



# Patterns of year-to-year variation in haemoglobin and glucose concentrations in the blood of nestling Pied Flycatchers *Ficedula hypoleuca*

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**Abstract** Physiological tools can be used to identify the sources and consequences of stressors on animals. Understanding the influences of variation in habitat quality and anthropogenic disturbance on organism condition and health may improve future management and conservation. We present results concerning variation in haemoglobin and glucose concentrations in the blood of about 14-day-old nestling Pied Flycatchers *Ficedula hypoleuca* in central Poland over a 4-year period, 2011–2014, in a deciduous forest. The most important findings of the study are: (1) the concentration of haemoglobin and glucose of the nestlings from the same brood tended to be consistently similar, with much variation occurring among broods; (2) repeatability of haemoglobin concentration was higher than repeatability of glucose concentration; (3) mean levels of haemoglobin and glucose varied among years; (4) haemoglobin and glucose concentrations were negatively correlated; and (5) there was a positive relationship between haemoglobin levels and breeding success.

**Keywords** Passerine · Body condition · Physiological condition · Hematology · Breeding success

## Zusammenfassung

### Muster der jährlichen Schwankungen in der Hämoglobin- und Glukose-Konzentration bei Nestlingen des Trauerschnäppers *Ficedula hypoleuca*

Physiologische Werkzeuge können genutzt werden, um Quellen und Auswirkungen von Stressoren auf Tiere zu identifizieren. Einflüsse von Schwankungen in Habitatqualität und anthropogene Störungen auf Lebensbedingungen und Gesundheit von Organismen zu verstehen, könnte zukünftig Management und Schutz verbessern. Wir stellen Ergebnisse einer vierjährigen Untersuchung (2011–2014) von Schwankungen in Hämoglobin- und Glukose-Konzentration im Blut von etwa 14 Tage alten Nestlingen des Trauerschnäppers (*Ficedula hypoleuca*) in einem Laubwald in Zentral-Polen vor. Die wichtigsten Befunde sind: (a) die Hämoglobin- und Glukose-Konzentration der Nestlinge derselben Brut waren nahezu immer ähnlich, mit hohen Unterschieden zwischen verschiedenen Bruten, (b) die Übereinstimmung der Hämoglobin-Konzentration bei Wiederholungsmessungen war höher als die der Glukose-Konzentration, (c) die mittlere Hämoglobin- und Glukose-Konzentration unterschied sich von Jahr zu Jahr, (d) Hämoglobin- und Glukose-Konzentration waren negativ korreliert, (e) es gab einen positiven Zusammenhang zwischen Hämoglobin-Konzentration und Bruterfolg.

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## Introduction

Physiological knowledge can improve predictions of organism responses to environmental change, and also provide tools to support evidence-based management

decisions (Cook et al. 2013); Prosser (1991) argued that there is a need for more physiological information on stress affecting individual species. Understanding the influences of variation in habitat quality and anthropogenic disturbance on organism condition and health could improve conservation (Cooke and O'Connor 2010; Ellis et al. 2012).

In altricial birds, during the critically demanding nesting stage, various components of reproductive effort accumulate and corresponding trade-offs become visible (Calow 1979). Thus, some parents cannot invest enough resources to raise offspring of high physiological quality (Sibly and Calow 1986). Nestlings that are in better condition have the chance to more effectively deal with various hazards, such as periods of food shortage, disease or parasitic infestation (Ots et al. 1998; Bańbura et al. 2004). Studies have shown that some blood parameters provide useful indicators of the body condition and state of health of animals, including birds (Atwal et al. 1964; Bradley and Threlfall 1974), when collected and interpreted with appropriate caution (Fair et al. 2007; Lill 2011; Lill et al. 2013). Blood components are good indicators for evaluating short-term stress in the environment (Brown 1996; Sergent et al. 2004). A high diagnostic value of the basic blood parameters results from the close connection of these parameters to factors such as age, physiological condition, circadian rhythms, nutritional status, and others (Yadava 1978; Gee et al. 1981; García-Rodríguez et al. 1987; Cerolini et al. 1990; Abelenda et al. 1993; Kostelecka-Myrcha 1997; Sergent et al. 2004).

