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Haematological parameters of pond bats (*Myotis dasycneme* Boie, 1825 Chiroptera: Vespertilionidae) in the Ural Mountains

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ABSTRACT
Haematological parameters of pond bats (*Myotis dasycneme,* Chiroptera), the most numerous bat species in the Ural Mountains, were investigated. Blood samples were collected from 40 wild bats. Ten haematological parameters were determined using an automated haematological analyser. Morphological differentiation of leukocytes was performed in blood smears. No significant differences were revealed in the main parameters of peripheral blood between adult males and females of *Myotis dasycneme* except for the content of haemoglobin ($p = 0.01$) and leukocytes ($p = 0.02$). Data show that significant differences exist between adult and subadult females in erythropoiesis (RBC, HGB, HCT, and MCV). Thus, with age, the number of erythrocytes and the haemoglobin level increase ($p < 0.05$), but the leukocytic chain remains relatively stable. Changes in haematological parameters were observed in the bats caught in the season of preparation for long hibernation (6–7 months). Conclusion: The blood system of *Myotis dasycneme* from natural populations in the Ural Mountains was investigated for the first time. It was found that bat growth and development are accompanied by significant changes in red blood cell indicators whereas the white blood cell count remains stable. Adaptive lability of red blood cells in bats allows them to respond adequately to seasonal environmental conditions.

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Myotis dasycneme; blood; sex; adult; subadult

Introduction

Chiroptera are a priority object of nature protection (Agreement 1991; The Convention 1992). Bats are often seen as indicators of the high homeostatic quality of biocenoses, viability and richness of ecological systems (Wilkinson and South 2002; Boye and Dietz 2005; Calisher et al. 2006; Kunz et al. 2011; Silva, Barreiro, and Alves 2014; Zidar and Zagmajster 2014; Zukal, Pikula, and Bandouchova 2015; Voigt and Kingston 2016). The most widespread bat species in the Urals region is *Myotis dasycneme.* Although ecological conditions in the Urals are not favourable, the area inhabited by *Myotis dasycneme* is wide. Mammals of this group carry out communication between aquatic, aerial and terrestrial ecosystems. For wintering, bats use caves, the ecological niche that is inaccessible to people. High bat activity is observed near cave entrances during spring and autumn migrations. Thus, the colony of more than 1700 bats hibernating in the cave Smolinskaya (Sverdlovsk region) was found in November (Bolshakov, Orlov, and Snitko 2005). It is very important to investigate blood as a functional system which unites the physiological system of an organism and is involved in the regulation of compensation and adaptive mechanisms (Kovalchuk and Yastrebov 2003; Kovalchuk 2008; Baker, Schountz, and Wang 2013; Kovalchuk, Mishchenko, and Chernaya 2014; Wawrocká and Bartonička 2014).

Aim

The main aim of this study was to deepen the knowledge of the blood system of *Myotis dasycneme* (Mammalia, Chiroptera), the most numerous species of natural bat populations in the Ural Mountains.

Materials and methods

Object of research

The current study into pond bats (*Myotis dasycneme*) of natural populations in the Ural Mountains was carried out between July and September 2013. The animals were caught in the Ilmensky Reserve (55°00′55″N, 60°09′30″E)

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on the shore of Lake Maloye Miassovo in the second half of July (at the average daily air temperature of +21 to +23 °C) and in the Smolinskaya karst cave (56°25′74″N, 61°36′74″E) in the second half of September (at the average daily air temperature of +5 to +7 °C). About 78% of the area is covered by woodland, with bogs and ponds occupying the rest of it. Arable land and pastures occupy an insignificant part. The height of the Ural mountains is 500–800 meters above the sea level. The climate is moderately continental.

According to the data reported by Bolshakov, Orlov, and Snitko (2005), the temperature in the cave throughout the year was constant (4–5 °C). Bats were captured when they returned to their roosts at dusk and before dawn between 5 and 7 am. All bats were caught with a hand net or a harp trap and were placed into light-weight drawstring cloth bags (12 cm × 16 cm) individually. Then these cloth bags were hung in a cooler. The temperature in the cooler was monitored with a thermometer so that it could be maintained similar to that in bat roosts. Morphometric measurements of bats were taken and sex of all individuals was identified. We also measured the body weight of all bats (with accuracy of 0.1 g) using an electronic balance (Acculab Pp-200dl1).

