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Sperm Motility in Bank (*Clethrionomys glareolus*) and Northern Red-backed Voles (*Cl. rutilus*) Exposed to Industrial Pollution

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Abstract—The motility of epididymal sperm cells in two closely related rodent species, bank vole (*Clethrionomys glareolus, n* = 71) and northern red-backed vole (*Cl. rutilus, n* = 52), from the vicinities of two copper smelters in the Middle Urals was studied with regard to the functional group of animals (mature young of the year and overwintered individuals). The proportion of motile cells (Motile) and parameters of their motility (VCL, VSL, VAP, ALH, BCF, STR, LIN) were determined using the MouseTraxx computer-aided sperm analysis (CASA) system (Hamilton Thorne, United States). These parameters proved to be species-specific: sperm velocity (VCL, VSL, VAP) and linearity of movement (LIN) in *Cl. glareolus* were higher, while head beat cross frequency (BCF) was lower than in *Cl. rutilus*. Sperm motility in both species showed no dependence on sampling region or functional group. Its parameters in *Cl. rutilus* did not differ between plots with different pollution levels, while in *Cl. glareolus* the proportion of motile cells (Motile) and their velocity (VCL) were lower in in animals from the impact than from the background zone. However, pollution accounted for only 8 and 9% of variation in test parameters, respectively; i.e., the parameters of sperm motility in rodents from natural populations have low sensitivity to pollution.

Keywords: sperm motility, fertility, reproductive success, rodents, copper smelters, heavy metals
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Small mammals inhabiting industrial regions are continuously exposed to the effect of polluted environment, both directly (through food objects and water) and indirectly, through pessimization of habitats [1–4]. It is considered that adaptation to toxic environmental factors is achieved based on compensatory demographic responses in populations [4, 5]. Populations of small insectivores and muroid rodents living in a technogenically degraded environment are characterized by a decrease in abundance and impoverishment in the composition of communities. A key role in adaptation aimed at the survival of species under adverse conditions is played by reproduction, but, despite the abundance of factual data, general trends of variation in animal fecundity depending on environmental quality have not yet been elucidated [4, 5]. It should be noted that most methods for assessing the fertility of animals from natural populations (laboratory experiments with poisoning, observations in natural biogeocenoses, mathematical models and extrapolation, etc.) are based on techniques developed in classical toxicology [5].

There are numerous ways to assess male fertility [6], with analysis of sperm motility being regarded as one of the most informative methods for evaluating the functional properties of these cells [7]. For example, the thresholds of reproductive failure, i.e., the lower levels of sperm motility leading to a sharp decline of male fertility, were determined In laboratory Swiss mice [8]. Sperm motility is measured both manually, in standard hemocytometers (e.g., Bürker, Neubauer, Goryaev, and Petroff–Hausser chambers) or specialized sperm counting chambers (Makler, MMC-SR, etc.), or using computer-aided systems such as IVOS and CEROS sperm analyzers (Hamilton Thorne, United States), SCA CASA System sperm class analyzer (Microptic, Spain), Androvision (Germany), MMS Sperm (Russia), SFA 500-2 (Russia), etc. Computer-aided analyzers have certain advantages: they allow cell movement to be separated into several components, thereby improving the information content of its description; moreover, such analysis is more accurate and reliable, which makes it possible to obtain reproducible results and, with certain corrections, compare them to those obtained by other authors [9].

Computer-aided systems of sperm analysis introduced in the mid-1980s were almost immediately tested in toxicological experiments aimed at revealing the effects of environmental pollution [10]. However, research in this field has mainly been performed on humans [11] and commercially important or laboratory animals [12, 13]. Relevant studies on animals from natural populations are much fewer [14, 15], and

those dealing with populations in polluted environments are very rare [16, 17].

Although several hundred studies using computer-aided sperm analysis (CASA) systems are published each year, parameters of sperm motility in many animal species have not been studied, since it is often difficult to collect samples of ejaculate. Sampling in muroid rodents is complicated because of small gonad size and ejaculate volume, and analysis is usually performed with sperm cells from the cauda epididymis, as their properties (viability and fertility) are the closest to those of ejaculated sperm or cells from the ejaculatory duct [18–20]. Epididymal sperm cells are initially immobile, and properties necessary for natural fertilization, such as motility and capacitance, are fully acquired after placing them in a medium containing analogs of substances contained in the seminal fluid and uterine tract [20, 21].

