J Ornithol (2016) 157:591–598
DOI 10.1007/s10336-016-1325-7

ORIGINAL ARTICLE

Spatial variation in haemoglobin concentration of nestling Blue Tits (*Cyanistes caeruleus*): a long-term perspective

Michał Glądalski[1] · Mirosława Bańbura[2] · Adam Kaliński[3] · Marcin Markowski[1] · Joanna Skwarska[1] · Jarosław Wawrzyniak[1] · Piotr Zieliński[4] · Jerzy Bańbura[1]

Received: 2 July 2015 / Revised: 29 December 2015 / Accepted: 4 January 2016 / Published online: 19 January 2016

Abstract An understanding of the influences of anthropogenic disturbance and variation in habitat quality on organism condition and breeding success may improve future management and conservation decisions. Some authors consider haemoglobin concentration to be a simple biochemical indicator of bird condition. The main goal of this paper is to examine if the level of haemoglobin displays any consistent pattern of variation across habitats differing in quality. We present results concerning long-term variation in haemoglobin concentration in the blood of about 14-day-old nestling Blue Tits (*Cyanistes caeruleus*) in central Poland in an 11-year period (2003–2013), in two landscapes (an urban parkland and a deciduous forest). The most important findings of the study are: (1) the concentration of haemoglobin of the nestlings from the same brood tended to be consistently similar, with most variation occurring between broods, (2) mean levels of haemoglobin varied between years, and were correlated with caterpillar abundance peaks in the forest study site, (3) mean haemoglobin concentration was significantly higher in the forest area than in the parkland area, (4) haemoglobin levels were positively correlated with breeding and fledging success. We confirmed that haemoglobin concentration displays a spatio-temporal pattern and that the level of haemoglobin is a reliable condition and habitat quality indicator in nestling Blue Tits in the study populations. Although, strictly speaking, the analysed differences are between two particular sites, we think that they reflect differences between urban and non-urban habitats.

Keywords Passerine · Wild populations · Urban populations · Environmental stress · Breeding success · Fledging success · Body condition · Physiological condition · Hematology

Zusammenfassung

Langzeitdaten zu lokalen Unterschieden in der Hämoglobinkonzentration von Nestlingen der Blaumeise (*Cyanistes caeruleus*)

Der Naturschutz profitiert vom Wissen um die Auswirkungen anthropogener Störungen und um die Unterschiede in der Qualität von Habitaten auf den Gesundheitszustand und den Fortpflanzungserfolg von Organismen. Die Messung der Hämoglobinkonzentration ist eine übliche biochemische Methode für die Bestimmung des Gesundheitszustands von Vögeln. Unser Hauptziel war es zu untersuchen, ob die Konzentration von Hämoglobin vorhersehbar mit der Qualität von Habitaten korreliert. Wir präsentieren

Communicated by L. Fusani.

Electronic supplementary material The online version of this article (doi:10.1007/s10336-016-1325-7) contains supplementary material, which is available to authorized users.

✉ Michał Glądalski
mglad@biol.uni.lodz.pl

1 Department of Experimental Zoology and Evolutionary Biology, Faculty of Biology and Environmental Protection, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland

2 Museum of Natural History, Faculty of Biology and Environmental Protection, University of Łódź, Kilińskiego 101, 90-011 Łódź, Poland

3 Department of Teacher Training and Biological Diversity Studies, Faculty of Biology and Environmental Protection, University of Łódź, Banacha 1/3, 90-237 Łódź, Poland

4 Department of Ecology and Vertebrate Zoology, Faculty of Biology and Environmental Protection, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland

Langzeitdaten (Zeitraum 2003 – 2013) zur Variation der Hämoglobinkonzentration in circa zwei Wochen alten Blaumeisennestlingen (*Cyanistes caeruleus*), die in Zentralpolen entweder in urbanen oder ländlichen Gegenden aufwuchsen. Zusammenfassend fanden wir heraus, dass (a) die Hämoglobinkonzentration mehr Variation zwischen Bruten als innerhalb einer Brut zeigte, (b) die durchschnittliche Hämoglobinkonzentration innerhalb des Studienzeitraums variierte und mit einem erhöhten Vorkommen an Raupen korrelierte, (c) die durchschnittliche Hämoglobinkonzentration im Wald signifikant höher als in Parks war, und (d) die Hämoglobinkonzentration positiv mit dem Brut- und Schlupferfolg korrelierte. Wir bestätigten damit, dass in unserer Population die Hämoglobinkonzentration in Blaumeisennestlingen sowohl eine zeitliche als auch eine räumliche Komponente aufweist und ein zuverlässiges Mittel ist, um Aussagen über den Gesundheitszustand der Vögel und die Qualität des Habitats zu treffen. Wir weisen darauf hin, dass die herausgefundenen Unterschiede zwei bestimmte Gebiete innerhalb unseres Studiengebiets betrafen, die für uns jedoch stellvertretend die Unterschiede zwischen urbanen und ländlichen Gebieten reflektieren.

