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The Method of Morphophysiological Indicators and Functional-Ontogenetic Approach to Solving Ecological Problems (Based on the Example of Splenomegaly in Rodents)

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Abstract—The productivity of the combined use of the method of morphophysiological indicators (MMPI) and functional-ontogenetic approach in population studies is shown based on the example of analysis of the phenomenon of splenomegaly (SM) in rodents. A solution of the problem of analysis of spleen with a giant variation range is proposed, namely, to analyze the spleen index separately for normal spleen and spleen with SM. It is shown that there is no morphophysiological peculiarity in individuals with different functional statuses in the studied states. It is established that SM does not significantly influence the processes of animal vital activity. The maximum proportion of animals with SM was recorded in reproducing groups, which is due to a high intensity of metabolic processes. It is proved that SM is infectious in the study area. Feral herd infection agents are identified and the mechanism of SM development is considered. The criteria for the correspondence of organs to morphophysiological indicators, as well as the use of spleen as an ecological indicator, are substantiated. All the obtained materials suggest the adaptation of the studied bank vole population to a long-term effect of the infectious factor, which was historically formed in the process of the long-term coevolution of the parasite–host system.

Keywords: population ecology, small mammals (rodents), population dynamics, ecophysiology, spleen, splenomegaly, feral herd infections, ecological indicator

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INTRODUCTION

Method of Morphophysiological Indicators

The variety and complexity of interactions of living systems at different organization levels with an environment determine the diversity of ecological study methods. Despite new high-technology methods, simple and available field survey methods are still relevant in animal population ecology. Among these methods is the method of morphophysiological indicators (MMPI) which was proposed by S.S. Shvarts [1] and flourished in the 20th century.

The MMPI is based on ecologically related morphological and physiological animal features, as well as on the natural relationship between the morph (the weight or size of organs) and their functions. According to the authors [1, p. 4], "While recognizing that the essential purpose of ecological research is to establish the patterns of the population dynamics, one should consider it particularly reasonable to use those methods which would make it possible to assess the physiological state of certain populations, taking into account ... the specific features of animals forming a population together." In other words, "the variability of individual morphological or physiological features forms the view of the biological peculiarity of study populations" [1, p. 5].

The body weight and relative weight of heart, liver, kidneys, adrenal glands, and thymus are proposed as main parameters and the relative internal organ weight, i.e., the weight index (the organ weight to body weight ratio, ‰), is calculated. The list of the above-proposed parameters is not complete. Parameters that were not included in the list, e.g., the relative brain weight, vitamins, and blood corpuscles, as well as data on the condition factor, muscular development, organ and body proportion, hypophysis, and pancreatic gland indices, and many other parameters, can also be used, depending on the objectives and goals of research.

The undeniable advantage of the MMPI is its accessibility. The method makes it possible to easily obtain mass data with native parameters under field conditions using the minimum set of tools, including

Abbreviations: MMPI, method of morphophysiological indicators; MPIs, morphophysiological indicators; SM, splenomegaly; FOA, functional-ontogenetic approach; PFG, physiological functional groups; HFRS, hemorrhagic fever with renal syndrome; ITBB, ixodid tick-borne borreliosis.

simple crush-traps that instantaneously kill small animals. Large sample volumes make it possible to compensate accidental variations. Slightly exaggerating, we can raise the following issue. Which way is the best: to measure the length of one individual with an accuracy up to 0.1 mm or of 100 individuals with an accuracy up to 1 cm? The answer is evident: only the second variant makes it possible to correctly characterize the value of the feature in the population. The method is intended for dealing with dominant species of terrestrial vertebrates that are available for trapping and do not have a nature protection or sociocultural value.

The opponents of the method sometimes consider (a) a low accuracy of individual measurement and (b) the absence of the definite dependence of the weight of the organ on its functional state as its disadvantages. Indeed, errors are inevitable when organs are taken under field conditions, which is sensitive when sample volumes are small. However, many successful studies based on the MMPI show the inconsistency of the second objection. For instance, the advantage of the eco-physiological method is the accuracy of the assessment of physiological animal features; however, it does not make it possible to use mass data directly in nature. As a rule, most of the contemporary high-technology methods require the delivery of living animals to the laboratory and it is necessary to use bulky live traps for trapping. The placement of animals into this traps and their further transportation to the laboratory cause their stress and selective death; as a result, animals that are actually nonrepresentative for the population and have a state that is far from the natural one are taken for analysis.

The initial enthusiasm on the development of the MMPI gradually decreased over 50 years. However, despite skepticism caused by an incorrect application, the method is widely used as a working field tool. The MMPI is traditionally used to solve ecological problems during the study of natural populations of small mammals that inhabit natural and anthropogenically exposed areas. It is prospective to use morphophysiological indicators (MPIs) as features that discriminate different biological phenomena (especially in combination with the FOA). For example, the study of the selectivity of the catch of rodents from different demographic groups by birds of prey used a discriminant analysis of morphophysiological features of voles from different functional-age groups. The results showed that animals from three PFGs with skull asymmetry are distinguished by general stable morphophysiological features [2].

Functional-Ontogenetic Approach

An important role in studies based on the MMPI is played by the identification of structural intrapopulation units. A conceptual functional-ontogenetic approach (FOA) has been developed for studying populations of cyclomorphic small mammals [3]; it is based on the possibility of the polyvariety of the ontogenetic development in the mammal class, which is carefully substantiated for the first time.

The first studies on the functional structuring of rodent populations (future types of ontogeny) should include the assumption of the existence of physiological races [4], as well as the idea of "seasonal generations" of rodents [5–8]. Shvarts et al. showed that rodents born in different seasons have a complex set of morphophysiological features[1]; individuals of different seasonal generations are specific and differ in the rate of aging [6]. Shvarts et al. used the term *generation* in most of their studies on the age structure; however, they used it in two meanings, which led to some terminological discrepancies. In the first case, the generation implies mass regular formations of droppings of young individuals from the beginning of spring reproduction. The modern literature [9, 10] uses the term of *cohort* as an equivalent to this notion, meaning an elementary structural unit of population. In the second case, the generation is a group of several mass spring or autumn droppings (cohorts) (*spring generation* or *autumn generation*, respectively); i.e., seasonal generations are groups of individuals that were born in different seasons, develop under different conditions, and have certain biological properties. It was axiomatic that almost all individuals, including underyearlings, reproduce in spring.

