

CRANIOMETRIC TRAITS OF *MICROTUS GREGALIS* IN ANTHROPOGENIC TERRITORIES OF NORTH-EASTERN KAZAKHSTAN

ZAKANOVA, A. N.* – YERZHANOV, N. T. – KRYKBAEVA, M. S.

*Biology and Ecology Department, Toraighyrov University
Lomov str. 64, 140008 Pavlodar City, Kazakhstan
(phone: +7-718-267-3685)*

**Corresponding author
e-mail: assel.biology@gmail.com; phone: +7-777-600-2206*

(Received 12th May 2024; accepted 23rd Sep 2024)

Abstract. The article delivers the results of craniometric analysis of *Microtus gregalis* Pall., 1779 from the anthropogenic areas of North-Eastern Kazakhstan. The aim of the study was to describe the impact of urbanization on craniometric traits of this rodent species. We compare craniometric traits of *M. gregalis* from anthropogenic and natural habitats. On drawing near the plant, the greater the anthropogenic impact on the environment becomes. We hypothesize that the craniometric measurements of animals from impact and buffer zones will have the most statistically significant differences from the control group. For comparing of the average values between all the groups, the equality of means was tested by using ANOVA (analysis of variance), which determines whether there are statistically significant differences in the average values of traits between the groups. Linear measurements of the skull were performed, since this part of the skeleton is conservative and performs a multitude of vital functions. Craniometric linear features illustrated that rodents in anthropogenic conditions have a statistically significant decrease in - the condylobasal length and length of the tooth row, but an increase in the height of the braincase, indicating physiological adaptation to life near humans and the need for rapid adaptation to new environmental conditions.

Keywords: *adaptation, urbanization impact, morphological changes, skull measurements, rodent, plasticity*

Introduction

Anthropogenic pressure on ecosystems

The constantly growing human population (Fraser, 2020) increases anthropogenic load on ecosystems. Polluted air, soil, surface and ground waters often negatively affect living organisms (Masindi and Muedi, 2018). The issues of the human activities impact on the environment are most relevant in regions with actively developing manufacture, such as North-Eastern Kazakhstan.

A significant contribution to environmental pollution has been made by non-ferrous metallurgy, particular the aluminum industry, that enterprises, due to technological specifics, discharges in air such as fluoride compounds and benzo(a)pyrene (Zaporozhets et al., 2020). The heavy industry of North-Eastern Kazakhstan began to develop actively in the middle of the last century. Coal mining, production of ferroalloys, aluminum production, electricity generation (Pearson, 1948) gave an economic impetus to the development of the region and the republic as a whole.

Mammals' representatives are of a particular concern in ecological forecasts and studies, since they are characterized by high metabolism (d'Havé et al., 2005). In natural habitats, animals which range includes areas near enterprises are exposed to

heavy metals in small doses for a long time (Hamers et al., 2006). Chronic exposure can affect the morphological features of the mammalian population. There are results of studies investigating the relationship between intraspecific differences in small mammal craniums and anthropogenic impact. The intraspecific variability of cranial parameters in waterfowl (*Myotis myotis* Borkhausen, 1797) under anthropogenic influence demonstrated changes in urban areas and regions with a high level of anthropogenic impact (Russo and Ancillotto, 2015). The research conducted in the United States on the species of white-breasted mouse (*Peromyscus leucopus*, Rafinesque, 1818) found differences in craniometric parameters in populations living in urban and suburban areas compared with forest areas. Intraspecific differences are associated with adaptation to changed conditions, including nutrition and environmental structure (Gryseels et al., 2016). Some researchers from China who studied the Chinese vole (*Apodemus chevrieri*, Milne-Edwards, 1868) discovered that the craniometric parameters of populations living in an human activity environment differ from populations in natural habitats. Intraspecific differences are associated with the influence of anthropogenic impact on the habitat and nutrition of animals (Zhang et al., 2008). Anthropogenic effects on the craniometric characteristics of borerfish (*Sorex* Linnaeus, 1758) in areas near cities in China lead to changes in the size and shape of borerfish craniums due to increased population density and the construction of urban infrastructure. (Zhang and Li, 2018). Thus, adaptation to altered nutritional conditions, landscape structures, and the presence of human activity affect the shape and size of craniums in small mammals. These studies emphasize the importance of studying the effects of anthropogenic factors on mammalian morphology in the context of biodiversity conservation.

The narrow-headed vole

The narrow-headed vole (*Microtus gregalis* Pall., 1779) was the dominant species in the anthropogenic territories of North-Eastern Kazakhstan among all small mammals. Therefore, this species was chosen as a bioindicator. *M. gregalis* is settled in the territories of Eurasia in the Subarctic region (northern regions) and forest-steppes, degrees and semi-deserts, therefore its wide distribution and dominance in the biotopes of Northeastern Kazakhstan is not surprising. *M. gregalis* is classified as a polyzonal species (Smirnov et al., 2007), it is capable of forming various types of adaptation within the same natural and climatic conditions. The high plasticity of the species explains the ability to live in areas with human-made loads and depletion of biotopes in these areas.