Introduction

The rapid growth of urban areas resulting in sudden changes in landscape spatial patterns has become one of the most important environmental issues in recent decades (Chamberlain et al. 2009). Therefore, an understanding of the influences of variation in habitat quality on organism condition and breeding success in association with physiological knowledge may provide instruments to support evidence-based management decisions (Cooke and O'Connor 2010; Bonier 2012; Ellis et al. 2012; Cook et al. 2013).

Conservation physiology is an emerging field that applies physiological tools and concepts to conservation in broad ways (Cook et al. 2013; Milenkaya et al. 2013). Fragmentation and degradation of natural habitats intensifies on a global scale, and it is important to assess the effects of changes in habitat on organisms (Gil and Brumm 2014). Urbanization leads to irreversible changes in natural landscapes. This process involves clearing much of the original vegetation to make way for urban infrastructure, habitat fragmentation, expansion of nonnative plant species, increasing amounts of easily accessible, but low quality urban food leftovers, light, noise, and chemical pollution (Shanahan et al. 2014). All those factors affect the condition of animals and may have significant impact on their breeding traits (Chamberlain et al. 2009). It was shown that a patchy environment makes foraging much more difficult for birds (Riddington and Gosler 1995; Hinsley et al. 2006). The paucity of natural food, access to urban food leftovers, and over-winter provisioning can lead to a reduction in fecundity and lower breeding success of birds in urban habitats (Harrison et al. 2010). Gorrisen et al. (2005) showed that male Great Tits (*Parus major*) that bred closest to a pollution source had a more limited song repertoire and were less frequent singers. An additional challenge for urban birds may be that any new vegetation planted by people is usually unlikely to resemble pre-settlement habitat (Cook et al. 2013; Thomson et al. 2003). Because the measuring of habitat quality in a direct way is often not feasible, the evaluation of individual condition offers an alternative method to assess habitat quality (Homyack 2010; Ellis et al. 2012). Body condition indices have been used to evaluate impacts of stressors and disturbances, assessing habitat fragmentation or to provide insights into the nutritional status of animals in different habitat types (Maron et al. 2012; Bańbura et al. 2013; Milenkaya et al. 2013; Kaliński et al. 2014; Minias 2015). Currently, relatively few landscape ecologists employ physiological approaches to evaluate habitat quality. The development of landscape physiology may help with identifying early warning signs of populations in trouble and may play an important role in monitoring and assessing the progress of habitat restoration effects (Ellis et al. 2012).

The concentration of haemoglobin was shown to be a relatively simple biochemical indicator of bird metabolism and condition (Sergent et al. 2004; Simmons and Lill 2006; Minias 2015; Johnstone et al. 2015). It reflects the presence of pathogens and parasites, as well as the nutritional status of organism (a drop in haemoglobin concentration suggests nutritional deficiency; Campbell 1995; Simon et al. 2004; Kasprzyk et al. 2006; Słomczyński et al. 2006). All those features support the use of the haemoglobin level as a relatively reliable indicator of physiological condition in field and experimental studies on birds (Stevens 1996; Kilgas et al. 2006a; Kaliński et al. 2009, 2012; Pyrke and Rollins 2012; Lill et al. 2013; Minias 2014; Minias et al. 2014; Glądalski et al. 2015a). But it is important to emphasize that some recent studies recommend caution when using blood characteristics to evaluate physiological condition in some species (Fair et al. 2007; Lill et al. 2013; Minias 2015; Johnstone et al. 2015). Also the use of haemoglobin concentration as a body condition index in a particular population should not be automatically extended to other populations (Fair et al. 2007; Smith and Barber 2012; Lill et al. 2013; Minias 2015; Glądalski et al. 2015a). Knowledge of phenotypic and ecological factors that directly influence the level of haemoglobin in free living birds is relatively poor (Minias et al. 2014; Minias 2015). Also, comparisons of hemoglobin concentrations between urban and non-urban populations of birds are scarce. A few existing studies refer usually to only 1–3 years of research.

Therefore, further long-term comparative studies between urban and non-urban populations of birds are needed (Chamberlain et al. 2009; Minias 2015).

The main aim of this study is to provide an analysis of long-term (11 seasons), year-to-year variation in haemoglobin concentration of nestling Blue Tits (*Cyanistes caeruleus*) in two structurally and floristically contrasting habitats (an urban parkland and a rich, deciduous forest). Our present study is aimed at (a) testing whether haemoglobin levels are consistent physiological characteristics of Blue Tit nestlings, with low variation within broods and high variation between broods, (b) presenting year-to-year variation in haemoglobin concentration during an 11-year period, (c) examining if the level of haemoglobin displays any consistent pattern of variation across habitats differing in quality, (d) testing if haemoglobin level tended to be related to peaks in caterpillar abundance in both study areas, and (e) testing whether breeding and fledging success are linked to haemoglobin concentration. The main hypotheses were formulated as follows: (1) assuming that hemoglobin concentration responds more to the factors acting on entire broods than individual nestlings within a brood, variation in haemoglobin concentrations within individual broods should be smaller than between different broods, (2) because trophic conditions and weather characteristics tend to be variable in time and space, which should influence the physiological condition of growing nestlings, mean level of haemoglobin should differ between sites and years, (3) since caterpillars are considered as the optimal food of breeding tits, haemoglobin level should be related to peaks in caterpillar abundance, (4) assuming that the level of haemoglobin is a reliable condition indicator, breeding success should be correlated with the level of haemoglobin.

