

Seasonal Variation in Hematological Indices in Bank Vole (*Clethrionomys glareolus*) in Different Reproductive States

E. A. Tarakhtii and Yu. A. Davydova

*Institute of Plant and Animal Ecology, Ural Division, Russian Academy of Sciences,
ul. Vos'mogo Marta 202, Yekaterinburg, 620144 Russia*

e-mail: tar@ipae.uran.ru

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Abstract—Hematological and hematopoietic indices were studied in various seasons in bank vole in different reproductive states. The seasonal variation in the indices demonstrated patterns typical of each state and reflecting the adaptive response to changed habitat conditions.

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Studies of the mechanisms of animal adaptation to environmental factors is a topical trend in ecophysiology. Seasonal variation in habitat conditions (climate, feeding, etc.) determines many indices of vital activity of animals and animal populations. In particular, population structure changes with season in many small mammal species, thus, providing for population stability. The long-term stationary monitoring in the Visim Reserve (Davydova and Kshnyasev, 2004) allowed us to clarify year-to-year and seasonal variation in demographic structure of bank vole population peculiar in the replacement of the generation of overwintered animals with the physiologically heterogeneous current-year generation. A fraction of underyearlings mature and reproduce, thus, providing for population growth, while the other fraction remains immature until next reproduction season. This pattern of gradual replacement of generations is observed during reproduction season and maintains the population. Immature individuals are more resistant to environmental factors as compared to mature ones (Olenev, 1989; Olenev and Grigorikina, 1998) and differ by some hematological indices (Tarakhtii *et al.*, 2005). The revealed differences in some characters among individuals of different reproductive states assume specific mechanisms of their adaptive responses to the factors in effect.

Approaches to the mechanisms of population stability include investigation of the hematopoietic system directly or indirectly responding to environmental changes. The changes in the structure and function of erythrocytes (Kalabukhov, 1969) closely associated with energy metabolism, which is the key step in adaptation (Baevskii, 1979), play the leading role in the evolution of body's oxygen supply.

The population of erythrocytes is heterogeneous (Kliorin and Tiunov, 1974). Erythrocyte properties such as the number, size, shape, and the ratio of blood hemoglobin concentration to erythrocyte surface area

allow us to describe hematopoietic status (Goldberg and Levina, 1969) and the rate of oxygen binding to hemoglobin (Kostelecka-Myrcha, 1967, 2002) as well as to predict body's resistance (Kliorin and Tiunov, 1974) and to describe animal state (Tarakhtii and Kardonina, 1995) and population health in radionuclide-contaminated areas (Tsyb *et al.*, 1996) including the identification of groups with structurally abnormal cells of the blood system undetectable by traditional laboratory techniques. Studies of the complex of quantitative morphological indices of the blood and hematopoietic tissue expose the mechanisms of their activity under the influence of natural and anthropogenic factors (Komar, 1992; Kozinets *et al.*, 1993).

Blood indices are known to considerably vary in small mammals. For instance, the leukocyte count is 1×10^3 – $5 \times 10^3/\mu\text{l}$ blood; erythrocyte count, 6.0×10^6 – 9.5×10^6 ; hemoglobin concentration, 12.0–22.9 g %; and hematocrit, 47.1–53.3%. The high variation in the mean values of these indices is attributed to animal age, habitat conditions, and season (Istomina *et al.*, 1971; Baragunova, 1994; Kovalchuk and Yastrebov, 2003). It can also be related to the reproductive state of animals (Chernyavskii and Lazutkin, 2004; Tarakhtii *et al.*, 2005). Evaluation of hematological indices can describe, on the one hand, the body's response to changing environmental conditions and, on the other hand, these conditions.

The goal of this work was to evaluate the hematological and hematopoietic indices in bank vole in different reproductive states as a function of the season and to evaluate the pattern of their changes in the animals.

MATERIALS AND METHODS

The study was carried out on bank vole (*Clethrionomys glareolus* Schreber, 1780), a dominant species in the community of small mammals with the proportion

in samples ranging from 29 to 100% depending on the year (Olenev, 1989; Davydova, 2004). The animals were captured in May, July, and September 2003 and 2004 and in February 2004 using live traps as described elsewhere (Karaseva and Telitsina, 1996) in primary fir spruce forests in the Visim State Biospheric Reserve (southern dark coniferous taiga, Central Urals, Sutuk Low Mountain Range Region, GPS coordinates: 57°22' N, 59°46' E, 538 masl).

Body weight, sex, state of generative organs (weight, size, and turgor of the testicles, epididymal fullness, and seminal vesicle size in males as well as vagina and uterus size, the presence of placental spots, embryos, yellow bodies in the ovaries, etc. in females), morphophysiological indices (Shvarts *et al.*, 1958), and age (by teeth examination; Olenev, 1989) were recorded for each individual 2–3 days after their arrival to the laboratory. Animals were assigned into three groups: overwintered animals, mature underyearlings, and immature underyearlings. The hematological indices were determined in 112 individuals (Table 1). Pregnant females and young individuals weighing less than 15 g were not analyzed.