However, the results of long-term mass individual marking [3, 11] showed that the "spring generation" (according to Shvarts) actually includes not only underyearlings that are involved in reproduction and embody the seasonal generation under consideration, but also underyearlings that are always present and do not reproduce in their birth year. The proportion of such animals is 10–30% in years with standard conditions and reaches 100% under extreme conditions: drought [12] or high density [13]. Seasonal generations consist of two groups of individuals, the functional state of which determines most of the study parameters [3, 11]. Therefore, early studies analyzed obviously heterogeneous samples, which led to significant errors in the results. The use of the functional approach allows us to avoid such errors and makes it possible to deal with strictly isolated intrapopulation groups.

The FOA is used in a wide range of studies. Owing to this approach, nonstrict terms, such as adultus and subadultus, are currently hardly used and samples have begun to be differentiated by their functional feature.

Although the FOA was previously discussed [3, 11], we provide a brief characteristic of its basis, i.e., types of ontogenetic development, here. Cyclomorphic mammals, including most species of small rodents, are characterized by cyclic changes in most biological

[1] Studies on the specific features of seasonal generations were prerequisite to the development of knowledge about the functional structuring of rodent populations. Our research continues and further develops these studies.

parameters over a period of approximately one year, serial reproduction, and generation overlap in the presence of two alternative development pathways. Both types of ontogeny are most clearly observed in rodent populations inhabiting the temperate zone of the Northern Hemisphere and its Arctic periphery, where the climate is sharply continental.

The essence of the FOA is that the main criterion for identifying structural units within a population is the functional unity of individuals in groups corresponding to the alternative types of growth and development (the two types of ontogeny) which functionally differ from each other in the population. These groups are identified on the basis of the functional status of animals (with respect to the specific features of growth, development, and reproductive state) and series of its time changes. Each group consists of individuals that are related by the functional role in the population reproduction. Animals that are characterized by one-phase growth (the first type of ontogeny, i.e., underyearlings that matured in the birth year of their birth) perform the function of growth in the population abundance on the basis of reproduction in the birth year. The function of the animals of the second type of ontogeny, i.e., underyearlings that do not reach maturity in the year of their birth (two-phase growth, second type, first phase) is to survive under unfavorable conditions, followed by the renewal of a population in the next phase of its development; i.e., these animals represent overwintered individuals (second type, second phase).

Animals with alternative types of ontogenetic development and with different functions in a population differ in their metabolic intensity [14], life span, rate of aging [3, 15], radioresistance [16], and MPI values [1]. One of the main advantages of the FOA is the opportunity to deal with pure intrapopulation groups of small mammals. This substantially improves the accuracy of analysis and permits logical interpretation of its results. The functional determination of ontogenetic changes in age [15] and biochemical [17] markers is shown. Differences in the indicators of mitotic activity [11] and cytogenetic instability [18], as well as in the accumulation of heavy metals [19] and responses to extreme natural [12, 13] and anthropogenic [16] effects, were revealed.

The objective of our study was to demonstrate the advantages of the combined use of the MMPI and FOA for analyzing the phenomenology and causes of a huge variability of a problem organ, such as spleen. We solved the following tasks: (1) to propose a method of arranging a huge variation range of spleen; (2) to analyze the SM phenomenon based on the MMPI and FOA; (3) to assess the degree of animal infection by feral herd as a factor of SM manifestation; (4) to assess the possibility of using spleen as a morphophysiological indicator and ecological indicator of a damaging factor in a population.

The relevance of the study of giant spleens (SM) is determined by a widespread distribution of this phenomenon in small mammal populations. Spleen functions are various: hemopoiesis, immune development, blood pool, and the involvement in stress responses. This indicates the organ reactivity and sensitivity to a wide range of effects [20–22]. In adult individuals of *Myodes glareolus*, the spleen index varies from 0.6 to 180‰ and weight varies from 0.02 to 5.5 g; i.e., the spleen weight sometimes exceeds the weight of the largest organ, i.e., liver. Presumably, spleen is not included in the list of classic MPIs due to its high variation [1]. SM was sought to be associated with species features [23], pollutions [24, 25], inflammatory processes, infections, and invasions [26–29]. Therefore, the published data on SM causes are contradictory, there are no data on the physiological adequacy of individuals with SM, and the mechanism of SM development has not been considered. All this seems to be due to the absence of proper methodology.

MATERIAL AND METHODS

Study area. The research was carried out in the specially protected area of the Ilmen State Nature Reserve (ISNR) (Chelyabinsk oblast, the Southern Urals), which is located in the pine–birch forest belt in the preforest steppe region of the Southern Urals [30] and is characterized by the absence of sources of technogenic pollution. Rodent trapping sites are near Lake Bolshoi Ishkul.

Objects and data set. The area is inhabited by forest voles (bank vole *Myodes glareolus* Schreber, 1780, northern red-backed vole *M. rutilus* Pallas, 1779, and red–gray vole *M. rufocanus* Sundevall, 1847); gray voles (field vole *Microtus agrestis* Linnaeus, 1761, common vole *M. arvalis* Pallas, 1779, and root vole *M. oeconomus* Pallas, 1776; and pygmy wood mouse *Sylvaemus uralensis* Pallas, 1811).

We used materials from the data set of G.V. Olenev (1975–2018) that were obtained in 1980–2014. In 1997–2000 and May 2014, a student of the Ural State University N.M. Salikhova (Pasichnik) participated in collecting the material. While working on her thesis, she digitized and partially processed materials on SM that were provided by Olenev. The research involved 4171 rodent specimens. Samples with different volumes were analyzed according to the tasks and logic of individual study blocks from the total data set. Their size is given in the tables. Bank vole was used as a model species, since it is the most numerous in the rodent community. Its proportion in traps is 60–80% in different years [3].

Methods. Three physiological functional groups (PFGs) of animals were distinguished for implementing the FOA [3, 11]: mature underyearlings (PFG 3); immature underyearlings (PFG 2); and overwintered individuals (PFG 1). Individuals were distributed over

Table 1. Variability of the spleen index and the proportion of individuals with SM in rodents of different taxa (1980–2013)

Species	Spleen index, ‰		Proportion of individuals with SM, %	*n*
	min	max		
Myodes glareolus	0.6	169.2	23.0	2600
M. rutilus	1.6	124.0	26.0	140
M. rufocanus	1.9	45.2	58.0	22
Sylvaemus uralensis	0.9	21.0	1.6	1283
Microtus agrestis	1.2	13.4	4.0	99
M. oeconomus	1.2	15.1	7.0	15
M. arvalis	2.9	10.4	0.0	12

PFGs by the aggregate of exterior and interior features: the state of the generative system; age markers (thymus and age changes in teeth); the trapping time was also taken into account.