Methods

Study area, field, and laboratory procedures

The present study, carried out during 11 breeding seasons (2003–2013) in two structurally and floristically contrasting habitats (an urban parkland and a rich deciduous forest, located only 10 km apart), is a part of a long-term project of research into the breeding biology of secondary cavity nesters around Łódź, central Poland. The parkland site (51°45′N; 19°24′E), about 80 ha (including the zoological garden of 16 ha and the botanical garden of 64 ha), has a highly fragmented tree cover (formed artificially) and is one of the major recreation and entertainment areas in Łódź, which is associated with strong human disturbance over the entire breeding season (Bańbura et al. 2013; Glądalski et al. 2015b). We assess that in 2008–2013 the average number of visitors to our parkland study site in May, as the main period of the nestling stage of Blue Tits, is about 75,000. The forest study area (51°50′N, 19°29′E), bordering on the NE part of the city, is about 120 ha area in the interior of mature mixed deciduous forest (1250 ha in total) with oaks (*Quercus robur* and *Q. petraea*) as predominating tree species. The study sites were supplied with ca. 500 standard wooden nestboxes (200 in the parkland and 300 in the forest) (Lambrechts et al. 2010). In every breeding season, the nestboxes are occupied first of all by Blue Tits and Great Tits, but also by Pied Flycatchers (*Ficedula hypoleuca*) and Nuthatches (*Sitta europaea*) and, incidentally, by Marsh Tits (*Poecile palustris*) and Coal Tits (*Periparus ater*).

The abundance of caterpillars was monitored over the Tit breeding seasons 2003–2013, using the frassfall collecting method. The amount of frassfall is directly associated with the ecological production of caterpillars and, therefore, is a reliable indicator of caterpillar numbers (Zandt 1994). The caterpillar frassfall was collected into tissue collectors, 1 m^2 on a metal framework, hanged below tree canopies, five in the parkland and nine in the woodland. The collectors were checked and emptied every 5 days. In the laboratory, the samples from frassfall collectors were inspected under the microscope to separate caterpillar frass from other particles, then the frass particles were dried (24 h in 60 °C) and weighed to the nearest 0.1 mg. For each study site, the resulting masses and counts were calculated and presented as peak values per trap per day in a given year (Marciniak et al. 2007). The minimum peak amount of frassfall in the parkland was 0.05 g/m^2/day (2007), while the maximum peak recorded was 0.41 g/m^2/day (2003), with mean ($\pm$ SD) 0.20 ± 0.11 g frass/m^2/day (2003–2013). The minimum peak amount of frassfall in the forest site was 0.07 g/m^2/day (2007) and the maximum peak was 2.13 g/m^2/day (2003), with mean ($\pm$ SD) 0.59 ± 0.55 g frass/m^2/day (2003–2013).

During the breeding season, the nestboxes were inspected at least once a week (or every day if needed) to record laying date, clutch size, and the number of nestlings at different stages. The Blue Tit nestlings were banded with individually numbered metal rings and measured (wing length, to the nearest 1 mm and body mass, to the nearest 0.1 g) after 13–14 (occasionally 12–16) days from the hatching of the first egg (Blue Tit nestlings fledge on about 19–20 day). A random subsample of five nestlings (2003–2006) or three nestlings (2007–2013) blind-drawn out of same-age nestlings from every Blue Tit brood were designated for blood sampling. Samples of 5 μL of blood were taken from the ulnar vein of nestlings to HemoCue cuvettes and analysed in the field using a portable HemoCue Hb 201+ photometer to measure haemoglobin concentration (g/L) (HemoCue AB, Angelholm, Sweden).

In the case of avian blood, this photometer shows haemoglobin values slightly higher than cyanomethaemoglobin spectrophotometry, as described by Eklom and Lill (2006) and Simmons and Lill (2006). All field procedures were carried out between 9.00 and 14.00. During 11 years of the study, 1711 Blue Tit nestlings from 461 broods (2003–27, 2004–52, 2005–42, 2006–37, 2007–63, 2008–51, 2009–50, 2010–43, 2011–21, 2012–35, 2013–40) were examined.