The majority of researchers consider that sperm cells are sensitive to pollution (e.g., in rodents [3, 22]), but data on sperm quality in animals from polluted areas are as yet incomplete and often contradictory: the authors do not regularly observe toxic effects such as an increase in sperm concentration and motility or an increase in the proportion of abnormal cells [20, 21, 23].

The purpose of this study was to estimate the effect of industrial pollution from copper smelters on sperm motility in two closely related sympatric rodent species, *Clethrionomys glareolus* (Schreber, 1780) and *Cl. rutilus* (Pallas, 1779).

To evaluate the relationship between epididymal sperm motility and pollution level, sperm samples were analyzed for the proportion of motile cells and seven parameters characterizing their movement. The initial hypothesis was that a high level of environmental pollution would have an adverse influence on sperm motility, with toxic effects being equally expressed in both *Cl. glareolus* and *Cl. rutilus*. The reproductive response to pollution was compared between these closely related species, and similarity of the results was evaluated.

Sperm motility in voles was analyzed with regard to differentiation of populations into functional groups (young of the year and overwintered animals), which is characteristic of muroid rodents inhabiting the temperate zone. This differentiation is due to the existence of two alternative variants of individual development (ontogenetic pathways), with part of the animals reaching maturity in the year of birth (variant I) and the other part, in the next year, after overwintering (variant II). It is considered that various animal traits, including reproductive parameters, may differ depending on ontogenetic pathway [24, 25].

MATERIAL AND METHODS

Study Region

Studies were performed in the vicinities of Middle Ural and Kirovgrad copper smelters (MUCS and KCS) located in the same landscape–climatic zone. In the study period, they produced comparable amounts of emissions containing similar spectra of priority pollutants, including sulfur dioxide (SO_2), metals (Cu, Zn, Pb, Cd, Fe, Hg, etc.), and metalloids (As). The smelters have been in operation for a long time (MUCS, since 1940; KCS, since 1914), with consequent formation of technogenic geochemical anomalies in their vicinities, where the contents of metals and other pollutants in the soil exceed the background level by one to two orders of magnitude. Despite overhauling of the smelters and reduction of emissions during the past decade, they still have a strong impact on ecosystems [6, 26].

Sampling plots were located in fir–spruce and mixed forests growing in zones with different levels of pollution and damage to ecosystems: the conditionally clean background zone (Bg, 20–40 km from the polluter) and heavily polluted impact zone (Imp, 1.5–6 km from the polluter). These zones were distinguished based on geobotanical relevés and data on the contents of metals in forest litter. When approaching the source of emissions, gradual transformation of various parameters of the environment was observed, including an increase in the contents of metals in substrates, pessimization of microenvironmental characteristics, a decrease in the diversity and productivity of herbaceous and tree layers, etc. [4, 26, 27].

Animal Sampling

Samples of small mammals were collected with wooden live traps from mid-May to late August in 2018–2020. Male bank and northern red-backed voles (*Clethrionomys glareolus* and *Cl. rutilus*) were delivered to the laboratory and kept in cages with bedding of wood shavings and hay at room temperature and natural photoperiod for 1–3 days, being fed oats, carrots, and cucumbers. The animals were then sacrificed by cervical dislocation and examined for a set of traits (body weight and size, the state of gonads, the presence of thymus and tooth roots) to differentiate them into two functional groups: mature young of the year and overwintered animals [24]. Only sexually mature males without any signs of involution of the testes and seminal vesicles were included in analysis. Differences related to other reproductive stages (puberty or senescence) were not considered in this study [25].

The structure of the total sample (n = 123) reflected the abundance and composition of rodent communities in the study years (Table 1). The bulk of the sample consisted of males trapped in years of high population abundance (2019–2020). The structure of rodent communities proved to differ between the two zones in the region of MUCS: *Cl. glareolus* dominated in the background plots but shared dominance with *Cl. rutilus* in the impact plots [4].

