Zhao *et al. Avian Res (2017) 8:18*
DOI 10.1186/s40657-017-0075-4

Avian Research

RESEARCH Open Access

Life-history dependent relationships between plasma alkaline phosphatase activity and body condition in male Eurasian Tree Sparrows

Yuliang Zhao[1†], Lingjuan Gong[1†], Baohua Zhao[1], Xuebin Gao[2], Yuefeng Wu[1] and Dongming Li[1*]

Abstract

Background: In temperate-breeding birds, individuals must adjust their physiological states from one life-history stage to another in response to changing conditions to maximize ecological fitness. Previous evidences have shown that body mass, size-corrected mass (SCM), and hematocrit (Hct) could be used as estimates of the energetic state of individuals to illustrate life-history trade-offs and individual quality in field physiology. Plasma alkaline phosphatase (ALP) plays critical roles in regulating the metabolism of energy but very limited information is known on its link with body mass or Hct.

Methods: We determined the changes of plasma ALP levels in both early breeding and wintering stages of male Eurasian Tree Sparrows (*Passer montanus*), and examined the relationships between ALP and body mass, SCM, and Hct of the birds.

Results: Our study showed that (1) in male Eurasian Tree Sparrows, body mass did not vary with life-history stage but plasma ALP activity significantly increased in the wintering stage compared to the breeding stage; (2) ALP activity was not correlated with individual body mass but was positively correlated with individual SCM and Hct. Such positive correlations, however, only occurred in the wintering but not in the breeding stages.

Conclusions: Our results suggest that plasma ALP activities in free-living birds can be used as one of the indicators of body condition or nutritional status for analyzing individual variation in the wintering but not in the breeding stages. The life-history dependent relationships between plasma ALP activity and body condition may contribute to our better understanding of the trade-off between individual survival and reproduction in free-living animals.

Keywords: Alkaline phosphatase, Size-corrected mass, Hematocrit, *Passer montanus*, Life history, Trade-off

Background

In temperate-breeding birds, individuals must be able to adapt to the annual cycle of changing environmental conditions by adjusting morphology, physiology and behavior (Wingfield 2008). Phenotypic flexibility, which means an individual switches its phenotype (e.g. morphological, physiological, and behavioral traits) from one life-history stage to another, is a critical way to maximize ecological fitness (Piersma and Drent 2003; Wingfield 2008).

Alkaline phosphatase (ALP) is an isozyme family in various tissues particularly concentrated in the liver, kidney, bone, and placenta (Iqbal 2011). ALP is a hydrolase enzyme responsible for the dephosphorylation from nucleotides, proteins, alkaloids etc., therefore, it plays critical roles in regulating the metabolism of energy and minerals (Lallès 2014). Previous studies have shown that plasma ALP activity is associated with bone growth and can be a sensitive indicator of skeletal development in

*Correspondence: lidngmng@gmail.com
†Yuliang Zhao and Lingjuan Gong contributed equally to the work
[1] Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology of Hebei Province, College of Life Sciences, Hebei Normal University, Shijiazhuang 050024, China
Full list of author information is available at the end of the article

various avian species, e.g. Spanish Imperial Eagle (*Aquila adalberti*) (Dobado-Berrios and Ferrer 1997), Black Vulture (*Aegypius monachus*) (Villegas et al. 2002), Pigeon Guillemot (*Cepphus columba*) (Seiser et al. 2000), White Stork (*Ciconia ciconia*) (Smits et al. 2007), and Great Tit (*Parus major*) (Tilgar et al. 2004, 2008). Furthermore, evidences have shown that plasma ALP activity is positively correlated with nutritional status, and can vary rapidly in response to the availability of food (Viñuela and Ferrer 1997; Villegas et al. 2002).

