

## Annual Variation in Hematological Indices in a Fluctuating Population of Bank Vole (*Clethrionomys glareolus*)

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**Abstract**—The quantitative and structural hematological indices were studied in bank vole in different reproductive states (overwintered individuals, mature underyearlings, and immature underyearlings) in different years corresponding to different phases of the population cycle. The annual changes in certain hematological indices have been revealed for each reproductive state irrespective of the population cycle phase.

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Stable existence of populations of many mammalian species is provided by the long-term and/or seasonal changes in their structure (Shvarts et al., 1964; Zhigal'skii and Kshnyasev, 2000; Olenov, 2000; Davydova and Kshnyasev, 2004). The reproductive and age structure of the population corresponds to a particular period in the seasonal and long-term cycles and reflects the proportion between mature and juvenile individuals with different resistance to environmental factors (Olenov and Grigorkina, 1998), specific morphophysiological (Shvarts et al., 1968), hematological (Tarakhtii et al., 2005), and other characters.

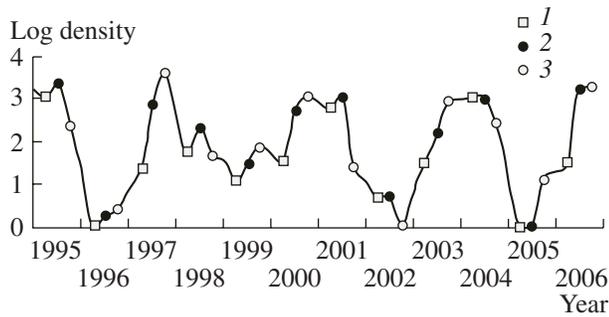
The mechanisms of the resistance of biological systems are a fundamental problem of biology studied at different levels of biological organization: from molecular to population. One of approaches to the mechanisms of adaptation at the population level is studying physiological properties of the body and its systems. Population dynamics-related changes in some physiological, biochemical (Chernyavskii et al., 2003), hematological, and body weight indices (Wolk and Kozlowski, 1989) have been reported. Since the neurohumoral system of the body responds to environmental changes (photoperiod, environmental temperature, and food resources; Kalabukhov, 1969), the multifunctional blood system can be used as a test system. Changes in blood reactivity are an early and sensitive marker of ecological impact on the body (Kozinets et al., 1993), which makes it possible to analyze the control mechanism of hematopoiesis (Komar, 1992). Evaluation of the size and shape of erythrocytes sheds light on the mechanism maintaining hemoglobin level of blood (Kostelecka-Myrcha, 1967, 2002) and body homeostasis in general and makes it possible to reveal early changes undetectable by standard techniques (Tarakhtii and Kardonina, 1995; Tsyb et al., 1996).

Seemingly simple and homogeneous, erythrocytes are not functionally uniform (Kliorin and Tiunov, 1974). The erythrocyte population composition varies in time: it is more complex in early ontogeny than later, since the cell maturation is modified as the body matures and the localization of erythropoiesis changes with age. The contribution of erythrocytes to oxygenation and gas-transfer function depends on the proportion between erythrocyte groups; hence, the functional activity of the whole cell population can vary, which represents a mechanism to regulate blood functions (*Fiziologiya sistemy...*, 1979).

The energy mechanism underlies the variations in erythrocyte population composition and cell structure (Kalabukhov, 1969); this mechanism triggers adaptation and allows us to predict the metabolic and structural changes at the whole organism level; i.e., adaptation is of structural significance (Baevskii, 1979).

In addition to erythrocyte variation, the indices of white blood cells vary in a wide range. The changes in hematological indices in small mammals are attributed to environmental conditions, animal age, and season of the year (Istomina et al., 1971; Baragunova, 1994; Koval'chuk and Yastrebov, 2003), population density (Chernyavskii and Lazutkin, 2003), reproductive state of animals (Tarakhtii et al., 2005), and other factors.