Studies were performed in laboratory conditions. The tested animal group included 40 *M. dasycneme* individuals. The young animals, *subadults*, were distinguished from *adults* visually by the ossification degree in the epiphysis of wing bones, metacarpals and phalanxes (Strelkov 1999). Seasonal and sexual distinctions were studied in adult bats. The animals were caught and maintained in the laboratory in compliance with the rules set out in the European Convention on Protection of Animals Used for Experimental and Scientific Purposes (The European 1986) and in the Oxford Handbook of Animal Ethics (Carbone 2004; Yarri 2005). Sampling of *M. dasycneme* was approved by the Committee for Ethics and by the Department of Federal Agency for Supervision and Management of the Environment in the Urals region [http://www.rpn-itfo.ru].

Methods

It is known that in many species, the size of bat individuals presents a challenge for obtaining large amounts of the blood necessary for long-term experiments (Baer and McLean 1972; Gustafson and Damassa 1985; Swann et al. 1997; Appleton, McKenzie, and Christidis 2004; Wimsatt et al. 2005; McLaughlin et al. 2007; Smith, De Jong, and Field 2010, 2011; Racey, Swift, and MacKie 2011; Schinnerl et al. 2011; Schneeberger et al. 2014; Strobel et al. 2015). Blood samples were collected from wild bats.

Blood samples (400–800 µl) were taken with sterile heparinised capillaries from the animals that, after etherisation, were killed by decapitation. The blood samples were collected into sterile vacutainer tubes (Becton Dickinson DP). Two hundred micro litre blood samples were analysed for haematological parameters, while part of blood plasma was stored for the further quantification of amino acids. Ten indicators of the periphery blood of the animals were estimated using the haematological analyser 'BC – 5800' (Mindrai, China): the total number of leukocytes (WBC), the total number of erythrocytes (RBC), haemoglobin (HGB), haematocrit (HCT), indicators: average erythrocyte volume (MCV), the mean corpuscular haemoglobin (MCH), the mean corpuscular haemoglobin concentration (MCHC), the quantity of thrombocytes (PLT), the average volume of thrombocytes (MPV), and thrombocrit (PCT). 50–100 µl of blood was used for preparing blood smears on glass slides. The leukocyte formula (percentage of various kinds of leukocytes) was calculated in the blood smears coloured using the Romanovsky-Himze method. Calculations were made for 100 leukocytes. The absolute ratio of leukocytes (G/L = 10^9 cells/L) was obtained by converting percentage to the total number of leukocytes (Kamyshnikov 2004).

Statistical methods

Results were processed using the package of the licensed programs 'Microsoft Excel 2003' and 'Statistica v. 6.0' (StatSoft, Inc., 1984–2001). Results were reported as $\bar{X}$ ± SE and as ranges. The analysis of peripheral blood indices between groups of bats (adults – subadults) and (males – females) was performed using the non-parametric Mann–Whitney *U*-test (Table 1). The statistical significance of differences between the compared samples was at $p < 0.05$. The analysis of seasonal changes in *Myotis dasycneme* peripheral blood was performed using the Principal Component Analysis (PCA) in the R environment (R 3.1.2, the ade4 package) (Chessel, Dufour, and Thioulouse 2004).

Results and discussion

The current study into the bat blood system revealed that there were no significant differences between the main blood parameters of adult males and females (Table 1). Significant distinctions were noted only in haemoglobin (HGB) (187.9 ± 3.2 g/L in males, 177.6 ± 2.1 g/L in females [$p = 0.01$]) and in leukocytes (WBC) (1.9 ± 0.1 G/L in males, 4.7 ± 0.5 G/L in females [$p = 0.01$]). We noted a tendency towards a lower number of thrombocytes (PLT) in females than in males, which is also characteristic of other mammals (Abrashova et al. 2010; Dannikov 2013).

We noted changes in white blood cells of *M. dasycneme* males and females.

A 2.5-fold increase in the number of leukocytes in the blood of females ($p = 0.01$) was caused by qualitative and quantitative changes in white blood cells. Also, a higher percentage ($p = 0.004$) and an absolute increase

Table 1. Indices of *Myotis dasycneme* peripheral blood.