The energy reserves and nutritional state of individuals in free-living birds can be reflected by body condition, including body mass and size-corrected mass (SCM; Bryant 1988; Jakob et al. 1996; Merila and Wiggins 1997; Zhao et al. 2017). Hematocrit (Hct), the volume of red blood cells within the total volume of blood, is used as an estimate of the extent and efficiency of oxygen-carrying capacity of individuals (Fair et al. 2007; Pap et al. 2015). Generally, a low Hct is generally associated with poor nutritional state (Richner et al. 1993; Fair et al. 2007). In birds, Hct has been commonly used as body condition index, and also regarded as an indicator of individual quality and fitness (Fair et al. 2007; Williams 2012; Zhao et al. 2017). Furthermore, body mass, SCM, and Hct all vary with life-history stage (Stearns 1989; Wojczulanis-Jakubas et al. 2015; Krause et al. 2016; Zhao et al. 2017), which is associated with plasma ALP activities (Villegas et al. 2002).

Recently, assessment of body condition and nutritional status has become more common in ecological studies as an approach to understanding the individual variations and life history trade-offs (Cam et al. 2016; Crates et al. 2016). In view of plasma ALP activity being considered as a physiological indicator, it may have useful applications in the assessment of the individual variability and physiological state from different life-history stages. The information of life-history dependent relationship between plasma ALP activity and body condition, is critical to understanding how free-living animals fine-tune the trade-off between reproduction and survival so as to optimize their chances of survival during nonbreeding stage, and successful reproduction during breeding stage (Stearns 1989; Cox et al. 2010). However, little information is available on the link between plasma ALP activity and body condition.

The Eurasian Tree Sparrow (*Passer montanus*) is a seasonally breeding species that is widely distributed across the Eurasian continent. Previous studies have demonstrated that the body mass or body condition of Eurasian Tree Sparrows vary with life-history stage (Li et al. 2008, 2011, 2012; Zheng et al. 2014) and living environment (Zhang et al. 2011; Sun et al. 2016, 2017). Furthermore, male sparrows during the early breeding stage had significantly greater Hct values compared to those from the early wintering stage, whereas there were no significant differences in SCM between the two life-history stages (Zhao et al. 2017). In the present study, we further determined the changes of plasma ALP levels in both the breeding and the wintering stages, and examined the relationships between ALP and SCM, and Hct in the same experimental animals (Zhao et al. 2017).

Methods

Experimental protocol and sample collection

Free-living Eurasian Tree Sparrows (*Passer montanus*) were captured opportunistically by mist nets in October 24, 2015 (the early wintering stage) and April 28, 2016 (the early breeding stage), on the campus of Hebei Normal University, Hebei Province, China (38°01.83′N, 114°31.50′E, elevation: 75 m). The birds were sexed by polymerase chain reaction following the procedures described in Round et al. (2007) since the Eurasian Tree Sparrow is sexually monomorphic. Subsequent measurements of body condition and plasma ALP activities were performed on male birds only.

Each individual was caged (50 cm × 34 cm × 33 cm) at an ambient temperature of 20 °C, and provided with food and water ad libitum. Birds sampled in the wintering stage were kept under a stimulated natural photoperiod (10L/14D; $n = 27$), whereas those sampled in the breeding stage were kept under a stimulated natural photoperiod (14L/10D; $n = 26$). Birds were acclimated under these conditions for 12–14 days.

After acclimation, approximately 80 μL blood were collected by piercing the alar vein with a 26-gauge needle and collecting blood into heparinized microhematocrit capillary tubes. Blood samples were stored on ice before being centrifuged at 855*g* for 10 min. Hct was measured as described in Zhao et al. (2017). Fresh plasma was split into several fractions and stores at −80 °C, and one of fractions was used for ALP assay.

Each bird was weighed to the nearest 0.1 g, and its wing length was measured to the nearest 1 mm. The SCM was calculated and reported in Zhao et al. (2017). After sampling, the birds were used for other experiments. All protocols were approved by the Institutional Animal Care and Use Committee (HEBTU2013-7), and the Ethics and Animal Welfare Committee (No. 2013-6) of Hebei Normal University, China, and were carried out under scientific collecting permits issued by the Departments of Wildlife Conservation (Forestry Bureau) of Hebei Province, China.

