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Splenomegaly in Small Mammals: Prevalence and Risk Factors

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Abstract—Sources of variation in the relative spleen weight have been studied in small mammals (Rodentia: Muridae, Cricetidae; Insectivora: Soricidae) from the Ural and Fennoscandian forest ecosystems differing in the level of industrial pollution (2004–2010). Formal threshold indices for the diagnosis of splenomegaly in rodents and shrews have been proposed and substantiated. Effects of several factors on the probability of splenomegaly have been estimated, and the "normal spleen" index has been determined.

Keywords: spleen, splenomegaly, rodents, insectivores, morphophysiological indicators, risk factors, industrial pollution, heavy metals

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The mammalian spleen—a multifunctional organ involved in hematopoiesis and immune reactions and highly variable in weight in size—has not yet been studied comprehensively. It is known that the spleen is sensitive to various influences, responding to them by changes in its macro- and micromorphology (*Atlas patomorfologicheskikh izmenenii* ..., 1994; Olenev and Grigorkina, 1998; Moskvitina, Paderov, and Prochan, 2000; Rodionova-Prochan and Paderov, 2004; etc.). The spleen has not been on the generally accepted list of morphophysiological indicators, or characters used to assess the state of natural populations (Shvarts, Smirnov, and Dobrinskii, 1968), but some authors consider this organ to be expedient as an indicator of environmental quality (Prochan, 2000).

Along with normal variation in the weight and size of the spleen (with the organ index ranging from 1 to 10‰), its pathological enlargement has been described in murine rodents, with the spleen index reaching or even exceeding 100‰ (*Evropeiskaya* ..., 1981; Ivanter, Ivanter, and Tumanov, 1985; Korosov, 2002; etc.). This phenomenon, often referred to as spleen hypertrophy or splenomegaly (SM), has also been proposed as an indicator of exposure of natural populations to certain deleterious factors (Olenev and Pasichnik, 2003). However, before considering SM as an indicator of population ill-being, it is first necessary to reveal the main causes of spleen enlargement.

Specialists in human and veterinary medicine consider that SM has a multiple etiology and, in most cases, is not a primary phenomenon but a pathology accompanying hepatic, hematological, neoplastic, inflammatory, and some other diseases (Hegglin, 1961; Vinogradov, 1980; *Vnutrennie bolezni*..., 2002). The causes of SM in humans and domestic animals are usually easy to determine by conventional methods of medical diagnosis (case history and clinical examination), but for obvious reasons this is inapplicable to small mammals from natural populations, since only materials from autopsies are usually available in the latter case.

A promising approach to determining the causes of SM in small mammals is based on retrospective analysis of risk factors, i.e., conditions that increase the probability of developing this pathology. These risk factors may include environmental conditions (extreme weather or climate events, industrial pollution, etc.), specific evolutionary and ecological features of the studed species, and the physiological condition of individuals (sex, age, reproductive status, infections and invasions, primary pathologies, etc.). The results of such an analysis make it possible to narrow down the range of suspected causes of pathology and quantitatively evaluate their effects. We have used this approach to estimate the effects of the following risk factors of SM in small mammals: species, sex, reproductive status, helminth infestation (cestodiasis), hepatomegaly (a symptom of some primary hepatic pathologies), and industrial pollution (emissions from copper–nickel smelters). Our interest in the last factor is not accidental: pollutants can cause toxic or allergic reactions, suppression of immunity, metabolic and genetic disturbances, etc. Industrial pollution can affect small mammal communities both directly and indirectly, via environment disturbance (Klosinska, 1996; Lukyanova and Lukyanov, 1998; Mukhacheva, 2001; Kataev, 2005; Phelps and McBee, 2010; etc.). Studies in a pollution gradient make it possible to evaluate parameters of interest at different levels of toxic load and can be regarded as a field ana-

Table 1. Characteristics of study regions and the amount of material included in analysis

Parameters	Middle Urals			Karabash, Southern Urals	Harjavalta, Finland
	Revda	Kirovgrad	Pripyshminskie Bory National Park		
Pollution source (operation start year)	Middle Ural Copper Smelter (1940)	Polymetals production (1910)	–	Karabashmed' (1914)	Norilsk Nickel Harjavalta (1944)
Distance from pollution source, km:					
impact areas	1–3	1–2.5	–	1–5	1–2
buffer areas	4–10	15–18*	–	8–12	3–6
background areas	20–34	34.5*	–	25–32	10–11
Type of forest community	Fir–spruce forest	Fir–spruce and birch forests	Pine forest	Birch forest	Pine forest
Included in analysis:					
species	12	9	5	10	4
individuals	1849	1133	54	374	70
liver samples	452	35	–	181	60
spleen samples	–	–	–	64	11

* Areas within the Visim State Biosphere Reserve

log of laboratory toxicological experiments (Vorobeichik and Kozlov, 2012).

