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Brief report

Weak correlation between haemoglobin concentration and haematocrit of nestling Great Tits *Parus major* and Blue Tits *P. caeruleus*

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The possible relationship between haemoglobin concentration and haematocrit were studied in 14-days-old Great Tit and Blue Tit nestlings in central Poland. From basic avian blood physiology it was expected that the correlation should be positive but not strong. Our results support this expectation, as both blood profile parameters proved to be significantly associated but their relationship was relatively weak. However, haemoglobin concentration and haematocrit may show different sensitivity to ecological factors and thus are worth studying in concert.

1. Introduction

The nestling stage of small passerines is demanding for parent birds in terms of parental care, but also for offspring that require rapid development of metabolic performance (O'Connor 1984, Bicudo *et al*. 2010). These demands have to be fulfilled in a relatively short period of nestling growth, which is strongly dependent on varying environmental conditions, including habitat-specific, seasonally and annually changing trophic conditions. Blood profile parameters generally provide reliable indices of different aspects of physiological state of individuals in ecological field studies (Ots *et al*. 1998, Simon *et al*. 2004, Bicudo *et al*. 2010). One of the most important conditions of successful fledging and being able to fly is the accomplishment of sufficient carrying capacity of oxygen by nestlings (Lutz 1980). Haematocrit (percentage of the packed volume of erythrocytes in the total volume of the blood) and haemoglobin concentration (mass of haemoglobin per unit of blood) are relatively simple measures of blood oxygen transportation performance (Bolotnikov & Solovev 1980, Lutz 1980, Campbell 1995).

The concentration of haemoglobin depends on its mean concentration in erythrocytes where it is located and the number of erythrocytes in the unit volume of blood. On the other hand, haematocrit depends on the number of erythrocytes in the blood, mature and immature, irrespective of the amount of haemoglobin. Because of a short lifespan of mature erythrocytes and their low level of macromolecule turnover, the amount of haemoglobin per cell is shaped during the process of erythrocyte growth and maturation (Stevens 1996). This results in different albeit inter-dependent growth trajectories of haemoglobin concentration and haematocrit (Kostelecka-Myrcha *et al.* 1973, 1993, Simmons & Lill 2006). However, taking into account potential changes in the amount of blood plasma, leads to a prediction that these blood profile parameters will be positively associated but not necessarily in a strict way. It is probable that cellular and haemoglobin synthesis components of the process of shaping blood oxygen affinity are sensitive to somewhat different factors (Lutz 1980, Eklom & Lill 2006, Simmons & Lill 2006), including certain ecological factors (Bańbura *et al.* 2007). In the case of haematocrit, the important role of both environmental and genetic effects was experimentally shown by Potti *et al.* (1999).

Experimental and observational studies have demonstrated that both haematocrit and haemoglobin concentration, when analysed separately, reflect ecological variation (Bech & Klaassen 1996, Potti *et al.* 1999, Dubiec & Cichoń 2001, O'Brien *et al*. 2001, Simon *et al*. 2004, Fair *et al*. 2007, Bańbura *et al.* 2008). Variation in nestling haemoglobin concentration reliably reflects differences in trophic conditions between habitats and seasons in Blue Tits and Great Tits (*Parus major* and *P. caeruleus*; Bańbura *et al*. 2007; Kaliński *et al*. 2009). Although some papers directly concern responses of these blood indices to ecological factors (O'Brien *et al*. 2001, Carleton 2008), the present paper is to our knowledge the first published report on correlations between these parameters in nestling birds.

The main aim of this study was to examine to what extent these two characteristics of blood oxygen transportation performance (i.e., haemoglobin concentration and haematocrit) are correlated. Specifically, we tested if both these parameters show consistency within broods of Great and Blue Tits, which would be expected from previously published reasoning (Nadolski *et al*. 2006).