In addition to the traditional hematological indices (leukocyte count, erythrocyte count, blood hemoglobin concentration (*HB*), hematocrit (*HT*), and blood count), bone marrow count in the shaft of femur was determined in a Goryaev's hemocytometer as well as the proportion between erythrocyte size fractions (3.5–8.9 μm , 10 points) using a Celloscope 401 counter (Lars Yungberg, Sweden) according to the manufacturer's instructions. The mean erythrocyte diameter (*D*), volume (*MCV*), surface area (*S*), mean cell hemoglobin (*MCH*), and mean cell hemoglobin concentration (*MCHC*) (*Laboratornye metody...*, 1987), as well as oxygen carrying capacity per unit volume of blood (*E*; Kostelecka-Myrcha, 1973) and per unit volume of blood with erythrocyte number normalized to body weight (*E*, cells/g body weight) were calculated. In addition to the standard counts, the erythrocyte and bone marrow counts were normalized to body weight considering that their number changes with age (Yushkov *et al.*, 1999).

The obtained data were subjected to discriminant analysis and analysis of variance using Statistica 6.0 package. All statistical tests were conducted at 5% significance level.

RESULTS AND DISCUSSION

Long-term stationary monitoring of the density and demographic (sex and age) structure of bank vole population in the Visim Reserve demonstrated cyclic seasonal and year-to-year variations in these indices. The population structure followed the season-specific phases of growth, peak, and depression in a three-year cycle. The bank vole population at the peak phase in 2004 had no mature underyearlings, while these peaked

at the growth phase in 2003 (Davydova, 2004; Davydova and Kshnyasev, 2004).

Discriminant analysis of all studied indices demonstrated the difference between the vole groups ($F_{(190,926)} = 3.468$, $p < 0.001$; Table 1), although the intergroup variation increased when the data for males and females were combined. According to the classification matrix, 78% of all animals correspond to the a priori classification. At the same time, a 90–100% correspondence was observed in groups 1, 5, and 10; while a 54–42% correspondence was observed in groups 7, 8, and 2. Overwintered individuals (3) indistinguishable from mature underyearlings (6, 7, and 8) as well as mature underyearlings (6) indistinguishable from overwintered animals (3, 4, and 5) by a set of characters cannot be represented by a common sample, since animal groups 6, 7, and 8 as well as 3, 4, and 5 differ from one another.

The analysis of variance demonstrated the effect of season ($R\text{-Pao}_{(75,553)} = 4.436$, $p < 0.001$, where $R\text{-Pao}$ is the test for equality of mean vectors) and individual reproductive state ($R\text{-Pao}_{(50,370)} = 4.328$, $p < 0.001$) on the indices. Accordingly, the variation of individual characters as a function of season is considered in voles in each reproductive state.

Age is known to affect hematological indices in humans and animals (Yuskov *et al.*, 1999). The changes in the hematological indices of bank voles as a function of their age was evaluated for each reproductive state using a covariance analysis model with age as a covariant and season as a factor. The age had effect only on the weight ($b = 0.548$, $s.e.(b) = 0.204$, $\beta = 0.61$, $t_{17} = 2.68$) and spleen index ($b = 0.024$, $s.e.(b) = 0.007$, $\beta = 0.676$, $t_{17} = 3.167$) in overwintered animals; season is considered as a factor below.

Qualitative and quantitative variation of the indices is a function of season ($R\text{-Pao}_{(23,27)} = 3.89$, $p < 0.001$) in overwintered animals representing the bulk of spring and autumn population of 2004 (Table 2). Summer animals demonstrated lower erythrocyte count, *HB*, and *E* compared to the spring and autumn ones. At the same time, *D*, *S*, and *MCV* were higher due to a greater proportion of cells with diameters of 7.3 and 6.1 μm and a lower proportion of cells with a diameter of 4 μm ($p < 0.1$). Autumn animals had the maximum erythrocyte count; cells were largely small (3.5 and 4.0 μm : 72% as against 66 and 59% in spring and summer animals, respectively), short-lived, and with a lower *MCV* and *MCH*. These animals had the maximum bone marrow count and *MCHC*. The revealed seasonal changes in the characters likely reflect the adaptive response of the blood system in overwintered animals targeted to maintain the physiological supply of tissues with oxygen.

In contrast to published data (Bol'shakov *et al.*, 1984), the obtained data point to the correlation between blood hemoglobin content and erythrocyte count in overwintered voles. A direct correlation was

Table 1. Discriminant analysis. Structure and number of analyzed animals and timing