Parameters	I. Autumn (n = 10) ♂adults $M \pm m$ (min–max)	II. Autumn (n = 8) ♀adults $M \pm m$ (min–max)	III. Summer (n = 5) ♀adults $M \pm m$ (min–max)	IV. Summer (n = 4) ♀subadults $M \pm m$ (min–max)	p-value (U-test, Mann–Whitney) I–II	II–III	III–IV
WBC, G/L	1.9 ± 0.1 (1.5–2.3)	4.7 ± 0.5* (3.9–6.0)	2.9 ± 0.4▲ (2.04–3.9)	2.7 ± 0.3 (2.1–3.1)	0.02	0.04	0.96
RBC, T/L	11.3 ± 0.5 (9.0–12.9)	11.1 ± 0.2 (10.6–11.6)	9.3 ± 0.5▲ (8.24–10.9)	5.7 ± 0.4¶ (4.6–6.5)	0.7	0.02	0.00005
HGB, g/L	187.9 ± 3.2 (177.0–200.0)	177.6 ± 2.1* (175.0–186.0)	152.6 ± 5.5▲ (136.0–166.0)	101.5 ± 6.5¶ (83.0–111.0)	0.01	0.01	0.00005
HCT, %	53.5 ± 1.7 (45.5–59.3)	51.17 ± 1.05 (46.3–54.0)	45.1 ± 1.8▲ (41.9–50.2)	30.0 ± 2.3¶ (23.4–33.6)	0.34	0.03	0.00005
MCV, fl	47.6 ± 0.4 (45.7–49.1)	46.7 ± 0.3 (45.9–47.7)	48.5 ± 0.8▲ (46.3–50.9)	52.3 ± 0.6¶ (51.5–54.0)	0.14	0.03	0.00005
MCH, pg	17.3 ± 0.7 (15.2–19.6)	15.6 ± 0.2 (14.8–16.2)	16.6 ± 1.1 (12.5–18.5)	17.8 ± 0.2 (17.2–18.2)	0.23	0.08	0.32
MCHC, g/L	357.0 ± 9.9 (331.0–394.0)	341.3 ± 3,5 (326.0–361.0)	358.8 ± 9.4 (341.0–377.0)	341.0 ± 5.4 (330.0–355.0)	0.51	0.09	0.14
PLT, G/L	319.6 ± 20.9 (267.0–368.0)	295.2 ± 17,2 (253.0–342.0)	112.8 ± 24.2▲ (63.2–179.0)	213.0 ± 32.3¶ (158.0–293.0)	0.31	0.01	0.04
MPV, fl	6.3 ± 0.2 (5.7–7.1)	6.6 ± 0.1 (5.9–7.1)	6.0 ± 0.3 (5.0–6.8)	6.6 ± 0.2¶ (6.2–6.9)	0.27	0.16	0.05
PCT, %	0.1 ± 0.02 (0.1–0.2)	0.2 ± 0.01 (0.1–0.2)	0.1 ± 0.02▲ (0.03–0.12)	0.1 ± 0.02 (0.1–0.19)	0.11	0.01	0.08

Notes: * – statistically significant differences between groups I and II ($p < 0.05$); ▲ – statistically significant differences between groups II and III ($p < 0.05$); ¶ – statistically significant differences between groups III and IV ($p < 0.05$).

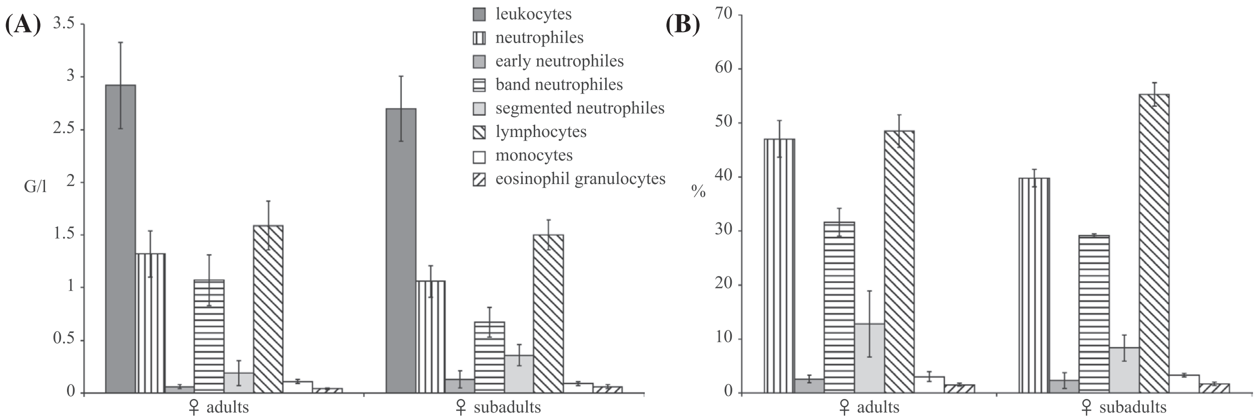

Figure 1. Leukocytic pool of peripheral blood in males and females of *Myotis dasycneme*.
Notes: * – differences between the groups are statistically significant ($p < 0.05$). A – absolute quantity; B – percentage.