Assays of plasma ALP activity

Plasma ALP activities were measured by an automatic biochemical analyzer (Mindray BS-180) with

commercially available kits (Mindray Corp., Shenzhen, China) after the plasma was diluted with dH_2O (1:39). All samples were run in duplicate. The intra- and inter-assay variations were 6.9 and 9.4%, respectively.

Statistical analyses

We ran Shapiro–Wilk normality test to examine the normal distributions of body mass, SCM, Hct, and ALP activity. All the variables met the normal distribution. The statistical significance of differences in body mass and plasma ALP activity between the breeding and the wintering stages were determined by Welch's *t*-tests (the data of SCM and Hct have been reported in Zhao et al. 2017). We ran a multiple regression analysis (MRA) using the *glm* function to obtain optimal models of plasma alkaline phosphatase (ALP) activity against life-history stage and mass, SCM, or Hct and their interactions in Program R v. 3.3.2. (R Core Team 2016). The homogeneity of variances was tested with three outliers removed before conducting statistical tests. All analyses were performed and all figures generated, using car, MASS, and ggplot2 packages in Program R v. 3.3.2. The data are shown as mean ± SD.

Results

Comparison of body mass and ALP activity between the wintering and the breeding stages

There were no significant differences in body mass of male Eurasian Tree Sparrows between the breeding (18.88 ± 0.19 g) and the wintering stages (19.36 ± 0.23 g; $t_{1,57} = 1.59$, $p = 0.118$), whereas the birds in the breeding stage had significantly lower ALP activities (442.9 ± 9.9 U/L) than those in the wintering stage (593.4 ± 18.0 U/L; $t_{1,51} = 7.2$, $p < 0.001$).

Correlations between ALP activity and body mass, SCM, or Hct

ALP activity was not correlated with individual body mass in both stages. However, ALP activity was positively correlated with individual SCM and Hct (Table 1). There were significant effects of the interaction 'season × SCM' and 'season × Hct', respectively (Table 1). Specifically, we found a positive relationship between ALP and SCM, or Hct in the wintering, but no significant relationships in the breeding stages (statistical results are shown in Fig. 1).

Table 1 Statistical results of best fitted regression models of plasma alkaline phosphatase (ALP) activity against life-history stage (stage, dummy variable) and body mass (Mass), size-corrected mass (SCM), or hematocrit (Hct) and their interactions in male Eurasian Tree Sparrows (*Passer montanus*) from the breeding and the wintering stages

Variable	Model	Standardized β value	SE	*t*	*p*
Mass	Intercept	0.658	0.148	4.461	<0.001
	Stage: breeding	–1.290	0.209	–6.179	*<0.001*
	Mass	0.187	0.106	1.774	0.083
SCM	Intercept	0.790	0.136	5.799	<0.001
	Stage: breeding	–1.456	0.190	–7.653	*<0.001*
	SCM	0.534	0.146	3.655	*0.001*
	Stage × SCM	–0.556	0.194	–2.868	*0.006*
Hct	Intercept	0.873	0.140	6.245	<0.001
	Stage: breeding	–1.587	0.200	–7.930	*<0.001*
	Hct	0.540	0.142	3.793	*<0.001*
	Stage × Hct	–0.506	0.202	–2.501	*0.016*

Italics mean that there is statistical significance in the results, in which significance is set at $p = 0.05$

Discussion

Seasonal differences in body mass and ALP activity

Body mass in birds is often affected by food availability and therefore it can reflect the amount of stored energy (Merila and Wiggins 1997; Cuthill et al. 2000). Loss of body mass is thought to reflect the mobilization of energy stores via protein catabolism during periods of increased energetic expenditure (Bryant 1988; Merila and Wiggins 1997). However, the absence of significant differences in body mass and SCM (reported in Zhao et al. 2017) of male Eurasian Tree Sparrows between the breeding and the wintering stages suggests that the energetic requirements might be constant in the two life-history stages.