Craniometry, the study of the size and shape of the skull, is a significant tool in biological research. This provides valuable information about intra- and interspecific variability and adaptation to the environment and evolutionary relationships.

Linear dimensions of animal skulls are used as indicators in environmental assessment. Craniometric signs directly depend on environmental parameters, such as ambient temperature, the sufficiency of the food supply, and the degree of movement within the range. These are fairly stable signs and usually their changes indicate permanent chronic deviations in environmental characteristics. Therefore, this feature was used to determine the effects of human activity on the animals' body.

The aim of the study was to determine the craniometric linear features of *M. gregalis* representatives in the anthropogenic areas of North-Eastern Kazakhstan.

Anthropogenic pressure on ecosystems requires constant environmental monitoring. Morphological and physiological characteristics of animals can change under the

influence of anthropogenic pressure (Kataev, 2005). The obtained results will assist in understanding how urbanization affects the morphology of this species and its adaptation to changing environmental conditions. The obtained data can be extrapolated to regions with similar natural conditions and anthropogenic impact. The study of the response to possible changes in the habitat at the population level is not only applied, but also of fundamental importance.

Materials and methods

Study area and sampling

The study of the craniometric parameters of *M. gregalis* took place in North-Eastern Kazakhstan, within the administrative territory of the Pavlodar region. A high concentration of industrial activities in the region is located near the city of Pavlodar, which is situated at 52°18'56" N, 76°57'23" E. The Pavlodar Aluminum Plant is the leading emitter of pollutants in the region and is located at 52°15'36" N, 77°03'07" E.

To reliably determine the abundance and changes in the morphophysiological state of *M. Gregalis*, monitoring sites were identified in the research area according to the wind rose. These sites included impact, buffer, and control zones, as shown in Figure 1.

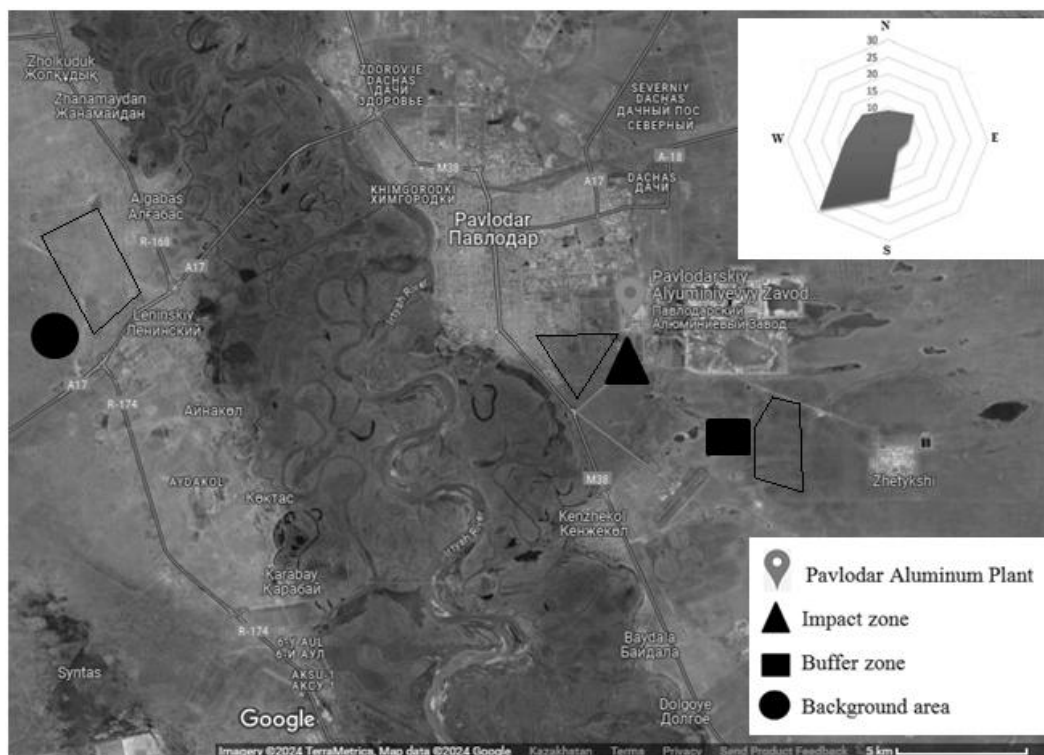


Figure 1. Location and the boundaries of monitoring sites in the vicinity of Pavlodar

The impact, buffer, and background monitoring sites were located on the windward side of the Pavlodar Aluminum Plant. The prevailing winds in the area are southwesterly and southerly, so the sites were located in this direction from the plant.