Statistical analyses

Repeatability of haemoglobin concentration within broods was calculated as intraclass correlation to test to what extent nestlings in broods tend to resemble one another (Zar 1996). A high repeatability indicates that variation within individual broods is much smaller than between broods. Repeatability is low if measurements within broods are very different. Because values of haemoglobin concentration in the blood of nestlings from the same brood were not independent, the individual nestling values were treated as unit records and analysed using mixed linear models, with brood ID being included as a random factor to control for clustering; degrees of freedom were approximated by the Satterthwaite method (Heck et al. 2010). Effects of year on the haemoglobin concentrations were modeled in an ANCOVA style by fitting a model that included wing length as an age-controlling covariate (Crawley 2002). Effects of nestling haemoglobin concentration on breeding success and fledging success (breeding success refers to the proportion of eggs resulting in fledglings, fledging success refers to the proportion of hatchlings that survived to fledging) were calculated using a generalized linear mixed model with binomial error distribution, the approximation of degrees of freedom by the residual method and applying logit link function (Crawley 2002; Heck et al. 2012). Years and sites were treated as random factors in these models.

Pearson's linear correlations between yearly mean haemoglobin concentrations and frassfall peaks as well as between haemoglobin concentrations at both study areas, were calculated using STATISTICA 10 (StatSoft Inc. 2011). Linear mixed and generalized linear mixed modeling was performed using IBM SPSS Statistics 22 software (Heck et al. 2010, 2012; IBM SPSS 2013).

Results

We found that the concentration of haemoglobin in the blood of Blue Tit nestlings from the same brood tended to be similar in a consistent way, with much variation occurring between broods. It resulted in significant within-brood repeatability of haemoglobin level ($R = 0.68 \pm 0.019$ (SE), $F_{460;1250} = 8.94$, $P < 0.001$).

Haemoglobin concentration differed between years and sites, and was 4 % higher in the forest area than in the parkland area, while the interaction between the site and year factors was nonsignificant (Table 1). Minimum yearly mean haemoglobin level in the park site was 113.7 g/L (2011) and maximum yearly mean was 134.0 g/L (2004). Minimum yearly mean haemoglobin concentration in the forest site was 120.5 g/L (2006), and maximum yearly mean was 134.2 g/L (2004) (Fig. 1). Post hoc (LSD Fisher test $P < 0.05$) examination of differences revealed significant differences between years, with 2004 being different from any other year of the study (see ESM for details). Blood haemoglobin levels were correlated with frassfall peaks in the forest site ($r = 0.92$, $N = 11$, $P < 0.001$), and there was no correlation in the parkland site ($r = 0.34$, $N = 11$, $P = 0.31$). There was a marginally nonsignificant positive correlation between year-to-year changes in mean haemoglobin concentration in both study areas ($r = 0.58$, $N = 11$, $P = 0.06$).

We also found a positive relationship between breeding success and fledging success of blue tits and the per-brood mean haemoglobin concentration in nestling blood (Table 2).

Discussion

In previous studies we found that variation in haemoglobin levels between individual nestlings within a brood was low in relation to differences among separate broods, resulting in significant repeatability for two Tit species [for Great Tits: 0.53 (breeding season 2004); Nadolski et al. 2006, and for Blue Tits: 0.69 (breeding seasons 2003–2005); Bańbura et al. 2007]. In nestling Pied Flycatchers (*Ficedula hypoleuca*) studied in our forest study site the repeatability of haemoglobin was assessed at 0.63 (Glądalski et al. 2015a). Potti et al. (1999) found that within-brood repeatability of haematocrit in Pied Flycatcher nestlings in Spain was 0.7. Our present long-term result for Blue Tits is high (0.68), which is consistent with previous findings. In general, high

Table 1 Results of a linear mixed model analysis for haemoglobin concentration in the blood of Blue Tit nestlings

Factor (covariate)	*df*	*F*	*P*
Year	10; 448	5.57	**<0.001**
Study area	1; 446	21.22	**<0.001**
Year × study area	10; 446	1.27	0.24
Wing length (cov)	1; 1117	114.19	**<0.001**

Effects of year, study area, and their interaction are given (mean wing length as covariate, significant values are in bold)

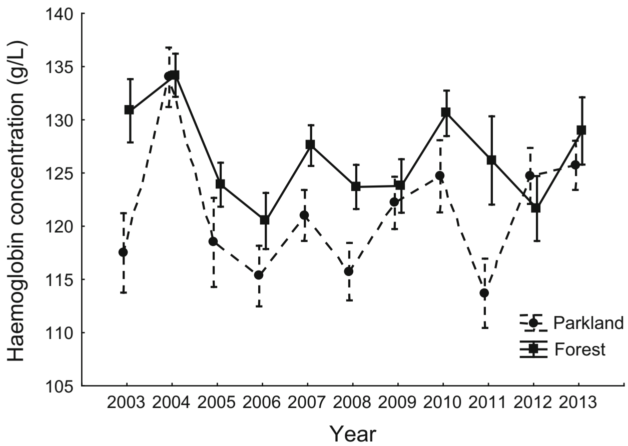

Fig. 1 Annual variation in mean haemoglobin concentration of Blue Tit nestlings in parkland and forest areas. Means ± standard errors corrected for wing length as covariate are shown

repeatability suggests that variation within individual broods is much smaller than variation between different broods. Repeatability is low if measurements within broods are very different (Bańbura and Zieliński 1990). The high repeatability of haemoglobin concentration we found suggests that hemoglobin responds first of all to the factors affecting entire broods, such as parasitic pressure in the nest (Glądalski et al. 2015a). More studies are needed to determine if this may be considered as a general pattern in wild populations of birds.