Animals	Overwintered										Underyearlings				
	Capture time										immature				
	May 2003	July 2003	May 2004	July 2004	September 2004	May 2003	July 2003	September 2003	July 2004	September 2004	February 2004				
Number of animals	2	1	12	5	5	3	5	12	19	24	18				
Group	1	2	3	4	5	6	7	8	10	11	12				
1		2.55*	1.04	1.34	1.06	0.76	1.29	1.27	1.64	2.08*	1.16				
2	106.75		3.48*	3.48*	3.60*	2.55*	3.64*	3.80*	4.22*	4.74*	4.35*				
3	23.29	78.21		2.26*	2.09*	0.93	2.41*	2.08*	6.24*	8.46*	4.50*				
4	35.02	91.10	15.25		2.98*	1.81*	2.01*	2.15*	3.05*	3.42*	3.91*				
5	27.74	94.39	14.08	31.21		1.24	2.24*	2.92*	3.49*	3.94*	2.34*				
6	23.77	80.29	11.09	28.54	19.50		1.23	1.13	1.68	2.45*	1.48				
7	29.86	83.91	8.67	14.73	16.47	15.45		2.40*	5.00*	6.79*	4.71*				
8	29.05	86.95	7.07	15.34	20.87	14.03	9.62		1.68	4.72*	3.12*				
10	35.37	91.20	13.44	17.99	20.60	18.71	13.63	4.30		3.76*	4.96*				
11	45.99	105.01	22.52	21.90	25.23	28.50	22.15	14.49	6.84		5.01*				
12	25.42	95.16	10.84	24.06	14.40	16.82	14.16	8.79	7.78	10.41					

Note: Mahalanobis distance square and $F_{(190,926)}$ test are given below and above the diagonal, respectively.

* $p < 0.05$ (for Tables 1–4).

Table 2. Analysis of variance. Mean indices in overwintered voles

Index	Result of analysis		Season			<i>p</i> < 0.05
	<i>MS</i> _{res}	<i>F</i> _(2,19)	spring (1)	summer (2)	autumn (3)	
Body weight, g	5.06	1.50	26.4	24.5	25.0	
Age, days	15.94	30.32*	362	463	513	1–2, 3
Spleen weight, g	1293.76	1.13	82.0	96.8	62.8	
Spleen index	1.96	1.29	3.1	3.9	2.5	
Bone marrow, 10 ⁶	23.82	6.94*	15.93	15.86	25.16	3–1, 2
Bone marrow, 10 ⁶ /g	0.04	8.17*	0.61	0.65	1.01	3–1, 2
Leukocytes, 10 ³	2.87	1.22	2.28	3.59	3.16	
Erythrocytes, 10 ⁶	6.47	5.77*	8.67	6.04	11.50	3–1, 2
Erythrocytes 10 ⁶ /g	0.01	5.36*	0.33	0.25	0.46	3–1, 2
<i>D</i> 9.6 μm, %	0.01	1.95	0.02	0.09	0.09	
<i>D</i> 8.9 μm, %	0.06	2.29	0.14	0.36	0.03	
<i>D</i> 8.2 μm, %	1.27	1.53	0.35	1.26	0.12	
<i>D</i> 7.3 μm, %	0.10	6.95*	0.54	1.03	0.32	2–1, 3
<i>D</i> 6.8 μm, %	1.19	0.68	1.63	1.46	0.96	
<i>D</i> 6.1 μm, %	2.19	5.41*	2.78	5.37	3.60	2–1, 3
<i>D</i> 5.4 μm, %	11.32	0.91	7.05	8.19	5.36	
<i>D</i> 4.7 μm, %	21.48	1.59	21.31	22.83	17.81	
<i>D</i> 4 μm, %	19.29	2.06	24.94	22.35	27.98	
<i>D</i> 3.5 μm, %	45.23	1.45	41.31	36.60	43.61	
Mean <i>D</i> , μm	0.01	5.76*	4.19	4.33	4.10	2–1, 3
<i>S</i> , μm ²	3.30	5.67*	34.2	36.6	32.8	2–1, 3
<i>MCV</i> , μm ³	945.61	3.86*	59.0	94.6	42.4	2–1, 3
<i>MCH</i> , pg	121.56	3.68*	20.3	33.8	16.2	2–1, 3
<i>MCHC</i> , %	10.87	2.77	34.6	33.8	38.3	
<i>HB</i> , g %	2.14	6.16*	16.4	15.0	18.2	3–1, 2
<i>HT</i> , %	20.17	0.71	47.3	44.7	47.6	
<i>E</i>	84.18	5.30*	25.3	16.7	35.4	2–3
<i>E</i> , cells/g body weight	0.12	5.95*	0.91	0.69	1.42	3–1, 2

Note: *MS*_{res}, residual mean square; *D*, erythrocyte diameter; *S*, erythrocyte surface area; *MCV*, mean erythrocyte volume; *MCH*, mean erythrocyte hemoglobin; *MCHC*, mean erythrocyte hemoglobin concentration; *HB*, blood hemoglobin concentration; *HT*, hematocrit; *E*, oxygen carrying capacity per unit volume of blood (for Tables 2–4).

observed in spring ($r = 0.45$) and autumn ($r = 0.56$) animals, while summer animals demonstrated a strong negative correlation between *HB* and the total number of erythrocytes ($r = -0.79$) and the number of 6.1 μm cells ($r = -0.92$), which consequently correlated with *E* ($r = 0.91$).

Overwintered spring and summer voles of 2003 differed only by *HT* (39.8 and 50%, respectively, $p < 0.05$), which can be due to a changed volume of the liquid blood fraction (Tembotova *et al.*, 1990). In addition, summer animals demonstrated a trend to increase in the erythrocyte count (1.74×10^7 versus

8.3×10^6 cells/μl), weight (666 versus 42 mg), and spleen index (21 versus 1.5).