The mean ALP activities in male Eurasian Tree Sparrows lay within the previously documented ranges in European Starlings (*Sturnus vulgaris*) but are higher than the values in Red-winged Blackbirds (*Agelaius phoeniceus*), Northern Bobwhites (*Colinus virginianus*, and Common Grackles (*Quiscalus quiscula*) (Hill and Murray 1987) and Black Vulture (Villegas et al. 2002). We found male Eurasian Tree Sparrows during the breeding stage had significantly decreased ALP activities compared to those in the wintering stage. Our results are in line with previous findings in captive Northern Bobwhites, Red-winged Blackbirds, Common Grackles but European Starlings (Hill and Murray 1987). Given that ALP activity could be affected by food availability or energetic condition, whether such seasonal differences are associated with life-history trade-offs of resources allocation warrants to be further determined.

Life-history dependent relationships between plasma ALP activity and body condition

The plasma ALP activities in male Eurasian Tree Sparrows were positively correlated with SCM and Hct during the wintering stage. Our results are in accordance with a positive relationship between ALP activity and condition

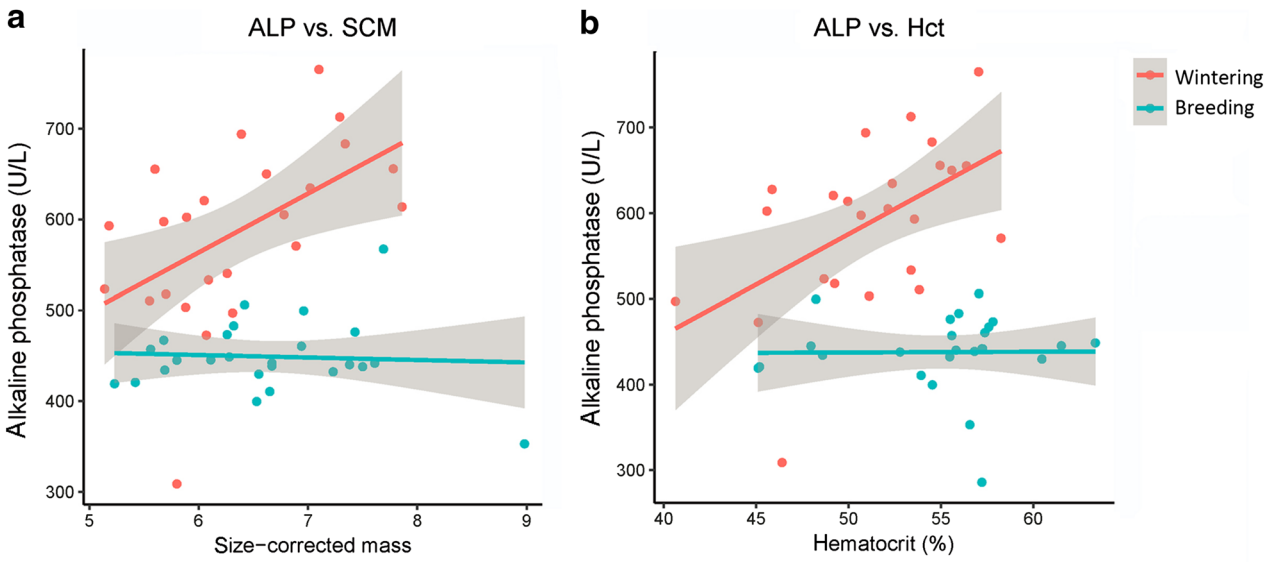

Fig. 1 Correlations between plasma alkaline phosphatase (ALP) and size-corrected mass (SCM) (**a** wintering: $df = 22$, $t = 2.858$, $p = 0.009$; breeding: $df = 23$, $t = -0.281$, $p = 0.781$), and hematocrit (Hct) (**b** wintering: $df = 22$, $t = 3.036$, $p = 0.006$; breeding: $df = 23$, $t = 0.372$, $p = 0.713$), of male Eurasian Tree Sparrows in the wintering or the breeding stages. Shaded areas are 95% confidence limits

index in Black Vulture nestlings (Villegas et al. 2002), and between ALP activity and nutritional status in rats (Martins et al. 1998).