The aim of this study was to estimate the prevalence and effects of risk factors of SM in small mammals. In particular, we determined a threshold for formally distinguishing between the normal spleen and SM, and then analyzed the dependence of SM frequency on different risk factors. Moreover, we compared the levels of heavy metal accumulation in normal and enlarged spleens, evaluated the relationship between SM and individual toxic loads, and analyzed the dependence of normal spleen index on the same set of risk factors.

MATERIAL AND METHODS

The small mammals ($n = 3453$) were captured in forest ecosystems of the Urals and Fennoscandia between 2004 and 2010. Census plots were located in undisturbed habitats (The Visim State Biosphere Reserve, Pripyshminskie Bory National Park) as well as in areas with different levels of industrial pollution (background, buffer, and impact zones) in the vicinities of Middle Ural, Kirovgrad, and Karabash copper smelters and the Norilsk Nickel Harjavalta copper–nickel smelter (Table 1). Degradation of phytocenoses and high levels of pollutant accumulation in the soil, forest litter and different organs (the liver, kidneys, and skeletal bones) of small mammals provided evidence for significant deterioration of environmental quality in plots within the buffer and, especially, impact areas (Vorobeichik, Sadykov, and Farafontov, 1994; Kozlov et al., 2009).

Small mammals (rodents and insectivores) were captured with snap- or live traps. The procedure of censuses and characteristics of investigated areas were described in detail previously (Vorobeichik et al., 2006; Mukhacheva, 2007; Kozlov, Zvereva, and Zverev, 2009; Mukhacheva, Davydova, and Kshnyasev, 2010). Each animal was examined to determine its species, sex, reproductive status, body weight, indices (organ-to-body weight ratios, m/M, ‰) of the spleen and liver, and the presence of cestode larvae in the liver. The total catch included representatives of eight rodent (Rodentia) species from two genera of the family Muridae (*Sylvaemus uralensis* and *Apodemus agrarius*) and two genera of the family *Cricetidae* (*Clethrionomys glareolus*, *Cl. rutilus*, *Cl. rufocanus*, *Microtus arvalis*, *M. oeconomus*, and *M. agrestis*) and four insectivore (Insectivora) species from the genus *Sorex* of the family Soricidae (*Sorex araneus*, *S. caecutiens*, *S. isodon*, and *S. minutus*).

The spleens from animals of seven species ($n = 75$) were analyzed for the concentrations of essential (Cu, Zn, Fe) and toxic (Cd, Pb, Ni) elements (μm/g dry weight), which were determined by atomic absorption spectrometry in an AAS 6 instrument (Analitik Jena AG, Germany). Individual toxic loads were evaluated on the basis of data on the levels of Cu, Zn, Cd, and Pb accumulation in the liver ($n = 728$). Quantitative determinations of elements ($n < 3400$) were carried out at the Laboratory of Ecotoxicology of Populations and Communities, Institute of Plant and Animal

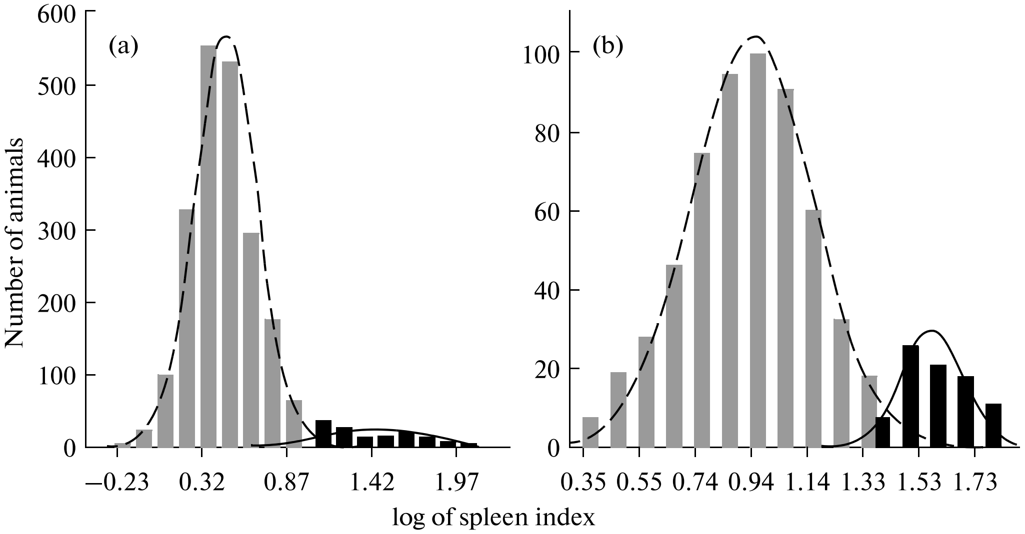

Fig. 1. Distributions of the logarithm of spleen index in (a) bank voles and (b) shrews: gray bars, animals with normal spleen weight; black bars, animals with splenomegaly; curves, approximation by the normal distribution.

