lake Forest, IL, USA)  
 273 before making a small incision between the last two ribs to expose  
 274 the left testis. Testis length and width were measured by position-  
 275 ing the tips of forceps at each end of the testis. Volume of the testis  
 276 was calculated from the formula for an ovoid sphere:  $V = 4/3 \pi a^2 b$ ,  
 277 where  $V$  is volume,  $a$  is the radius of the testis at its widest point  
 278 and  $b$  is half the long axis. All fat scores and testis measurements  
 279 were made by the same observer (SD). After the procedure, we  
 280 closed the incision using cyanoacrylate adhesive (3 M, St. Paul,  
 281 MN, USA) and applied a topical antibiotic (Alpharma, Baltimore,  
 282 MD, USA). Each bird then received a uniquely numbered aluminum  
 283 US Geological Survey leg band and was released at the capture site.

### 284 2.6. Innate immunity assay

285 To assess immune performance, we quantified lytic and agglu-  
 286 tination capacity using the hemoagglutination-hemolysis assay.  
 287 We modified a previously described protocol that quantifies the  
 288 most dilute plasma concentration that can lyse and agglutinate for-  
 289 eign red blood cells, and has been used in Abert's Towhees (Butler  
 290 et al., 2013). This assay quantifies two aspects of the innate  
 291 immune system and is an ideal candidate to test habitat-related  
 292 intraspecific differences in immune performance for two reasons.  
 293 First, it does not rely on a single, antigen-specific response in indi-  
 294 viduals that may have been exposed to different antigens. Second,  
 295 it is the primary method of host defense for most vertebrate taxa  
 296 (Litman et al., 2005). We serially diluted 30  $\mu$ l of each plasma sam-  
 297 ple from 1:2 to 1:2048 with phosphate-buffered saline (PBS) along

rows of round bottom 96-well plates. As a negative control, the final well of each row contained only PBS. We then added 10  $\mu$ l of 50% heparinized whole sheep blood diluted 1:100 (HemoStat Laboratories, Dixon, CA; SBH050) to each well. After gently vortexing each plate, we covered the plates with Parafilm and incubated them at 39 °C for 90 min. We then tilted plates for 20 min at room temperature before scanning them using a flatbed scanner at 600 dots per inch to quantify agglutination. Following a 70 min incubation at room temperature, we rescanned each plate to quantify lysis. Plate images were independently scored by HB and MG, without knowledge of the sample identity. Scores obtained independently by two observers were repeatable (determined via the intraclass correlation coefficient) for both lysis ( $F_{62,62} = 7.26$ ,  $r^2 = 0.86$ ,  $P < 0.0001$ ) and agglutination ( $F_{62,62} = 39.2$ ,  $r^2 = 0.98$ ,  $P < 0.0001$ ). Thus, average values were used in the analysis. Lysis and agglutination were defined as the lowest plasma concentration at which RBCs were ruptured and at which a compact pellet of RBCs formed, respectively (Butler et al., 2013).

### 2.7. Hormone assays

We quantified plasma LH using a micromodification of a previously described radioimmunoassay (Sharp et al., 1987). This radioimmunoassay has been used extensively to quantify plasma LH in a wide range of avian species (e.g., Fraley et al., 2013; Schaper et al., 2012a), including many emberizid sparrows (Deviche et al., 2012a, 2012b; Meddle et al., 2002; Wingfield et al., 2012). Briefly, 20  $\mu$ l of plasma sample or standard was mixed with 20  $\mu$ l of primary rabbit LH antibody and 20  $\mu$ l of  $I^{125}$ -labeled LH for a total reaction volume of 60  $\mu$ l. The primary antibody was precipitated to separate free and bound  $I^{125}$  label using 20  $\mu$ l of donkey anti-rabbit precipitating serum and 20  $\mu$ l of non-immune rabbit serum. All samples were measured in a single assay, for which the intra-assay coefficient of variation was 3.6% and the minimum detectable limit was 0.2 ng/ml.

We quantified plasma T using a commercial competitive enzyme-linked immunoassay, according to the manufacturer's instructions (Enzo Life Sciences, Farmingdale, NY, USA). We validated this assay in the Abert's Towhee by demonstrating parallelism of a serially diluted plasma sample (4 $\times$  to 64 $\times$  dilutions) with the standard curve using GraphPad Prism 5 (La Jolla, CA, USA). Before assay, plasma was diluted 8 $\times$  with assay buffer containing steroid displacement reagent (Enzo Life Sciences; designed to eliminate interference of binding globulins with antibody binding in the assay) at a concentration equal to 1% of plasma volume. Samples were assayed in duplicate and randomly assigned to assay plates, with a standard curve on each plate. The average detection limit was 21.1 pg/ml. The average intra- and inter-assay coefficients of variation were 6.1% and 17.3%, respectively ( $n = 3$  plates; 94 samples).

### 2.8. Statistical analysis

To test whether LULC differed between our study sites, we calculated the proportion of each LULC classification in the 1 km radius area surrounding each study site. We then arcsine transformed these values and used independent samples Student's  $t$ -tests to examine whether sites differed in the amount of impervious surface, vegetation, and open water. To test if body condition differed between the two habitats and/or changed over the course of spring, we used an ANCOVA with habitat (urban vs. desert) as a fixed factor and day of year (Day 1 = January 1st) as a covariate. We analyzed agglutination capacity using a similar ANCOVA model. Because furcular fat score was an ordinal variable and lytic capacity deviated from normality, we analyzed these data using a generalized linear model with a Poisson distribution. Testis volume,

plasma T, and CP width data were log transformed prior to analysis to attain normality. To test whether urban and desert birds differ in vernal reproductive development, we used ANCOVA with testis volume, plasma LH and T, or CP width as dependent variables, habitat as a fixed factor, and day of year as a covariate. The interaction between habitat and the covariate was non-significant for all ANCOVA tests, demonstrating homogeneity of regression slopes. We also included year (2011 vs. 2012) as a fixed factor in the full models, but found no effect of this factor in any of the tests and so removed it from the models. Linear regression analysis found no diurnal pattern in either plasma LH ( $r^2 = 0.01$ ,  $P = 0.59$ ) or plasma T ( $r^2 = 0.01$ ,  $P = 0.45$ ), so time of capture was not included in either model. For all statistical analyses we used PASW version 20.0 (SPSS Inc., Chicago, Illinois, USA) with an alpha of 0.05. Data are presented as means  $\pm$  standard errors of the mean (SEM) and all graphs depict untransformed data.