Given that SCM and Hct are defined as the status of metabolic reserves (Newton 1993; Fair et al. 2007) and plasma ALP activity is involved in regulating energetic metabolism (Lallès 2014), the positive relationships between plasma ALP activity and SCM, or Hct may provide reliable evidences for direct link of morphological indices and hematologic parameters. Our results suggest that the plasma ALP activity can be used as an indicator of the physiological state for analyzing phenotypic flexibility and individual variation related to body condition or nutritional status of free-living animals in the wintering stage. Whether individuals with better body condition and higher ALP activities have increased fitness requires further investigation.

However, in the breeding stage, plasma ALP activities of male Eurasian Tree Sparrows were neither correlated with SCM nor Hct. Breeding is considered as an energetically expensive activity that requires high levels of oxygen delivery to active tissues (Williams 1966), which can be reflected by elevated Hct in this period relative to nonbreeding stage, e.g. in male Eurasian Tree Sparrow (Zhao et al. 2017), and White-crowned Sparrow (*Zonotrichia leucophrys*) (Krause et al. 2016). Compared to the nonbreeding stage, male Eurasian Tree Sparrows in breeding stage require sufficient energy and nutrients to meet the demands of maintaining reproduction-related physiological and behavioral activities, e.g. exhibiting peak T levels, competing for territories, building nests, etc. (García-Navas et al. 2008; Li et al. 2012). In the present study, our results indicate that individuals with greater value of SCM and Hct do not necessarily have higher plasma ALP activities in breeding stage.

Therefore, we found the relationships between plasma ALP activity and SCM, or Hct varied with life-history stage, which is consistent with the relationships between plasma immunological indices and SCM, or Hct in male Eurasian Tree Sparrows (Zhao et al. 2017). Whether such life-history dependent relationships between hematologic parameters and body condition can reflect a strategy of a shift in energy allocation from self-maintenances to reproduction in free-living birds during the breeding stage, i.e. trade-off between individual survival and reproduction, remains to be further investigated.

Conclusions

In summary, male Eurasian Tree Sparrows exhibited elevated plasma ALP activity but lower Hct values during the wintering compared to the breeding stages. The positive correlations between plasma ALP activities and individual SCM or Hct occurred in the wintering but not in the breeding stages, which suggests that plasma ALP activities can be used as one of the indicators of body condition or nutritional status in free-living birds for analyzing individual variation in nonbreeding stage. Such life-history dependent relationships between plasma ALP activity and body condition may contribute to our better understanding of the trade-off between individual survival and reproduction in free-living animals.

Authors' contributions
DML conceived the research project and led the writing, YLZ and LG collected the data, BHZ, XBG, and YFW analyzed the data. All authors read and approved the final manuscript.

Author details
[1] Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology of Hebei Province, College of Life Sciences, Hebei Normal University, Shijiazhuang 050024, China. [2] Shaanxi Institute of Zoology, Xi'an 710032, China.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (Grant Nos. 31672292, 31372201), and the Natural Science Foundation of Hebei Province (C2017205059).

Competing interests
The authors declare that they have no competing interests.