Haemoglobin is a simple biochemical indicator of bird metabolism (Sergent et al. 2004; Nadolski et al. 2006; Simmons and Lill 2006) that reflects the nutritional status of the animal, its hydration, and the presence of parasites and pathogens, and allows for the estimation of mineral deficiency (Campbell and Dein 1984; Campbell 1995). Stressors also contribute to changes in the level of haemoglobin, e.g., increasing the ambient temperature causes changes in the concentration of haemoglobin (Wilson 1971), and nutritional deficiencies cause a noticeable drop in the level of haemoglobin (Kasprzyk et al. 2006). Studies showed also that changes in haemoglobin could be caused by natural factors that include age, sex, energy expenditure, parasite pressure and genetics (Clark and Mason 1988; Dufva and Allander 1996; Simon et al. 2004; Słomczyński et al. 2006; Fair et al. 2007).

Glucose is also considered to be an indicator of the nutritional condition of birds (Fairbrother et al. 1990; Ramage-Healey and Romero 2001; Casado et al. 2002; Dunbar et al. 2005; Kaliński et al. 2014). The level of this monosaccharide in passerine birds usually ranges from 200 to 500 mg/dL (Lewandowski et al. 1986; Harris 1991), and is more than 800 mg/dL in hummingbirds (Diamond et al. 1986). Stress factors also raise the level of energy expenditure by the animal and reduce its availability for other

physiological processes. As a result of severe environmental stress, the blood glucose level rises (Graczyk et al. 2003).

Our previous findings on tits showed that the level of concentration of haemoglobin corresponds positively with body condition (on the Great Tit *Parus major*: Nadolski et al. 2006, on the Blue Tit *Cyanistes caeruleus*: Bańbura et al. 2007), while glucose is an inverse index of condition (on the Blue Tit: Kaliński et al. 2014). Therefore, a negative correlation between the concentration of haemoglobin and glucose would be expected, as recently reported by Minias (2014).

In this paper, we analyse year-to-year variation in haemoglobin and blood glucose concentrations of nestling Pied Flycatcher *Ficedula hypoleuca*. Our analysis of the data is aimed at (1) investigating whether haemoglobin and blood glucose concentrations are consistent physiological characteristics of Pied Flycatcher broods, with low variation within broods and high variation among broods; (2) presenting year-to-year variation in levels of both of these condition indicators; (3) analysing the relation of haemoglobin level to glucose level; (4) examining the impact of weather parameters on both of these condition indicators; and (5) showing whether Pied Flycatcher breeding success is linked to both of these physiological condition indicators and to mass of nestlings. It is worth mentioning that we are not aware of any published results analysing differences in haemoglobin and glucose concentrations between years, or their relation to each other in Pied Flycatcher nestlings.

## Materials and methods

This study, carried out during four breeding seasons from 2011 to 2014, is part of a long-term project of research into the breeding biology of secondary cavity nesters around Łódź, central Poland. The forest study area (51°50'N, 19°29'E), bordering on the NE part of the city, is a c. 130 ha area in the center of mature mixed deciduous forest (1250 ha in total) with oaks *Quercus robur* and *Q. petraea* as predominating tree species.

The study site was supplied with 300 standard wooden nestboxes (Lambrechts et al. 2010). For every breeding season, the nestboxes were first occupied by Blue Tits and Great Tits. Pied Flycatchers arrived at the breeding area later, and occupied free nestboxes (Skwarska et al. 2012). During the breeding season, the nestboxes were visited at least once a week (or every day if needed) to record laying date, clutch size, the number of nestlings and the nestlings' basic developmental traits. The Pied Flycatcher 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) 13–14 days after the hatching of the first egg (nestlings fledge on c. the 18th day in our study population). A random subsample of three nestlings, blind-drawn out of same-age nestlings from every Pied Flycatcher brood, was designated for blood sampling. Samples of c. 5 µL of blood were taken from the ulnar vein of nestlings, placed in HemoCue cuvettes, and analysed in the field using a portable HemoCue Hb 201+ photometer to measure haemoglobin concentration (g/L). A portable HemoCue Glucose 201+ photometer (HemoCue AB, Angelholm, Sweden) was used to establish blood glucose concentration (mg/dL) in another sample of blood. All field procedures were carried out between 9.00 AM and 2.00 PM. During the 4 years of the study, 111 nestlings (2011–27, 2012–36, 2013–30, 2014–18) from 37 broods of Pied Flycatcher were examined.