Overwintered animals and mature underyearlings are functionally similar and can be assigned to reproducing animals. Mature underyearlings, which comprise the bulk of the 2003 population at the stage of growth (Davydova, 2004), also demonstrated the effect of season on the indices ($R\text{-Pao}_{(120,232)} = 7.224$, $p < 0.001$). Summer animals had a considerably higher erythrocyte count compared to autumn animals and a lower *HT* compared to spring ones. The proportion of 6.8 μm cells gradually decreased from spring to autumn (Table 3). At the background of the minimum volume

Table 3. Analysis of variance. Mean indices in mature underyearlings

Index	Result of analysis		Season			$p < 0.05$
	MS_{res}	$F_{(2,24)}$	spring (1)	summer (2)	autumn (3)	
Body weight, g	12.00	1.72	25.7	22.35	21.51	
Age, days	271.5	8.97*	82	65	94	2–3
Spleen weight, g	23117.41	0.52	66.7	73.25	122.41	
Spleen index	42.56	0.37	2.0	3.28	5.53	
Bone marrow, 10^6	27.18	2.04	18.43	17.36	13.45	
Bone marrow, $10^6/g$	0.06	1.19	0.71	0.79	0.62	
Leukocytes, 10^3	0.87	7.49*	3.73	3.46	2.15	3–1
Erythrocytes, 10^6	12.37	5.64*	9.20	11.10	6.17	2–3
Erythrocytes $10^6/g$	0.03	4.61*	0.36	0.51	0.29	2–3
D 9.6 μm , %	0.05	1.14	0.27	0.07	0.08	
D 8.9 μm , %	0.02	0.02	0.10	0.08	0.09	
D 8.2 μm , %	0.07	0.09	0.15	0.20	0.22	
D 7.3 μm , %	0.25	0.81	0.48	0.64	0.38	
D 6.8 μm , %	0.64	4.70*	2.24	1.22	0.68	1–3
D 6.1 μm , %	9.31	0.80	2.39	3.90	2.38	
D 5.4 μm , %	14.68	0.84	6.13	7.46	5.41	
D 4.7 μm , %	19.19	0.75	15.19	18.54	17.20	
D 4.0 μm , %	37.49	0.42	22.09	23.11	24.99	
D 3.5 μm , %	77.91	1.61	51.32	43.14	48.54	
Mean D , μm	0.04	0.33	4.1	4.19	4.14	
S , μm^2	23.62	0.06	34.4	36.14	33.33	
MCV , μm^3	544.10	9.72*	57.31	41.35	84.15	2–3
MCH , pg	61.82	7.29*	18.93	15.37	27.80	2–3
$MCHC$, %	11.61	5.45*	32.80	37.42	33.31	2–3
HB , g %	1.72	0.90	16.53	15.64	15.36	
HT , %	25.38	3.84*	50.67	42.37	46.55	1–2, 3
E	16118.45	4.5*	26.04	31.46	16.38	2–3
E , cells/g body weight	3644.33	3.65*	101.06	144.74	76.61	2–3

and hemoglobin content ($p < 0.05$), erythrocytes in summer voles provided for the highest E . Apparently, this was due to their high numbers.

Analysis of the seasonal variation in mature underyearlings and overwintered animals demonstrated a lower number of changing indices in underyearlings and a different dynamics of certain indices from spring to autumn, which can underlie the observed differences in the corresponding indices, which were particularly pronounced between the autumn samples. For instance, overwintered autumn animals had higher erythrocyte count, bone marrow count, and hemoglobin concentration in the blood and erythrocytes compared to mature autumn underyearlings. One can propose that these differences were due to different reproductive period duration—animal reproduced in winter at the stage of pop-

ulation growth in 2003, which was also confirmed by the proportions of population groups of 2003 and 2004, while the total abundances were comparable (Davydova and Kshnyasev, 2004). It is also possible that the indices observed in overwintered autumn voles are typical of aging organism when body weight decreases with age ($r = -0.97$). Alternatively, this can represent a specific response of the animals to environmental factors.

The effect of season on the studied indices was demonstrated in immature animals of 2004 ($R-P_{(46,66)} = 3.835$, $p < 0.001$), while the effect of sex was insignificant ($R-P_{(23,33)} = 0.864$, $p < 0.637$). The proportion of 5.4 and 4.7 μm erythrocytes changed with seasons being lower in autumn than in summer animals (Table 4). In autumn, the proportions of other size frac-