2. Material and methods

This study was carried out during the breeding season of 2010 as part of a long-term research project on the breeding biology of tits in central Poland. The two study sites represent parkland and woodland (for details, see Marciniak *et al.* 2007, Bańbura *et al.* 2010). The sites are 10 km apart, located in the urban area of the city of Łódź. The woodland site (51°50' N, 19°29' E) is ca. 120 ha in size, located in the central sections of a 1,250-ha mature deciduous forest dominated by oaks (*Quercus* spp.). The parkland site (51°45' N, 19°24' E) is ca. 80 ha in size, has a scattered tree-canopy cover and including botanical and zoological gardens, characterised by abundant alien plant species and intensive human disturbance during the whole breeding season.

The two study sites were supplied with standard wooden nest boxes, 300 in the woodland site and 200 in the parkland (M. Bańbura *et al.* 2010).

Table 1. Within-brood repeatability of Great Tit and Blue Tit nestlings' blood haemoglobin and haematocrit.

		Haemoglobin				Haematocrit			
Species	df	*R*	SE	*F*	*p*	*R*	SE	*F*	*p*
Great Tit	40/67	0.57	0.09	4.44	<0.0001	0.45	0.103	3.19	<0.0001
Blue Tit	37/65	0.65	0.08	5.98	<0.0001	0.52	0.098	3.92	<0.0001

During the breeding season, the boxes were inspected at least once a week to record breeding traits of nesting birds. For this paper, in 2010, data from 47 first broods and 112 individual Blue Tit nestlings, and from 50 broods and 117 Great Tit nestlings were obtained. The nestlings of both species were individually ringed and measured at the age of 14 days from the hatching of the first egg. Three of the nestlings with the same age, from each nest, were randomly chosen for blood sampling. Samples of ca. 5μL of blood were taken from ulnar vein of the nestling directly to HemoCue cuvettes and analysed in the field using a portable HemoCue Hb 201+ photometer (HemoCue AB, Angelholm, Sweden) to establish haemoglobin concentration (g/L), following Burness *et al.* (2000). In the case of avian blood, this photometer shows haemoglobin values slightly higher than cyanomethaemoglobin spectrophotometry (Simmons & Lill 2006, Eklom & Lill 2006). Simultaneously, another blood sample was taken from the same vein in a 19-μL heparynised capillary tube and, following most frequently applied procedures (Dawson & Bortolotti 1997, Simmons & Lill 2006), stored in an ice-based cooler and then processed in the laboratory. It was not possible to conduct the centrifugation immediately in the field, as practiced in some studies (e.g., Potti *et al.* 1999). After 10 min of centrifugation at 12,000 g, the haematocrit was assessed as being the proportion of capillary length occupied by packed red blood cells in relation to capillary length occupied by the entire blood sample. The length was measured with a caliper to the nearest 0.1 mm. Some capillaries were rejected because of air bubbles, blood clotting or due to damages during centrifugation.

Within-brood repeatability was calculated using intra-class correlations, based on analysis of variance for variation among broods (Zar 1984, Lessells & Boag 1987, Bańbura & Zieliński 1990). Standard errors of repeatability were calculated according to Becker (1984). All calculations were done using standard statistical methods, such as Pearson's correlation between haematocrit and haemoglobin concentration, using STATISTICA 9 (StatSoft Inc. 2009). To avoid an effect of within-brood interdependence of blood-profile values, the relationship between haemoglobin and haematocrit was also analysed using mixed linear modeling procedure in SPSS 18 software, with broods as subjects (random factor) and degrees of freedom approximated by Satterthwaite method (West *et al.* 2007).

3. Results

No significant differences between the two study sites in nestling haematocrit values or haemoglobin levels were found for the two species ($F_{2,114}$ = 1.271 and $F_{2,109}$ = 0.806, respectively, p >0.05 for both species). Therefore, data from these areas were combined within each species. Both hamatocrit and haemoglobin were significantly repeatable within broods (Table 1), suggesting that nestlings in particular broods were non-randomly similar to each other with respect to these blood characteristics. A significant positive correlation between haemoglobin concentration and haematocrit value was found for nestlings of both species (Fig. 1, Table 2). High standard errors of regression coefficients indicate that the relationship is weak (Table 2).