A three-year population cycle has been revealed for bank voles living in southern taiga forests of the Central Urals (Davydova and Kshnyasev, 2004). In this case, each phase of the cycle (depression, growth, and peak) demonstrated specific seasonal dynamics of the density and demographic structure (Davydova, 2004). It seems worthwhile to study variation in hematological indices in small mammals of different reproductive state and age, which can both describe the individual states and evaluate the factors underlying cyclic population dynamics.



**Fig. 1.** Dynamics of bank vole population density (1995–2006); 1, spring; 2, summer; 3, autumn.

The goal of this work was to evaluate the indices of blood and hematopoietic tissue in bank voles (*Clethrionomys glareolus* Schreber, 1780) of different reproductive state and age in different years of the population cycle, when the abundance and structure of the population vary.

## MATERIALS AND METHODS

Bank vole is a widespread and dominant species in the community of small mammals in the southern taiga zone of the Central Urals.

The demographic indices of the population and annual and seasonal population dynamics were studied in spring, summer, and autumn from 1995 to 2004 using the standard techniques of trap lines (Karaseva and Telitsina, 1996) in conventional primary and primary fir spruce forests in the Visim State Biospheric Reserve (southern dark coniferous taiga, Central Urals, Sutuk Low Mountain Range Region, 57°22' N, 59°46' E, 538 masl).

The hematological indices were studied in animals captured in May, July, and September from 2001 to 2004. Body weight, morphophysiological indices (Shvarts et al., 1968), sex, state of generative organs, and age (Olenev, 1989) were determined in each individual. According to these characters, the animals were assigned into three reproductive/age groups: overwintered animals, mature underyearlings, and immature underyearlings. Leukocyte count, erythrocyte count, blood hemoglobin concentration (*HB*), hematocrit (*HT*), blood count, and bone marrow count in the shaft of femur were determined using the standard techniques and the manufacturers' protocols. The proportion between erythrocyte size fractions (3.5–8.9  $\mu\text{m}$ , 10 points) was evaluated using a Celloscope 401 counter (Lars Yungberg, Sweden); *HT*, using a Hawksley Micro Haematocrit Centrifuge and Hawksley Micro Haematocrit Reader (United Kingdom); *HB*, using a Linson 3 Photometer (Sweden). 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 of blood (*E*) (Kostelecka-Myrcha, 1973) were calculated. The erythrocyte and bone marrow counts were normalized to body weight considering that their number changes with age (Yushkov et al., 1999). These indices were evaluated in 186 individuals. Pregnant females and young individuals weighing less than 15 g were not analyzed.

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 observations of the dynamics of bank vole population allowed us to reveal a three-year cycle with three phases, depression, growth, and peak (Fig. 1), each of which has specific dynamics of the density and demographic structure (Davydova and Kshnyasev, 2004). Based on the population structure in 2001–2004 (three-year cycle; Table 1) with the peak in 2001, depression in 2002, growth in 2003, and peak in 2004, twelve animal groups were identified by the reproductive state and age for comparative evaluation of the hematological indices. These included overwintered individuals captured in spring (1) and autumn (2) 2001; spring (3) and summer (4) 2003; spring (5), summer (6), and autumn (7) 2004; mature underyearlings captured in autumn 2002 (8) and 2003 (9); and immature underyearlings captured in autumn 2001 (10), 2003 (11), and 2004 (12).

Discriminant analysis has demonstrated significant differences between the groups (Table 2). According to the classification matrix, 78% of all animals correspond to the a priori classification. The greatest correspondence (50–100%) was observed in the groups of overwintered (1, 4, 5, 6, and 7) and immature individuals (10, 11, and 12); while the least correspondence (below 35%) was observed in bred underyearlings.