Ecology (Ural Branch, Russian Academy of Sciences), accredited in the State System of Analytical Laboratories (certificate no. ROSS.RU0001.515630).

Formal discrimination between normal and enlarged (SM) spleens was based on analyzing the distribution of common logarithms of spleen index values, with the antimode of this bimodal distribution being taken as a conditional threshold. The conclusion concerning its bimodality was drawn from the analysis of plots made on normal probability paper and the results of kernel smoothing a sample histogram (Wessa, 2012). Thereafter, the continuous variable (the logarithm of the spleen index) was dichotomized: $Y_i = 0$ (normal spleen), $Y_i = 1$ (SM).

Data analysis was performed using the generalized linear model (GLM) approach (McCullagh and Nelder, 1989). The dependence of SM probability on a certain risk factor (with regard to the effects of other factors) was estimated by the additive logit regression $\ln\{Pr(Y_i = 1)/Pr(Y_i = 0)\} = b_0 + \Sigma b_i X_i$; or linear regression for the logarithm of the normal spleen index $\log(Y) = b_0 + \Sigma b_i X_i$. Factors included in analysis (X_i): animal sex (female, 0; male, 1), reproductive status (immature, 0; mature, 1), and cestodiasis (negative, 0; positive, 1). The level of pollution, taken as an ordinal variable, was ranked 0 in the background, 1 in the buffer, and 2 in the impact areas. Taxa were encoded by K–1 indicator variables (0; 1). Odds ratios (OR) and their 95% confidence intervals (CI) are shown after back transformation: $OR = \exp(b_i)$ or $OR = 1/\exp(b_i)$, where b_i are parameters of logit regression (logarithms of the odds ratio). Odds ratios for rare events (less than 10% frequency) can be directly interpreted as risk ratios (Agresti, 2007). Statistical data processing and visualization were performed with the Statistica program (StatSoft inc., 2001).

RESULTS

Analyzing distributions of the logarithm of spleen index, we found that its threshold values discriminating between the normal spleen and SM were different in rodents and insectivores: 10‰ ($\log(m/M) = 1$) in the former and 26‰ ($\log(m/M) = 1.4$) in the latter. These distributions and their approximation by the normal distribution are shown in Fig. 1 for the bank vole, the most abundant specie in catches (n_{norm} = 2069, $N(0.48; 0.20)$; n_{SM} = 127, $N(1.37; 0.27)$), and for shrews (n_{norm} =568, $N(0.94; 0.21)$; n_{SM} = 84, $N(1.58; 0.11)$). On the basis of threshold values, the animals were divided into two groups, with normal spleens and with SM (Table 2).

Cases of SM were recorded in all reproductive groups of rodents and insectivores (Table 2). Exterior and interior characters in animals with normal spleens and SM were similar and, therefore, could not be regarded as symptomatic of pathological spleen enlargement (data not presented here). Among them, of special interest to us was hepatosplenomegaly, i.e., the simultaneous pathological enlargement of both the liver and spleen. As shown by examining bank voles and shrews, SM was not accompanied by the enlargement of the liver (Fig. 2).

Rodents of the family Muridae were excluded from subsequent analysis of risk factors of SM in small mammals, because the prevalence of this pathology in them was only 1.1%. Statistical modeling of SM prevalence by means of multiple logit regression analysis allowed us to estimate the adjusted effect of each factor, with all other factors being controlled. All the effects are statistically significant (Table 3); the effects of "reproductive status," "taxon," and "industrial pollution" could be classified as strong, while "sex" and "cestodiasis" as moderate ($OR < 2$). Thus, the proba-

Table 2. Numbers of small mammals with splenomegaly (numerator) and with normal spleens (denominator) and their percent ratio (in the parentheses) in habitats with different levels of industrial pollution