Animals were trapped using the method of irreversible removal with Gero traps in stationary plots. Three–five rounds were annually carried out from May to September (additionally in December and February in some years). The data on animals were processed according to the MMPI [1]. From 1975 to 2001, individual marking with repeated traps was simultaneously carried out (using the CMR method), which made it possible to trace the dynamics of the age structure and functional state of individuals. The age of voles was determined by the level of age changes in their teeth [15].

Animals with the normal state of spleen and with SM are characterized using the main morphophysiological indicators (MPIs): body weight and relative heart, liver, adrenal gland, kidney, and thymus weights. The index of wellbeing, i.e., the hematosuprarenal (H/S) coefficient, was used as an integrated index of the total physiological state of animals [31]. Analysis was carried out at the PFG level with the division of individuals into subsamples with normal spleen and SM as two qualitatively different states.

Virusological and bacteriological studies. The blood, lungs, liver, and spleen were analyzed at the accredited laboratory of the Hygienic and Epidemiological Center for Tyumen oblast (Tyumen) to determine feral herd infection agents as a possible cause of SM. The list of identified agents was compiled based on the published data on the incidence of zoogenous infections in the Ural Region. Thirty-nine individuals trapped in May and July 2013–2014 were used. The laboratory research was carried out using the PCR method, microscopic technique, and microagglutination test.

Statistical analysis. The distribution of variables was tested for normality with the Kolmogorov–Smirnov test and Shapiro–Wilk *W*-test. Paired comparisons were carried out using the Student's *t*-test (when the variances were equal) and Mann–Whitney *U*-test (when the variances were unequal). The inequality of variances is typical when dealing with animals from PFG 2. This is due to different sample volumes: the number of individuals with normal spleen is 16 times higher than that of individuals with SM. The relationship between MPIs was analyzed using the regression modeling method and the contribution of MPIs to sample differences (norm–SM) was determined using the discriminant analysis. The data on the occurrence of feral herd infection agents were analyzed using generalized linear models (GLMs).

RESULTS

Arrangement of the variation range of spleen. The spleen weight is highly variable and significantly differs in rodents of different genera (Table 1). The largest size and frequency of SM incidence are characteristic of forest voles. The proportion of *M. glareolus* with SM is always significant, being 11 to 42%, and the variation range of its spleen index is maximal compared to other species. Therefore, the further analysis was carried out based on the example of bank vole.

The distribution of the number of *M. glareolus* individuals with different values of the spleen index showed a high heterogeneity with two unimodal distributions with respect to this feature (Fig. 1). The threshold limit is up to 10‰ for the normal state of spleen and over 10‰ for the state of spleen with SM [22, 23].

Splenomegaly and features used in the MMPI. The results of the combined analysis of the body and spleen weight using the regression modeling method showed a significant positive relationship between these parameters for all PFGs (Fig. 2). This is confirmed by the results of the study of both sex-combined samples and samples including only males (Table 2).

Similar results were provided by analysis of the liver index which is used as a food availability index, the indicator of energy balance intensity. An increase in the liver index determined by the animal condition factor indicates the accumulation of nutrients [1, 32]. A considerably larger liver weight was established for

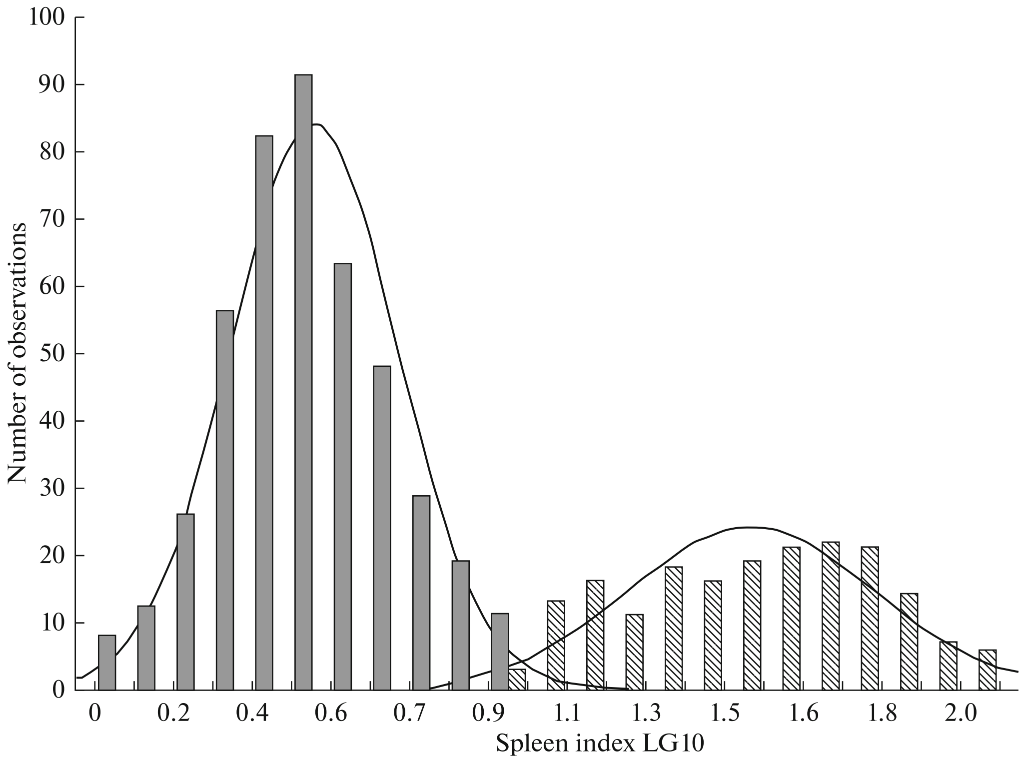

Fig. 1. Bimodal distribution of the spleen index logarithm for *M. glareolus*: norm (black columns)–SM (dashed columns). The unit in the logarithmic scale corresponds to 10‰ of the spleen index.

animals with SM in all PFGs. There is a linear relationship between spleen and liver indices in voles from reproducing groups (Table 3). This suggests the presence of hepatosplenomegaly, i.e., a simultaneous increase in the size of liver and spleen. These organs are anatomically interrelated and have general innervation and lymph pathways.

According to the MMPI [1, 32], the adrenal gland index displays the level of metabolic intensity. The highest values of this parameter are characteristic of animals in reproducing groups (PFGs 1 and 3), especially females. No differences were found in individuals of norm–SM subsamples of PFGs 1 and 3 (see Fig. 2). In PFG 2, underyearlings with SM had a significantly higher adrenal gland index than individuals with normal spleen.