Yearly means of haemoglobin levels and glucose levels were tested for correlation with the following average weather variables for the same years: mean daily minimum temperature and mean rainfall. The above correlations with weather variables were analysed for a 14-day period, beginning on the first hatching day in a given year for a given nest. The weather data for Łódź were obtained from TuTiempo.net climate data base (<http://www.tutiempo.net/en/Climate/LODZ/124650.htm>). The repeatability of haemoglobin and blood glucose concentrations 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 among different broods (Bańbura and Zieliński 1990). Repeatability is low if measurements within broods are very different. Because haemoglobin and glucose concentrations 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 controlling for clustering; restricted maximum likelihood estimates were used and degrees of freedom were approximated by the Satterthwaite method (Heck et al. 2010). Effects of year on the haemoglobin and glucose concentrations were modelled in an ANCOVA style by fitting a model that included wing length as an age-controlling covariate (Crawley 2002). Relationships between haemoglobin concentration, blood glucose level, nestling mass and breeding success (breeding success refers to the proportion of eggs resulting in young that left the nest) were examined using a generalized linear model with binomial error distribution, logit link function, and Wald Chi squared test statistics (Crawley 2002). Relations between yearly mean haemoglobin concentration, yearly mean blood glucose level and weather variables were examined using Pearson’s linear correlation. Pearson correlations were calculated in STATISTICA 10

(StatSoft Inc. 2011). Mixed linear models and generalized linear models were calculated using IBM SPSS 15.0 software (Heck et al. 2010; SPSS 2006).

## Results

The concentration of haemoglobin of the nestlings from the same brood tended to be consistently similar, with much variation occurring among broods, resulting in significant within-brood repeatability ( $R = 0.63 \pm 0.08$  (SE),  $F_{38;72} = 6.19$ ,  $p < 0.001$ ). The concentration of the blood glucose of the nestlings from the same brood also tended to be consistently similar, with much variation occurring among broods, resulting in significant within-brood repeatability ( $R = 0.32 \pm 0.11$  (SE),  $F_{38;70} = 2.39$ ,  $p < 0.001$ ).

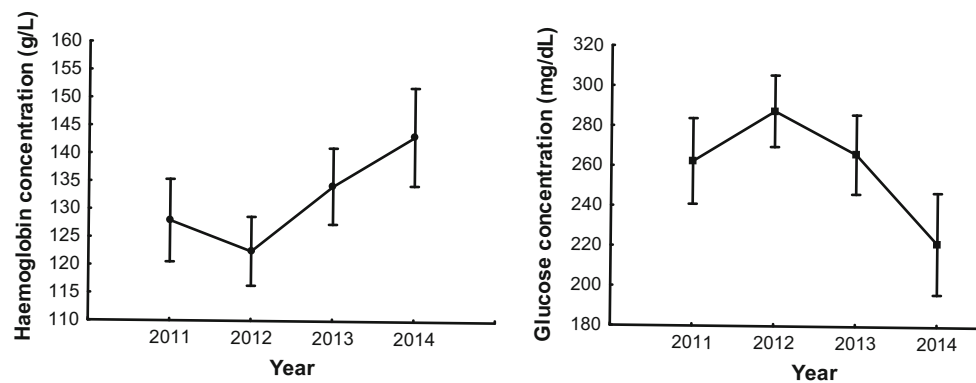
Haemoglobin concentration and blood glucose concentration in nestling Pied Flycatchers differed between years (Table 1). The minimum individual haemoglobin level was 101 g/L (2012), and the maximum individual value was 167 g/L (2014). Corresponding brood mean values were 103.67 g/L (2012) and 157 g/L (2014, Fig. 1). The minimum individual glucose concentration was 161 mg/dL (2014), and the maximum individual value was 394 mg/dL (2012). Corresponding brood mean values were 175.67 mg/dL (2014) and 341 mg/dL (2012, Fig. 1). The mean haemoglobin level and the mean blood glucose level were negatively correlated ( $R = -0.34$ ,  $N = 37$ ,  $P = 0.04$ ).

For the 14-day-long period beginning on the first hatching day, mean daily minimum temperatures significantly affected mean haemoglobin levels ( $R = 0.34$ ,  $N = 37$ ,  $p = 0.04$ , Fig. 2). There was a marginally non-significant positive correlation between the mean haemoglobin levels and mean rainfall ( $R = 0.31$ ,  $N = 37$ ,  $p = 0.06$ ). There was also a marginally nonsignificant negative correlation between the mean blood glucose levels and mean rainfall ( $R = -0.30$ ,  $N = 37$ ,  $p = 0.07$ ).