Order, family, genus, species	Reproductive status*	Industrial pollution zone		
		background	buffer	impact
Rodentia, Muridae,	**0**	**0/50 (0)**	**2/171 (1.2)**	**0/20 (0)**
***Sylvaemus, Apodemus* ((mice))**	**1**	**0/43 (0)**	**3/123 (2.4)**	**0/33 (0)**
Sylvaemus uralensis –	0	0/44 (0)	2/169 (1.2)	0/20 (0)
pygmy wood mouse	1	0/41 (0)	3/123 (2.4)	0/22 (0)
Apodemus agrarius –	0	0/6 (0)	0/2 (0)	–
striped field mouse	1	0/2 (0)	–	0/11 (0)
Rodentia, Cricetidae,	**0**	**21/596 (3.4)**	**9/623 (1.4)**	**3/108 (2.7)**
***Clethrionomys* (forest voles)**	**1**	**54/367 (12.8)**	**45/353 (11.3)**	**5/92 (5.2)**
Cl. glareolus –	0	14/486 (2.8)	7/487 (1.4)	5/60 (7.7)
bank vole	1	44/268 (14.1)	36/261 (12.1)	7/48 (12.7)
Cl. rutilus –	0	6/77 (0.7)	1/84 (1.2)	0/48 (0)
northern red-backed vole	1	7/71 (9.0)	5/41 (10.9)	1/42 (2.3)
Cl. rufocanus –	0	1/33 (2.9)	1/52 (1.9)	–
large-toothed red-backed vole	1	3/28 (9.7)	4/51 (7.3)	–
Rodentia, Cricetidae,	**0**	**1/14 (6.7)**	**2/14 (12.5)**	**0/6 (0)**
***Microtus* (gray voles)**	**1**	**7/15 (31.8)**	**5/4 (55.6)**	**1/5 (16.7)**
M. oeconomus –	0	1/1 (50.0)	1/4 (20.0)	–
root vole	1	6/10 (37.5)	4/0 (100)	–
M. arvalis –	0	0/8 (0)	1/6 (14.3)	1/2 (33.3)
common vole	1	0/2 (0)	1/3 (25.0)	1/1 (50.0)
M. agrestis –	0	0/5(0)	0/4 (0)	0/4 (0)
field vole	1	1/3 (25.0)	0/1 (0)	0/4 (0)
Insectivora, Soricidae,	**0**	**55/314 (14.9)**	**10 /143 (6.5)**	**0/44 (0)**
***Sorex* (shrews)**	**1**	**20/36 (35.7)**	**1/25 (3.8)**	**0/10 (0)**

* Reproductive status: (0) immature, (1) mature, (–) no data; values in boldface refer to families or genera.

bility of SM was estimated to be 4.2 (2.3–7.7) times higher in *Microtus* voles and 3.6 (2.6–4.9) times higher in shrews (*Sorex*) than in *Clethrionomys* voles (Table 3); 4.0 (3.0–5.5) times higher in mature than in immature individuals; 1.4 (1.02–1.8) times higher in males than in females; and 1.7 (1.01–2.9) times higher in animals with cestodiasis than in uninfested animals. A decrease in pollution load by unit step (the transition from the buffer to background or from the impact to buffer zones) was found to increase the odds ratio for SM by a factor of 1.8 (1.4–3.0), and the transition between the extreme (impact and background) zones, by a factor of 3.2 (2.0–5.1) (Table 3).

We also analyzed the dependence of the logarithm of normal spleen index on the same set of factors ($R^2 = 0.50$, $F(8; 3165) = 394.8$, $p < 0.001$) (Table 4). The relative spleen weight was found to be maximum in the family Soricidae and minimum in Muridae, with genera of the family Cricetidae (*Clethrionomys* and *Microtus*) having similar mean spleen indices. The normal spleen index was, on average, 8 (6–9)% higher in mature than in immature animals and, similar to SM frequency, higher in the background than in the buffer and impact areas. The statistical significance of the interaction of “taxon” and “industrial pollution” ($F(4, 3405) = 7.43$, $p < 0.001$) is explained by the fact

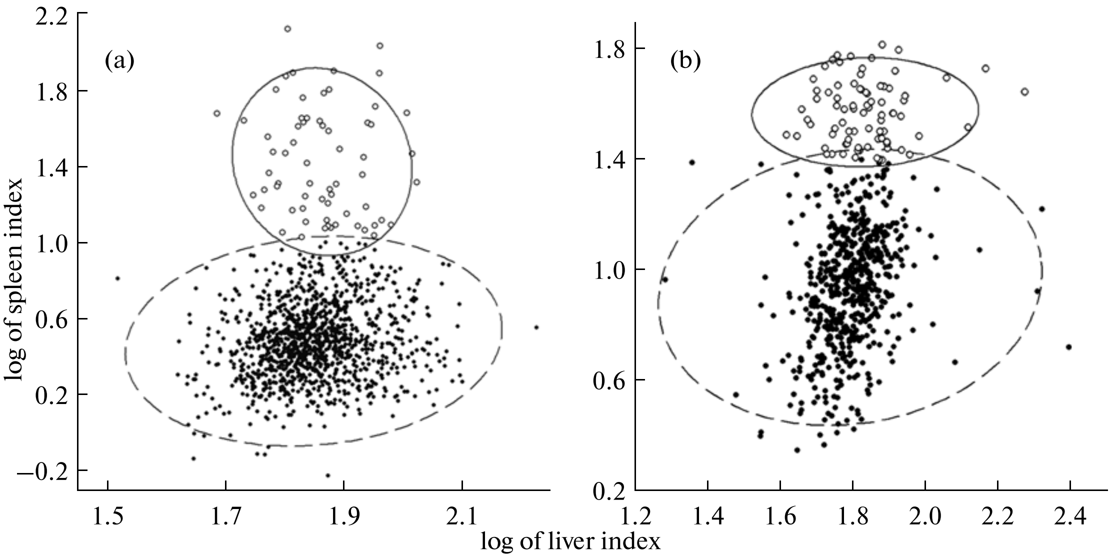

Fig. 2. Distribution of individuals (with confidence ellipsoids) in the plane of factors "logarithm of liver index" and "logarithm of spleen index": (a) bank voles (n = 1245), (b) shrews (n = 652); dots and circles refer to animals with normal and enlarged spleens, respectively.

that the average spleen weight in species of the family Muridae was similar in all habitats studied, whereas that in Soricidae and Cricetidae was higher in animals from background areas (Fig. 3, Table 4). The "sex" and "cestodiasis" had no statisticaly significant effect on the normal spleen index (Table 4).