4. Discussion

Significant values of repeatability of nestling blood haemoglobin concentration and haematocrit within broods of Great Tits and Blue Tits suggest that both these blood-profile characteristics show consistent intra-brood and inter-brood variation.

Table 2. Summary of mixed linear models for the relationship between haemoglobin concentration and haematocrit in nestling Great Tits and Blue Tits.

Species	Effect	Estimate ± SE	df	*F*	*p*
Great Tit	Intercept	100.66 ± 8.29	1; 108.662	147.36	<0.0001
	Haematocrit	0.579 ± 0.190	1; 106.244	9.31	0.003
Blue Tit	Intercept	107.77 ± 6.98	1; 103.813	238.13	<0.0001
	Haematocrit	0.507 ± 0.165	1; 102.203	9.49	0.003

Such a pattern, resulting from a tendency of nestlings within particular broods to resemble one another, with most variation occurring among broods, has previously been postulated to be an important property of any indicator of nestling physiological condition (Nadolski *et al*. 2006, Bańbura *et al*. 2007). Consequently, both haemoglobin concentration and haematocrit meet this requirement.

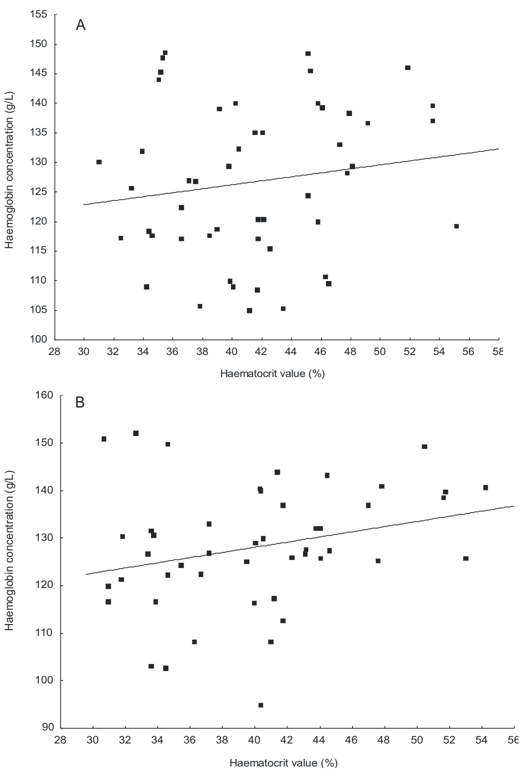

Fig. 1. Relationship between haematocrit value (%) and haemoglobin concentration (g/L) in Great (A) and Blue Tit (B) nestlings. The scatters are based on per-brood mean values (Great Tit *n* = 50, Blue Tit *n* = 47).

Most importantly, we found a correlation between the concentration of haemoglobin and haematocrit values. The assumption that such a correlation exists is widely accepted as a basis for recalculations in human clinical diagnostics (e.g., Thomas 1998). In physiological studies on birds, the validity of this assumption has rarely been tested, and has been explicitly examined only in specific circumstances. Soevik *et al*. (1979), working on chicks of the domestic hen, found a high correlation between haematocrit and haemoglobin concentration. However, the chicks were subjected to a food-supplementation experiment and the response in blood characteristics was analyzed across several stages of chick age. Because the values of different blood variables, including haemoglobin concentration and haematocrit, increase with the growth of body size and age of chickens, and nestling birds in general (Kostelecka-Myrcha 1976, Kostelecka-Myrcha *et al*. 1973, Kostelecka-Myrcha & Chołostiakow-Gromek 2001), the correlation reported by Soevik *et al*. (1979) must arise for methodological reasons through a consideration of chicks of very different size and age. Apart from the present paper, we are not aware of any ecophysiological studies on nestlings of wild birds testing this correlation.