Table 4. Analysis of variance. Mean indices in immature underyearlings

Index	Result of analysis		Season			<i>p</i> < 0.05
	<i>MS</i> _{res}	<i>F</i> _(2,58)	summer (1)	autumn (2)	winter (3)	
Body weight, g	6.24	27.02*	17.1	19.6	23.1	1–2–3
Age, days	3061.4	62.32*	90	157	292	1–2–3
Spleen weight, g	295.25	1.48	38.6	46.7	39.5	
Spleen index	0.89	2.89	2.3	2.4	1.7	
Bone marrow, 10 ⁶	18.67	0.21	13.05	12.64	13.53	
Bone marrow, 10 ⁶ /g	0.05	3.22*	0.76	0.65	0.59	1–3
Leukocytes, 10 ³	8.75	4.75*	4.55	3.20	1.56	1–3
Erythrocytes, 10 ⁶	9.30	17.95*	6.15	10.69	11.70	1–2, 3
Erythrocytes 10 ⁶ /g	0.03	8.14*	0.36	0.56	0.51	1–2, 3
<i>D</i> 9.6 μm, %	0.01	0.46	0.05	0.03	0.03	
<i>D</i> 8.9 μm, %	0.01	6.95*	0.14	0.12	0.02	3–1, 2
<i>D</i> 8.2 μm, %	0.05	3.25*	0.35	0.18	0.20	
<i>D</i> 7.3 μm, %	0.61	4.71*	0.97	0.86	0.24	3–1, 2
<i>D</i> 6.8 μm, %	1.14	9.12*	2.09	1.48	0.60	3–1, 2
<i>D</i> 6.1 μm, %	2.90	9.20*	4.96	3.87	2.56	1–3
<i>D</i> 5.4 μm, %	6.25	14*	9.06	6.61	4.73	1–2, 3
<i>D</i> 4.7 μm, %	15.31	15.02*	21.58	19.54	14.71	1–2, 3
<i>D</i> 4.0 μm, %	28.47	3.45*	23.05	27.11	23.98	
<i>D</i> 3.5 μm, %	50.49	24.78*	37.89	40.19	53.06	3–1, 2
Mean <i>D</i> , μm	0.02	23.07*	4.3	4.2	4.0	1–2–3
<i>S</i> , μm ²	5.31	21.38*	36.2	34.0	31.2	1–2–3
<i>MCV</i> , μm ³	1229.62	17.10*	102.5	45.9	45.8	1–2, 3
<i>MCH</i> , pg	55.81	26.99*	31.1	16.2	15.6	1–2, 3
<i>MCHC</i> , %	11.45	1.82	36.6	35.3	34.6	
<i>HB</i> , g %	1.47	1.86	16.2	16.9	16.3	
<i>HT</i> , %	56.06	0.94	44.5	45.6	47.8	
<i>E</i>	79.50	12.33*	18.5	32.0	30.4	1–2, 3
<i>E</i> , cells/g body weight	0.24	7.71*	1.1	1.7	1.3	1–2

tions of erythrocytes demonstrated a trend to decrease and significantly differed from those in February animals (4 and 3.5 μm, 77 versus 60%; greater than average diameter, 23 versus 39%). Due to the changed proportions between size fractions of erythrocytes, wintering animals demonstrated the minimum mean diameter and surface area of erythrocytes (Table 4), which agrees with published data (Kostelecka-Myrcha, 1967; Wolk and Kozlowski, 1989; *Obyknovennaya polevka...*, 1994; Baragunova, 1994). In this case, these publications considered the seasonal variation in the whole population rather than in its physiological and functional groups, although immature animals are known to predominate in autumn. Autumn and wintering animals had higher erythrocyte count (the total number and the

number per g body weight), *E*, and bone marrow count compared to summer ones. The oxygen carrying capacity per g body weight demonstrated a trend to decrease from summer to winter (7.6×10^5 , 6.5×10^5 , and 5.9×10^5 ; $p > 0.05$), which can be attributed to the increasing body weight or accelerated cell release into blood (Table 4). The correlation of *HB* to erythrocyte count in autumn animals ($r = 0.47$) and *HB* to *MCH* ($r = 0.54$) and *MCHC* ($r = 0.55$) in wintering animals confirms the specific adaptive response of these animals to changed environmental conditions. *HB*, spleen weight, and spleen index did not significantly ($p > 0.05$) vary with season in these animals.

Distinguishable groups of immature animals of 2003 ($R\text{-Pao}_{(23,51)} = 5.883$, $p < 0.001$) also demonstrated

seasonal variation in the proportion between size fractions of erythrocytes: summer animals had more 7.3–5.4 μm cells but less 3.5 μm cells, which was similar to immature animals of 2004.

The analysis of the published data on seasonal variation in hematological indices (*Obyknovennaya polevka...*, 1994) demonstrated correlation of *HB* with neither erythrocyte count ($r = 0.47$) nor erythrocyte size ($r = -0.45$). According to our data, all overwintered voles demonstrated a correlation of *HB* with erythrocyte count ($r = 0.96$) and diameter ($r = 0.97$), while mature underyearlings demonstrated a correlation of *HB* with erythrocyte size only ($r = 0.97$). The published example of seasonal variation in the erythrocyte count (*Obyknovennaya polevka...*, 1994) demonstrates similarity with our data combined for overwintered animals and mature underyearlings (Fig. 1) in both the value and dynamics but was different from that for overwintered animals and mature underyearlings separately. The revealed differences support a specific adaptive response to changing conditions in animals in different reproductive state, which should be taken into account to understand the response mechanism.