Table 1. Size and structure of *Cl. glareolus* and *Cl. rutilus* samples used to analyze sperm motility

Species	Polluter	Pollution zone	Numbers of young of the year/overwintered voles in different years			
			2018	2019	2020	All years
Cl. glareolus	MUCS	Bg	0/2	0/5	0/17	0/24
		Imp	3/2	5/0	0/9	8/11
	KCS	Bg	0/0	7/1	0/11	7/12
		Imp	0/0	0/0	5/4	5/4
Cl. rutilus	MUCS	Bg	1/0	0/0	0/1	1/1
		Imp	2/0	8/1	6/8	16/9
	KCS	Bg	0/0	6/1	0/5	6/6
		Imp	0/0	4/3	2/4	6/7

Pollution zones: Bg, background (20–40 km from polluter); Imp, impact (1.5–6 km from polluter).

Assessment of Sperm Motility

Males were dissected to remove epididymides. The cauda epididymis was punctured, and a Proline 1–2.5 µL pipette (Sartorius AG, Finland) was used to collect 0.5 µL of epididymal fluid, which was mixed with 2 mL of nutrient medium and exposed for 10–15 min. The resulting suspension was then used to fill both 80-µm chambers of 2X-CEL slides (Hamilton Thorne, United States), 10 µL per chamber, which were placed on a MiniTherm slide warmer (Hamilton Thorne) under the objective lens. All manipulations with epididymides were performed under an MS-2 stereomicroscope (Biomed, Russia).

The medium for sperm cells was prepared by mixing DMEM (Dulbecco's Modified Eagle's Medium with L-glutamine and glucose, 4.5 g/L) with 2% BSA (bovine serum albumin) (BioloT, Russia). This mixture is a modification of the media used to analyze sperm motility in Crlj:CD1 (ICR) laboratory mice [28] and bank voles from a laboratory colony [29]. It was dispensed into sterile microtubes and placed in a Microstat thermostated rack (Tekhnom, Russia) at 37°C.

Sperm motility was analyzed using cells from the right epididymis, except for two cases of obvious asymmetry (with the right epididymis being twice smaller than the left one) and two cases of technical mistake in sampling. Analysis was performed using the CEROS CASA system with MouseTraxx v. 12.3 software (Hamilton Thorne) and Olympus CX41 microscope with Sony XC-ST50 camera (Japan). Sperm cell tracks were captured in 30 consecutive frames at 60 frames per second in no less than three microscopic fields at 4× objective lens magnification. Cells imaged in only part of the frames were excluded from analysis. The number of recorded tracks per animal varied from 240 to 1782, averaging 450. Sperm samples were analyzed for the proportion of motile cells and parameters of their movements (Table 2, Fig. 1) without differentiating between cells with progressive and nonprogressive motility.

Statistical Analysis

Experimental data were processed statistically in Statistica v. 8 (StatSoft Inc., 2007) and RStudio IDE v. 1.4 (R Core Team, 2020). In all cases, an animal was taken as a statistical unit, with arithmetic means per individual being used in calculations. Most parameters of sperm motility showed normal distribution (Shapiro–Wilk test). The structure of correlations between

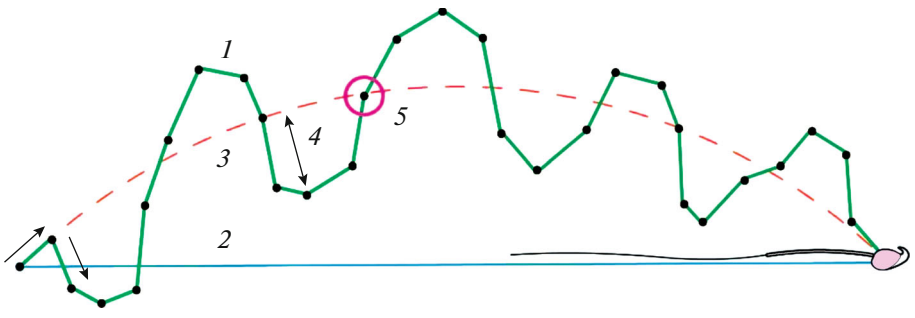

Fig. 1. Scheme illustrating parameters of sperm motility: (*1*) curvilinear line velocity (VCL, µm/s), (*2*) straight line velocity (VSL), (*3*) average path velocity (VAP), (*4*) amplitude of lateral head displacement, (*5*) beat cross frequency. Dots indicate cell location at the moment of capture.