The correlations we found for nestling Great and Blue Tits were significantly positive but rather low, suggesting that the two variables tend to covary – but with high background variation. However, a relatively weak correlation between haemoglobin concentration and haematocrit in nestling birds is not unexpected. A difference between the two blood-profile variables in their response to some ecological factors is a potential source of the weakening of their association. For example, O'Brien *et al*. (2001) and Carleton (2008) found that haemoglobin concentration was influenced by ectoparasites in nestling House Wrens (*Troglo-*

dytes aedon) and Eastern Bluebirds (*Sialia sialis*), but haematocrit level was not. Nyholm (1998) reported that haemoglobin concentration and haematocrit responded differently to heavy-metal exposure in nestling Pied Flycatchers (*Ficedula hypoleuca*). The development of both these characteristics in nestlings is strongly influenced by nest and microhabitat conditions (Potti *et al*. 1999, Norte *et al*. 2009) and proceeds according to different, character-specific growth trajectories (Kostelecka-Myrcha *et al*. 1973). Haemoglobin concentration depends on the amount of haemoglobin and the volume of blood, while haematocrit depends on the number of erythrocytes, the average volume of the cell and the total volume of blood. Therefore, the value of haematocrit will be sensitive also to the number of immature erythrocytes (reticulocytes) in the blood stream, which is not the case with haemoglobin concentration (O'Brien *et al*. 2001, Słomczyński *et al*. 2006, Bańbura *et al*. 2007, Carleton 2008). All this may result in covariation that is widely distributed rather than very strict.

Another important factor that could potentially affect haematocrit values is dehydration, which can cause a decrease in the total volume of the plasma (Fudge 2000). Landys-Ciannelli *et al*. (2002) presented such an effect for the long-distance migrant Bar-tailed Godwits (*Limosa lapponica taymyrensis*). It is possible that dehydration could similarly affect Great and Blue Tit nestlings in nest boxes, especially on hot days when temperature inside a nest box may at least occasionally exceed 30° C. However, weather conditions in our study area during spring 2010 were rather rainy with relatively low temperatures (authors' pers. obs.). Under such circumstances dehydration due to dry and hot weather should not play a significant role; however, low temperatures may potentially lead to increased haematocrit due to thermoregulation (Potti *et al.* 1999, Fair *et al.* 2007). In addition, experiments by Carmi *et al*., (1993) on three different bird species belonging to three different orders revealed that despite the body-mass losses of several per cent, plasma volumes remained constant.

Variation in haemoglobin concentration and haemoatocrit is likely to indicate overlapping but slightly different environmental and physiological causal effects. Therefore, it is important to consider both of these as response variables for different ecological factors wherever possible. The conclusion of Fair *et al*. (2007) that no single physiological parameter should be considered as the best indicator of physiological condition or health is undisputable. However, separately the blood characteristics analysed in this paper were found to display interesting patterns of variation linked with different ecological factors (e.g., Thomas *et al*. 2007, Sanchez *et al*. 2007, Bańbura *et al*. 2007, Kaliński *et al*. 2009), which indicates their practical importance as simple indices of physiological performance.

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Hemoglobiinipitoisuuden ja hematokriitin heikko korrelaatio tali- ja sinitiaisella

Hemoglobiinipitoisuuden ja hematokriitin mahdollista yhteyttä toisiinsa tarkasteltiin 14 päivän ikäisillä tali- ja sinitiaisen (*Parus major* ja *P. caeruleus*) poikasilla Keski-Puolassa. Lintujen yleisen fysiologian mukaisesti tekijöiden tulisi korreloida heikon positiivisesti. Tulokset tukivat tätä näkemystä, sillä molemmat veriprofiiliparametrit olivat merkitsevässä yhteydessä toisiinsa, joskin heikosti. Kuitenkin tekijät voivat reagoida ekologisiin tekijöihin eri tavoin, mistä syystä niitä on syytä tutkia samanaikaisesti.

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