White blood counts were analyzed in mature underyearlings in the population at the stage of growth in 2003. No differences in the proportion of white blood cells were observed in overwintered animals in different seasons. Mature underyearlings in summer demonstrated the highest number of segmented neutrophils (15%) and the lowest number of lymphocytes (71%), which significantly differed from the corresponding indices in immature autumn animals (6 and 83%, respectively). The number of lymphocytes was similar in the blood of immature underyearlings in summer and autumn, while the number of neutrophilic leukocytes (stab neutrophils, metamyelocytes, and myelocytes) was higher in summer than in autumn immature underyearlings as well as the stab neutrophil/segmented neutrophil ratio (0.25 and 0.15, respectively). The proportions of other not numerous cell types did not significantly differ.

Overall, the white blood count demonstrated a leftward shift and increase in the number of leukocytes in summer underyearlings is typical of other small rodent species (*Obyknovennaya polevka...*, 1994) since they are more exposed to infections and invasions in summer. In autumn, the number of neutrophils decreases, while the number of lymphocytes increases (from 71 to 83%, $p < 0.05$) to indistinguishable levels in immature animals (83% in summer and 87% in autumn), which does not seem to be incidental. Lymphocytes are not just immune cells. Their stimulating effect on hematopoietic stem cell proliferation and differentiation was reported many times (Yastrebov *et al.*, 1988; Yushkov *et al.*, 1999; Chereshnev *et al.*, 2002). Under conditions of erythroid bone marrow hypoxia, they induce proliferation of erythroid bone marrow cells (Chereshnev *et al.*, 2002), which can also take place at low temperatures. More-

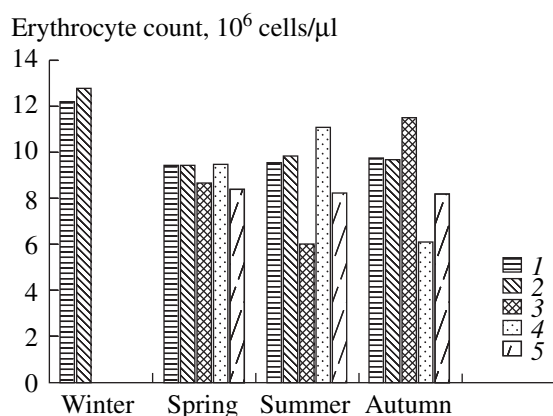


Fig. 1. Erythrocyte concentration in the blood of male (1) and female (2) voles in different seasons according to published (*Obyknovennaya polevka...*, 1994) and original data for overwintered animals (3), mature underyearlings (4), and a combination of overwintered animals and mature underyearlings (5).

over, lymphocyte count in males correlates with testosterone level (Moshkin, 2004), a reproductive function marker. The seasonal changes in the proportion of white blood cells notably increased the lymphocyte/neutrophilic leukocyte ratio in autumn mature and immature animals (from 5.6 to 12.5 and from 10.4 to 19.6, respectively), which was significant between mature summer and immature autumn animals. This index was proposed as a marker of organism reactivity (Mashneva *et al.*, 1984). With this in mind, immature autumn animals can be considered as more resistant, which is important for the population under autumn and winter conditions. A similar blood cell proportions were observed in the animals in summer 1994 with the same population cycle phase as in 2003.

The changes in the white and red cell indices correlated. The content of erythrocytes with a greater than average size (5.4 μm and more) is directly related with the numbers of metamyelocytes, stab neutrophils, and monocytes and inversely related with the numbers of lymphocytes and eosinophils. At the same time, the increased proportion of small (3.5 μm) erythrocytes and decreased proportion of large ones is accompanied by an increase in the proportion of lymphocytes and a decrease in the proportion of metamyelocytes, stab neutrophils, and monocytes.

The correlation between the hematological and hematopoietic indices reflected the interrelated changes in them and was observed at the cell, tissue, and organism levels. The erythrocyte count correlated ($p < 0.05$) with the spleen weight ($r = 0.62$) and the number of bone marrow cells ($r = 0.53$), which consequently correlated with the body weight ($r = 0.62$). The proportion of 5.4–7.5 μm erythrocytes inversely correlated with the proportion of 3.5 μm cells ($r = -0.61 \dots -0.81$). The proportion of 5.4 μm erythrocytes correlated with spleen weight ($r = 0.64$) and spleen index ($r = 0.62$),

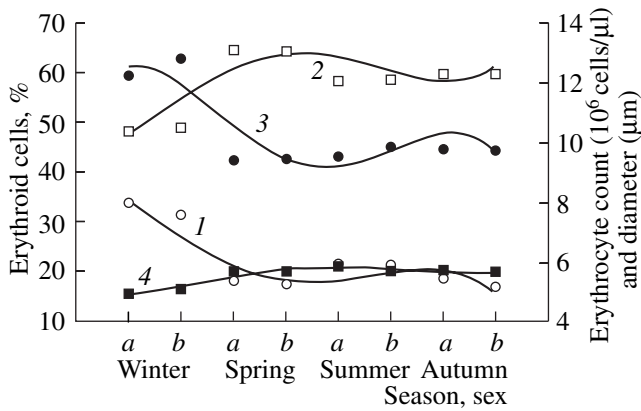


Fig. 2. Relative content of all erythroid (1) and oxyphilic (2) bone marrow cells as well as erythrocyte concentration (3) and mean diameter (4) in male (a) and female (b) voles in different seasons (*Obyknovennaya polevka...*, 1994).