Table 2. Parameters of sperm motility included in analysis

Parameter	Unabbreviated name	Measurement unit	Description
Motile	Motile cells	%	The proportion of motile sperm cells
VCL	Curvilinear line velocity	μm/s	The velocity measured over the actual point-to-point track followed by the sperm cell
VSL	Straight line velocity	μm/s	The velocity of cell movement along the straight line between the first and last points of the track
VAP	Average path velocity	μm/s	The velocity of cell movement along its smoothed pathway
ALH	Amplitude of lateral head displacement	μm	Twice the maximum displacement of a sperm head from its spatial average path
BCF	Beat cross frequency	Hz	The frequency of sperm head movement across the middle plane of its averaged path
STR	Straightness	%	Percentage straightness of the average path (VSL/VAP) × 100
LIN	Linearity	%	Percentage linearity of the curvilinear track (VSL/VCL) × 100

the parameters was evaluated by principal component analysis (PCA).

Since the initial dataset was unbalanced (see Table 1), the effect of relevant factors (species, functional group, region, pollution zone) on sperm motility was estimated by Student's *t*-test. General linear models (LM) were used to analyze the proportions of explained variance in R^2. Factor "year" was not taken into account. The expected false discovery rate in multiple comparisons of statistical hypotheses was controlled using the Benjamini–Yekutieli procedure (adjusted *q*-value significance levels are presented).

RESULTS

The proportion of motile sperm cells did not differ between the species, but all parameters of their velocity were higher, while all DCF values were lower in *Cl. glareolus* than in *Cl. rutilus* (Table 3). In view of species specificity of test parameters, subsequent analysis was performed for each species separately.

The first two principal components (PC1 and PC2) of variation in sperm motility parameters accounted for 76.9% of the total variance in *Cl. glareolus* and for 76% in *Cl. rutilus* (Table 4). In both species, parameters contributing most to the principal axes were those characterizing the velocity of sperm cells (to PC1) and directionality of their movement (to PC2). Further analysis was performed for two clearly interpretable parameters with high factor loadings that did not correlate with each other—VCL (PC1) and STR (PC2)—and also for the proportion of motile cells (Motile). These parameters showed no dependence either on the functional group or on the sampling region (for *Cl. glareolus,* $|t| = 0.31 - 1.65$, $p = 0.103–0.756$; for *Cl. rutilus,* $|t| = 0.25–1.66$, $p = 0.103–0.805$), which made it possible to pool animals of different groups and from different regions.

The effect of pollution level on Motile and VCL in *Cl. glareolus* was statistically significant: both parameters in the impact plots proved to be lower than in *Cl. rutilus* : $t_{Motile} = -2.60$, $p = 0.011$; $t_{VCL} = -2.43$, $p = 0.018$ (Fig. 2). However, factor "pollution zone" explained only 9% of the total variance in Motile and 8% in VCL. Both these parameters in *Cl. rutilus* did not differ between the zones ($t_{Motile} = -0.21$, $p = 0.837$; $t_{VCL} = -1.87$, $p = 0.067$), and the same was true of the straightness of sperm cell movement (STR) in both species (in *Cl. glareolus,* $t = 0.60$, $p = 0.548$; in *Cl. rutilus,* $t = -0.76$, $p = 0.451$).

Even though no effects depending on pollution zone were observed in *Cl. rutilus,* variation in VCL proved to be higher in the impact than in the background plots ($CV_{Bg} = 10.43$; $CV_{Imp} = 13.18$). Voles of both species from the impact plots were also characterized by higher variation in Motile: in *Cl. glareolus,* $CV_{Bg} = 14.85$, $CV_{Imp} = 25.18$; in *Cl. rutilus,* $CV_{Bg} = 19.02$, $CV_{Imp} = 23.37$. Moreover, animals with extremely low parameters of sperm motility occurred only in the impact plots (Fig. 2).