in the number of lymphocytes (by 2.4 times, p = 0.01) was observed in females (Figure 1).

Lymphocytes are responsible for immune surveillance and genetic constancy of the inner medium. A similar tendency was revealed in the content of neutrophils (1.0 ± 0.1 G/L in males, 1.8 ± 0.2 G/L in females, p = 0.02). Neutrophils are responsible for the non-specific form of leukocytic protection. In the blood of females, more mature forms of neutrophils were found to prevail: band neutrophils (1.8 times more than in males, p = 0.006) and segmented neutrophils (2.1 times more than in males, p = 0.03). They are able to perform their physiological functions more effectively than immature cells (Figure 1).

We noted a significant decrease in the percentage (B) of monocytes (1.1% in females, 4.6% in males, p = 0.001) and a tendency towards reduction of their absolute (A) quantity (Figure 1). We also reported the redistribution of leukocytes: more lymphocytes and neutrophils. Monocytes play a role in the regulation of erythrocytopoiesis by participating in formation of erithroblastic islands and Fe utilisation (Zaharov and Rassohin 2002). This might explain the connection between a decrease in the haemoglobin count and a decrease in percentage and absolute numbers of monocytes in females.

No statistically significant sex-related differences were found in the percentage of eosinophils and neutrophils (p > 0.05) (Figure 1). The observed sex-related differences in the leukocyte pool confirmed the greater adaptability of female bats.

Age variability

We investigated peculiarities of morphological and physiological parameters of erythrocytes in bats of various age groups. The study into erythrocyte indices in females (adults, subadults) revealed significant differences in erythrocytopoiesis (Tepliy, Nesterov, and Kuperman 2012).

Thus, the number of erythrocytes in the blood of subadults was 1.6 times lower than that in adult females (p = 0.00005). In adults, the average volume of erythrocytes (48.5 ± 0.8) was found to be lower than in young

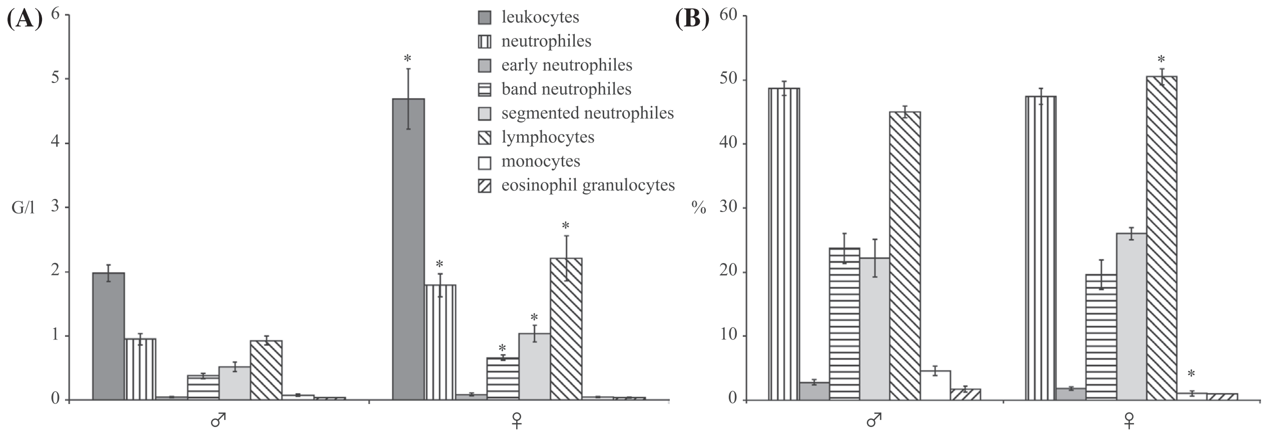

Figure 2. Age-dependent patterns of leukocytic pool in peripheral blood of *Myotis dasycneme* females.
Notes: * – statistically significant differences between the groups ($p < 0.05$). A – absolute quantity; B – percentage.