The heart index is the indicator of locomotor activity and energy expenditures [1]. Changes in life conditions or the life style due to an increase in energy expenditures, as well as biological features (growth, reproduction, etc.), lead to an increase in the size of heart and an intensification of its functions [1, 32]. The highest heart index was established for underyearlings with SM (see Fig. 2), while animals from PFGs 1 and 2 did not have differences.

In addition to the heart index, the kidney index is also elevated in underyearlings of PFG 3 with SM (see Fig. 2). Kidneys are sensitive to metabolic changes, and the intensification of metabolic processes is accompanied by an increase in their index [1, 32]. The increased values of kidney and heart indices in underyearlings of PFG 3 with SM reflect a high metabolic rate.

Table 2. Relationship between the spleen index logarithm and body weight (Pearson correlation coefficients and regression coefficients: $y = kx + b$) in PFGs 1 and 3 (subsample: males) (1981–1983 and 1997–2000)

PFG	r (correlation coefficient)	k (angular coefficient)	t	p	b (free term)	t	P
1	0.59	0.10	7.79 (115)*	<0.001	–1.64	–4.48 (115)*	<0.001
3	0.54	0.07	4.72 (54)	<0.001	–0.55	–1.70 (54)	0.094

* Figures in parentheses show the number of degrees of freedom.

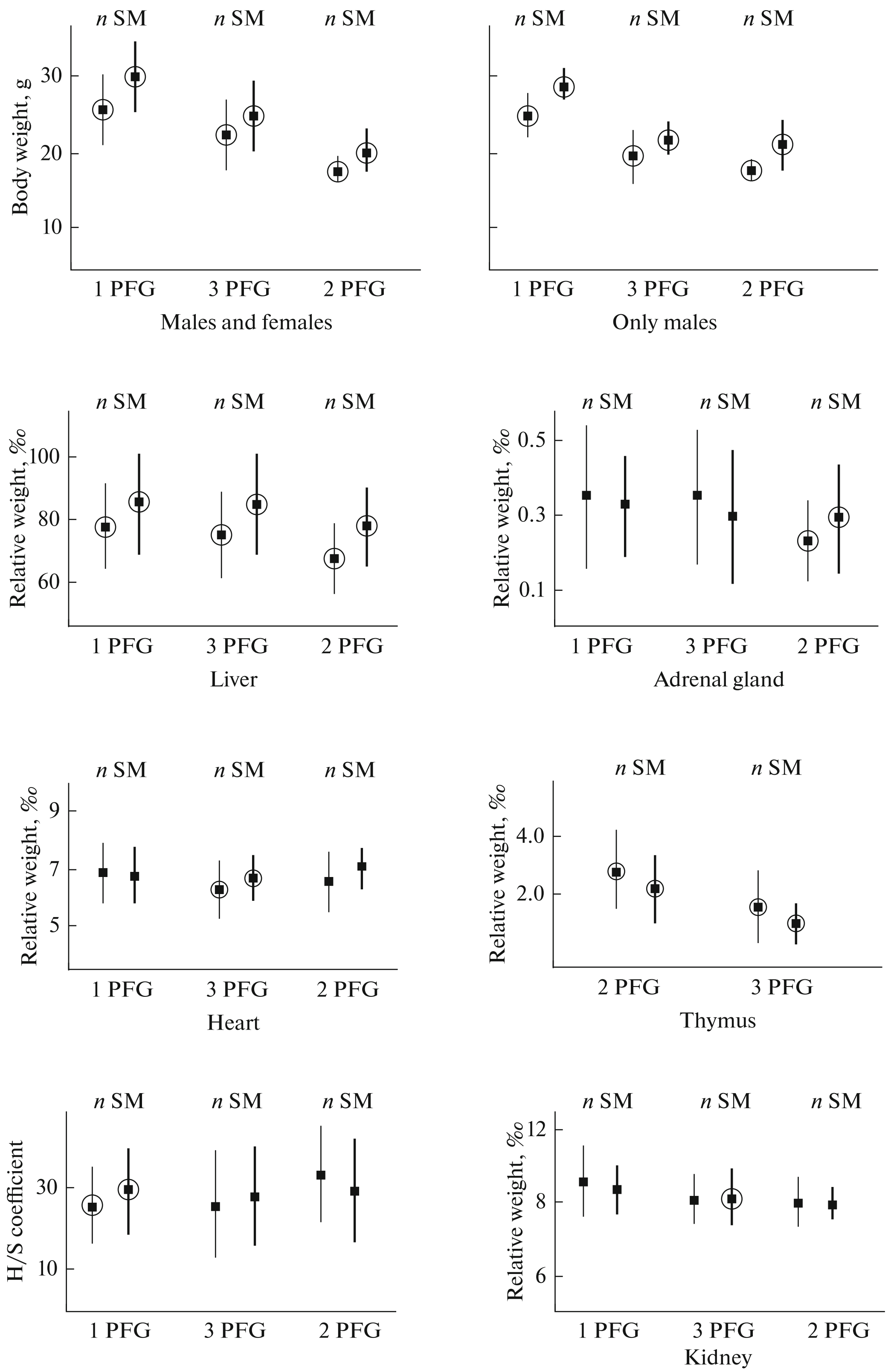

Fig. 2. Morphophysiological indicators and H/S coefficient ($\overline{X} \pm$ SD) in animal subsamples: norm (*n*)—SM in individuals of different PFGs (the circle shows the values that significantly differ within PFGs).

Table 3. Relationship between the spleen index logarithm and liver index logarithm (Pearson correlation coefficients and regression coefficients: $y = kx + b$) in PFGs 1 and 3 (subsample: males) (1981–1983 and 1997–2000)

PFG	*r* (correlation coefficient)	*k* (angular coefficient)	*T*	*p*	*b* (free term)	*t*	*P*
1	0.30	0.04	3.41 (115)*	<0.001	1.84	130.9 (115)*	<0.001
3	0.66	0.16	6.48 (54)	<0.001	3.02	115.08 (54)	<0.001

* Figures in parentheses show the number of degrees of freedom.

Table 4. Statistics for discriminant function models in PFGs (1981–1983 and 1997–2000)

PFG	Eigen values (Eigenval)	Wilks' lambda (λ)	F	*p*	χ^2	*P*
1	0.3134	0.761	8.83 (6, 169)*	<0.001	46.61 (6)*	<0.001
2	0.2147	0.823	7.76 (7, 253)*	<0.001	49.69 (7)*	<0.001
3	0.3876	0.721	5.24 (7, 95)*	<0.001	31.88 (7)*	<0.001

* Figures in parentheses show the number of degrees of freedom.