DISCUSSION

Species-specific Reference Values of Sperm Motility Parameters

Although a considerable number of studies on sperm motility with computer-aided analyzers have been performed to date [9, 30], the data on relevant parameters in many small mammal species are still absent. The results of this study could be compared with published data only for *Cl. glareolus*, namely, with those on sperm motility in 2-month-old males from a laboratory colony reported by Kotula-Balak et al. [31] and in four males from a natural population reported by Tourmente et al. [32]. Compared to their data, the average parameters of sperm velocity (VCL, VSL, VAP) recorded in this study are 1.5–2 times higher,

Table 3. Parameters of sperm motility in *Cl. glareolus* and *Cl. rutilus* from background areas

Parameter	*Cl. glareolus*, $n = 43$	*Cl. rutilus*, $n = 14$	q
Motile, %	59.7 ± 1.35 (40.6–74.4)	53.4 ± 2.84 (36.0–70.9)	0.130
VCL, μm/s	251.2 ± 4.88 (183.2–345.5)	223.6 ± 6.23 (185.1–267.7)	0.024
VSL, μm/s	198.5 ± 3.62 (145.2–245.6)	173.3 ± 6.82 (147.4–230.0)	0.001
VAP, μm/s	216.6 ± 3.89 (153.5–264.4)	188.7 ± 6.62 (156.9–244.2)	0.001
ALH, μm	9.12 ± 0.27 (6.88–12.96)	9.0 ± 0.53 (6.23–13.4)	1.000
BCF, Hz	35.5 ± 0.48 (28.9–41.9)	38.9 ± 0.92 (29.5–46.4)	0.001
STR, %	91.1 ± 0.43 (83.3–96.3)	91.3 ± 0.93 (83.2–96.5)	1.000
LIN, %	79.0 ± 0.69 (68.0–87.0)	78.1 ± 1.73 (65.9–86.2)	1.000

Abbreviations of parameters are deciphered in Table 2. The data are presented as mean ± SD (min–max).

while head beat cross frequency (BCF) is similar. Various factors may be responsible for these differences, from specific features of samples and keeping conditions for animals to the methods of sperm sampling and analysis. The most probable factors related to these methods are the depth of counting chambers, the composition of nutrient medium, the maintenance of temperature regime during analysis, the time of preincubation, the experience of researcher, and differences in software used in the system [9].

The influence of medium composition on sperm cell motility is not always apparent. As shown by comparing different media, the addition of calcium, magnesium, or glucose does not improve the parameters of sperm cell movement on a short time scale, while albumin is necessary for the long-term maintenance of sperm motility [28]. On the other hand, the authors cited above [31, 32] used different media—IVF with glucose (≈ 1 g/L) and albumin and mT-H with glucose (6 g/L) without albumin—but parameters of sperm motility obtained in their studies were generally similar.

A high degree of correlation between parameters of sperm velocity (VCL, VSL, VAP) observed in both vole species (Table 4) was also previously reported by Gómez Montoto et al. [32] for 11 rodent species and by Tourmente et al. [33] for 16 species, including *Cl. glareolus*. Similarity between correlation patterns described by these authors may be indirect evidence for the validity of analysis performed in in this study. Moreover, a strong correlation between VCL, VSL, and VAP is one more piece of evidence for the straightness of sperm cell movement, in addition to the calculated parameters (STR and LIN).

As shown by Valverde et al. [34], VSL and ALH in boar sperm are stable independently of settings for capturing sperm cell tracks. This result is important, since it shows that the list of potential factors influencing sperm motility parameters may be reduced to two main factors: specific features of the procedure of sperm sampling and of animal samples. If sperm sampling and analysis are performed by standard procedures, the recorded parameters of cell motility can be directly interpreted as characteristic of animals from the study sample (population group). Since the procedure of sperm sampling from small mammals has not yet been standardized, it is only possible to obtain reference values of sperm motility parameters that are specific for a given laboratory.