## 3. Results

### 3.1. Land use and land cover

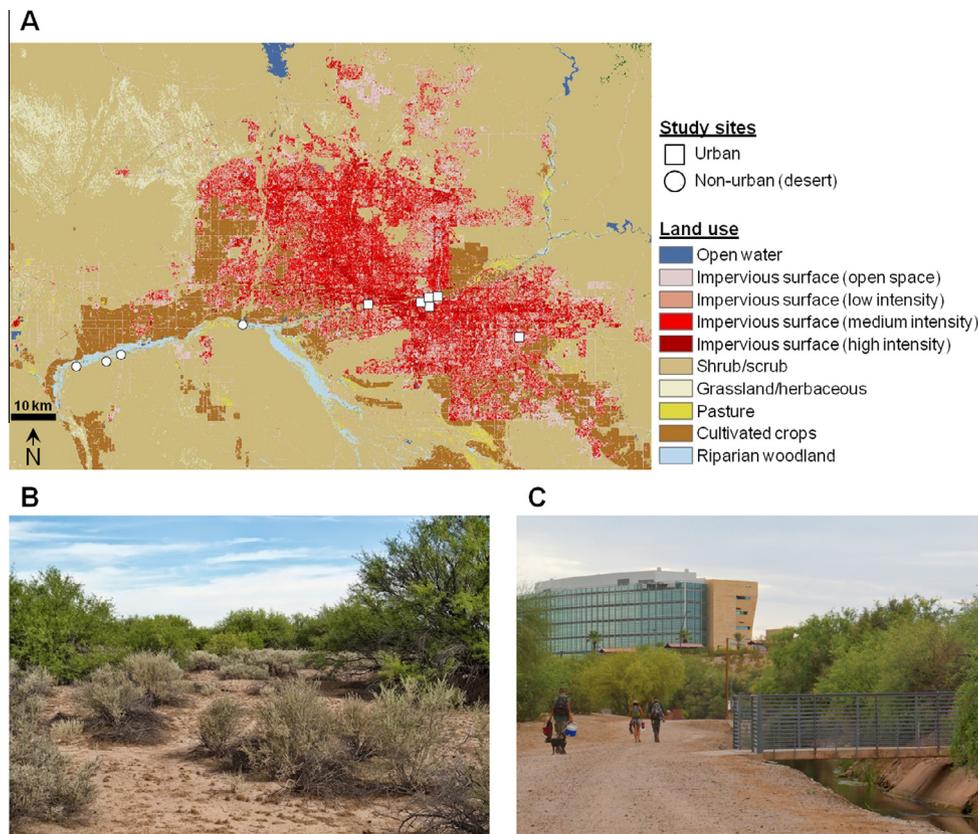
Compared to desert sites, urban sites had a higher proportion of impervious surface (urban:  $38.8 \pm 6.1\%$ , desert:  $0.9 \pm 0.8\%$ ;  $t_8 = 8.79$ ,  $P < 0.0001$ ) and a lower proportion of vegetation (urban:  $21.4 \pm 4.7\%$ , desert:  $35.7 \pm 2.0\%$ ;  $t_8 = -2.55$ ,  $P = 0.034$ ). However, the proportion of open water was similar between the two study site types (urban:  $6.6 \pm 3.7\%$ , desert:  $14.1 \pm 3.7\%$ ;  $t_8 = -1.55$ ,  $P = 0.16$ ). This difference persisted when swimming pools were excluded from the analysis (urban:  $6.5 \pm 3.2\%$ , desert:  $14.1 \pm 3.7\%$ ;  $t_8 = -1.71$ ,  $P = 0.13$ ). The desert sites had a higher – although not significantly different – proportion of open water due to the presence of river beds, which were classified as seasonal river. When river beds were removed from the analysis, there was still no detectable difference in the proportion of open water (urban:  $6.4 \pm 3.3\%$ , desert:  $2.5 \pm 1.9\%$ ;  $t_8 = 0.79$ ,  $P = 0.45$ ) (see Fig. 1).

### 3.2. Reproductive development

Testis volume increased over the course of spring ( $F_{1,76} = 143.52$ ,  $P < 0.0001$ ), and the slopes of the regression lines were similar between the two habitats ( $F_{1,75} = 0.10$ ,  $P = 0.32$ ; Fig. 2), indicating that the rate of testicular development did not differ between urban and non-urban birds. However, the intercept of the regression lines with the horizontal axis (sampling date) when  $y$  is equal to the overall mean testis volume of the two populations was less for urban than desert birds ( $F_{1,76} = 69.01$ ,  $P < 0.0001$ ), indicating that urban birds began testicular development earlier than did non-urban birds. Similarly, CP width increased over the course of spring ( $F_{1,76} = 87.84$ ,  $P < 0.0001$ ; Fig. 2) and the slopes of the regression lines were similar for birds sampled in the two habitats ( $F_{1,75} = 2.39$ ,  $P = 0.13$ ). The intercepts of the CP width regression lines with the horizontal axis when  $y$  is equal to the overall mean CP width of the two populations was less for urban than rural birds ( $F_{1,79} = 34.92$ ,  $P < 0.0001$ ), indicating that urban birds developed CPs earlier than did non-urban birds.

### 3.3. Reproductive physiology

Plasma LH increased over the course of the spring ( $F_{1,73} = 35.73$ ,  $P < 0.0001$ ), and the regression line slopes were similar between the two habitats ( $F_{1,72} = 1.46$ ,  $P = 0.23$ ), indicating that the rate of rise in LH secretion did not differ between urban and non-urban birds (Fig. 3). However, the intercepts of the plasma LH regression lines with the horizontal axis when  $y$  is equal to the overall mean plasma LH of the two populations indicated that the timing of the



**Fig. 1.** A map of the study area with land use and the study sites indicated (A), as well as representative pictures of the non-urban (B) and urban (C) study sites. The map was made using data from the USGS National Map (<http://nationalmap.gov/>). Photographs B and C taken by P. Deviche.

418 rise in plasma LH was earlier in urban than non-urban birds  
419 ( $F_{1,73} = 6.49, P = 0.01$ ; Fig. 3). Plasma T also increased over the  
420 course of the spring ( $F_{1,77} = 9.39, P = 0.003$ ), but did not differ  
421 between urban and desert birds ( $F_{1,77} = 0.02, P = 0.90$ ; Fig. 3).

422 **3.4. Further characterization of reproductive development**

423 To test whether the relationships between plasma LH, testis  
424 volume, CP width, and plasma T differed between urban and  
425 non-urban birds, we used ANCOVAs with habitat as a fixed factor.  
426 However, habitat was not a significant factor in any of the models  
427 (plasma LH vs. testis volume:  $F_{1,76} = 1.09, P = 0.30$ ; plasma LH vs.  
428 plasma T:  $F_{1,76} = 1.95, P = 0.16$ ; plasma LH vs. CP width:  
429  $F_{1,76} = 0.46, P = 0.50$ ; testis volume vs. CP width:  $F_{1,76} = 0.002,$   
430  $P = 0.97$ ; testis volume vs. plasma T:  $F_{1,76} = 1.47, P = 0.23$ ; plasma  
431 T vs. CP width:  $F_{1,76} = 2.56, P = 0.11$ ), indicating that the two groups  
432 were homogenous. We, therefore, combined data from the two  
433 habitats. Linear regression revealed that plasma LH was positively  
434 related to testis volume ( $r^2 = 0.31, P < 0.0001$ ), plasma T ( $r^2 = 0.06,$   
435  $P = 0.034$ ), and CP width ( $r^2 = 0.36, P < 0.0001$ ; Fig. 4). Furthermore,  
436 CP width was positively related to testis volume ( $r^2 = 0.59,$   
437  $P < 0.0001$ ; Fig. 4); however, plasma T was related to neither CP  
438 width ( $r^2 = 0.01, P = 0.50$ ) nor testis volume ( $r^2 = 0.04, P = 0.079$ ;  
439 Figs. 4 and 5).