Comparisons of heavy metal concentrations in normal and enlarged spleen from animals of different species showed that SM did not have an effect on the accumulation of most elements, e.g., cadmium ($t(70) = 0.13, p = 0.89$), but the concentration of iron in the organ slightly increased by a factor of 1.01 (1.001–1.013) ($t(70) = 2.37, p = 0.021$) (Table 5). On the other hand, the concentrations of investigated elements (except nickel) in both normal and enlarged spleens were found to be significantly higher in animals from polluted areas. In particular, the concentrations of cadmium were increased by a factor of 4.3 (2.6–7.1) ($t(70) = 26.11, p < 0.001$), and those of iron, by a factor of 1.5 (1.2–1.8) ($t(70) = 4.01, p < 0.001$). The concentrations of heavy metals in the liver of *Clethrionomys* voles and shrews were used as indicators of individual toxic loads. Using the example of cadmium (one of the most toxic metals), it was found that shrews accumulated greater amounts of this metal, compared to voles, and that there is no relationship between SM and the level of cadmium accumulation in the liver within the two trophic groups (Fig. 4).

Table 3. Effects of risk factors on the probability of splenomegaly in small mammals as evaluated by logit regression analysis ($G(6) = 167.52$)

Factor	b	se(b)	Wald's $X^2(1)$ test	Odds ratio		
				OR	95% CI	
b_0 [a]	**−35.33**	**15.24**	**5.38**			
Microtus [b]	**1.43**	**0.31**	**20.93**	**4.19**	**2.27**	**7.73**
Sorex [b]	**1.27**	**0.17**	**58.78**	**3.55**	**2.57**	**4.91**
Reproductive status	**1.39**	**0.16**	**80.26**	**4.03**	**2.97**	**5.47**
Cestodiasis	**0.54**	**0.27**	**4.05**	**1.72**	**1.01**	**2.92**
Sex	**0.31**	**0.15**	**4.46**	**1.37**	**1.02**	**1.83**
Industrial pollution	**−0.58**	**0.12**	**22.94**	**0.56**	**0.44**	**0.71**
		$\mathrm{OR_R}$		**0.31**	**0.19**	**0.50**

Note: (b_0) the reference group of *Clethrionomys* voles (immature females without parasites from background areas); (OR_R) the odds ratio for extreme categories (background and impact areas); (a, b) uniform groups are designated by the same symbols; $p < 0.05$.

Table 4. Dependence of the logarithm of normal spleen index on different factors as evaluated by generalized linear regression analysis

Factor (predictor)	b	se(b)	t(3165)	95% CI	
b_0 ^{b}	0.89	0.74	1.21	–0.56	2.34
Sorex ^{c}	**0.47**	**0.01**	**48.42**	**0.46**	**0.49**
Muridae ^{a}	**–0.13**	**0.01**	**–12.08**	**–0.15**	**–0.11**
Microtus ^{b}	–0.01	0.03	–0.50	–0.07	0.04
Sex	0.01	0.01	–0.62	–0.02	0.01
Cestodiasis	–0.01	0.02	–0.65	–0.05	0.02
Reproductive status	**0.08**	**0.01**	**9.86**	**0.06**	**0.09**
Industrial pollution					
background	**0.06**	**0.01**	**9.99**	**0.05**	**0.07**
buffer	–0.01	0.01	–2.15	–0.02	0.00

Note: (b_0) the reference group of *Clethrionomys* voles (immature females without parasites from impact areas); (a, b) uniform groups are designated by the same symbols; values significant at $p < 0.05$ are boldfaced.

DISCUSSION

In the paper by Olenev and Pasichnik (2003) dealing with spleen hypertrophy in murine rodents, the phenomenon well known to theriologists was first considered as an independent research object. On the basis of long-term studies on seven species of rodents in the Il'men Nature Reserve (the Southern Urals) and analysis of published data, these authors estimated the prevalence of spleen hypertrophy, its correlation with the reproductive status of animals, and dependence on certain ecological factors, such as population structure and density, specific biotopic features, technogenic impact, and exposure to infections and parasites. The ecological analysis of this phenomenon failed to reveal its causes, but the authors considered that their results will promote future studies aimed at identification of factors responsible for spleen hypertrophy. The "medical" aspects of this phenomenon (not considered here) have become a kind of starting point for our research, and the retrospective approach has been used as a methodology allowing us to consecutively exclude less probable causes of SM.