Received: 24 January 2017 Accepted: 24 June 2017
Published online: 06 July 2017

References

Bryant DM. Energy expenditure and body mass changes as measures of reproductive costs in birds. Funct Ecol. 1988;2:23–4.
Cam E, Aubry LM, Authier M. The conundrum of heterogeneities in life history studies. Trends Ecol Evol. 2016;31:872–86.
Cox RM, Parker EU, Cheney DM, Liebl AL, Martin LB, Calsbeek R. Experimental evidence for physiological costs underlying the trade-off between reproduction and survival. Funct Ecol. 2010;24:1262–9.
Crates RA, Firth JA, Farine DR, Garroway CJ, Kidd LR, Aplin LM, Radersma R, Milligan ND, Voelkl B, Culina A, Verhelst BL, Hinde CA, Sheldon BC. Individual variation in winter supplementary food consumption and its consequences for reproduction in wild birds. J Avian Biol. 2016;47:678–89.
Cuthill IC, Maddocks SA, Weall CV, Jones EKM. Body mass regulation in response to changes in feeding predictability and overnight energy expenditure. Behav Ecol. 2000;11:189–95.
Dobado-Berrios PM, Ferrer M. Age-related changes of plasma alkaline phosphatase and inorganic phosphorus, and late ossification of the cranial roof in the Spanish imperial eagle (*Aquila adalberti* C.L. Brehm, 1861). Physiol Zool. 1997;70:421–7.
Fair J, Whitaker S, Pearson B. Sources of variation in haematocrit in birds. Ibis. 2007;149:535–52.
García-Navas V, Arroyo L, José Sanz J. Nestbox use and reproductive parameters of tree sparrows *Passer montanus*: are they affected by the presence of old nests? Acta Ornithol. 2008;43:32–42.
Hill EF, Murray HC. Seasonal variation in diagnostic enzymes and biochemical constituents of captive northern bobwhites and passerines. Comp Biochem Physiol B. 1987;87:933–40.
Iqbal J. An enzyme immobilized microassay in capillary electrophoresis for characterization and inhibition studies of alkaline phosphatases. Anal Biochem. 2011;414:226–31.
Jakob EM, Marshall SD, Uetz GW. Estimating fitness: a comparison of body condition indices. Oikos. 1996;77:61–7.
Krause JS, Németh Z, Pérez JH, Chmura HE, Ramenofsky M, Wingfield JC. Annual Hct profiles in two subspecies of white-crowned sparrow: a migrant and a resident comparison. Physiol Biochem Zool. 2016;89:51–60.
Lallès JP. Intestinal alkaline phosphatase: novel functions and protective effects. Nutr Rev. 2014;72:82–94.
Li DM, Wang G, Wingfield JC, Zhang Z, Ding CQ, Lei FM. Seasonal changes in adrenocortical responses to acute stress in Eurasian tree sparrow (*Passer montanus*) on the Tibetan Plateau: comparison with house sparrow (*P. domesticus*) in North America and with the migratory *P. domesticus* in Qinghai Province. Gen Comp Endocrinol. 2008;158:47–53.
Li DM, Wu JZ, Zhang XR, Ma XF, Wingfield JC, Lei FM, Wang G, Wu YF. Comparison of adrenocortical responses to acute stress in lowland and highland Eurasian Tree Sparrows (*Passer montanus*): similar patterns during the breeding, but different during the prebasic molt. J Exp Zool. 2011;315A:512–9.
Li DM, Zhang XR, Li YQ, Hao CY, Zhang J, Wu YF. Stress responses of testosterone and corticosterone-binding globulin in a multi-brooded species, Eurasian Tree Sparrows (*Passer montanus*): Does CBG function as a mediator? Horm Behav. 2012;61:582–9.
Martins MJ, Dias PO, Hipólito-Reis C. Rat serum alkaline phosphatase electrophoretic fractions: variations with feeding, starvation and cellulose fibre ingestion. Clin Nutr. 1998;17:279–85.
Merila J, Wiggins DA. Mass loss in breeding blue tits: the role of energetic stress. J Anim Ecol. 1997;66:452–60.