Analysis of variance has demonstrated that the indices varied as a function of the year of capture ( $R\text{-Pao}_{54,226} = 3.51$ ;  $p < 0.001$ ), individual reproductive state ( $R\text{-Pao}_{54,226} = 3.28$ ;  $p < 0.0001$ ), and their combination ( $R\text{-Pao}_{108,451} = 2.39$ ;  $p < 0.0001$ ). The impact of the sex was insignificant ( $R\text{-Pao}_{25,189} = 0.842$ ;  $p = 0.685$ ); accordingly, the data for males and females were represented for the common mean value. The statistical model cannot simultaneously include independent correlated variables and animal reproductive state. The analysis demonstrates that correct evaluation of annual variation in the indices requires the individual reproductive state to be taken into account. Based on the specific population structure (Table 1), the annual variation in individual indices was evaluated in overwintered spring and autumn individuals and immature underyearlings using two-way analysis of variance; in

**Table 1.** Reproductive and age structure of the bank vole population (2001–2004)

Year (cycle phase)	Capture date	Reproductive and age group			Total
		overwintered	underyearlings		
			mature	immature	
2001 (peak)	15.05	23	0	0	23
	19.07	10	0	31	41
	16.10	0	0	3	3
2002 (depression)	19.05	2	0	0	2
	22.07	0	1	1	2
	23.08	0	0	0	0
2003 (growth)	20.05	3	4	0	7
	21.07	0	16	0	16
	10.10	0	6	16	22
2004 (peak)	11.05	20	0	0	20
	05.08	9	0	9	18
	06.10	1	0	21	22

mature autumn underyearlings, using one-way analysis of variance.

**In overwintered individuals** captured in May 2001, 2003, and 2004, which amounted to 100, 43 and 100% in the population and were clearly distinguishable (Table 2), the bone marrow count, *HB*, leukocyte count, and erythrocyte count remained unaltered (Table 3). The dynamics of erythrocyte count (including that normalized to body weight) demonstrated a trend to increase, which became significant in 2004 only in males ( $10^7$  versus  $7.7 \times 10^6$  cells/ $\mu\text{l}$  in 2001). Throughout the observation period, the overwintered voles demonstrated different size proportions of erythrocytes, which was also clear between animals at the same population phase. For instance, the animals had more 5.4–7.3  $\mu\text{m}$  erythrocytes and less 3.5  $\mu\text{m}$  erythrocytes in 2001; accordingly, the mean erythrocyte diameter and surface area were higher in this year. In 2003, the proportion of 6.1 and 5.4  $\mu\text{m}$  erythrocytes was lower compared to 2001 (8.2 versus 20.9%), while the proportion of smaller than average erythrocytes (4.0 and 3.5  $\mu\text{m}$ ) was higher (73%) than in 2001 (51%) and 2004 (66%). At the same time, the *MCV*, *MCH*, *MCHC* and *E* demonstrated no significant annual variation.

The leukocyte count was also variable and peaked in overwintered individuals in 2003. Lymphocytes predominated among leukocytes in bank vole. Their count was higher in 2001 (72%) than in 2003 (64%) and 2004 (66%) as well as the lymphocyte/neutrophil ratio (7.98, 2.78, and 4.53, respectively), which reflects the body reactivity (Mashneva et al., 1984). The blood of individuals in 2004 contained more stab neutrophils and had the maximum stab cell/segmented cell ratio

(0.65 versus 0.15 and 0.54, respectively). One can see the changes in both leukocyte count and the proportion of different white blood cell types. The voles in 2004 and 2001 had more neutrophilic leukocytes and lymphocytes, respectively. Atypical cells occurred in the smears: large neutrophils with basophilic cytoplasm often containing two nuclei of different maturity, one stab and one transitional segmented. Six to ten cells with large cytoplasmic inclusions resembling monocytes and/or large lymphocytes and described as a new leukocyte type, azurocyte (Mihok et al., 1987), occurred per 100 leukocytes. Each cell type fulfills specific functions and, considering different proportions of erythrocytes, one can propose different physiological state of the animals in these years.