440 **3.5. Energetic status**

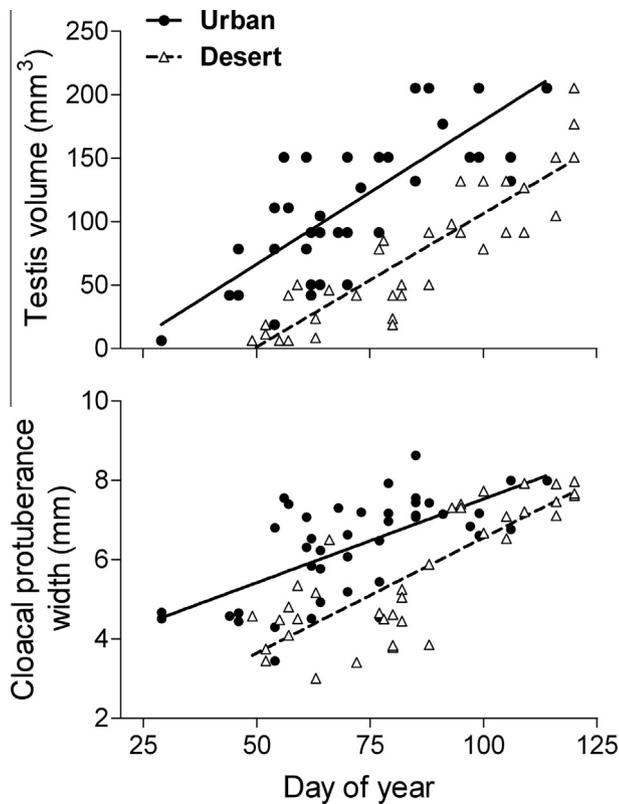
441 Furcular fat score was similar between urban and desert birds  
442 ( $\chi^2_1 = 0.24, P = 0.63$ ) and did not change over the course of spring  
443 ( $\chi^2_1 = 0.76, P = 0.38$ ; Fig. 6). Body condition was also similar between  
444 habitats ( $F_{1,76} = 0.051, P = 0.82$ ) and did not change over the course  
445 of spring ( $F_{1,76} = 0.53, P = 0.47$ ; Fig. 6).

446 **3.6. Immune performance**

447 Lytic capacity was similar between habitats ( $\chi^2_1 = 1.97, P = 0.16$ )  
448 and did not change over the course of spring ( $\chi^2_1 = 0.02, P = 0.89$ ;  
449 Fig. 7). Agglutination capacity was also similar between habitats  
450 ( $F_{1,56} = 0.60, P = 0.44$ ), but there was a (non-significant) trend toward  
451 an increase in capacity over the course of spring ( $F_{1,56} = 3.826,$   
452  $P = 0.055$ ; Fig. 7).

453 **4. Discussion**

454 We found that urban male Abert's Towhees activated their HPG  
455 axis, as assessed by plasma LH, testis volume, and CP width, earlier  
456 than their desert conspecifics. This observation is consistent with  
457 previous studies on other urban species of birds (Deviche et al.,  
458 2010; Partecke et al., 2005; Schoech and Bowman, 2003).  
459 Assuming that the testes are functional (i.e., produce sperm) at  
460 approximately half maximum size (Partecke et al., 2005; Young  
461 et al., 2001), the results suggest that urban male Abert's Towhees  
462 are capable of breeding approximately 32 days earlier than their  
463 desert conspecifics (as estimated by the intercepts of the testis vol-  
464 ume data). Although we did not measure lay date and further  
465 research is, therefore, necessary to demonstrate that the two pop-  
466 ulations differ in their actual onset of breeding, the present obser-  
467 vations are consistent with the proposition that earlier gonad  
468 development plays a role in advancing the breeding phenology of  
469 urban bird populations (Chamberlain et al., 2009; Deviche and  
470 Davies, 2014; Partecke et al., 2005). Whether differences in the  
471 timing of reproductive development and/or onset of breeding in  
472 urban birds are adaptive remains unclear and potential  
473 population-level effects of this phenomenon require scrutiny.



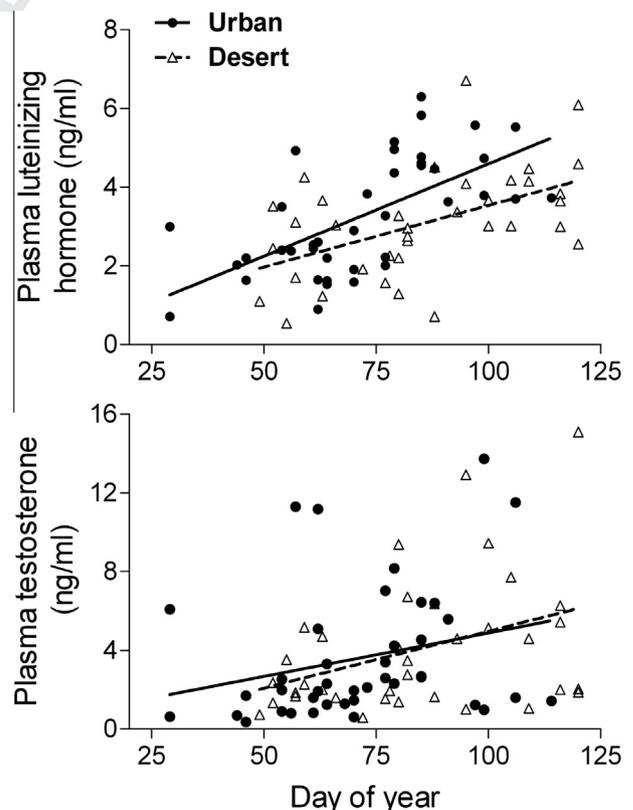
**Fig. 2.** The vernal development of testis volume and cloacal protuberance width was advanced in free-ranging Abert's Towhees, *Melospiza aberti*, inhabiting urban (filled circles) localities in Phoenix, AZ, USA compared to their non-urban, desert conspecifics (open triangles). Each point represents one individual. On the horizontal axes, 1 = January 1st.