Newton SF. Body condition of a small passerine bird: ultrasonic assessment and significance in overwinter survival. J Zool. 1993;229:561–80.
Pap PL, Vágási CI, Tökölyi J, Czirják GÁ, Barta Z. Variation in haematological indices and immune function during the annual cycle in the great tit *Parus major*. Ardea. 2015;98:105–12.
Piersma T, Drent J. Phenotypic flexibility and the evolution of organismal design. Trends Ecol Evol. 2003;18:228–33.
R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2016.
Richner H, Oppliger A, Christe P. Effect of an ectoparasite on reproduction in great tits. J Anim Ecol. 1993;62:703–10.
Round PD, Hansson B, Pearson DJ, Kennerley PR, Bensch S. Lost and found: the enigmatic large-billed reed warbler *Acrocephalus orinus* rediscovered after 139 years. J Avian Biol. 2007;38:133–8.
Seiser PE, Duffy LK, David McGuire A, Roby DD, Golet GH, Litzow MA. Comparison of pigeon guillemot, *Cepphus columba*, blood parameters from oiled and unoiled areas of Alaska eight years after the Exxon Valdez oil spill. Mar Pollut Bull. 2000;40:152–64.
Smits JE, Bortolotti GR, Baos R, Jovani R, Tella JL, Hoffmann WE. Disrupted bone metabolism in contaminant-exposed white storks (*Ciconia ciconia*) in southwestern Spain. Environm Pollut. 2007;145:538–44.
Stearns SC. Trade-offs in life-history evolution. Funct Ecol. 1989;3:259–68.
Sun YF, Li M, Song G, Lei FM, Li DM, Wu YF. The role of climate factors in geographic variation in body mass and wing length in a passerine bird. Avian Res. 2017;8:1.
Sun YF, Ren ZP, Wu YF, Lei FM, Dudley R, Li DM. Flying high: limits to flight performance by sparrows on the Qinghai-Tibet Plateau. J Exp Biol. 2016;219:3642–8.
Tilgar V, Kilgas P, Mägi M, Mänd R. Age-related changes in the activity of bone alkaline phosphatase and its application as a marker of prefledging maturity of nestlings in wild passerines. Auk. 2008;125:456–60.
Tilgar V, Ots I, Mänd R. Bone alkaline phosphatase as a sensitive indicator of skeletal development in birds: a study of the great tit nestlings. Physiol Biochem Zool. 2004;77:530–5.
Viñuela J, Ferrer M. Regulation of growth in red kites and imperial eagles. Wilson Bull. 1997;109:92–101.
Villegas A, Sánchez J, Costillo E, Corbacho C. Blood chemistry and haematocrit of the black vulture (*Aegypius monachus*). Comp Biochem Physiol A. 2002;132:489–97.
Williams GC. Natural selection, the costs of reproduction, and a refinement of lack's principle. Am Nat. 1966;100:687–90.
Williams TD. Physiological adaptations for breeding in birds. Princeton: Princeton University Press; 2012.
Wingfield JC. Organization of vertebrate annual cycles: implications for control mechanisms. Phil Trans R Soc Lond B. 2008;363:425–41.
Wojczulanis-Jakubas K, Jakubas D, Chastel O, Kulaszewicz I. A big storm in a small body: seasonal changes in body mass, hormone concentrations and leukocyte profile in the little auk (*Alle alle*). Polar Biol. 2015;38:1203–12.
Zhang SP, Lei FM, Liu SL, Li DM, Chen C, Wang PZ. Variation in baseline corticosterone levels of Tree Sparrow (*Passer montanus*) populations along an urban gradient in Beijing, China. J Ornithol. 2011;152:801–6.
Zhao YL, Li M, Sun YF, Wei W, Kou GQ, Guo LL, Xing DN, Wu YF, Li DM, Zhao BH. Life-history dependent relationships between body condition and immunity, between immunity indices in male Eurasian Tree Sparrows. Comp Biochem Physiol A. 2017;210:7–13.
Zheng WH, Li M, Liu JS, Shao SL, Xu XJ. Seasonal variation of metabolic thermogenesis in Eurasian tree sparrows (*Passer montanus*) over a latitudinal gradient. Physiol Biochem Zool. 2014;87:704–18.