Considering the problem at issue, we have used the term "splenomegaly," which in medicine refers to pathological spleen enlargement. A primary histological analysis of enlarged spleen in the bank vole has revealed hyperplastic processes (i.e., an increase in the number of cells) in the organ; therefore, the term "hypertrophy" (referring mainly to an increase in cell volume) in this case can only be used in a very broad sense (Davydova et al., 2011). The histological analysis of the enlarged spleen in small mammals allows a diagnosis of certain pathology, although does not always reveal its causes. If such an analysis is impossible, it is important to know the formal threshold indicating the conventional boundary between normal and enlarged spleens. Olenev and Pasichnik (2003) proposed a threshold value of 10‰ based on a visual analysis of rank distributions of spleen indices. Our calculations formally substantiated this value as the threshold between the normal spleen and SM in rodents. On the other hand, they showed that this value in insectivores is close to the median, with the threshold determined for these animals being 2.5 times higher than that for rodents (Fig. 1). The use of specific threshold values for the two orders of small mammals makes it possible to analyze the phenomenon of SM as a dichotomous trait within the framework of the same statistical model.

On the basis of their observations, Olenev and Pasichnik (2003) arrived at the conclusion about the genus specificity of spleen hypertrophy (in particular,

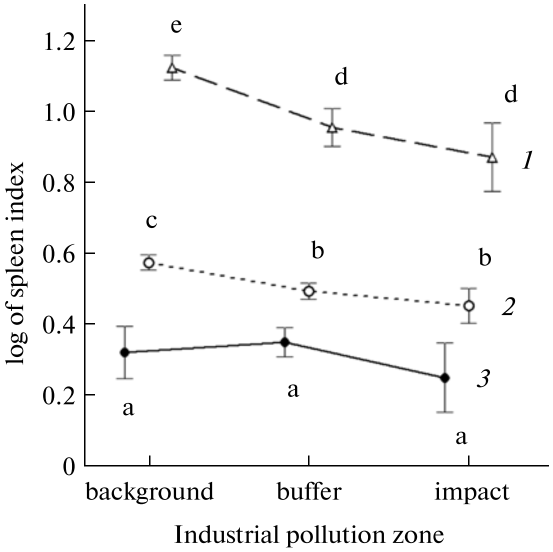

Fig. 3. Normal spleen indices (means and 95% CIs) in small mammals from areas with different levels of industrial pollution: (*1*) Soricidae, (*2*) Cricetidae, (*3*) Muridae. Uniform groups are indicated by the same letters.

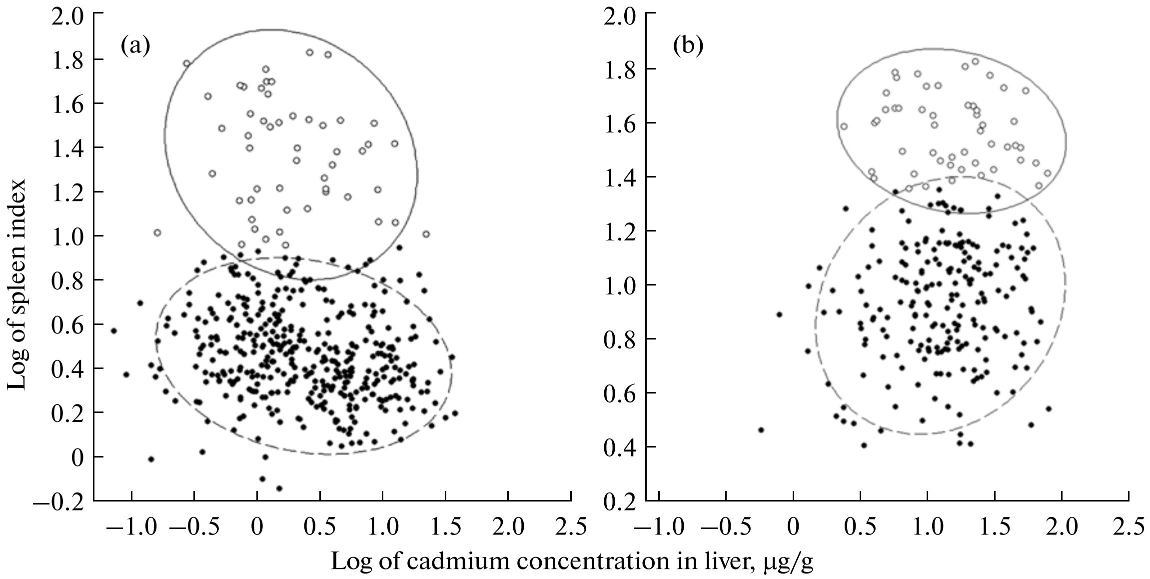

Fig. 4. Distribution of individuals (with confidence ellipsoids) in the plane of factors "logarithm of cadmium concentration in the liver" (μg/g) and "logarithm of spleen index": (a) forest voles ($n = 466$), (b) shrews ($n = 262$); dots and circles refer to animals with normal and enlarged spleens, respectively.

for *Clethrionomys* voles) but did not exclude the possibility of its occurrence in any other rodent species from different localities. Our data indicate that this phenomenon is fairly widespread among small mammals (Table 2), with its prevalence markedly differing between taxa. In this respect, species of the family Muridae may be regarded as a reference because SM is very rare among them. Differences between rodents and insectivores may be due to their distinctive ecological features, such as trophic peculiarities and the existence of specific ecto- and endoparasites and infections. Differences in the prevalence of SM within the family Cricetidae may also be accounted for by species- or genus-specific factors, e.g., differences in the response to pathogenic factors or in the way of life, which is of primary importance in cases of infectious or parasitic diseases.