In overwintered spring animals of nearly identical age, the mean body weight varied from year to year (Table 3). It peaked in 2001 and was significantly higher than in 2004 ( $p < 0.05$ ). Covariance analysis with age as a covariant and season as a factor has demonstrated the impact of age on body weight (coefficient  $\pm$  error =  $0.017 \pm 0.007$ ,  $p = 0.022$ ). In 2001, the highest spleen weight and index (spleen weight, mg, to body weight, g) were observed; however, no significant annual differences in the mean values have been revealed due to high dispersion of the organ weight (Table 3).

The samples of overwintered autumn voles were not numerous due to the decrease in the abundance of bred individuals from summer to autumn (Table 1). These individuals were 14–17-month-old, which is the limiting life span under natural conditions (Shvarts et al., 1964). Overwintered animals in autumn 2001 and 2004 were distinguishable (Table 2). The animals in 2001 as

**Table 2.** Structure of bank vole population and discriminant analysis

Reproductive and age group	Overwintered												Underyearlings					
	mature						immature						mature			immature		
	May 2001	September 2001	May 2003	July 2003	May 2004	July 2004	September 2004	September 2002	September 2003	September 2001	September 2003	September 2004	September 2002	September 2003	September 2001	September 2003	September 2004	
Number of animals	33	8	5	2	15	5	5	5	4	12	38	35	24					
Group	1	2	3	4	5	6	7	8	9	10	11	12						
1	6.29*	6.29*	2.26*	7.40*	27.46*	9.03*	18.16*	21.38*	63.44*	129.70*	154.95*	89.87*						
2	9.00*	6.70	0.90	6.64*	5.12*	1.27	6.79*	14.30*	24.30*	27.97*	35.97*	25.22*						
3	14.45*	6.70	2.75*	2.75*	0.08	1.27	1.80	4.57*	4.45*	5.36*	5.90*	3.67*						
4	47.19*	49.3*	33.99*	32.02*	4.83*	5.90*	3.35*	10.34*	11.53*	13.05*	13.68*	9.55*						
5	17.45*	8.60*	0.50	4.88*	9.71*	4.88*	6.38*	10.88*	26.32*	52.18*	54.05*	32.01*						
6	15.71*	3.53	9.79	45.62*	30.30*	3.45*	3.45*	14.07*	23.35*	25.62*	31.05*	21.84*						
7	31.61*	18.90*	13.93	25.89*	12.69*	10.68*	84.29*	18.16*	32.51*	42.40*	43.46*	26.97*						
8	70.27*	61.92*	42.38*	95.95*	38.45*	65.32*	70.40*	0.66	0.66	2.99*	0.72	5.25*						
9	51.52*	45.11*	30.30*	78.48*	27.92*	50.57*	72.67*	2.43	4.31*	5.48*	1.60	12.65*						
10	46.77*	39.29*	34.05*	82.93*	31.79*	43.91*	75.62*	9.74*	4.31*	3.95*	10.95*	40.96*						
11	59.92*	51.46*	37.62*	87.28*	34.34*	54.03*	48.97*	2.35	1.30	17.87*	11.59*	25.06*						
12	41.55*	37.99*	23.66*	61.66*	22.76*	39.66*		17.67*	11.23*									

Notes: Mahalanobis distance square and  $F_{66,861}$  test are given below and above the diagonal, respectively.\*  $p < 0.05$  (for Tables 2 and 3).