**Table 1** Linear mixed model tests for the effects of year on haemoglobin and glucose concentrations in the blood of nestling Pied Flycatchers

Y-variable and effects	df	F	p
<b>Haemoglobin</b>			
Year	3; 33.96	6.30	<b>0.002</b>
Wing length (cov)	1; 57.14	12.09	<b>0.001</b>
<b>Glucose</b>			
Year	3; 31.74	6.30	<b>0.002</b>
Wing length (cov)	1; 37.30	8.56	<b>0.006</b>

Wing length was used as a covariate (significant *p* values in the model are in bold)



**Fig. 1** Annual variation in mean haemoglobin and mean blood glucose concentrations in the blood of Pied Flycatcher nestlings. Mean  $\pm$  standard errors corrected for wing length as covariate are shown

There was a positive relationship between breeding success of Pied Flycatchers and the per-brood mean haemoglobin concentration in nestling blood and mean nestling mass (Table 2). There was no significant relation between breeding success and mean blood glucose level (Table 2). The same relationships were revealed when mean haemoglobin and mean glucose were analysed in separate models (generalized linear models: Wald  $\chi^2_1 = 4.00$ ,  $p = 0.046$  for haemoglobin model; Wald  $\chi^2_1 = 0.54$ ,  $p = 0.46$  for glucose model).

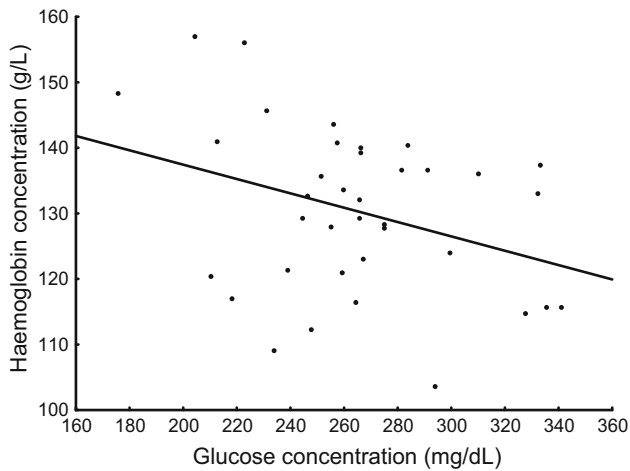
## Discussion

We are not aware of any published results showing differences in haemoglobin and glucose concentrations between years, or the relationship between haemoglobin and glucose levels in Pied Flycatcher nestlings. The differences in haemoglobin and glucose levels between individual nestlings within a brood being low in relation to differences among separate broods have been described in tit species (repeatability of haemoglobin for Great Tits: 0.53, Nadolski et al. 2006, for Blue Tits: 0.53–0.77, Bańbura et al. 2007 and repeatability of glucose: 0.43, Kaliński et al. 2014). Repeatability of glucose (0.32) in our study is lower than that for haemoglobin (0.63), which is in agreement with our previous findings on tits, but more studies are needed to determine whether it may be considered as a general pattern in wild populations of birds. A value of glucose repeatability that is lower than that for haemoglobin repeatability suggests that blood glucose fluctuates more. Glucose concentrations (in contrast to haemoglobin) probably show rapid changes in response to food intake, so that if all the chicks are not fed by the parents simultaneously, then glucose concentrations are expected to show more intrabrood variation. It could also be that haemoglobin concentration responds more to the

factors acting on entire broods during the whole nestling stage, such as general trophic conditions and parasitic pressure in the nest, which would further explain higher intrabrood repeatability of this measure. Potti et al. (1999) assessed within-brood repeatability of haematocrit in Pied Flycatcher nestlings and found it to be 0.7, which is similar to our result for haemoglobin.

Haemoglobin and glucose concentrations were negatively correlated. Our previous findings on tits foresaw the possibility of such a relationship (Bańbura et al. 2007; Kaliński et al. 2014), and it would be expected, assuming that hyperglycaemic states are likely to co-occur with anaemic symptoms in vertebrates. Minias (2014) found a strong negative correlation between the concentrations of haemoglobin and glucose in adult Whiskered Tern *Chlidonias hybrida*. This author stated that he was not aware of any other studies with empirically evaluated associations between blood concentrations of haemoglobin and glucose in wild populations of birds, and that more empirical studies were necessary to determine whether it could be considered as a general pattern in wild-living avian species. Our data confirm the existence of this pattern, being presumably the second study that shows this relationship, and the first to show it in Pied Flycatcher.