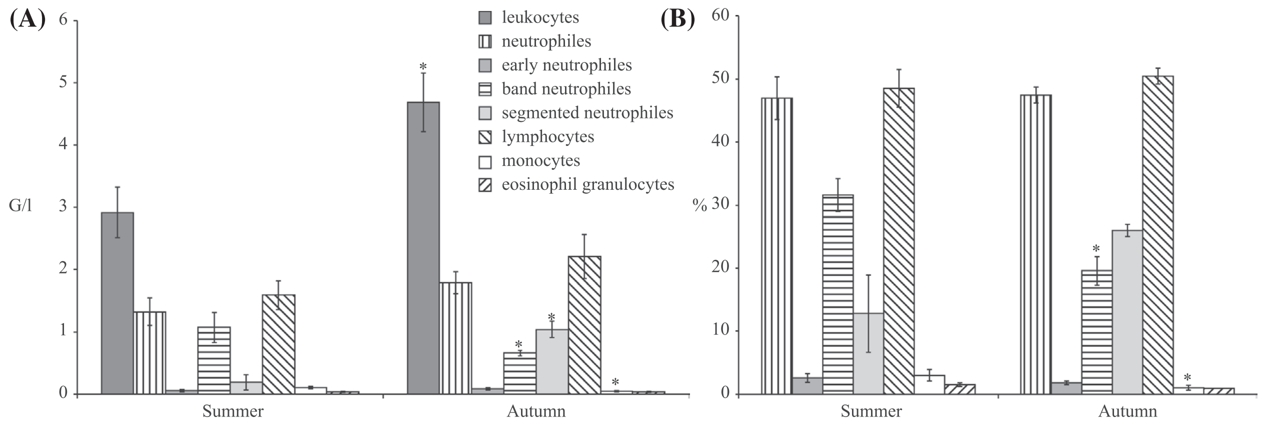

Figure 3. Seasonal changes in leukocytic pool in peripheral blood of *Myotis dasycneme* females.
Notes: * – statistically significant differences between the groups ($p < 0.05$). A – absolute quantity; B – percentage.

bats (MCV = 52.3 ± 0.6) (p = 0.00005). In adults, HGB was 152.6 ± 5.5, which was higher than that in the young: HGB = 101.5 ± 6.5 (p = 0.00005) (Table 1).

The greater number of erythrocytes in mature females was due to the 1.5-fold increase in haematocrit (p = 0.01). The examined specimens exhibited a tendency towards a 1.9-fold higher content of thrombocytes (p = 0.01). With age, thrombocyte aggregation increased and fibrillation processes accelerated. Involution of blood platelets was observed. These processes led to a decreased amount of these cellular elements in peripheral blood (Kuznik, Vitkovsky, and Ljul'kina 2005). The growth and development of bats was accompanied by significant changes in red blood cells, white blood cells retaining stability. No statistically significant age-related differences were found in white blood cells of *M. dasycneme* females (Figure 2).

Seasonal dynamics

In the blood of the animals caught in autumn, during the period of preparation for hibernation, fluctuations were recorded in the erythrocyte pool with a significant increase in the number of erythrocytes (11.1 ± 0.2 in autumn, 9.3 ± 0.5 in summer, p = 0.02), in haematocrit (51.7 ± 1.1% in autumn, 45.1 ± 1.8% in summer, p = 0.03), and in haemoglobin (177.6 ± 2.1 in summer, 152.6 ± 5.4 in autumn, p = 0.01) (Table 1). There was also a 3.7% decrease in MCV (p = 0.03) observed. These changes show that in autumn, during preparation for hibernation, the blood system adapts itself to temperature changes in the environment and to possible further hypoxia.

In autumn, the thrombocyte number in females was 2.6-fold (p = 0.01) higher than in summer, but still within reference values (Table 1). The twice higher thrombocrit recorded (PCT) (p = 0.01) in animals in autumn showed that thrombocytes constitute the major part of the total blood volume. Activation of thrombocytopoiesis was observed at a constantly low ambient temperature. The functional activity of thrombocytes correlated directly with processes of biologically active substances in granulocytes of thrombocytes, which is a very important fact during preparation for hibernation during the winter period (Schiffman 2000).