The low prevalence of SM among animals inhabiting impact area, compared to population from buffer and background, may be explained by their low density, which prevents host density-dependent transmission of infections and invasions (probable causes of SM). In turn, the low density of small mammals in impact areas may result from the impact of toxic pollution, either direct (poisoning and elimination of weakened and diseased animals) or indirect (transformation of the environment leading to a deficit of food and shelters and/or deterioration of their quality).

In this study, we obtained, for the first time, data on the concentrations of chemical elements in the spleen of different animal species, with regard to the presence or absence of SM. Analysis of these and published data on the accumulation of heavy metals in the normal and enlarged spleens has shown that their contents are highly variable, which may be due to variation in spleen weight and size conditioned by specific morphofunctional features of this organ (in particular, the ability to hold a reserve of blood and, when necessary, to quickly release it into the circulation). It appears that the spleen, in contrast to the liver and kidneys, does not accumulate toxic elements in large amounts (Mukhacheva and Bezel', 1995). The only exception is iron. This essential metal is a degradation product of hemoglobin from red blood cells, and its high concentration in the organ is evidence for active hemolysis, which may have some causes. Elevated levels of other elements in the spleen of mammals from polluted areas (Table 5) are apparently a consequence of spleen involvement in metabolic processes against the background of general toxic exposure.

Individual levels of toxic exposure are usually estimated from the concentrations of certain elements in depot organs, in particular, heavy metals in the liver. We have found that the levels of test elements in the liver do not depend on spleen size and weight, although a false correlation between the spleen index and heavy metal contents in the liver may be observed when rodents and insectivores are pooled in one group. The observed differences between animals representing different trophic levels (shrews, compared to rodents, have a higher spleen index and accumulate greater amounts of heavy metals) confirm the necessity for differentiating between these groups in the course of analysis (see Fig. 4).

All known to us examples illustrating the relationship between changes of the spleen in small mammals and industrial pollution of the environment concern the organ remaining within the normal weight range.

Table 5. Concentrations of chemical elements in the spleens of small mammals from background and industrially polluted areas, μg/g dry weight (average or min–max).

Study region	Species	*n*	Element						Data source
			Cu	Zn	Fe	Cd	Pb	Ni	
Background areas									
Finland	*Cl. glareolus*	1*	4.9	93	933	0.29	1.6	0.3	[1]
Southern Urals (vicinities of Il'men State Nature Reserve)	*Cl. glareolus*	7*	6.9/3.6–11.1	90/85–98	839/435–1302	0.14/0.04–0.30	0.2/0.02–0.3	0.9/0.03–2.0	
		1	4.6	100	403	0.07	–	2.8	
	Cl. rutilus	1	6.5	107	706	0.05	–	0.8	
	Ap. uralensis	2*	4.2	70	757	0.89	0.05	1.7	
		9	3.7/2.6–4.9	78/61–91	596/507–733	0.13/0.02–0.29	0.2/0.08–0.2	1.6/0.6–2.5	
	M. arvalis	13*	6.2/3.1–10.2	81/77–90	816/474–1648	0.04/0.01–0.09	0.2/0.03–0.4	1.0/0.2–2.3	
		13	5.7/3.4–8.3	82/59–89	771/558–1531	0.05/0.01–0.14	0.2/0.02–0.8	1.8/0.3–3.2	
	M. oeconomus	1*	9.3	81	785	0.08	0.01	0.5	
		3	9.2/5.6–14.6	82/64–90	824/640–1085	0.08/0.05–0.12	0.1/0.01–0.2	1.0/0.4–2.2	
	M. agrestis	1*	7.6	77	1912	0.13	0.2	1.1	
Borecka Forest, Poland	*Cl. glareolus*	10	8.4	113	526	0.23	–	–	[2]
Central Slovakia	*Cl. glareolus*	22	0.1–6.0	30–275	241	0.1–1.0	0.1–3.0	–	[3]
	Ap. flavicolis	23	0.1	21–477	559	2.0	0.1–1.0	–	
Southeastern Portugal	*Mus spretus*	1–7	6.6	137	–	0.3	–	0.8	[4]
	Rattus rattus	3–5	11.0	60	748	0.5	16–83	0.8	
Industrially polluted areas									
Harjavalta, Finland; 4–6 km from Norilsk Nickel plant	*Cl. glareolus*	8*	4.7/3.1–6.5	90/84–100	2066/688–5031	0.99/0.04–2.62	6.0/0.7–14.4	2.4/0.1–7.5	[1]
		1	4.1	96	1631	0.80	14.1	1.4	
	Sorex araneus	1	5.2	87	564	1.41	6.6	1.9	
Karabash, Southern Urals; 1–5 km from Karabashmed' plant	*Cl. glareolus*	1*	7.5	91	4223	0.41	4.4	0.1	
		1	9.6	93	420	0.43	0.8	2.1	
	Ap. uralensis	3	5.4/5.1–5.7	81/69–87	948/601–1258	0.24/0.06–0.53	0.8/0.6–1.4	1.2/0.5–1.3	
	M. arvalis	1*	13.9	89	562	0.06	0.6	1.6	
	M. oeconomus	2*	15	83	845	0.09	0.8	0.6	
		5	11.7/7.3–19.8	88/84–93	761/600–1220	0.12/0.07–0.19	0.5/0.2–0.8	0.9/0.2–1.8	
Krakow, Poland	*Cl. glareolus*	65	11.2–18.8	72–106	288–638	0.93–2.98	–	–	[2]
Central Slovakia	*Cl. glareolus*	4	0.1–1371	61	952	5.0	122	–	[3]
	M. arvalis	34	0.1–75	15–248	208–454	0.1–36	0.1–229	–	
	Ap .flavicolis	54	1.0–37	19–570	281–672	0.1–20	0.1	–	
Southeastern Portugal	*Mus spretus*	3–8	21.8	1340	1919	1.2	–	12.5	[4]
	Rattus rattus	1–4	14	120	1836	0.8	2.7	0.6	