**Table 3.** Analysis of variance and mean indices of bank voles

Index	Result of analysis				Mean index									<i>p</i> < 0.05
	<i>MS</i> <sub>res</sub>	year (1)	maturity (2)	1 × 2	2001			2003			2004			
					May	September		May	September		May	September		
		<i>F</i> (2.139)	<i>F</i> (2.139)	<i>F</i> (4.139)	a	a	b	a	a	b	a	a	b	
			1	2	3	4	5	6	7	8	9			
Body weight, g	5.91	9.61	75.46	0.90	29.7	27.1	21.0	27.3	23.1	19.0	26.4	25.0	19.6	1-7; 3-6
Age, days	2078	8.7*	347.5*	4.26	370	423	103	363	480	84	361	513	157	3, 5 ≠ all; 6-9
Spleen weight, g	49732	2.68	1.15	1.18	260.4	229.8	48.9	42.0	94.0	45.0	82.0	62.8	46.8	
Spleen index, mg/g body weight	61.92	2.01	0.62	0.88	7.97	8.43	2.35	1.53	4.07	2.39	3.11	2.53	2.43	
Bone marrow, 10 <sup>6</sup>	27.84	2.38	5.02*	2.83*	19.20	18.77	13.93	14.75	10.50	12.92	15.93	25.16	12.64	2-8
Bone marrow, 10 <sup>6</sup> /g body weight	0.05	2.27	0.80	2.25	0.65	0.70	0.66	0.55	0.45	0.68	0.61	1.01	0.65	
Leukocytes, 10 <sup>3</sup>	1.21	2.59	4.99*	6.62*	3.07	1.70	2.19	4.93	1.70	2.09	2.28	3.16	3.20	4-7; 3, 6-9
Erythrocytes, 10 <sup>6</sup>	3.44	56.94*	3.95*	14.72*	7.90	4.87	3.93	8.31	5.60	5.88	8.67	11.50	10.69	3-6, 9; 6-9; 2-8
Erythrocytes 10 <sup>6</sup> /g body weight	0.01	51.85*	2.48	13.22*	0.27	0.18	0.19	0.32	0.24	0.31	0.33	0.46	0.56	3-6, 9; 6-9; 2-8
HB, g %	5.91	7.10*	3.92*	4.34*	17.2	14.0	13.8	17.5	17.3	15.1	16.4	18.2	16.9	3-9
HT, %	32.90	0.07	0.99	0.51	46.3	48.1	46.9	50.0	48.0	45.3	47.3	47.6	45.6	
Erythrocyte fractions, %														
9.6 μm	6.02	0.43	0.05	0.12	0.61	0.10	1.00	0.22	0.00	0.07	0.02	0.09		
8.9 μm	0.37	2.28	0.76	0.83	0.73	0.19	0.25	0.18	0.00	0.07	0.14	0.03	0.12	
8.2 μm	1.09	4.63*	1.44	1.26	1.65	0.60	0.53	0.37	0.00	0.17	0.35	0.12	0.18	1-7
7.5 μm	1.53	8.37*	2.71	8.72*	3.33	0.99	0.63	0.41	0.00	0.44	0.54	0.32	0.86	1-4, 7
6.8 μm	2.49	13.84*	6.64*	8.63*	5.71	2.10	1.70	1.50	0.86	0.80	1.63	0.96	1.48	1-4, 7
6.1 μm	3.29	25.16	4.56*	17.72*	9.37	4.91	4.07	3.04	1.72	2.65	2.78	3.60	3.87	1-4, 7; 3-6
5.4 μm	6.81	19.39*	1.52	3.10*	12.18	9.06	8.26	5.16	5.60	5.76	7.05	5.36	6.61	1-4, 7; 3-6
4.7 μm	18.38	0.11	0.18	1.34	18.14	19.62	19.44	17.55	22.41	17.79	21.31	17.81	19.54	
4.0 μm	28.40	11.10*	2.67	3.66*	21.08	24.54	22.43	40.67	28.88	27.00	24.94	27.98	27.11	4-1, 7; 3-6, 9
3.5 μm	62.36	4.42*	7.70*	5.25*	28.04	37.97	42.41	32.28	40.95	45.29	41.31	43.61	40.19	1-7
<i>D</i> , μm	0.07	11.86*	4.96*	6.63*	4.8	4.3	4.2	4.3	4.1	4.1	4.2	4.1	4.2	1-7
<i>S</i> , μm <sup>2</sup>	28.83	8.85*	3.90*	4.84*	45.10	36.28	34.95	35.37	33.27	33.08	34.19	32.76	33.96	1-7

Table 3. (Contd.)