a resource allocation trade-off between reproduction and self-maintenance (Stearns, 1989), in which allocation to reproduction comes at a cost to somatic processes, such as immune function (Zera and Harshman, 2001). If a disparity in energetic status plays a role in the difference in timing of reproductive development between urban and desert male towhee populations, we, therefore, also predicted population differences in lysis and agglutination capacity. Again, this was not the case. Therefore, multiple lines of evidence provide no support for the hypothesis that energetic constraints limit reproductive development of desert male Abert's Towhees compared to their urban conspecifics. We cannot exclude the possibility that there was indeed a difference in body condition that our measurement of body mass did not detect. However, such a difference would be small (i.e., less than 0.5 g or 1% of the average Abert's Towhee body mass). We also stress that plasma lysis and agglutination capacity does not provide a comprehensive evaluation of all aspects of immune performance, and we cannot exclude the possibility that urban and desert towhees may differ in other aspects of their immune system. Furthermore, we cannot exclude a role for energetic status in female Abert's Towhees, whereby energetic status may be more important in modulating reproductive development of females than males. A study of Florida Scrub-jays, *Aphelocoma coerulescens*, opposes this proposition, however, because urban females bred earlier than their non-urban conspecifics and had higher plasma protein levels, but did not differ in body condition, total body lipids, or plasma calcium (Schoech and Bowman, 2003). Similarly, urban male and female European Blackbirds, *Turdus merula*, developed their gonads earlier than non-urban conspecifics, but body mass did not differ between the populations and fat stores were actually lower in the urban blackbirds (Partecke et al., 2005). Taken together, these

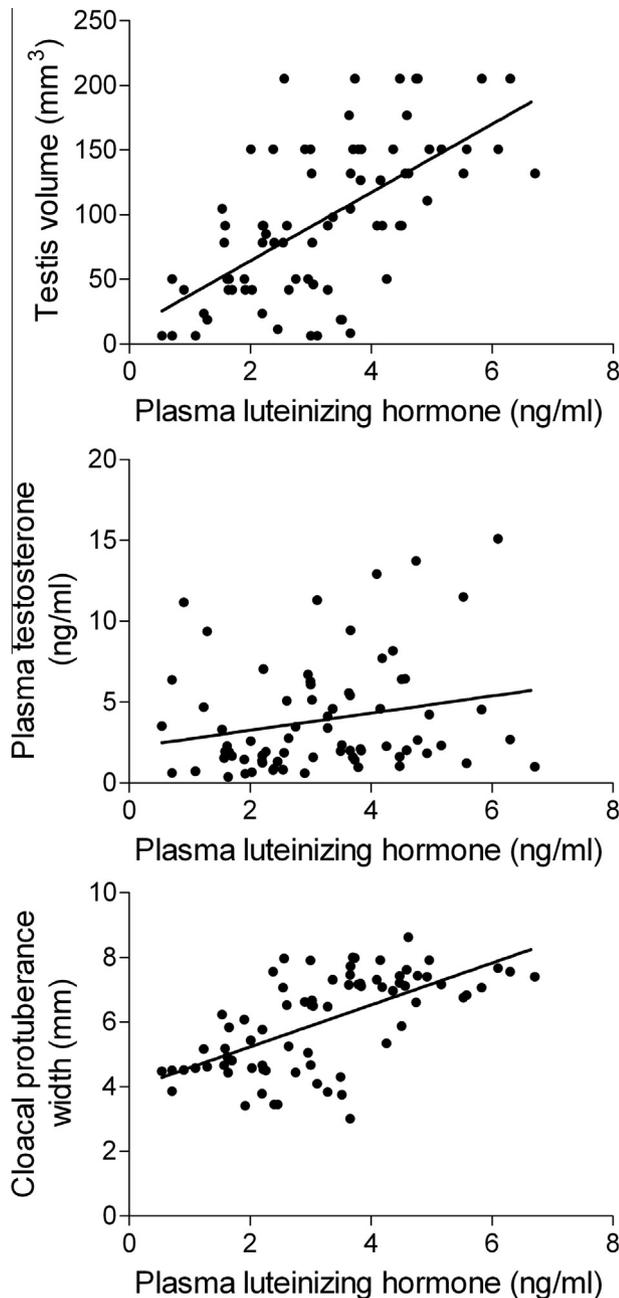
Indeed, the few studies that have examined reproductive success in urban versus non-urban birds suggest that it is highly variable and additional research is needed on this topic (reviewed by Chamberlain et al., 2009). We are not aware of any study that has tested whether vertebrate classes other than birds also advance the timing of reproductive development or breeding in urban areas. Many fish, amphibian, reptile and mammal species inhabit urban areas around the world. Since the timing of breeding has a strong influence on reproductive success, differences in the timing of breeding associated with inhabiting urban areas may determine the presence and abundance of vertebrates in urban areas. These vertebrate classes warrant investigation to reveal whether modified timing of reproductive development or breeding is a general response of vertebrates to urban areas, as well as to enable an evidence-based approach to conservation of urban populations.

#### 4.1. Energetic constraints

To investigate whether reproductive development is constrained by a bird's energetic status, we examined whether urban and desert Abert's towhee populations differed in fat stores, body condition, and innate immune performance, as assessed by plasma lysis and agglutination capacity. If desert towhee populations develop their reproductive system later than their urban conspecifics due to an energetic constraint, we predicted corresponding disparities in fat stores and body condition between the two populations. However, this was not the case. Furthermore, neither fat stores nor body condition changed over the course of the spring. Life history theory predicts that when resources are limited there is



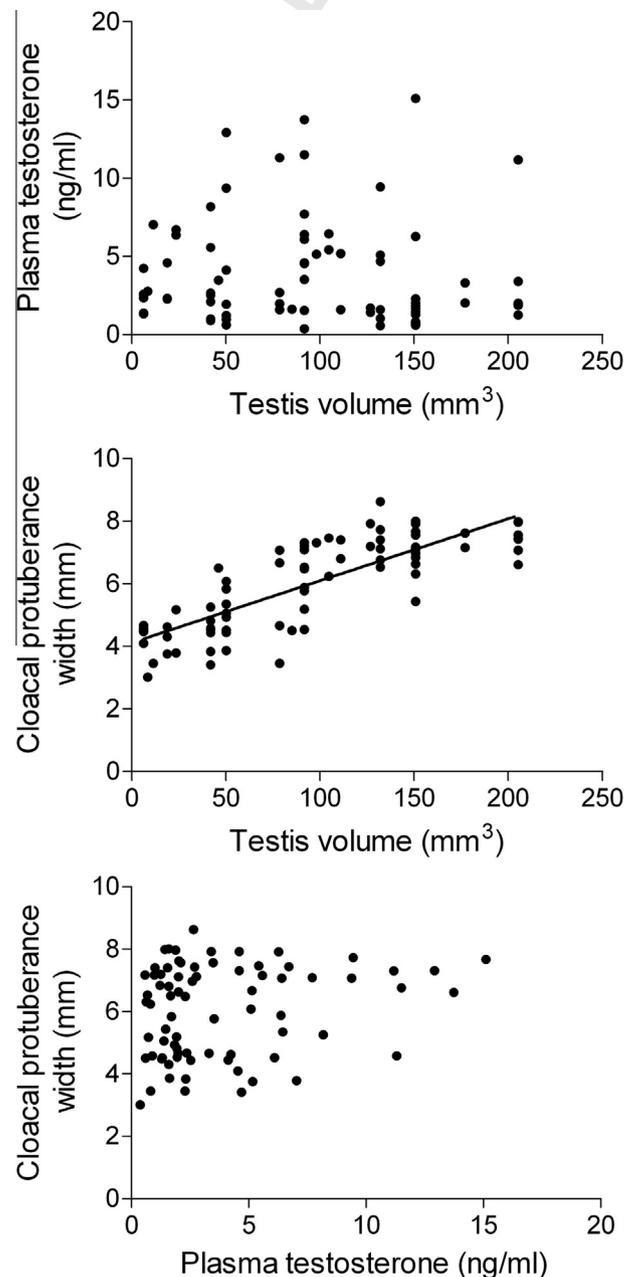
**Fig. 3.** Free-ranging urban male Abert's Towhees, *Melospiza aberti*, (filled circles) increased plasma levels of luteinizing hormone, but not plasma testosterone, earlier than did non-urban towhees (open triangles). Each point represents one individual. On the horizontal axes, 1 = January 1st.