which showed a trend to increase in immature animals of 2004 and mature underyearlings of 2003 (Tables 3, 4). Body weight correlated with the proportion of $6.8 \mu\text{m}$ ($r = 0.55$), $6.1 \mu\text{m}$ ($r = 0.52$), and $3.5 \mu\text{m}$ ($r = -0.58$) erythrocytes as well as with erythrocyte surface area ($r = 0.60$) and diameter ($r = 0.57$). A correlation was found between the proportions of $5.4\text{--}6.8 \mu\text{m}$ ($r = 0.29\text{--}0.31$) and $3.5 \mu\text{m}$ ($r = -0.24$) erythrocytes and population abundance.

Published data indicate that the spleen index is higher in animals living in industrially contaminated areas than in more ecologically clean areas ($3.4\text{--}7.4$ versus $1.3\text{--}3.1$, respectively; cited from Olenev and Pasichnik, 2003). The values observed in mature summer and autumn underyearlings of 2003 as well as overwintered animals of 2004 fit within these values describing the industrial impact (Tables 2, 3). At the same time, even higher values ($6.4\text{--}12.3$) are sometimes attributed to active reproduction of animals (Yastrebov *et al.*, 1988), high storage function of the spleen, and active erythrocyte lysis (Vasil'ev and Zakharov, 1992). According to our data, the spleen weight and spleen index changed with age in overwintered animals of 2004 ($b = 0.548$, $s.e.(b) = 0.204$, $\beta = 0.610$, $t_{17} = 2.684$ and $b = 0.024$, $s.e.(b) = 0.007$, $\beta = 0.676$, $t_{17} = 3.167$, respectively), which was not observed in the voles in other reproductive states.

Thus, the analysis of seasonal variation in the hematological indices of the voles in different reproductive states demonstrated higher erythrocyte counts in overwintered animals and immature autumn underyearlings compared to the corresponding summer animals (1.15×10^7 and 1.06×10^7 cells/ μl versus 6.0×10^6 and 6.2×10^6 cells/ μl , respectively). Small cells comprised the bulk of erythrocytes (72 and 67% versus 59 and 61%, respectively), which seem to underlie the changes in the erythrocyte mean diameter and surface area. The latter indices did not vary with season in mature under-

yearlings due to insignificant changes in the proportions of size fractions of erythrocytes. Overwintered autumn animals had a higher weight compared to immature autumn ones, which required a higher hemoglobin concentration (Tables 2, 4) provided by an increased proportion of erythrocytes relative to the corresponding summer animals (4.6×10^4 versus 2.5×10^4 cells/g and 5.6×10^4 versus 3.6×10^4 cells/g, respectively), which was clearly higher in immature animals. In addition, a doubled proportion of smaller than average erythrocytes (13 versus 6%) as well as of short-lived ones (Pospishil and Vakha, 1996) notably increased the number of bone marrow cells in overwintered autumn animals (Table 2).

The presented seasonal variation in the indices as an adaptive response of the blood system in the voles in different states is consistent with the published data (*Obyknovennaya polevka...*, 1994). Analysis of these data demonstrated that erythrocyte count was directly related to the number of erythroid bone marrow cells ($r = 0.95$) and inversely related to erythrocyte size ($r = -0.95$). Indeed, wintering animals demonstrate the highest number of erythroid bone marrow cells and the lowest erythrocyte size, which is illustrated by the polynomial curves approximating the experimental points (Fig. 2). The erythrocyte size irrespective of season directly correlated with the number of oxyphilic normoblasts ($r = 0.86$) and inversely correlated with the numbers of basophilic ($r = -0.76$) and polychromatophilic ($r = -0.95$) normoblasts. These observations suggest specific patterns in hematopoiesis in animals in different reproductive states and the identification of these patterns can shed light to the mechanism of the revealed hematological changes.

In addition to the above-mentioned indices varying with season, overwintered animals demonstrated increasing concentration of hemoglobin in erythrocytes in autumn (Table 2, $p < 0.01$). Active biosynthetic processes including synthesis of nucleic acids and proteins, which require high energy expenditures and deplete the synthetic components, are hardly beneficial under autumn and winter conditions. It interferes with the adaptive mechanisms and can cause disease and death (Baevskii, 1979), which seems real for old overwintered animals. This raises the question of whether high body weight and erythrocyte count increasing with age (Yushkov *et al.*, 1999) indicate animal population health (Wolk and Kozlowski, 1989)? Are erythrocyte volume and hemoglobin content age-dependent (Yushkov *et al.*, 1999)? In our case, the latter indices were indistinguishable in different age groups of autumn overwintered animals and immature underyearlings; however, they were higher in mature autumn underyearlings, which can be due to different reproductive activity from year to year rather than to age.