In autumn, leukocytosis was observed in *M. dasycneme* (1.6 times more leukocytes than in summer, p = 0.04) (Figure 3). The leukocytic pool may respond to the ambient temperature by intensive production of leukocyte

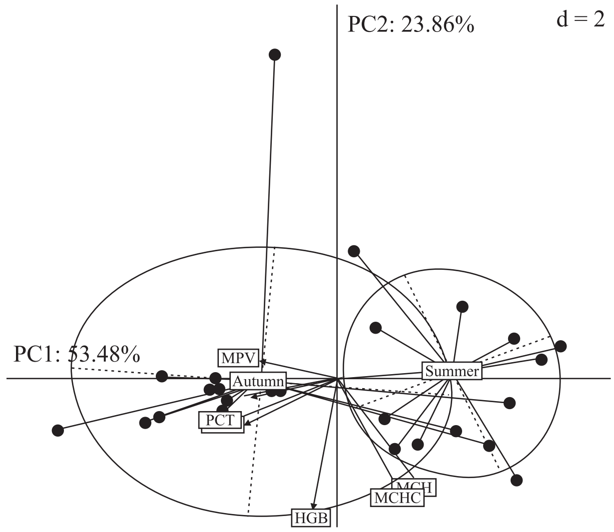

Figure 4. Seasonal changes in peripheral blood of *Myotis dasycneme* (♂adults + ♀adults) [summer (n = 18), autumn (n = 18).

Notes: PC1, PC2 – Principal Components; % – percentage of data dispersion (Table 2); direction and length of arrows show the quality of correlation between variables and principal components; ellipses – 95% confidential areas and gravity centre of classes (main graph).

groups and their redistribution in blood (Abatchikova and Kostesha 2010) (Figure 3). A significant redistribution takes place among neutrophils groups in autumn resulting in a greater number of mature forms.

The percentage and absolute content of band neutrophils decreased 1.6-fold (p = 0.02 and p = 0.01, respectively) in autumn. Possibly, this is an adaptive response of the blood system to the unfavourable conditions of the environment (Speakman and Thomas 2003; Wojciechowski, Jefimow, and Tęgowska 2007; Encarnacao, Otto, and Becker 2012).

A 2.8-fold decrease in percentage (B) and a 2.2-fold decrease in the absolute (A) number of monocytes (p = 0.02 and p = 0.03, respectively) in autumn prevented organisms from the excessive activity of the leukocyte system (Figure 3). The observed tendency for the number of lymphocytes to increase in response to the changing environmental conditions suggests that the qualitative structure of leukocytes changes over time.

As is well known, most insectivorous bats of the family Yespertilionidae in high-latitude and temperate regions are hibernators (Audet and Fenton 1988; Stawski, Willis, and Geiser 2014). The observed maximal differences in haematological indices of *M. dasycneme* were related to the metabolic reorganisation of the organism during preparation for long hibernation (6–7 months).

Conclusions

Bats belong to Chiroptera, and are one of the largest groups in the class Mammalia. For the first time, the blood system of natural *Myotis dasycneme* populations in the Ural Mountains was investigated.

During puberty, higher indices of haemoglobin and erythrocytes were recorded in males than in females. In the earlier study by Woz, Wiesz, and Bogdanowicz (1987), haematological parameters of male and female bats were found to differ only with respect to the number and volume of erythrocytes. Areìvalo, Peìrez-Suaìrez, and Loìpez-Luna (1992) revealed sex-related differences in erythrocyte number, hematocrit and haemoglobin concentration in the bat species Rhinolophus.

This study revealed that bat growth and development are accompanied by significant changes in the count of red blood cells, the number of white blood cells remaining stable (Table 1). The comparison of adult and subadult females' blood revealed significant differences with regard to erythropoiesis. This preliminary comparison showed that the number of erythrocytes in the blood of subadults was 1.6 times lower than that in adult females (p = 0.00005). It was found that the growth and development of bats (females) are accompanied by significant changes in red blood cell indices (p = 0.00005), whereas the count of white blood cells remains stable (p = 0.96). Thus, with age, the number of erythrocytes

Table 2. Coefficients of correlation between 9 indices of *Myotis dasycneme* peripheral blood and principal components: PC1 and PC2 (PCA plot was generated using the Ade4 R package).