Index	Result of analysis				Mean index									$p < 0.05$
	$MS_{res}$	year (1)	maturity (2)	$1 \times 2$	2001			2003			2004			
					May	September		May	September		May	September		
	$F$ (2.139)	$F$ (2.139)	$F$ (4.139)	a	a	b	a	a	b	a	a	b		
<i>MCV</i> , $\mu\text{m}^3$	633.69	31.29*	7.55*	13.39*	58.51	107.06	124.66	60.75	85.70	90.16	59.03	42.44	45.89	3–6, 9; 6–9; 2–8
<i>MCH</i> , pg	58.90	20.96*	3.90*	8.04*	21.33	30.80	36.03	21.40	30.91	29.00	20.29	16.20	16.19	3–6, 9; 6–9; 2–8
<i>MCHC</i> , %	29.21	5.27*	1.99	4.67*	37.44	29.02	29.36	35.05	36.06	33.72	34.62	38.27	35.28	3–6, 9
<i>E</i>	46.21	32.66*	9.74*	23.03*	30.63	13.19	9.86	26.33	16.53	15.15	25.31	35.38	32.01	3–6, 9; 6–9; 2–8
<i>E</i> , cells/g body weight	0.10	39.92*	0.39	20.78*	1.03	0.49	0.47	1.01	0.72	0.81	0.96	1.42	1.68	3–6, 9; 6–9; 2–8
Number of animals					27	8	36	2	1	33	12	5	24	

Notes:  $MS_{res}$ , residual mean square (evaluation of intragroup variance); a, overwintered individuals; b, immature underyearlings; 1–9, group number.

well as overwintered spring ones had the highest spleen weight and index, high *MCV* and *MCH*, but low bone marrow count, erythrocyte count (both overall and normalized to body weight), and *E* (Table 3).

The samples of overwintered voles captured in summer 2003 and 2004 proved different (Table 2; groups 2 and 5). The animals in 2003 had more erythrocytes ( $1.74 \times 10^7$  versus  $6.0 \times 10^6$  cells/ml) as well as higher body weight (30.3 versus 24.5 g), spleen weight (665.5 versus 96.8 mg), and spleen index (21.2 versus 3.9); and the spleen was abnormally high (1.2 g) in one individual.

The obtained data indicate that overwintered voles in 2001 and 2004 at the same population phase differed by hematological indices and body weight (and integral index), which reflect both the physiological state of the body and, indirectly, the environmental conditions. The factors of variation of the indices remain unclear considering that the population density and the proportion of such individuals in the population were similar in these years.

**Mature underyearlings** were similar to overwintered voles by the reproductive state; at the same time, the autumn underyearlings in 2002 and 2003 differed from overwintered animals by the set of indices but were mutually indistinguishable (Table 2). Despite the similar time of animal capture, the voles in 2002 were younger than in 2003 (63 versus 94 days,  $p < 0.002$ ). The erythrocyte count was similar, while *HT* (39.7 ver-