**Fig. 4.** Plasma luteinizing hormone was positively related to testis volume, plasma testosterone, and cloacal protuberance width in free-ranging Abert's Towhees, *Melospiza aberti*. Each point represents one individual.

proposition is also supported by the results of captive studies in which towhees developed their testes in response to long artificial photoperiod (S. Davies, unpublished data). Urban and desert sites in the present study are located at similar latitudes and the advancement in reproductive development of urban relative to desert towhees is, therefore, not due to differences in natural day length. A wealth of studies indicate that supplementary environmental cues, such as artificial light, ambient temperature, plant growing seasons, and food supply, differ between urban and non-urban areas. However, urban areas are heterogeneous, comprising of various habitat types (residential, industrial, riparian, parks, etc.) that differ with respect to their environmental characteristics and, therefore, also potential cues that urban birds use to

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**Fig. 5.** Relationships between testis volume, cloacal protuberance width, and plasma testosterone in free-ranging Abert's Towhees, *Melospiza aberti*. Testis volume was not related to plasma testosterone, but was related to cloacal protuberance width. There was also no relationship between plasma testosterone and cloacal protuberance width. Each point represents one individual. For the day of year axis, 1 = January 1st.

results provide little support for the hypothesis that the delay in reproductive development observed in non-urban birds results from these birds being energetically constrained.

#### 4.2. Supplementary environmental cues

Our findings are, however, consistent with the hypothesis that differences in information provided by environmental cues in urban versus desert areas account for the advanced timing of reproductive development in urban birds. The pattern of seasonal reproductive development during increasing spring day lengths is consistent with the Abert's Towhee being photoperiodic and using increasing day length as the initial predictive cue to initiate endocrine activity of the HPG axis and reproductive development. This

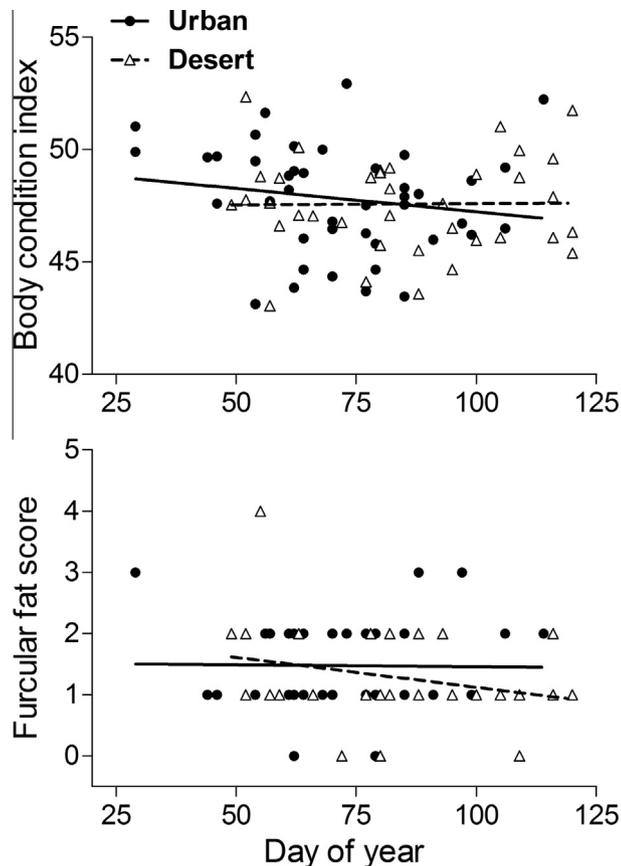


Fig. 6. Body condition and furcular fat stores were similar in free-ranging Abert's Towhees, *Melospiza aberti*, inhabiting urban (filled circles) and non-urban, desert (open triangles) localities in and around Phoenix, AZ, USA. Each point represents one individual. On the horizontal axis, 1 = January 1st. Note that the trend lines are not significant.

many arthropods are temperature-dependent (van Asch and Visser, 2007). Accordingly, evidence suggests that the urban heat island effect advances the timing of the vernal increase in plant growth in urban areas (Buyantuyev and Wu, 2009, 2012; Imhoff et al., 2004). Warmer spring temperatures are associated with earlier seasonal breeding in wild birds (Visser et al., 2006; Williams, 2012). Studies in controlled laboratory settings also suggest that ambient temperature plays a role in the timing of reproductive development (Schaper et al., 2012b). It is conceivable, therefore, that the elevated ambient temperatures in urban areas advance reproductive development of urban birds. However, the average increase in urban ambient temperature is relatively small (i.e., just a few degrees; Imhoff et al., 2010) and we are not aware of any evidence that such small increases by themselves suffice to cause differences in the phenology of reproductive development of birds (DeViche and Davies, 2014). We are also not aware of studies with sufficient temporal resolution to test whether urban areas are associated with advances in the phenology of arthropod emergence, but long-term studies of caterpillar phenology demonstrate that the peak in caterpillar abundance is highly synchronized with tree phenology (van Asch and Visser, 2007; Visser et al., 2006). If the timing of arthropod emergence parallels the advanced plant growing season in urban areas, we predict that arthropod abundance will increase earlier in the year in urban areas compared to non-urban areas. Bird populations synchronize breeding with the peak in food availability (Visser et al., 2006), and may use the timing of plant and/or arthropod phenology as environmental cues to optimally time reproduction to local conditions. We, therefore,

regulate their reproductive system activity. Whether the latter is the case cannot be determined from the present data and warrants further research.

Artificial light is a ubiquitous characteristic of urban areas (Gaston et al., 2013) and may contribute to advancing the timing of seasonal reproductive development (Kempnaers et al., 2010), such as observed in the current study. This difference may result from direct stimulation of the HPG axis, whereby artificial light directly activates hypothalamic encephalic receptors and leads to a false perception of longer days earlier in the season. However, as yet, there is limited evidence for such a direct effect and findings from studies in controlled laboratory conditions are contradictory (DeViche and Davies, 2014; Dominoni et al., 2013; Schoech et al., 2013; Spoelstra and Visser, 2014). Furthermore, the spectral sensitivity of avian hypothalamic encephalic receptors suggests that direct activation of the HPG axis by urban light is unlikely (DeViche and Davies, 2014). Additional studies are necessary, however, to elucidate whether artificial lights in urban areas directly stimulate the HPG axis and cause the earlier reproductive development observed here or, instead, exert indirect effects on reproductive development (e.g., by changing behavior; DeViche and Davies, 2014; Spoelstra and Visser, 2014).