Note: (*) Enlarged spleen; data sources: [1] original data; [2] Topolska, Sawicka-Kapusta, and Cielik, 2004; [3] Andráš, Križáni, and Šlezárovaá, 2008; [4] Pereira et al., 2006.

The authors of these studies describe changes in the weight (index) of the spleen and specific features of its size, shape, and histomorphology under increasing pollution levels and consider it possible to regard the normal spleen as an organ indicating environmental quality, although data on its weight (index) are contradictory: according to some authors, the spleen index increases under such conditions (Ignatova, 1998; Olenev and Pasichnik, 2003), while others note its decrease, or "spleen hypotrophy" (Prochan, 2000). An increase or decrease in the relative size and weight of the spleen is interpreted as evidence for the "strained" functional condition of animals exposed to technogenic impact.

The results of studies on industrial pollution as a risk factor for the development of SM show that neither normal nor enlarged spleen can serve as an organ reliably indicating the level of toxicant accumulation in the body. The frequency of SM and the value of normal spleen index negatively correlate with the level of industrial pollution of the environment (at least with heavy metals), and, therefore this index cannot be recommended as a direct indicator of industrial pollution.

Another "medical" aspect concerns the close anatomical and functional interrelation of the spleen and liver, which accounts for the simultaneous enlargement of both organs in some pathological states. The results of this study show, however, that changes in the spleen and liver indices do not correlate with each other (Fig. 2), which allows us to exclude diseases of the liver accompanied by its enlargement from the list of probable causes of SM. In general, the spleen and liver are not equal in their significance for the organism. The spleen appears to be a "victim organ" that is one of the first to respond to various disturbances (it is not for nothing that the spleen is the first to be examined during pathomorphological analysis of abdominal organs) and, unlike the liver, is not indispensable for life: in many diseases of the spleen, its surgical removal is prescribed.

Analysis of SM risk factors in small mammals has shown that both the prevalence of SM and the spleen index depend on the same factors, namely, taxonomic features and reproductive status (or biological age). In addition, cestodiasis and sex have a slight effect on SM probability. Risk factors understood in conventional biological terms have proved to be factors accounting for variation in the relative weight (indices) of internal organs. In this sense, the results of this study are quite expectable and in agreement with views on the patterns of variation in morphophysiological characters. Thus, the "medical" approach to the study of SM, unlike the ecological approach, can help to get a deeper insight into the causes of this phenomenon in small mammals.

We can conclude that SM is almost absent in the studied species of the family Muridae. In other groups, the probability of SM is higher (1) in *Microtus* voles and shrews than in *Clethrionomys* voles, (2) in mature than in immature animals, (3) in animals with cestodiasis than in uninfested animals, (4) in males than in females, and (5) in animals from unpolluted areas than from areas polluted with heavy metals. The relative spleen weight (within the normal range) is greater in mature animals and animals from unpolluted areas. The normal spleen index is found to be maximum in shrews and minimum in mice, with its values in *Clethrionomys* and *Microtus* voles being similar. The absence of correlation between SM or normal spleen index and the contents of heavy metals in the body suggests that the observed differences in these parameters among animals from areas with different pollution levels have resulted from changes in ecological conditions and small mammal population density in these areas, rather than from the direct impact of toxic pollutants.

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