Some recent studies suggest caution when using haemoglobin concentration as a simple body condition index (Lill et al. 2013). However, our previous studies showed that the level of haemoglobin is a reliable condition indicator for Tits (Nadolski et al. 2006; Kaliński et al. 2009; Kaliński et al. 2012) and for Pied Flycatcher (Glądalski et al. 2015a). Kilgas et al. (2006a, b) also found that the concentration of haemoglobin is strongly positively related to the physiological condition of nestling great tits. But the usefulness of this condition indicator should not be automatically extended to other bird species and other populations. In the present study mean haemoglobin concentration was significantly higher in the forest area than in the parkland area. This difference in condition between both study areas reflects differences in trophic conditions, as described by Naef-Daenzer and Keller (1999). Our study areas considerably differ in caterpillar productivity, with the forest site being characterized by 2–5 times higher abundance of caterpillars than the parkland site (Marciniak et al. 2007; Bańbura et al. 2013). Additionally, long-term year-to-year changes in haemoglobin concentration were correlated with frassfall peaks in the forest site. Bańbura et al. (2007) found a strong association between haemoglobin concentration in Blue Tit nestlings and the amount of caterpillars during the time of extremely high abundance of caterpillars in seasons 2003-2005. Chamberlain et al. (2009) showed in their meta-analysis that urban bird populations are characterized by lower productivity (lower clutch sizes, lower fledging success) and worse body condition (lower nestling weights) than non-urban populations. On the other hand, in our study, the long-term year-to-year changes in haemoglobin concentration were not correlated with frassfall peaks in the parkland site. The abundance of caterpillars is almost always relatively low in the parkland site and, therefore, Blue Tits have to use a greater proportion of alternative prey sources, which could obscure a potential relation between physiological indices and year-to-year differences in the amount of caterpillar frassfall. Michalski et al. (2011) who analysed droppings of tits in both our study areas, showed that a proportion of non-caterpillar prey delivered to nestlings is higher in the parkland area than in the forest. This may suggest that caterpillar abundance may be a more important determinant factor for Blue Tit breeding parameters and physiological condition in the forest than in the parkland area.

Data on the effects of blood parasites on the level of haemoglobin in nestlings are consistent for different study species and indicate negative effects of parasitism on the haematological condition of chicks and adult birds

Table 2 Results of generalized linear mixed models showing a positive relationship between two binomial response variables, fledging success and breeding success, and per-brood mean haemoglobin concentrations in the blood of nestling Blue Tits (mean wing length as covariate, significant values are in bold)

Dependent variable	Effect (cov)	Est ± SE	*df*	*F*	*P*
Fledging success					
	Mean haemoglobin	0.057 ± 0.025	1; 451	5.26	**0.022**
	(Mean wing length)	0.167 ± 0.082	1; 451	4.20	**0.041**
Breeding success					
	Mean haemoglobin	0.058 ± 0.015	1; 452	14.13	**<0.001**
	(Mean wing length)	0.169 ± 0.051	1; 452	11.12	**0.001**

(Johnstone et al. 2015; Minias 2015). Krams et al. (2013) showed experimentally that in the absence of blood parasites, Great Tit nestlings had higher concentrations of haemoglobin. Furthermore, they survived at higher rates thorough the nestling and fledgling phase. Słomczyński et al. (2006) replaced natural Blue Tit nests with clean artificial nests (twice during the nestling stage). This treatment caused an increase of 7–10.5 g/L in haemoglobin concentration of nestlings in comparison with control nestlings. This confirms that the level of haemoglobin may be treated as an indicator of environmental harshness. Our study sites (the parkland and the forest) may differ in the numbers of parasite vectors and a probability of infection by blood parasites. Further studies should examine whether tits at both study areas differ in parasite loads.

Another important factor influencing lower body condition in the parkland area may be patchy environment, which, among other effects, makes foraging more difficult for parents (Riddington and Gosler 1995; Hinsley et al. 2006; Sandstrom et al. 2006). Additionally, it is important to emphasize that the parkland area analysed in this study is a major recreation and entertainment area in the city of Łódź, with tens of thousands of visitors in May every year. Birds have been shown to respond to the presence of humans or predatory animals with a behavioral reaction (disturbing the regularity of feeding), or with a physiological response (increasing the level of stress hormones), or with both those effects at the same time (Holberton et al. 1996; Romero and Remage-Healey 2000; Verhulst et al. 2001; Jarvis 2005). In small birds such as Blue Tits, the presence of humans near the nest may provoke a strong behavioral response. A parent emits alarm calls and hesitates to approach the nest (Müller et al. 2006). Bańbura et al. (2013) examined the heterophil-to-lymphocyte ratio (H/L) in Blue Tits at both our study areas and found that nestlings had on average higher level of the H/L in the urban parkland study area than in the forest study area. The H/L is considered as a reliable indicator of chronic stress reaction in birds (Gross and Siegel 1983; Ots et al. 1998; Moreno et al. 2002; Davis et al. 2008).