The process of urbanization, particularly the replacement of vegetation and evaporative soil surfaces with impervious, low-albedo paving and buildings, causes urban areas, including Phoenix (Buyantuyev and Wu, 2010), to be warmer than non-urban areas, and creates 'urban heat islands' (Imhoff et al., 2010). The seasonal growth of many plants and the emergence of

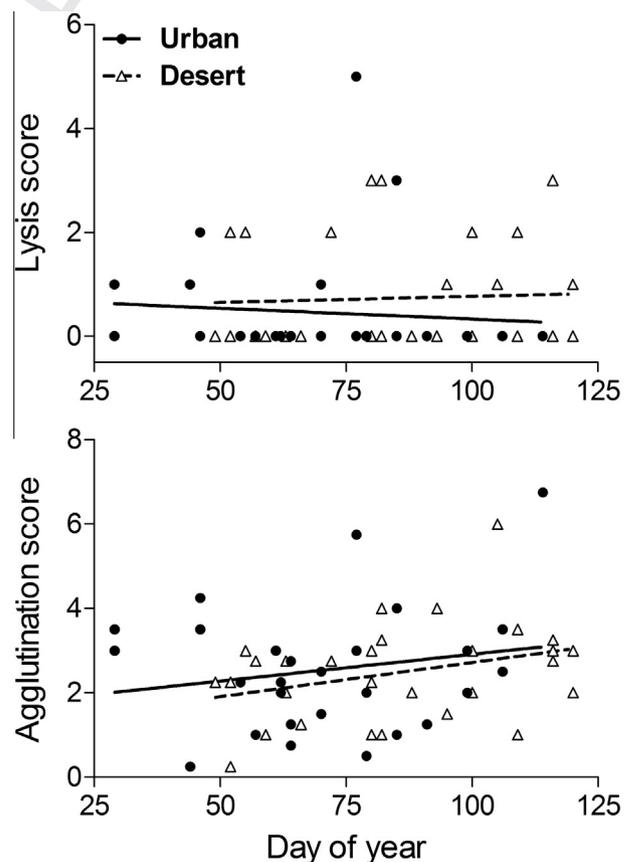


Fig. 7. Immune performance, as measured by lysis and agglutination capacities, was similar in free-ranging Abert's Towhees, *Melospiza aberti*, inhabiting urban (filled circles) and non-urban, desert (open triangles) localities in and around Phoenix, AZ, USA. Each point represents one individual. On the horizontal axis, 1 = January 1st. Note that the trend lines are not significant.

suggest that the advances in reproductive development of urban male Abert's Towhees are associated with advances in the timing of plant growth, which itself may cause early arthropod emergence. Human-provided food, such as from feeders and discarded food waste, available in urban areas may constitute an additional environmental cue, giving birds the perception that local food availability peaks earlier in urban areas compared to non-urban areas (Deviche and Davies, 2014).

#### 4.3. Physiological mechanisms

To shed light on the physiological mechanism responsible for modulating reproductive development to urban areas, we examined whether the advanced reproductive development of male Abert's Towhees in Phoenix is associated with an earlier increase in endocrine activity of the HPG axis. Consistent with its pivotal role in gonad development, plasma LH increased earlier in urban than desert towhees, and levels of this hormone were related to testis volume. Our results suggest, therefore, that the earlier increase in plasma LH of urban towhees contributes to the advanced reproductive development of this population. However, plasma T was similar between the populations and was not correlated with testis volume. This finding suggests that the timing of reproductive development in male Abert's Towhees is adjusted to urban areas, at least partly, as a result of an earlier increase activity of the anterior pituitary gland and/or hypothalamus. Furthermore, we suggest two non-mutually exclusive hypotheses to account for the finding that plasma T was similar between urban and desert towhees – despite plasma LH being higher in urban towhees. First, plasma levels of LH are more temporally stable than are levels of T, and, hence, plasma LH is a better predictor of gonad development. In support of this proposition, plasma T has a shorter half-life and is more responsive to stimuli such as acute stress than plasma LH (Deviche et al., 2012a, 2012b; Norris, 2007). Our second hypothesis is that there may be a habitat-related difference in the regulation of gonadal endocrine function in response to LH stimulation. That is, a given plasma concentration of LH may elicit more plasma T production in desert towhees, due to, for example, differences in LH receptor density and/or the activity of enzymes involved in T production between the populations.

The endocrine control of reproductive development and/or lay date of urban and non-urban bird populations have been compared in just two other species, to our knowledge, and the findings of these studies are inconsistent. For example, despite developing testes 20 days earlier than non-urban European Blackbirds, urban male blackbirds had lower plasma LH and T (Partecke et al., 2005). Urban female European Blackbirds, on the other hand, which developed follicles 28 days earlier than non-urban blackbirds, had similar plasma LH and  $E_2$  to non-urban blackbirds (Partecke et al., 2005). By contrast, a 20 day earlier initiation of first clutches in urban female Florida Scrub-jays was mirrored by an earlier increase in plasma LH, but not  $E_2$  (Schoech and Bowman, 2003). Thus, the association between reproductive development (i.e., testis volume and CP width) and endocrine activity of urban and non-urban bird populations appears to be variable. Although few studies simultaneously examine reproductive development, endocrine activity, and lay date, the available evidence suggests that a lack of association between these parameters is widespread (Caro et al., 2006; Schaper et al., 2012a). The apparent lack of association between plasma T and testis development may be accounted for by differences in the effects of photoperiod on plasma LH versus plasma FSH. In the White-crowned Sparrow, *Zonotrichia leucophrys*, and the Great Tit, *Parus major*, plasma LH and, in turn, plasma T peak substantially before the testes reach their maximum development, and levels of these hormones fall at the time of breeding (Silverin, 1984). In the Great Tit, plasma

FSH, on the other hand, increases later than plasma LH and reaches it peaks around the time of breeding (Silverin et al., 1997). The available evidence, therefore, suggests that, although plasma levels of reproductive hormones are broadly indicative of the timing of breeding in birds, these levels do not consistently reflect fine-scale (i.e., a month or less) differences in breeding between populations. Future research on avian reproductive ecology – including populations in urban areas – may benefit from measuring not only plasma levels of reproductive hormones, but also hormone receptor densities in target tissues and factors downstream of hormone binding. In support of this, studies on the endocrine control of behavior in birds have found that steroid receptor density often predicts the occurrence of steroid-dependent behaviors better than do plasma hormone levels (e.g., Horton et al., 2014).