It is likely that one of the factors responsible for variation in the level of haemoglobin between years was the weather. Our results suggest that in springs with lower temperatures, mean haemoglobin levels are lower and there was a tendency (marginally nonsignificant positive correlation) suggesting that moderate rainfall may have some positive impact on haemoglobin concentration and a negative impact on glucose concentration. A low temperature inhibits activity of insects (Mellanby 1939; Bale 2002), and very low temperatures may partly reduce prey accessibility for birds (Kaliński et al. 2014). Low temperatures may also reduce female capacity for warming chicks efficiently (Kaliński et al. 2014). Thus a



**Fig. 2** Relationship between concentrations of haemoglobin and glucose in nestling Pied Flycatchers. The *line* represents the fitted regression

**Table 2** Results of generalized linear binomial models showing correlative relationship of mean haemoglobin concentration, mean glucose concentration and mean nestling mass on breeding success (the number of fledglings in relation to clutch size) of Pied Flycatcher broods

Effects	Est ± SE	Wald $\chi^2$	df	p
Mean haemoglobin	0.038 ± 0.019	4.00	1	<b>0.046</b>
Mean glucose	−0.004 ± 0.006	0.54	1	0.463
Mean mass	0.467 ± 0.238	3.86	1	<b>0.049</b>

Significant *p* values in the model are in bold

combination of mild temperatures and regular but not heavy rainfall provides good conditions and stimulates the development of rich arthropod communities (Southwood 1984; Bolger et al. 2005; Kaliński et al. 2009). Extreme rainfall would probably cause the reverse situation, due to hindered foraging (Zajac 1995; Radford et al. 2001).

Haemoglobin concentration and body mass have turned out to be predictors of breeding success. Broods that were characterized by levels of haemoglobin and higher mass had higher breeding success. This result is in tune with our previous findings on tit species (Nadolski et al. 2006; Kaliński et al. 2009). In many bird species, the condition of nestlings is often linked to the probability of survival to the fledging stage, and later for final individual success in recruiting to the breeding population (Brown 1996). Haemoglobin concentration is functionally related to oxygen carrying capacity, which is sensitive to the nutritional state, as well as hydration and mineral deficiencies (Amand 1986; Campbell 1995). Body weight, frequently used as a reliable and easy-to-obtain condition index (Schulte-Hostedde et al. 2005; Labocha and Hayes 2012), depends

on many environmental and individual factors (van Noordwijk et al. 1988; Acquarone et al. 2002; Cucco et al. 2002). One of the important environmental factors affecting condition indicators is the amount and quality of food, as well as diversity of nutrients, affecting daily pattern and body mass index (Owen et al. 2005; Labocha and Hayes 2012). Naef-Daenzer and Keller (1999) showed for tits that the pattern of variation in the abundance of caterpillars (main food during the breeding season for tits) reflects average body condition of nestlings. Bańbura et al. (2007) showed for Blue Tits that haemoglobin concentration correlates directly with food-abundance pattern differences between years. Thus, it would seem reasonable that food-abundance is partly responsible for variations in haemoglobin levels in Pied Flycatcher nestlings.

Kaliński et al. (2014) showed in tits that high glucose levels negatively affected fledging and breeding success. We found no such a pattern in Pied Flycatcher nestlings. Kern et al. (2001) analyzed glucose concentrations in Pied Flycatcher nestlings. Blood glucose was little affected by fasting, but increased throughout development. Their results showing that glucose concentration was not a very good indicator of nutritional status is consistent with our findings from a Polish population. Blood glucose also tends to show rapid changes within a very short period of time, and may show circadian rhythms and seasonal variation, and also depends on other factors (Bairlein 1983; Totzke and Bairlein 1998; Ramage-Healey and Romero 2000; Prinzing and Misovic 2010; Scanes and Braun 2013). Additionally, perhaps this relationship was more subtle than haemoglobin-breeding success, and a relatively small sample size and only four breeding seasons made it impossible to detect.

Our data support the idea that haemoglobin concentration may be considered a reliable condition indicator and is useful in field studies of the ecophysiology of Pied Flycatchers. While glucose concentration is probably also indicative of condition, it is usually much more difficult to interpret, and it may fluctuate more due to uncontrollable factors. More studies are needed to examine whether negative haemoglobin–glucose relations and differences between haemoglobin and glucose repeatabilities may be considered to be general patterns in wild populations of birds.

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