Thymus indicates the age during the initial period of ontogeny. The direct relationship between the relative thymus weight and viability of young animals allows us to consider the thymus index as an indicator of growth intensity of young animals [1, 5]. The thymus of underyearlings of PFG 3 after maturation almost completely involutes for 1–2 months; the thymus weight also decreases during the growth of underyearlings in PFG 2; overwintered individuals have no thymus [11]. Underyearlings of different functional statuses (PFGs 2 and 3) with SM have a lower thymus index than the norm (see Fig. 2). On the one hand, this can be determined by a higher body weight of animals with SM, which gives an impression of a decrease in the calculated values of all indices. On the other hand, animals with SM are, on average, older than those with normal spleen in each PFG; accordingly, older individuals have a more pronounced thymus involution. It is improbable that a decrease in the thymus index in individuals with SM can also be determined by the animal immune status.

The H/S coefficient indicates the intensity of metabolic and mobilization processes, as well as the level of energy resource availability. Its value is directly proportional to the level of body energy potential and inversely proportional to the energy intensity of regulatory and compensatory mechanisms [31]. High values of the H/S coefficient were established for wintered individuals (PFG 1) with SM, while the differences between underyearling subsamples (PFGs 2 and 3) were absent (see Fig. 2). Consequently, animals with normal spleen and SM are characterized by the same resistance and energy potential.

The assessment of the influence of the MPI contribution to the discrimination of animals with normal spleen and SM showed the significance of the model for all PFGs (Table 4). The body weight, liver index, and related H/S coefficient contribute most significantly to the discrimination of subsamples of individuals of all PFGs with normal spleen and SM (Table 5).

Seasonal dynamics of the spleen index. Individuals of PFG 3 with normal spleen are characterized by a high index during reproduction, followed by the death of animals that finished reproduction. For underyearlings of PFG 2, the spleen index varies insignificantly throughout the vegetation period; they are characterized by a pronounced autumn–winter depression. After overwintering (the transition to PFG 1), the relative spleen weight naturally increases due to maturation. The index slightly decreases upon the completion of reproduction; however, the animals that were involved in reproduction die by the beginning of winter [11]. It is impossible to observe the complete seasonal dynamics of the index of spleen with SM due to the absence of individuals of PFG 2 with SM in winter.

The most informative analysis is the analysis of seasonal changes in the proportion of individuals with SM in PFGs (Fig. 3). In underyearlings of PFG 3, SM is recorded even among individuals of the first cohorts. The proportion of these individuals varies from 30% in June to 56% by the end of their life cycle. The proportion of individuals of PFG 2 with SM is low (10%). After overwintering (PFG 1), individuals with SM emerge in the population again; they are single in April samples, while the abundant incidence of SM (60%) begins to be observed as early as the beginning of May. Simultaneously with spring maturation, the spleen index reaches high (over 100‰) values. The proportion of such animals is maximal in July (over 80%) and remains high till the end of their life cycle.

SM and feral herd infection. The proportion of individuals infected by a HFRS agent in the bank vole

Table 5. Standardized coefficients of models and the factor structure of features in PFGs

Features (variables)	Standardized coefficients			Factor structure		
	PFG 1	PFG 2	PFG 3	PFG 1	PFG 2	PFG 3
Weight, g	–0.99	–0.85	0.81	**–0.81***	**–0.70**	**0.44**
Heart (index), ‰	–0.45	–0.08	0.68	0.07	–0.02	**0.40**
Liver (index), ‰	–0.20	–0.47	0.68	**–0.46**	**–0.39**	**0.53**
Kidney (index), ‰	0.17	0.09	–0.01	0.22	0.06	–0.09
Adrenal gland (index), ‰	–0.28	–0.52	–0.11	0.10	**–0.20**	–0.01
Thymus (index), ‰	–	0.31	–0.04	–	0.17	–0.07
H/S coefficient	–0.42	–0.46	0.06	**–0.31**	**–0.13**	**0.19**
Relative cumulative proportion of variance (Cum. Prop)	1.0	1.0	1.0	–	–	–

*Boldface indicates values statistically significant at $p < 0.05$.

population is 27%, while the degree of ITB infection is 2 times higher (59%). The frequency of incidence of human monocytic ehrlichiosis does not exceed 5%. Agents of tick-borne encephalitis, babesiosis, tularaemia, and leptospirosis were not revealed.

The effect of ITB infection on the spleen index of bank voles with different functional statuses was estimated in the course of statistical modeling (Table 6). On average, the probability of the development of SM is 2.3 (95% CI 1.02–5.0) times higher for animals infected by an ITB agent than for intact animals. The average probability of the SM development is 2.9 (95% CI 1.1–7.8) times higher for overwintered voles and 3.3 (95% CI 1.06–10.42) times higher for mature underyearlings (PFG 3) than for immature individuals (PFG 2). The significance of the interaction between the spleen index and an ITB infection agent is illustrated in Fig. 4. The correlation between SM and the infection of voles in the studied population confirms the assumption of the infectious nature of SM.

DISCUSSION

The key aspects of our analysis were as follows: (1) strict distribution of animals within interpopulation functional groups [23]; (2) separate analysis of the spleen index in the normal state and in SM. The latter provided a general possibility of studying this phenomenon. The nececessity of differentiating the values of the spleen index is due to its unusually high variability. The total variation range of the relative spleen weight is three orders of magnitude. Previously, this did not make it possible to perform a correct analys.

The ranges of spleen index values differ in representatives of *Myodes*, *Microtus*, and *Sylvaemus*. The largest number of individuals with SM was revealed among species of the genus *Myodes*, while the lowest

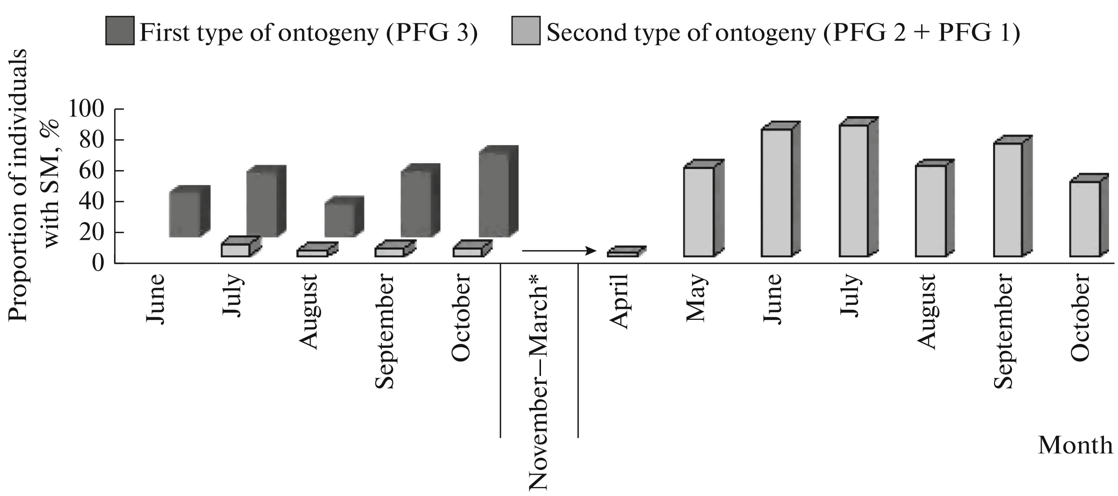

Fig. 3. Seasonal dynamics of the proportion of individuals with SM for animals with different types of ontogeny (*—no animals with SM from November to March).