#### 4.4. Conclusions

A burgeoning body of research demonstrates that birds adjust to urban areas by breeding earlier than their non-urban conspecifics at the same day length. However, the underlying cause of this difference is unclear. We found that urban male Abert's Towhees developed their gonads and secondary sexual organs earlier than did their desert conspecifics. These results add to the mounting evidence indicating that urban birds develop their reproductive system earlier than do their nearby non-urban conspecifics. Our results suggest that urban and desert towhees are in similar energetic status and that the earlier reproductive development of urban birds is potentially due to differences in the timing and/or strength of information provided by environmental cues in urban versus desert areas. We, therefore, encourage future research to focus on potential environmental cues that differ between urban and non-urban areas. Prominent candidates include the timing of seasonal increase of plants and food supply. The physiological mechanism responsible for differences in timing of reproductive development remains unclear, but our results suggest that the advanced reproductive development of urban towhees arises from an earlier increase in endocrine activity at the anterior pituitary gland and/or hypothalamic level.

#### 5. Data accessibility

Data are permanently deposited and available in the NSF LTER Network database (link: <http://caplter.asu.edu/data/data-catalog/?id=613>).

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#### References

- Butler, M.W., Stahlschmidt, Z.R., Ardia, D.R., Davies, S., Davis, J., Guillette Jr., L.J., Johnson, N., McCormick, S.D., McGraw, K.J., DeNardo, D.F., 2013. Thermal sensitivity of immune function: evidence against a generalist-specialist trade-off among endothermic and ectothermic vertebrates. *Am. Nat.* 181, 761–774.