Table 4. Factor loadings of *Cl. glareolus* and *Cl. rutilus* sperm motility parameters on the first two principal components (PC1 and PC2)

Parameter	*Cl. glareolus*		*Cl. rutilus*	
	PC1	PC2	PC1	PC2
VCL, μm/s	0.89	–0.25	0.90	–0.35
VSL, μm/s	0.95	0.24	0.97	0.20
VAP, μm/s	0.98	0.02	0.98	–0.09
ALH, μm	0.24	– 0.93	0.35	–0.92
BCF, Hz	– 0.45	0.34	–0.28	0.27
STR, %	0.07	0.96	0.00	0.97
LIN, %	0.53	0.78	0.12	0.93
Proportion of total variance, %	43.2	33.7	38.9	37.1

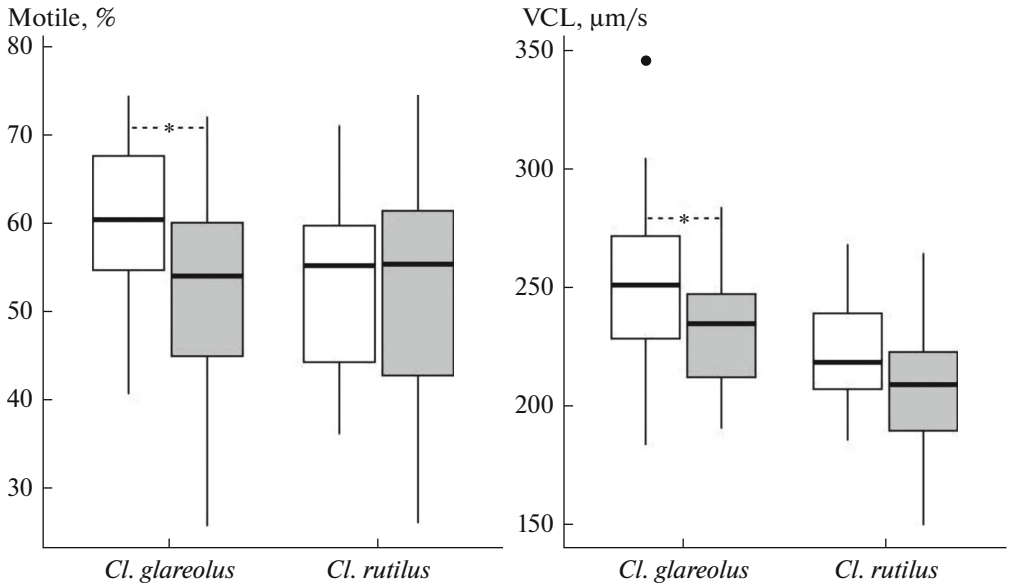

Fig. 2. Boxplots of the proportion of motile sperm cells (Motile, %) and their curvilinear line velocity (VCL, μm/s) in *Cl. glareolus* and *Cl. rutilus* from background plots (clear boxes) and impact plots (gray boxes). The horizontal line shows the median; box height, the interquartile range; whiskers, the minimum and minimum values beyond 1.5 interquartile ranges; the dot is an outlier. An asterisk indicates that differences are statistically significant at $p < 0.05$.

Interspecific Differences in Sperm Motility Parameters

The form, size, and motility of sperm cells are determined by sexual selection and reflect their basic function of delivering the male genome to the egg cell [35–37]. Many authors consider that inter- and intraspecific competition is a driver of evolution in sperm size and shape [33, 38–40]. Thus, Tourmente et al. [41] showed that increasing competition leads to the growth of all structural elements of sperm cells, with their heads becoming more elongated. According to the same authors, the increasing length of sperm cells is correlated with higher velocity of their movement, which is an adaptive feature under conditions of competition.

As shown in this study, the proportion of motile sperm cells in *Cl. glareolus* and *Cl. rutilus* were equal, by parameters of sperm velocity in the former species were higher (Table 3). However, some morphometric traits—head length, midpiece length, and total cell length—were found to be higher in *Cl. rutilus* than in *Cl. glareolus* [42]. The shape of sperm head also differs slightly between the species: it is thickened in the basal part in *Cl. glareolus* and is more elongated in *Cl. rutilus*.