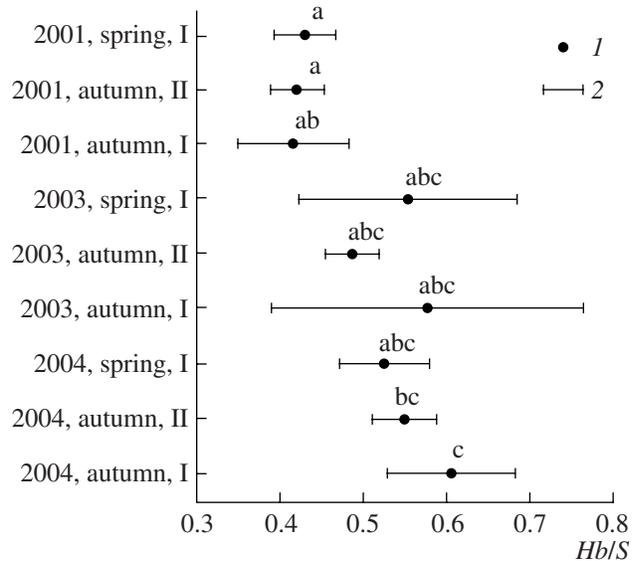
sus 46.6%,  $p < 0.002$ ) and *MCH* (44.4 versus 33.1%,  $p < 0.02$ ) were higher in 2002 than in 2003. Note significantly ( $p > 0.05$ ) lower spleen weight (35.3 and 122.4 mg) and index (1.76 and 5.53), which correlated with the *MCHC* ( $r = 0.60$ ). A slightly higher *HB* (16.2 and 15.4 g%) could be due to hemoglobin-rich erythrocytes. The latter indices also correlated ( $r = 0.73$ ).

**Immature individuals** constituted the bulk of the population (100, 73, and 95%) in autumn 2001, 2003, and 2004. These animal samples were distinguishable by both the set of indices (Table 2) and individual indices (Table 3). For instance, the erythrocyte count (total and normalized to body weight), *HB*, *MCHC*, and *E* increased from year to year. The erythrocyte population in 2001 individuals included more 8.9, 8.2, and 6.8–5.4  $\mu\text{m}$  cells and less cells below 4  $\mu\text{m}$  compared to the voles in 2003 and 2004, and this pattern was reproduced in overwintered spring individuals. Conversely, the proportion of small (3.5  $\mu\text{m}$ ) cells was high and the proportion of large erythrocytes was low (6.8 and 6.1  $\mu\text{m}$  as compared to 2001 and 2004 individuals and 5.4  $\mu\text{m}$  as compared to 2001 ones). High *D* and *MCV* values in erythrocytes of 2001 individuals were due to different proportions of erythrocyte size fractions and do not reflect increased size of all cells. According to Wolk and Kozłowski (1989), the mean erythrocyte diameter in yellow-necked mouse remains stable from year to year. The inconsistency between the obtained and published data can be attributed to the differences between animal species or between techniques used.

The leukocyte count peaked in 2004 individuals and different monocyte counts were observed (11% in 2004, 6% in 2001, and 5% in 2003). Individuals in 2004 demonstrated a higher stab cell/segmented cell ratio (0.44 versus 0.17 and 0.13, respectively) and a lower lymphocyte/neutrophil ratio (4.5 versus 12 and 10, respectively). The large and medium size fractions of lymphocytes were more common, while some small lymphocytes were cytoplasm-free or had barely visible cytoplasm ring. Azurocytes were less common (1–2 per 100 leukocytes) than in overwintered animals. The body weight in immature underyearlings peaked in 2001 similar to overwintered ones.

The revealed variation of the indices among animals of different reproductive state and age had common and distinct properties from year to year. Most likely, this variation reflects the response of the blood system and body as a whole to environmental conditions in these years. The overwintered spring individuals and immature autumn underyearlings in 2001 shared an altered proportion between erythrocyte size fractions towards large cells. However, the degree of index variation was different between them: the overwintered animals had a wider range of large cells, which corresponded to the higher  $D$  and  $S$ . In 2003 corresponding to the growth phase of the population cycle, the proportion of smaller than average erythrocytes (4 and 3.5  $\mu\text{m}$  both in overwintered and immature underyearlings) was higher and the proportion of larger cells (6.8–6.1  $\mu\text{m}$  in overwintered and 6.1–5.4  $\mu\text{m}$  in immature underyearlings) was lower than in 2001 corresponding to the peak of the cycle. Although the maximum leukocyte count was observed in different years (2003 for overwintered spring and 2004 for autumn overwintered and immature underyearlings), the relative content of individual leukocyte types was comparable in both of them in 2003. Only the lymphocyte/neutrophil ratio was higher in immature underyearlings than overwintered spring underyearlings (12 versus 2.8, respectively). It is common knowledge that lymphocytes have an effect on erythropoiesis under hypoxic conditions (Chereshnev et al., 2002) and are related to sexual activity (Moshkin, 2004). Likewise, their count was higher in immature underyearlings exposed to severe conditions in autumn and winter, when small and short-lived erythrocytes require active reproduction.