Year-to-year changes in mean haemoglobin concentration tended to be similar in both study areas. This may suggest that some factors that influence the level of haemoglobin in both study areas are similar. The weather seems to fit as such a factor. Effects of temperatures per se, as described by Kaliński et al. (2014), in some cases could be too complex to interpret and can influence many processes also in an indirect way. It was shown for nestling Pied Flycatchers that year-to-year variation in the level of haemoglobin correlated with daily minimum temperature (Glądalski et al. 2015a). Low temperatures could reduce female capacity for warming chicks efficiently and a combination of mild temperatures and regular, but not heavy rainfall provides good conditions which enable the development of rich arthropod communities (Kaliński et al. 2009; Kaliński et al. 2014).

Haemoglobin concentration have turned out to be a reliable predictor of breeding success and fledging success. Variation in nestling body performance could be expected to have consequences for breeding success (Nadolski et al. 2006; Kaliński et al. 2014). Body condition of nestlings is usually connected to the chance of survival to the fledging stage and, later, for ultimate individual success in recruitment to the breeding population (Brown 1996). Haemoglobin, being related to oxygen carrying capacity, is sensitive to the hydration, mineral deficiencies and nutritional state (Campbell 1995). Our present findings are consistent with other studies with respect to a positive relationship between the level of haemoglobin and breeding performance (for Great Tits Kaliński et al. 2009, for Pied Flycatchers Glądalski et al. 2015a). Although strictly speaking, the analysed differences are between only two particular sites, we think that they reflect differences between urban and non-urban habitats and our results are comparable to other similar studies.

Acknowledgments All procedures were approved by the local ethical committee and the State Office for Environment Protection. We thank A. Jaksa, D. Mańkowska, M. Janiszewska, and J. Białek for their help and consent to conducting research in the areas under their administration. The study was founded by a grant from the Polish Ministry of Science and Higher Education No. N N304 045136 and University of Łódź (No. 506/1145). We also thank B. Marciniak, J. Nadolski, M. Michalski, B. Loga, and M. Nowakowska for collecting data on caterpillar abundance. We are obliged to P. Procter for linguistic consultation. We are very grateful to the two reviewers and the Subject Editor for their constructive suggestions and for their proposed corrections to improve this paper.

References

Bańbura J, Zieliński P (1990) Within-clutch repeatability of egg dimensions in the black-headed gull Larus ridibundus. J Ornithol 131:305–310

Bańbura J, Bańbura M, Kaliński A, Skwarska J, Słomczyński R, Wawrzyniak J, Zieliński P (2007) Habitat and year-to-year variation in haemoglobin concentration in nestling blue tits Cyanistes caeruleus. Comp Biochem Physiol A 148:572–577

Bańbura J, Skwarska J, Bańbura M, Glądalski M, Hołysz M, Kaliński A, Markowski M, Wawrzyniak J, Zieliński P (2013) Spatial and temporal variation in heterophil-to-lymphocyte ratios of nestling passerine birds: comparison of blue tits and great tits. PLoS One 8(9):e74226