It is also known that *Cl. glareolus,* compared to *Cl. rutilus*, is characterized by higher testicular index (the ratio of testis weight to body weight) and testicular and blood testosterone levels [43]. These parameters are very important for the reproductive physiology of animals, including voles, since they can indicate the production level of sex hormones and sperm cells, consequently reflecting the level of inter- and intraspecific competition between males (i.e., the higher the testicular index, the more competitive the sperm of a species or an individual) [39, 44–46]. Kruzcek et al. [29] revealed correlation of sperm motility with the weights of the body, testes, and accessory glands in *Cl. glareolus* from a laboratory colony. These authors explained increasing sperm motility by the secretory activity of accessory sex glands, which are under direct control by androgens. In turn, the level of androgens (including testosterone) depends on the growth and development of gonads. However, high concentrations of testosterone can suppress sperm production and secretion of pituitary hormones (FSH and LH) involved in control of steroidogenesis [47].

In the first sight, males with a larger size of sex organs and sperm cells should have a competitive advantage, but reproductive success of a species depends not so much on their size as on more complex structural and biochemical interactions between components of the reproductive system. It should also be noted that differences in sperm cell shape, size, and motility between *Cl. glareolus* and *Cl. rutilus* do not prevent their hybridization either under laboratory conditions [48] of in nature [49].

Sperm Motility in Males of Different Functional Groups

The dependence of sperm motility on age, as many other aspects of sperm quality, has been studied mainly in humans [50], commercially important and laboratory animals [52, 53] and in experiments on wild animals [29, 53]. Such studies on animals from natural

populations are fewer, but there are data on age-related variation in the parameters of sperm motility in the Namibian cheetah (*Acinonyx jubatus*) [54] and Iberian red deer (*Cervus elaphus hispanicus*) [55]. The seasonal component of age-related variation in sperm motility has been studies in some long-lived species of agricultural and wild animals [51, 56].

Kruzcek et al. [29] revealed the effect of absolute age on the proportion of progressively motile sperm cells in *Cl. glareolus* from a laboratory colony: their proportion in males aged 4 months proved to be higher than in younger or older males. These authors also found that, beginning from the age of 6 months, sperm quality, including parameters of motility, markedly deteriorated by the end of reproductive period at the age of 15 months.

Muroid rodents inhabiting natural zones of the temperate belt show two variants of individual development (ontogenetic pathways), with part of animals in the population reaching maturity in the year of birth (variant I, young of the year with a life span of 3–5 months) and the other part, in the next year (variant II, overwintered animals with a life span of 13–14 months) [24]. There are several hypotheses as to what is responsible for specific physiological features of these population groups (the occurrence of winter diapause, the cumulative action of different environmental factors, etc.), but most authors (with rare exceptions [57]) still describe the phenomenology of differences between the two variants of development rather than mechanisms underlying their existence.

The results of our previous study on *Cl. glareolus* [25] show that when the morphology of normal spermatozoa is analyzed, it is of no significance at what stage of maturity or senescence the animal is and when it has sexually matured However, the frequency of sperm cells with defective heads proved to be significantly lower in mature young of the year than in overwintered males, indicating that the variant of individual development should be taken into account when analyzing abnormal sperm cells. Moreover, we revealed no differences in parameters of sperm motility between mature males from different functional groups, but it cannot be excluded that this is due to a small size of animal samples and their unbalanced age composition (Table 1).

Effect of Toxic Exposure on Sperm Motility

As a rule, a dose dependence between the level of toxic exposure and parameters of sperm motility is observed in toxicological experiments Thus, the experimental group of *Cl. glareolus* voles from a laboratory colony that were treated with copper sulfate and aluminum chloride showed a decrease in the proportion of motile sperm cells, compared to control (from 82 to 61%), while the proportion of cells with abnormal heads increased [3, 22]. The authors hypothesized that copper at high concentrations could cause oxidative stress resulting in damage to sperm cells, with consequent decrease in their motility and fertilization capacity.