Published data on the development of generations of small mammals and bank vole, in particular (Shvarts *et al.*, 1964; Olenev, 1989) allow us to propose that

immature autumn underyearlings of 2003, wintering animals of 2004, and spring, summer, and autumn overwintered animals of 2004 are the same animals considered in different time intervals and adapted to the corresponding conditions. These animals and the descendants of overwintered animals of 2004 can be distinguished by all indices as demonstrated by discriminant analysis ($F_{(102,548)} = 6.36, p < 0.001$) and analysis of variance ($F_{(4,90)} = 165.4, p < 0.001$). Autumn immature animals of 2003 can be distinguished from autumn overwintered animals of 2004 as well as from quite similar autumn immature voles of 2004 destined to maintain the population in winter. As compared to the former ones, they have lower body weight (18.9 versus 25 g), bone marrow count (1.29×10^7 versus 2.52×10^7 cells/ μl or 7×10^5 versus 1×10^6 cells/g body weight), blood hemoglobin concentration (15.1 versus 18.2 g %), and erythrocyte count (5.9×10^6 versus 1.15×10^7 cells/ μl). The age-related changes in erythrocyte count in this sequence of animals (Fig. 3) demonstrated a significantly higher erythrocyte concentration in immature voles, which likely reflects a higher energy capacity of the body. As compared to the second ones, a slightly lower body weight (18.9 versus 19.6 g, $p > 0.05$) was due to a different age (84 and 157 days, respectively) ($F_{(1,71)} = 7.59, p = 0.007$), which was considered as a covariant ($b = 0.015, s.e.(b) = 0.005, \beta = 0.311, t_{71} = 2.755, p = 0.007$). No age dependency was observed for other indices; the animals born in 2003 had a lower erythrocyte concentration (3.1×10^5 versus 5.6×10^5 cells/g body weight) but higher erythrocyte volume (90 versus $45 \mu\text{m}^3$) and hemoglobin content (29 versus 16.2 pg), which likely compensated their low numbers. Distinguishable indices between immature autumn animals born in 2003 and 2004 corresponding to population growth and peak phases, respectively, confirm different states of the animals. In this pair, a higher energy potential was observed in animals of 2004, which can reflect a stress of the system also indicated by some other physiological and biochemical indices in populations at the density peak (Chernyavskii and Lazutkin, 2004). These animals going to winter determined the population structure of the next reproduction season.

Thus, complex investigation of hematological and hematopoietic indices in bank vole in different reproductive states—overwintered animals as well as mature and immature underyearlings—shed a new light on seasonal character variation.

The degree and timing of the seasonal changes in the studied indices are specific for each animal reproductive state and can be considered as adaptive response to particular habitat conditions.

The revealed shift to the increased proportion of large erythrocytes in spring, and the more so in summer voles, and the opposite shift to the decreased proportion of small cells in autumn and, particularly, in wintering

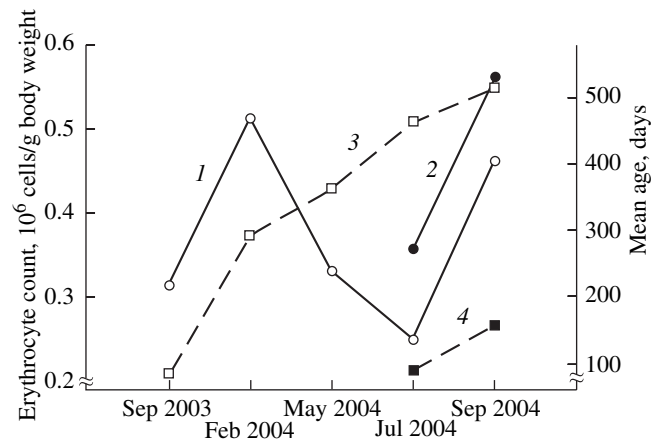


Fig. 3. Erythrocyte concentrations (1, 2) and age (3, 4) of voles born in September 2003 (1, 3) and July 2004 (2, 4).

animals, not only indicates the changes in the mean diameter and surface area of erythrocytes but also reflects a mechanism of HB blood maintenance and oxygen supply of tissues under changing conditions.

The revealed changes in the proportions of white blood cells and size fractions of red blood cells as well as the associated variation in the hematological and hematopoietic indices reflect the interaction between the blood and hematopoietic organs, which can be considered as a way to control hematopoietic function, since quantitative changes in each element of the blood system cannot be realized without the involvement of other components. The balanced and closely linked complex of changing hematological indices (erythrocyte count and hemoglobin concentration in the blood, numbers of all and subtypes of bone marrow cells, spleen weight, etc.) has been demonstrated in experiments on body exposure to extreme conditions (Yushkov *et al.*, 1999).

The analysis of variation in individual indices in the course of organism maturation allowed us to demonstrate correlations between the indices and to evaluate energy potential of animal body in different reproductive states.

Studies of the animal blood system with an account of their reproductive state and age as well as of the seasonal variation in the indices can contribute to understanding the mechanisms of variation in population parameters. In addition, the proposed approach to study variation of hematological indices in response to changing factors as well as the obtained data can be used to evaluate or refine the state of biocenoses using mouse-like rodents.

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