Bonier F (2012) Hormones in the city: endocrine ecology of urban birds. Horm Behav 61:763–772
Brown ME (1996) Assessing body condition in birds. Curr Ornithol 13:67–135
Campbell TW (1995) Avian hematology and cytology, 2nd edn. Iowa University Press, Ames
Chamberlain DE, Cannon AR, Toms MP, Leech DI, Hatchwell BJ, Gaston KJ (2009) Avian productivity in urban landscapes: a review and meta-analysis. Ibis 151:1–18
Cook CN, Mascia MB, Schwartz MW, Possingham HP, Fuller RA (2013) Achieving conservation science that bridges the knowledge–action boundary. Conserv Biol 27:669–678
Cooke SJ, O'Connor CM (2010) Making conservation physiology relevant to policy makers and conservation practitioners. Conserv Lett 3:159–166
Crawley MJ (2002) Statistical computing: an introduction to data analysis using S-Plus. Wiley, Chichester
Davis AK, Maney DL, Maerz JC (2008) The use of leucocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol 22:760–772
Eklom K, Lill A (2006) Development of parameters influencing blood oxygen-carrying capacity in nestling doves. Emu 106:283–288
Ellis R, McWhorter TJ, Maron M (2012) Integrating landscape ecology and conservation physiology. Landscape Ecol 27:1–12
Fair J, Whitaker S, Pearson B (2007) Sources of variation in haematocrit in birds. Ibis 149:535–552
Gil D, Brumm H (eds) (2014) Avian urban ecology. Oxford University Press, Oxford
Glądalski M, Skwarska J, Kaliński A, Bańbura M, Markowski M, Wawrzyniak J, Zieliński P, Bańbura J (2015a) Patterns of year-to-year variation in haemoglobin and glucose concentrations in the blood of nestling Pied Flycatchers, Ficedula hypoleuca. J Ornithol 156:811–817
Glądalski M, Bańbura M, Kaliński A, Markowski M, Skwarska J, Wawrzyniak J, Zieliński P, Cyżewska I, Bańbura J (2015b) Inter-annual and inter-habitat variation in breeding performance of blue tits (Cyanistes caeruleus) in central Poland. Ornis Fennica 92:34–42
Gorrisen L, Snoeijs T, Van Duyse E, Eens M (2005) Heavy metal pollution affects dawn sinning behavior in a small passerine bird. Oecologia 145:504–509
Gross WB, Siegel H (1983) Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis 34:759–761
Harrison T, Smith J, Martin G, Chamberlain D, Bearhop S, Robb G, Reynolds S (2010) Does food supplementation really enhance productivity of breeding birds? Oecologia 164:311–320
Heck RH, Thomas SL, Tabata LN (2010) Multilevel and longitudinal modeling with IBM SPSS. Routledge, New York
Heck RH, Thomas SL, Tabata LN (2012) Multilevel modeling of categorical outcomes using IBM SPSS. Routledge, New York
Hinsley SA, JrPF Doherty, Bellamy PE, JrTC Grubb (2006) Consequences of habitat fragmentation for birds: comparison between Europe and North America. Acta Zool Sin 52:187–190
Holberton RL, Helmuth B, Wingfield JC (1996) The corticosteron-estress response in gentoo and king penguins during the nonfasting period. Condor 98:850–854
Homyack JA (2010) Evaluating habitat quality of vertebrates using con—servation physiology tools. Wildl Res 37:332–342
IBM SPSS Statistics 22. (2013) SPSS for Windows, Release 22.0, IBM Corporation
Jarvis PJ (2005) Reactions of animals to human disturbance, with particular reference to flight initiation distance. Recent Res Devel Ecol 3:1–20
Johnstone CP, Lill A, Reina RD (2015) Use of erythrocyte indicators of health and condition in vertebrate ecophysiology: a review and appraisal. Biol Rev. doi:10.1111/brv.12219
Kaliński A, Wawrzyniak J, Bańbura M, Skwarska J, Zieliński P, Bańbura J (2009) Haemoglobin concentration and body conditions of nestling Great Tits Parus major: a comparison of first and second broods in two contrasting seasons. Ibis 151:667–676
Kaliński A, Bańbura M, Skwarska J, Wawrzyniak J, Zieliński P, Glądalski M, Markowski M, Bańbura J (2012) Parallel variation in haemoglobin concentration in nestling-rearing blue tits Cyanistes caeruleus and great tits Parus major. Acta Ornithol 47:129–136
Kaliński A, Bańbura M, Glądalski M, Markowski M, Skwarska J, Wawrzyniak J, Zieliński P, Cyżewska I, Bańbura J (2014) Landscape patterns of variation in blood glucose concentration of nestling blue tits (Cyanistes caeruleus). Landscape Ecol 29:1521–1530
Kasprzyk M, Hetmański T, Kulczykowska E (2006) Changes in hematological parameters In free-living pigeons (Columbia livia f. urbana) during the breeding cycle. J Ornithol 147:599–604
Kilgas P, Mänd R, Mägi M, Tilgar V (2006a) Hematological parameters in brood-rearing great tits in relation to habitat, multiple breeding and sex. Comp Biochem Physiol A 144:224–231
Kilgas P, Mänd R, Tilgar V (2006b) Hematological health sate indices predict local survival in a small passerine bird, the great tit (Parus major). Physiol Biochem Zool 79:565–572
Krams IA, Suraka V, Rantala MJ, Sepp T, Mierauskas P, Vrublevska J, Krama T (2013) Acute infection of Avian malaria impairs concentration of haemoglobin and survival in juvenile altricial birds. J Zool 291:34–41
Lambrechts M et al (2010) The design of artificial nestboxes for the study of secondary hole-nesting birds: a review of methodological inconsistencies and potential biases. Acta Ornithol 45:1–26
Lill A, Rajchl K, Yachou-Wos L, Johnstone ChP (2013) Are haematocrit and haemoglobin concentration reliable body condition indicators in nestlings: the Welcome Swallow as a case study. Avian Biol Res 6:57–66
Marciniak B, Nadolski J, Nowakowska M, Loga B, Bańbura J (2007) Habitat and annual variation in arthropod abundance affects blue tit Cyanistes caeruleus reproduction. Acta Ornithol 42:53–62
Maron M, Goulding W, Ellis RD, Mohd-Taib F-S (2012) Distribution and individual condition reveal a hierarchy of habitat suitability for an area-sensitive passerine. Biodivers Conserv 21:2509–2523
Michalski M, Nadolski J, Marciniak B, Loga B, Bańbura J (2011) Faecal analysis of nestling diet determination in insectivorous birds: a case study in blue tits Cyanistes caeruleus and great tits Parus major. Acta Ornithol 46:164–172
Milenkaya O, Weinstein N, Legge S, Walters JR (2013) Variation in body condition indices of crimson finches by sex, breeding stage, age, time of day, and year. Conserv Physiol 1:1–14
Minias P (2014) High glucose concentrations are associated with symptoms of mild anaemia in Whiskered Terns: consequences for assessing physiological quality in birds. J Ornithol 155:1067–1070
Minias P (2015) The use of haemoglobin concentrations to assess physiological condition in birds: a review. Conserv Physiol. doi:10.1093/conphys/cov007
Minias P, Włodarczyk R, Piasecka A, Kaczmarek K, Janiszewski T (2014) Ecological, physiological, and morphological correlates of blood hemoglobin concentration in a migratory shorebird. Physiol Biochemical Zool 87:771–781
Moreno J, Merino S, Martinez J, Sanz JJ, Arriero E (2002) Heterophil/lymphocyte ratios and heat-shock protein levels and related to growth in nestling birds. Ecoscience 9:434–439
Müller C, Jenni-Eiermann S, Blondel J, Perret P, Caro SP, Lambrechts M, Jenni L (2006) Effect of human presence and handling on circulating corticosterone levels in breeding blue tits (Parus caeruleus). Gen Comp Endocrinol 148:163–171