Tannenbaum et al. [16, 17] used the method of rodent sperm analysis (RSA) in comparative studies on different small rodent species trapped in sites with high contents of heavy metals, trinitrotoluene, cyclonite (RDX), and other dangerous substances and in reference (background) sites. The results showed that this method successfully discriminated between clean and polluted areas. However, even though differences in sperm parameters between animals from these areas were statistically significant, their magnitude did not reach the thresholds for classifying the polluted are as dangerous (40–50% for sperm motility parameters and 60% for the proportion of motile cells) [17]. The main reason for setting these threshold so high is that the amount of sperm produced by rodents is very high, 10–20 times higher than is necessary for successful reproduction.

Almost all authors cited above studied a complex of sperm parameters. Thus, Kotula-Balak et al. [31] showed that photoperiod and xenoestrogens have differently directed influence on parameters of *Cl. glareolus* sperm motility, with both factors affecting the proportion of cells with defective tails: their proportion increased upon reduction in the daylight period and treatment with a xenobiotic. In experiments on treating *Cl. glareolus* voles with copper sulfate and aluminum chloride, a decrease in the proportions of both motile cells and morphologically normal cells [3, 22].

In contrast, Tannenbaum et al. [16], who performed sperm analysis in white-footed mice (*Peromyscus leucopus*) from the vicinity of Ravenna army ammunition plant (RVAAP; Ravenna, OH), revealed no effect of pollution on the proportion of either motile sperm cells (it remained in the range of 94–99%) or pathological cells (they were absent). Another study by the same authors on several rodent species from different regions showed that the proportion of motile sperm cells was not correlated with the proportion of pathological cells: the magnitude of change in in the proportion of motile cells in the background and impact areas reached 47%, while the frequency of pathologies did not exceed 0.1% [17].

Despite the inconsistency of published data, an integrated analysis of sperm parameters appears to be important, because it makes it possible to estimate the concordance of changes in them. For example, Osadchuk and Kleshchev [58] showed in the study on CBA/Lac mice that low contents of sperm cells in both epididymides (the epididymal reserve) was accompanied by an increase in their motility and a decrease in the proportion of abnormal cells. The authors consider that male fertility may be maintained at the optimal level due to compensatory variation in sperm parameters.

We so far managed to collate motility parameters of sperm cells with their morphology only indirectly and for only one species, *Cl. glareolus*. In the region of MUCS, the frequency of abnormal cells and sperm motility in the impact zone were lower than in the background zone [59]. To understand whether this is a compensatory mechanism, it is necessary to compile data on the morphology, concentration, and motility of sperm cells in the same animals, which we plan to do in the near future.

CONCLUSIONS

The hypothesis that sperm motility decreases under the impact of pollution was tested in two closely related sympatric rodent specie, *Cl. glareolus* and *Cl. rutilus*, inhabiting the vicinities of two copper smelters. The motility of epididymal sperm cells in *Cl. rutilus* was studied for the first time. Likewise, analysis of sperm mobility parameters in rodents was performed for the first time with regard to their functional group. As in our previous study on the morphology of normal sperm cells [25], it was found that in analyzing sperm motility it is of no significance when the animals have reached maturity, in the year of birth or in the next year, after overwintering.

The above hypothesis was confirmed only partly. Toxic effects—reduction in the proportion of motile sperm cells and their velocity in animals from polluted plots—were observed in only one species, *Cl. glareolus*. The absence of such effects in *Cl. rutilus* samples may be explained by their relatively small size.

On the whole, the results of this study show that parameters of sperm motility in rodents from natural populations are low responsive to the impact of pollution. This may be due to several factors, including the mosaic spatial pattern of toxic load; high mobility of rodents, which allows them to avoid habitats with adverse conditions; and the low recorded level of pollution, which is insufficient for causing substantial shifts in reproductive parameters included in analysis.

Despite that the observed effects proved to be weak, analysis of sperm cells and parameters of their motility allows a direct assessment of the health and reproductive quality of animals under changing environmental conditions, in contrast to indirect estimations of risk for natural populations.

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COMPLIANCE WITH ETHICAL STANDARDS

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interests. The author declares that he has no conflict of interest.

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