Table 6. Estimates of GLM parameters (on the assumption of gamma distribution for a random component and log transformation) for the spleen index $se = \exp(\Sigma b_i X_i)$

Predictors	b	Se	Wald χ^2	$P \leq$	exp	95% CI	
b_0*	1.35	0.43	9.76	0.001	3.9	1.66	9.06
PFG 1	1.06	0.51	4.41	0.04	2.9	1.07	7.82
PFG 3	1.20	0.58	4.25	0.04	3.3	1.06	10.42
ITB	0.82	0.40	4.08	0.04	2.3	1.02	5.01

* b_0 is the reference group: individuals of PFG 2 that are not infected by an ITBB agent.

number was recorded among species of the genus *Microtus*. Therefore, it is reasonable to state the genus specificity of SM manifestation in the area of our surveys. This does not rule out the possibility of detecting SM in small mammals of different taxa in other regions [23, 24, 33]. It should be noted that the relationship between the manifestation of SM and degree of technogenic pollution was not revealed [24]. At the same time, the threshold limit of the spleen index norm that we established (10‰) [23] was confirmed [24]. We assumed that the study of SM in different systematic groups would made it necessary to reveal the specific threshold values for differentiating the norm and SM. Indeed, this value is 2.5 times higher in insectivores than in rodents [24]. The value of 5‰ was used as a threshold value for Muridae in dark-coniferous forests of the Yenisei Ridge [29]. In any case, it is essential to divide the variation range of the relative spleen weight when studying SM, thereby allowing its investigation in combination with other characteristics.

Seasonal changes in the normal spleen index are subject to the common patterns for most of the MPIs [1]. The seasonal dynamics of the index of spleen with SM does not have clear patterns. The common aspect for seasonal changes in the index of normal spleen and spleen with SM is that the highest median values are observed in the reproduction period, followed by their decrease in autumn. The seasonal change in the proportion of individuals with SM in PFGs proved to be most informative. The maximum number of animals with SM is observed in reproducing groups, especially in the group of wintered individuals. The latter is confirmed by the results of analysis of the ecological factors of the SM development risk: the strongest effect is related to the functional state factor [34]. The probability of the SM development is 7.4 times higher for reproductively active animals (PFGs 1 and 3) than for immature underyearlings (PFG 2). The calendar age related to the functional status is also an important risk factor, since the probability of the SM development increases by over 10 times by the end of the life cycle of an individual.

It is believed that infections are the main cause of the development of SM [26–29, 35]. According to our results, the ITB and HFRS are significantly widespread among bank voles. Over 80% of individuals with SM are infected by a certain agent or have mixed infection. One should also note the detection of ITB agents in some reproducing animals with normal spleen. This is possibly due to the fact that the development of the physiological response to the SM state in the form of an enlargement of the organ should take some time from the date of infection. Here, it is important to note that agents were identified using a reliable method, namely, the PCR analysis, which makes it possible to detect agents at the initial stage.

One should note the difference between the revealed feral herd infections in their infection routes: ITB is transferred by ixodic ticks, while the main route of HFRS infection is respiratory. It can be assumed that mixed infections cause a decrease in the immune status of the organism due to the infection by one agent and the related increased probability of infection by another.

The comparison of the data on the relationship between the level of infection of individuals and presence of SM shows that animals with the spleen index of over 20‰ were always infected by an ITB agent. Our data indicate the SM association with the pres-

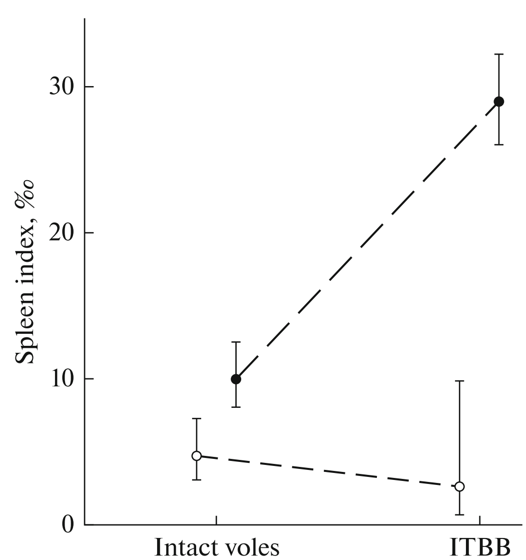

Fig. 4. Spleen index ($\bar{X} \pm 93\%$ CI) of bank voles with different functional statuses (intact voles and ITBB-infected voles: light circles, PFG 2, black circles, PFGs 1 and 3.

ence of ITB agents. This allows us to consider SM a diagnostic feature of ITB infection in the study area. However, one cannot rule out the effect of agents, such as bartonellosis, ricketsial diseases, and brucellosis (the identification of which was outside the scope of our research), on the SM development.

Presumably, SM develops due to infection by feral herd infection agents. The infection proper results from infected tick stings; the transplacental route of transmission of infective matter is also possible [28]. The spleen is enlarged with growth in the level of parasitemia due to the intensification of its functions and changes in the histological and morphological structure [29, 33]. Animals of the second type of ontogeny are probably infected at the first development phase (PFG 2), thereby becoming infection carriers. The low metabolic rate (the state that Shvarts figuratively termed *conserved youth*) hinders the reproduction of an agent and SM development. The intensification of metabolism in wintered animals triggers the mechanism of physiological response to infection in spring in the form of the mass SM development. The proportion of animals with SM is 37% in samples as early as May, increasing to 80% in the course of time; i.e., the accumulative effect is observed. In addition, the seasonal maximum increase in disease incidence may be due to the immunosuppressive effect of sexual hormones, the production of which significantly increases at sexual maturation [36].