- Buyantuyev, A., Wu, J., 2009. Urbanization alters spatiotemporal patterns of ecosystem primary production: a case study of the Phoenix metropolitan region, USA. *J. Arid Environ.* 73, 512–520.
- Buyantuyev, A., Wu, J., 2010. Urban heat islands and landscape heterogeneity: linking spatiotemporal variations in surface temperatures to land-cover and socioeconomic patterns. *Landscape Ecol.* 25, 17–33.
- Buyantuyev, A., Wu, J., 2012. Urbanization diversifies land surface phenology in arid environments: interactions among vegetation, climatic variation, and land use pattern in the Phoenix metropolitan region, USA. *Landscape Urban Plan.* 105, 149–159.
- Caro, S.P., Lambrechts, M.M., Chastel, O., Sharp, P.J., Thomas, D.W., Balthazart, J., 2006. Simultaneous pituitary–gonadal recrudescence in two Corsican populations of male blue tits with asynchronous breeding dates. *Horm. Behav.* 50, 347–360.
- Chamberlain, D.E., Cannon, A.R., Toms, M.P., Leech, D.I., Hatchwell, B.J., Gaston, K.J., 2009. Avian productivity in urban landscapes: a review and meta-analysis. *Ibis* 151, 1–18.
- Cook, W.M., Faeth, S.H., 2006. Irrigation and land use drive ground arthropod community patterns in an urban desert. *Environ. Entomol.* 35, 1532–1540.
- Davies, S., Rodriguez, N.S., Sweazea, K.L., Deviche, P., 2013. The effect of acute stress and long-term corticosteroid administration on plasma metabolites in an urban and desert songbird. *Physiol. Biochem. Zool.* 86, 47–60.
- Davies, S., Deviche, P., 2014. At the crossroads of physiology and ecology: food supply and the timing of avian reproduction. *Horm. Behav.* 66, 41–55.
- Davies, S., Cros, T., Richard, D., Meddle, S.L., Tsutsui, K., Deviche, P., in press. Food availability, energetic constraints, and reproductive development in a wild seasonally breeding songbird. *Funct. Ecol.*
- Dawson, A., 2008. Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. *Philos. Trans. R. Soc. B* 363, 1621–1633.
- Deviche, P., Davies, S., 2014. Reproductive phenology of urban birds: environmental cues and mechanisms. In: Gil, D., Brumm, H. (Eds.), *Avian Urban Ecology: Behavioral and Physiological Adaptations*. Oxford University Press, Oxford, UK, pp. 98–115.
- Deviche, P., Dawson, A., Sabo, J., Fokidis, B., Davies, S., Hurley, L., 2012a. Up to the challenge? Hormonal and behavioral responses of free-ranging male Cassin's sparrows, *Peucaea cassinii*, to conspecific song playback. *Horm. Behav.* 61, 741–749.
- Deviche, P., Gao, S., Davies, S., Sharp, P.J., Dawson, A., 2012b. Rapid stress-induced inhibition of plasma testosterone in free-ranging male rufous-winged sparrows, *Peucaea carpalis*: characterization, time course, and recovery. *Gen. Comp. Endocrinol.* 177, 1–8.
- Deviche, P., Hurley, L.L., Fokidis, H.B., 2010. Avian testicular structure, function, and regulation. In: Norris, D.O., Lopez, K.H. (Eds.), *Hormones and Reproduction of Vertebrates*. Academic Press, San Diego, CA, pp. 27–70.
- Deviche, P., Small, T., Sharp, P., Tsutsui, K., 2006. Control of luteinizing hormone and testosterone secretion in a flexibly breeding male passerine, the Rufous-winged sparrow, *Aimophila carpalis*. *Gen. Comp. Endocrinol.* 149, 226–235.
- Dominoni, D., Quetting, M., Partecke, J., 2013. Artificial light at night advances avian reproductive physiology. *Proc. R. Soc. Lond. B Biol. Sci.* 280, 20123017.
- Fokidis, H.B., Orchinik, M., Deviche, P., 2011. Context-specific territorial behavior in urban birds: no evidence for involvement of testosterone or corticosterone. *Horm. Behav.* 59, 133–143.
- Fraleigh, G.S., Coombs, E., Gerometta, E., Colton, S., Sharp, P.J., Li, Q., Clarke, I.J., 2013. Distribution and sequence of gonadotropin-inhibitory hormone and its potential role as a molecular link between feeding and reproductive systems in the Pekin duck (*Anas platyrhynchos domestica*). *Gen. Comp. Endocrinol.* 184, 103–110.
- Gaston, K.J., Bennie, J., Davies, T.W., Hopkins, J., 2013. The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev.* 88, 912–927.
- Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X., Briggs, J.M., 2008. Global change and the ecology of cities. *Science* 319, 756–760.
- Hahn, T.P., Pereyra, M.E., Katti, M., Ward, G.M., MacDougall-Shackleton, S.A., 2005. Effects of food availability on the reproductive system. In: Dawson, A., Sharp, P.J. (Eds.), *Functional Avian Endocrinology*. Narosa, New Delhi, pp. 167–180.
- Halfwerk, W., Slabbekoorn, H., 2013. The impact of anthropogenic noise on avian communication and fitness. In: Gil, D., Brumm, H. (Eds.), *Avian Urban Ecology: Behavioral and Physiological Adaptations*. Oxford University Press, Oxford, UK, pp. 84–97.
- Horton, B.M., Hudson, W.H., Ortlund, E.A., Shirk, S., Thomas, J.W., Young, E.R., Zinzow-Kramer, W.M., Maney, D.L., 2014. Estrogen receptor alpha polymorphism in a species with alternative behavioral phenotypes. *Proc. Natl. Acad. Sci. USA* 111, 1443–1448.
- Imhoff, M.L., Bounoua, L., DeFries, R., Lawrence, W.T., Stutzer, D., Tucker, C.J., Ricketts, T., 2004. The consequences of urban land transformation on net primary productivity in the United States. *Remote Sens. Environ.* 89, 434–443.
- Imhoff, M.L., Zhang, P., Wolfe, R.E., Bounoua, L., 2010. Remote sensing of the urban heat island effect across biomes on the continental USA. *Remote Sens. Environ.* 114, 504–513.
- Kempnaers, B., Borgström, P., Loës, P., Schlicht, E., Valcu, M., 2010. Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds. *Curr. Biol.* 20, 1735–1739.
- Kuenzel, W.J., 2000. Central nervous system regulation of gonadal development in the avian male. *Poult. Sci.* 79, 1679.
- Li, X., Myint, S.W., Zhang, Y., Galletti, C., Zhang, X., Turner II, B.L., 2014. Object-based land-cover classification for metropolitan Phoenix, Arizona, using aerial photography. *Int. J. Appl. Earth Obs.* 33, 321–330.
- Litman, G.W., Cannon, J.P., Dishaw, L.J., 2005. Reconstructing immune phylogeny: new perspectives. *Nat. Rev. Immunol.* 5, 866–879.
- Meddle, S.L., Romero, L.M., Astheimer, L.B., Buttemer, W.A., Moore, I.T., Wingfield, J.C., 2002. Steroid hormone interrelationships with territorial aggression in an arctic-breeding songbird, Gambel's white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Horm. Behav.* 42, 212–221.
- Meijer, T., Drent, R., 1999. Re-examination of the capital and income dichotomy in breeding birds. *Ibis* 141, 399–414.
- Murton, R.K., Westwood, N.J., 1977. *Avian Breeding Cycles*. Clarendon Press, Oxford.
- Nakwa, A., Sitasuwan, N., Jatisatein, A., Chantaramongko, P., Pupichit, W., Srisak, P., 2008. The effects of tourists on bird diversity in tourist area compared to restricted area of seasonal evergreen forest at Tung Salang Luang National Park, Phetchabun province. *Thai. Int. J. Zool. Res.* 4, 96–105.
- Norris, D.O., 2007. *Vertebrate Endocrinology*. Academic Press, Boston, USA.
- O'Brien, S., Hau, M., 2005. Food cues and gonadal development in neotropical spotted antbirds (*Hylophylax naevioides*). *J. Ornithol.* 146, 332–337.
- Partecke, J., Van't Hof, T., Gwinner, E., 2005. Underlying physiological control of reproduction in urban and forest-dwelling European blackbirds *Turdus merula*. *J. Avian Biol.* 36, 295–305.
- Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* 118, 1883–1891.
- Rosenberg, K.V., Ohmart, R.D., Hunter, W.C., Anderson, B.W., 1991. *Birds of the Lower Colorado River Valley*. Univ. Arizona Press, Tucson.
- Schaper, S.V., Dawson, A., Sharp, P.J., Caro, S.P., Visser, M.E., 2012a. Individual variation in avian reproductive physiology does not reliably predict variation in laying date. *Gen. Comp. Endocrinol.* 179, 53–62.
- Schaper, S.V., Dawson, A., Sharp, P.J., Gienapp, P., Caro, S.P., Visser, M.E., 2012b. Increasing temperature, not mean temperature, is a cue for avian timing of reproduction. *Am. Nat.* 179, E55–E69.
- Schoech, S.J., Bowman, R., 2003. Does differential access to protein influence differences in timing of breeding of Florida scrub-jays (*Aphelocoma coerulescens*) in suburban and woodland habitats? *Auk* 120, 1114–1127.
- Schoech, S.J., Bowman, R., Hahn, T.P., Goymann, W., Schwabl, I., Bridge, E.S., 2013. The effects of low levels of light at night upon the endocrine physiology of western scrub-jays (*Aphelocoma californica*). *J. Exp. Zool. A* 319, 527–538.
- Sharp, P.J., Ciccone, N., 2005. The gonadotropin releasing hormone neurone: key to avian reproductive function. In: Dawson, A., Sharp, P.J. (Eds.), *Functional Avian Endocrinology*. Narosa, New Delhi, pp. 59–72.
- Sharp, P.J., Dunn, I.C., Talbot, R.T., 1987. Sex differences in the LH responses to chicken LHRH-I and -II in the domestic fowl. *J. Endocrinol.* 115, 323–331.
- Silverin, B., 1984. Annual gonadotropin and testosterone cycles in free-living male birds. *J. Exp. Zool.* 232, 581–587.
- Silverin, B., Kikuchi, M., Ishii, S., 1997. Seasonal changes in follicle-stimulating hormone in free-living great tits. *Gen. Comp. Endocrinol.* 108, 366–373.
- Spoelstra, K., Visser, M.E., 2014. The impact of artificial light on avian ecology. In: Gil, D., Brumm, H. (Eds.), *Avian Urban Ecology: Behavioral and Physiological Adaptations*. Oxford University Press, Oxford, UK, pp. 21–28.
- Stearns, S.C., 1989. Trade-offs in life-history evolution. *Funct. Ecol.* 3, 259–268.
- Tweit, R.C., Finch, D.M., 1994. Abert's towhee (*Melospiza aberti*). In: Poole, A. (Ed.), *The Birds of North America*. Cornell Lab of Ornithology, Ithaca.
- van Asch, M., Visser, M.E., 2007. Phenology of forest caterpillars and their host trees: the importance of synchrony. *Annu. Rev. Entomol.* 52, 37–55.
- Visser, M.E., Holleman, L.J.M., Gienapp, P., 2006. Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia* 147, 164–172.
- Watts, H.E., Hahn, T.P., 2012. Non-photoperiodic regulation of reproductive physiology in the flexibly breeding pine siskin (*Spinus pinus*). *Gen. Comp. Endocrinol.* 178, 259–264.
- Wilder, S.M., Raubenheimer, D., Simpson, S.J., in press. Moving beyond body condition indices as an estimate of fitness in ecological and evolutionary studies. *Funct. Ecol.*
- Williams, T.D., 2012. *Physiological Adaptations for Breeding in Birds*. Princeton University Press, Princeton, NJ, USA.
- Wingfield, J.C., Sullivan, K., Jaxion-Harm, J., Meddle, S.L., 2012. The presence of water influences reproductive function in the song sparrow (*Melospiza melodia morphna*). *Gen. Comp. Endocrinol.* 178, 485–493.
- Young, K.A., Ball, G.F., Nelson, R.J., 2001. Photoperiod-induced testicular apoptosis in European starlings (*Sturnus vulgaris*). *Biol. Reprod.* 64, 706–713.
- Zera, A.J., Harshman, L.G., 2001. The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* 32, 95–126.

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