	Loadings, a_{ij}			Contribution to principal components = $(a_{ij}^2 \times 100)/\lambda_j$, %)		
	(Principal components – PC), j = 1, 2, 3					
Indices (I = 9)	1	2	3	1	2	3
RBC	**–0.92*****	–0.12	–0.33	**17.61*****	0.72	12.1
HGB	–0.23	**–0.93*****	–0.22	1.08	**40.03*****	5.12
HCT	**–0.79****	–0.13	–0.56	**12.89****	0.78	34.02
MCV	**0.76****	0.04	–0.15	**12.06****	0.07	2.32
MCH	**0.68***	**–0.69***	0.13	**9.64***	**22.24***	1.99
MCHC	0.53	**–0.77****	0.12	5.83	**27.27****	1.63
PLT	**–0.84****	– 0.32	0.26	**14.81****	4.73	7.44
MPV	**–0.71***	0.12	0.45	**10.45***	0.7	22.3
PCT	**–0.87*****	–0.27	0.35	**15.62*****	3.46	13.08
		(Eigenvalues, λ_j) PC			Dispersion: PC (%)	
	4.81	2.15	0.91	53.48	23.86	10.13

Notes: % – percentage of data dispersion; coefficients of correlation statistically significant.
$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

and haemoglobin in the blood of adults increased. The lower HGB concentration in subadults was most likely caused by immaturity of the blood system in the early ontogenetic period (p = 0.00005) (Table 1). Our results are in agreement with those reported by Bassett and Wiederhielm (1984).

The analysis of the obtained data has proved that during hibernation the number of erythrocytes in *M. dasycneme* blood considerably increases (p = 0.02). Similar changes were observed in haemoglobin (p = 0.01) and haematocrit (p = 0.03). The average erythrocyte volume was found to be lower in autumn than in summer (p = 0.03). These changes revealed that in autumn, during the preparation for hibernation, the blood system adapts itself to ambient temperature changes and the possible further hypoxia.

Based on the present study results, we suggest that maximal differences in the haematological parameters recorded during the research into seasonal variability of the blood system are connected with metabolic changes in the organism during preparation for protracted hibernation (6–7 months). It was found that bat growth and development are accompanied by significant changes in red blood cell indices. Our findings and those of the authors Areìvalo, Peìrez-Suaìrez, and Loìpez-Luna (1992), Buffenstein, Maloney, and Bronner (1999), Korine, Zinder, and Arad (1999), Rodriquez-Duran and Padilla-Rodriquez (2008), Schinnerl et al. (2011) indicate that due to adaptive lability of haematological parameters bats are able to respond adequately to seasonal environmental conditions.

The presented classification based on Euclidean distances, allows comparing and making quantitative decisions concerning seasonal variations (summer and autumn) in 9 indices of *M. dasycneme* peripheral blood. Finally, the multivariate analysis (Ade4-generated PCA plot) visualized differences between groups of bats, confirming the above results of the peripheral blood statistical analyses (Figure 4; Table 2). We used the Ade4 multivariate analysis to identify differences between the groups of bats tested in summer and autumn periods, and also, to identify the main elements of *M. dasycneme* peripheral blood that determine essential differences between bat groups at a confidence level of 95%. Figure 4 is a graphical representation of this analysis in the form of a PCA plot. The performed analysis shows that 53.48% of the general dispersion of *Myotis dasycneme* peripheral blood lies on the first Principal Component (PC1) and 23.86% on the second Principal Component (PC2) (Figure 4). The greatest contribution to seasonal variability in blood on the Principal Component (PC1) was made RBC, PLT, PCT, HCT, and MCV (Table 2). The greatest contribution to seasonal variability in blood on the Principal Component (PC2) was made by HGB, MCH, and MCHC (Table 2).

It is necessary to note high percentage of HGB, MCH, and MCHC in the general volume of bat blood and their strong correlation with the second Principal Component (PC2). The correlation coefficient between HGB (−0.93***), MCH (−0.69*), MCHC (−0.77**) and the second Principal Component (PC2) is high (Table 2). The Second Principal Component (PC2) indicates certain differences in the basic elements of bat blood, based on which bats can be divided into two distinct summer and autumn period groups.

We hope that the present study will significantly deepen the knowledge of the biology, ecology and conservation of bats.

Disclosure

The authors declare that they have no conflicts of interest regarding the content of this study.

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