Different indices of the blood system include the increasing erythrocyte counts with age in immature individuals including the count normalized to body weight,  $HB$ ,  $MCHC$ , and  $E$ . The erythrocyte shape and size depend on the cell gas-transport function (Kostecka-Myrcha, 1967). The erythrocyte surface area is the principal factor of tissue oxygenation; the  $HB/S$  ratio is a more informative index than  $HB$ , which is quite similar in many mammalian species (Kostecka-Myrcha, 2002). The analysis of this index variation has revealed only one significant factor—observation year ( $F_{2,143} = 19.34$ ). No significant sexual, reproductive, or seasonal properties have been revealed. The greatest



**Fig. 2.** Blood hemoglobin concentration ( $HB$ , g %) to erythrocyte surface area ( $S$ ,  $\mu\text{m}^2$ ) ratio in overwintered bank voles (I) and immature underyearlings (II) in spring or autumn; the mean (1) and 95% confidence interval (2); heterogeneous groups according to Tukey's test include no same characters (a, b, c).

difference in this index (Fig. 2) was observed in 2001 and in 2004 for one phase of the population cycle.

Thus, the study of hematological and hematopoietic indices in bank voles of different reproductive state and age in different years corresponding to different phases of the population cycle has demonstrated the quantitative and structural variation of erythrocytes, which agrees with published data (Kliorin and Tiunov, 1974) as well as the variation in leukocyte count and proportion of different leukocyte types ( $R\text{-Pao}_{50,267} = 2.461$ ,  $p < 0.0001$ ). The hematopoietic indices varied to a lesser extent. The coordinated variation in the hematological indices and body weight specific for each state of animals reflects adaptive responses of the blood system maintaining the stable functioning of this system as well as the whole body. Since the hematological indices in both overwintered individuals and immature underyearlings differed even within the same phase of the population cycle (in 2001 and 2004), the observed differences in the indices are not likely due to the effects dependent on the population density or cycle stage. Indeed, the discriminant analysis has revealed annual differences in the indices between the overwintered spring animals (1 and 5) at the same phase of the population cycle and population density (3 and 22 ind/100 trap-days in 2001 and 2004, respectively), and finally has found no differences between overwintered animals in spring (3 and 5) and mature underyearlings in autumn (8 and 9) in years with different cycle phase and population density (7 and 20 ind/100 trap-days in 2003 and 2004 (groups 3 and 5) and 0.6 and 22 ind/100 trap-days in 2002 and 2003 (groups 8 and 9), respectively). Previously, the depen-

dence of the physiological and biochemical indices on population density has been demonstrated (Chernyavskii et al., 2003). In this case, the authors outlined the importance of animal selection for experiments. Evaluation of the effects on annual variation in the studied indices with an account of individual reproductive state and age using two-way multivariate analysis of variance of the data shown in Table 3 has demonstrated the effect of reproductive state of animals ( $R\text{-}P_{56,230} = 5.845$ ;  $p < 0.0001$ ). The effect of the population cycle phase was insignificant ( $R\text{-}P_{28,115} = 0.920$ ;  $p > 0.964$ ). The results obtained for homogeneous samples of animals suggest other not yet identified factors contributing to the variation of both hematological and whole body indices together with the factors considered.

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