The extent of the phenomenon and a high proportion of animals with a giant spleen raise the issue of the physiological and reproductive adequacy of individuals with SM. Do animals with SM and those with normal spleen differ with respect to their life span, morphophysiological features, and reproduction success? This study investigated the morphophysiological peculiarity of animals of different structure-functional units (PFGs) with normal spleen and SM. Our data indicate the existence of these differences with respect to a number of MPIs. In all PFGs, individuals with SM have a larger body weight and relative liver weight, while underyearlings with different functional statuses (PFGs 2 and 3) have a lower relative thymus weight. Individuals with normal spleen and with SM have a high resistance and adequate energy potential, which is also indicated by similar values of the index of well-being. It is important that SM does not significantly influence the animal life span estimated by age markers [15], as well as their reproductive function. Individuals with SM are actively involved in the population reproduction; there is no difference in the fecundity of individuals with SM and with normal spleen [22, 34].

Therefore, one can conclude that the bank vole population has adapted to the long-term effect of the infectious factor causing SM and assume that this adaptation was formed in the process of coevolution in the parasite–host system. It is known that the highest intensity of the reproduction of an agent is characteristic of organisms with a high metabolic rate. This is confirmed by our results: the maximum number of individuals with SM and the relationship of SM with infection are recorded for reproductively active individuals of PFGs 1 and 3. The proportion of individuals with SM is low in PFG 2; however, they are constantly present in the population except winter. This interesting fact can be interpreted as a “dormant” stage of an agent. Individuals of PFG 2 are more resistant to unfavorable natural and technogenic impacts. Their high radioresistance owing to the metabolic minimization is impressive [16]. The winter immunity intensification in rodents from natural populations and the role of the seasonal variation of the state of the immune system of rodents in the circulation of zoogenous infections are also documented [37].

CONCLUSIONS

We have considered the possibility of using the spleen state index and SM phenomenon from in terms of the MMPI by analyzing the correspondence of the spleen state to the criteria for indicator organs in the MMPI. The general criteria of indicator organs are as follows: (1) they should be vitally important; (2) it should be definitely established what they indicate; (3) they should have quite a high reactivity and clearly respond to environmental changes; (4) they should reflect the physiological state of animals, thereby performing specific functions; (5) the existing variation should provide a possibility for carrying out statistical processing; (6) it should be possible to obtain data under field conditions without complex equipment; (7) the indicator organs should permit the investigation of mass data.

The spleen is an organ, the surgical removal of which does not cause the animal death. There is no definite view of what exactly the spleen indicates. It performs various functions and is characterized by a high reactivity; however, it is unclear what it responds to. The spleen has a giant variation; however, the division into the ranges of normal variation and pathological state makes it possible to correctly perform a statistical analysis. Mass data on the spleen state can be obtained without using complex equipment.

Therefore, the spleen does not meet four of the seven criteria of the morphophysiological indicator. However, owing to a high sensitivity to unfavorable impacts, as well as to the simplicity of SM detection, the spleen can be considered an indicator of ecological ill-being. SM indicates the influence of damaging factors which presumably differ in different regions and populations. Our materials allow us to recommend SM in small mammals as a marker of feral herd infections. The detection of SM is a basis for epidemiological alertness. Therefore, we recommend Rospotrebnadzor bodies (Federal Service for Supervision of Consumers Protection) to use our results during target surveys of areas by zoologists of a sanitary epidemiological station, who carry out inventories of the abundance of ticks and their feeders in their natural and anthropogenic habitats, as well as during predictions and the elaboration of strategies for the optimization of epidemiological risks for humans.

It has been 50 years since publication of the book *The Method of Morphophysiological Indicators in the Ecology of Terrestrial Vertebrates*. This method remains a reliable, simple, and effective tool of population ecology. The MMPI combined with other, more sophisticated methods is applicable for a wide range of population studies, including the assessment of aftereffects of different anthropogenic interferences and population responses to environmental pessima. The MMPI is effective for the discrimination of different phenomena, especially when used in combination with the functional-ontogenetic approach [2]. In particular, the combined use of these methods makes it possible to successfully solve even nontraditional ecological problems, which was exemplified in this study. We have shown that the MMPI and FOA supplement each other; their combined use makes it possible to successfully solve even nontraditional tasks related to the analysis of problem organs and is prospective for solving other ecological problems.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest. *Statement on the welfare of animals*. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