Nadolski J, Skwarska J, Kaliński A, Bańbura M, Śniguła R, Bańbura J (2006) Blood parameters as consistent predictors of nestling performance in great tits (Parus major) in the wild. Comp Biochem Physiol A 143:50–54

Naef-Daenzer B, Keller L (1999) The foraging performance of great and blue tits (Parus major and P. caeruleus) in relation to caterpillar development, and its consequences for nestling growth and fledging weight. J Anim Ecol 68:708–718

Ots I, Murumägi A, Hõrak P (1998) Haematological health state indices of reproducing great tits: methodology and sources of natural variation. Funct Ecol 12:700–707

Potti J, Moreno J, Merino S, Frias O, Rodriguez R (1999) Environmental and genetic variation in the haematocrit of fledgling pied flycatchers Ficedula hypoleuca. Oecologia 120:1–8

Pyrke SR, Rollins LA (2012) Mothers adjust offspring sex to match the quality of the rearing environment. Proc R Soc Lond B 279:4051–4057

Riddington R, Gosler AG (1995) Differences in reproductive success and parental qualities between habitats in the great tits (Parus major). Ibis 137:371–378

Romero M, Remage-Healey L (2000) Daily and seasonal variation in response to stress in captive starlings (Sturnus vulgaris): corticosterone. Gen Comp Endocrinol 119:52–59

Sandstrom UG, Angelstam P, Mikusiński G (2006) Ecological diversity of birds in relation to the structure of urban green space. Landscape Urban Plann 77:39–53

Sergent N, Rogers T, Cunningham M (2004) Influence of biological and ecological factors on hematological values in wild Little Penguins, Eudyp-tula minor. Comp Biochem Physiol A 138:333–339

Shanahan DF, Strohbach MW, Warren PS, Fuller RA (2014) The challenges of urban living. In: Gil D, Brumm H (eds) Avian urban ecology. Oxford University Press, Oxford

Simmons P, Lill A (2006) Development of parameters influencing blood oxygen carrying capacity in the welcome swallow and fairy martin. Comp Biochem Physiol A 143:459–468

Simon A, Thomas DW, Blondel J, Per P, Lambrechts MM (2004) Physiological ecology of Mediterranean blue tits (Parus caeruleus L). Physiol Bioch Zool 77:492–501

Słomczyński R, Kaliński A, Wawrzyniak J, Bańbura M, Skwarska J, Zieliński P, Bańbura J (2006) Effects of experimental reduction in nest micro-parasite and macro-parasite loads on nestling hemoglobin level in blue tits Parus caeruleus. Acta Oecol 30:223–227

Smith KD, Barber C (2012) Hematocrit does not indicate condition in nestling or adult european starlings. Wilson J Orn 124:788–792

StatSoft Inc (2011) STATISTICA (data analysis software system), version 10. http://www.statsoft.com

Stevens L (1996) Avian biochemistry and molecular biology. Cambridge University Press, Cambridge

Thomson K, Austin KC, Smith RM, Warren PH, Angold PG, Gaston KJ (2003) Urban domestic gardens (I): putting small-scale plant diversity in context. J Veg Sci 14:71–78

Verhulst S, Oosterbeek K, Ens BJ (2001) Experimental evidence for effects of human disturbance on foraging and parental care in oystercatchers. Biol Conserv 101:375–380

Zandt HS (1994) A comparison of three sampling techniques to estimate the population size of caterpillars in trees. Oecologia 97:399–406

Zar JH (1996) Biostatistical analysis. Prentice Hall, Upper Saddle River