1. Shvarts, S.S., Smirnov, V.S., and Dobrinskii, L.N., *Metod morfofiziologicheskikh indikatorov v ekologii nazemnykh pozvonochnykh* (The Method of Morphophysiological Indicators in the Ecology of Terrestrial Vertebrates), Sverdlovsk: Ural. Fil. Akad. Nauk SSSR, 1968.
2. Vasil'ev, A.G., *Epigeneticheskie osnovy fenetiki: na puti k populyatsionnoi meronomii* (Epigenetic Foundations of Phenetics: On the Way to Population Meronomy), Yekaterinburg: Akademkniga, 2005.
3. Olenev, V.G., Alternative types of ontogeny in cyclomorphic rodents and their role in population dynamics: An ecological analysis, *Russ. J. Ecol.,* 2002, vol. 33, no. 5, pp. 321–330.
4. Stieve, H., Untersuchungen uber die Wechselbeziehungen zwischen Gesamtkorper und Keimdrusen. Mitt. 2, *Arch. Mikrosk. Anat. Entw.-mech.,* 1923, vol. 99, no. 2, pp. 390–560.
5. Olenev, V.G., Seasonal changes in some morphophysiological characters of rodents in relation to dynamics of the age structure of populations, *Extended Abstract of Cand. Sci. (Biol.) Dissertation,* Sverdlovsk, 1964.
6. Shvarts, S.S., Ishchenko, V.G., Ovchinnikova, N.A., et al., Alternation of generations and life span in rodents, *Zh. Obshch. Biol.,* 1964, vol. 25, no. 6, pp. 417–431.
7. Pokrovskii, A.V., Seasonal fluctuations of body weight in voles, *Tr. Inst. Biol. Ural. Fil. Akad. Nauk SSSR,* 1967, no. 51, pp. 95–106.
8. Amstislavskaya, T.S., Mitotic activity of surface epithelium in seasonal generations of *Clethrionomys* voles, *Extended Abstract of Cand. Sci. (Biol.) Dissertation,* Sverdlovsk, 1975.
9. Gliwicz, J., Why do some young voles growing?, *Pol. Ecol. Stud.,* 1995, vol. 20, nos. 3–4, pp. 335–341.
10. Shilov, I.A., The biosphere, life organization levels, and ecological problems, *Ekologiya,* 1981, no. 1, pp. 5–11.
11. Olenev, G.V., Functional–ontogenetic approach in studies on populations of cyclomorphic rodents, *Extended Abstract of Doctoral (Biol.) Dissertation,* Yekaterinburg, 2004.
12. Olenev, G.V., Population mechanisms of adaptation to extreme environmental factors (the exmple of bank vole), *Zh. Obshch. Biol.,* 1981, no. 4. pp. 506–511.
13. Kolcheva, N.E. and Olenev, G.V., Concordance of population changes in the wood mouse and the bank vole in forest biogeocenoses of the Southern Urals, *Ekologiya,* 1991, no. 1, pp. 43–52.
14. Novikov, E.A., Kondratyuk, E.Yu., and Petrovskii, D.V., Effect of the life history pattern on bioenergetic parameters of northern red-backed voles (*Myodes rutilus* Pall.) in a mountain taiga population from the south of Western Siberia, *Russ. J. Ecol.,* 2015, vol. 46, no. 5, pp. 476–480.
15. Olenev, G.V., Determining the age of cyclomorphic rodents: Functional-ontogenetic determination, ecological aspects, *Russ. J. Ecol.,* 2009, vol. 40, no. 2, pp. 93–104.
16. Grigorkina, E.B. and Olenev, G.V., Role of multiversality of animal development in evaluating the consequences of radiation impact, *Radiats. Biol. Radioekol.,* 2015, vol. 55, no. 1, pp. 16–21. doi 10.7868/S086980311501004X
17. Gulyaeva, I.P. and Olenev, G.V., On changes in the electrophoretic pattern of blood serum transferrins in bank voles depending on the physiological status of animals, *Ekologiya,* 1979, no. 6, pp. 47–52.
18. Rakitin, S.B., Cytogenetic instability in bank voles from different structural–functional group, in *Biosfera i chelovechestvo* (The Biosphere and Humanking), Yekaterinburg, 2000, pp. 219–224.
19. Bezel', V.S. and Olenev, G.V., Intrapopulation structure in rodents under technogenic pollution of the environment, *Ekologiya,* 1989, no. 3, pp. 40–45.
20. Gilbert, C.W., A double minus log transformation of mortality probabilities, *Int. J. Radiat. Biol.,* 1974, vol. 25, pp. 633–634.

21. Inra, C.N., Zhou, B.O., Acar, M., et al., A perisinusoidal niche for extramedullary haematopoiesis in the spleen, *Nature,* 2015, vol. 527, no. 26, pp. 466–470.
22. Salikhova, N.M., Ecological analysis of splenomegaly in populations of cyclomorphic mammals, *Extended Abstract of Cand. Sci. (Biol.) Dissertation,* Yekaterinburg, 2015.
23. Olenev, G.V. and Pasichnik, N.M., Ecological analysis of spleen hypertrophy in cyclomorphic rodents taking into account the type of ontogeny, *Russ. J. Ecol.,* 2003, vol. 34, no. 3, pp. 188–197.
24. Davydova, Yu.A., Mukhacheva, S.V., and Kshnyasev, I.A., Splenomegaly in small mammals: Prevalence and risk factors, *Russ. J. Ecol.,* 2012, vol. 43, no. 6, pp. 466–475.
25. Gagarskaya (Ignatova), N.K. and Chernova, E.N., Monitoring the elemental composition of birch, oak, and hazel leaves and the state of small mammal fauna in a technogenic geosystem, *Vestn. Krasnoyarsk. Gos. Agrarn. Univ.,* 2013 no. 1, pp. 74–79.
26. Wiger, R., Hematological, splenic and adrenal changes associated with natural and experimental infections of *Trypanosoma lemmi* in the Norwegian lemming, *Lemmus lemmus* (L.), *Folia Parasitol.* (Praha), 1978, no. 25, pp. 295–230.
27. Yurdakul, P., Dalton, J., Beattie, L., et al., Compartment-specific remodeling of splenic micro-architecture during experimental visceral leishmaniasis, *Am. J. Pathol.,* 2011, vol. 179, no. 1, pp. 23–29.
28. Korenberg, E.I., Pomelova, V.G., and Osina, N.S., *Prirodno-ochagovye infektsii, peredayushchiesya iksodovymi kleshchami* (Infections with Natural Focality Transmitted by Ixodid Ticks), Moscow, 2013.
29. Ekimov, E.V., Shishikin, A.S., and Borisov, A.N., On the causes of mass splenomegaly in natural populations of voles, *Russ. J. Ecol.,* 2015, vol. 46, no. 2, pp. 189–194.
30. Gorchakovskii, P.L., The vegetation of the Urals, in *Ural i Priural'e* (The Urals and Transural Region), Moscow, 1968, pp. 211–262.
31. Puzanskii, V.N., On some criteria for assessing the viability of water vole populations, *Ekologiya,* 1974, no. 2, pp. 81–83.
32. Ivanter, E.V., Ivanter, T.V., and Tumanov, I.A., *Adaptivnye osobennosti melkikh mlekopitayushchikh* (Adaptive Features of Small Mammals), Leningrad: Nauka, 1985.
33. Davydova, Yu.A., Mukhacheva, S.V., Kshnyasev, I.A., et al., Spleen hypertrophy in small mammals: An ecological and histological analysis, *Dokl. Biol. Sci.,* 2011, vol. 440, pp. 297–299.
34. Olenev, G.V., Salikhova, N.M., Grigorkina, E.B., and Kolcheva, N.E., The phenomenon of splenomegaly in populations of cyclomorphic rodents: Manifestation, ecological risk factors, and causes, *Vestn. Tver. Gos. Univ., Ser. Biol. Ekol.,* 2014, no. 4, pp. 160–168.
35. Bol'shakov, V.N., Belyaev, P.A., and Popova, E.F., On the epidemiological significance of *Clethrionomys* voles in a natural focus of hemorrhagic fever with renal syndrome in the Urals, *Tr. Inst. Poliomielita Virus.* Entsefalitov, Moscow, 1965, vol. 7, pp. 101–106.
36. Moshkin, M., Gerlinskaya, L., and Evsikov, V., The role of the immune system in behavior strategies of reproduction, *J. Reprod. Dev.,* 2000, vol. 46, no. 6, pp. 341–365.
37. Lokhmiller, R.L. and Moshkin, M.P., Ecological factors and adaptive significance of immunity variations in small mammals, *Sib. Ekol. Zh.,* 1999, no. 1, pp. 37–58.

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