The impact monitoring site is the most transformed and influenced by plant emissions. The degree of impact decreases in the buffer zone, and it is assumed that the amount of emissions in the background area is minimal, compared with other studied territories (Kulczycka, 2016).

The cranial remains of *M. gregalis* were collected at these sites in compliance with ethical standards and rules. As control samples, the museum skulls of *M. gregalis*, which lived in the Bayanaul National Park from 2007-2015, were used. The Bayanaul National Park is located at 51°00'00" N, 75°40'00" E, and its location is shown in *Figure 2*. The collections were kindly provided by the staff of the Museum of Institute of Systematics and Animal Ecology, Novosibirsk, Russia.

Figure 2. Study area and location of Bayanaul National Park

The inhabitants of the Bayanaul National Park represent a natural population of mammals that is not significantly affected by human activity. This makes it possible to explore the natural variations and craniometric characteristics of a given population.

Craniometric analysis

After collection, the cranial remains were thoroughly cleaned of biological particles and contaminants. To study the craniometric features, samples were selected without visible deformations or damage. The craniometric study of *M. gregalis* was conducted over three spring and summer seasons, from 2021 to 2023. The material for the study was cranial remains seized from monitoring sites in the Pavlodar region during the specified period. Measurements were carried out using an electronic vernier caliper with an accuracy of 0.01 mm, and a stereoscopic microscope MBS-9 was also used.

The skulls of adult mature individuals (adultus), male and female, were studied. On the upper third molar (M3) of the studied skulls belonging to the C4 group (Ben Saleh et al., 2013), an almost complete fusion of the first and second lobes of the longitudinal connection is observed. This connection is very wide, but still remains noticeable on the tips of other molars.

To obtain data on linear measurements of Rodent orders representatives (Ikbali et al., 2019), the following indicators were used: condylobasal length (CL), rostrum width, rostrum length, facial length (FL), cerebral length (BL), the largest width of the skull, height of the cerebral section (BH), subglacial width, interglacial width (IW), occipital width (BW), length of molars, length of nasal bones (NBL). These indicators can be useful for studying the shape and size of the cranium in rodents of anthropogenic territories, as well as for comparison between populations of Northeastern Kazakhstan.

Statistical analysis

The data obtained were processed by a statistical software package such as Past 4.0 (Hammer, 2001). For each parameter, the average values, standard deviation, and other variation parameters were calculated. To determine significant differences ANOVA test (Cuevas, 2004), Mann-Whitney test (Dai, 2022), median equality test, coefficient of variation, T-tests were used.

Results and discussion

The *M. gregalis* of northeastern Kazakhstan is defined as a southern subspecies. The animals in this region are characterized by a smaller size and a shorter tail. In anthropogenic territories, the body length of these voles does not exceed 9-10 cm, and their tail length is up to 3.9 cm. The animals have a narrow muzzle and a small distance between the eye sockets, which helps them to move more safely in dense vegetation and even in frozen ground (Cui et al., 2019).

The biotopes where the materials have been collected had vegetation that is favorable for *M. gregalis*. The animals feed on cereals, roots, seeds, and the green parts of wild and cultivated plants. Plants from the monocotyledonous sedge family (*Cyperaceae*) are common in these biotopes, and *M. gregalis* readily eats species such as common fluff (*Eriophorum latifolium* Honck., 1782) and hairy sedge (*Carex hirta* L., 1753).

In adverse conditions, the stored food that the animals prepare for winter helps them to survive. A laboratory study has shown that these rodents can also exhibit hunting behavior and eat animal-based food. This hunting behavior increases the adaptive traits of populations by expanding their food spectrum to include mobile insects (Bardos et al., 2022).

Craniometric features of *M. gregalis*

To determine the craniometric features of *M. gregalis*, 17 skulls of rodents living in impact zones, 30 skulls from buffer zones, 11 skulls from background zones, and 102 skulls from the Bayanaul National Park were studied. Human economic and industrial activities are prohibited in the national park, so the craniometric measurements from this population have been used as controls.

The cranium and mandibles of small mammals are complex, multidimensional structures that are easy to collect, preserve, and analyze. A set of linear measurements of the skull is used as a standard method of craniometric analysis (Gureeva, 2021).

Craniometric changes of M. gregalis inhabiting at different distances from the plant

The cranium of the narrow-headed voles in the anthropogenic territories of northeastern Kazakhstan had certain common features. The skulls were narrow and elongated, with a well-defined interocular constriction. The width between the orbital cavities was no more than 2 mm. The skulls were of medium size, with a length of usually 25-30 mm. They were large and swollen, with well-developed ridges. The facial section was short and narrow, with protruding incisors. The incisors were chisel-shaped, longer on the outside and shorter on the back. They were located in one pair on each jaw and separated from the main row by a diastema. The teeth were relatively large, with high crowns. The incisors were wide and curved, and the molars had a complex chewing pattern. The nasal bones were long and narrow, forming an acute angle at the anterior end of the skull. The zygomatic arches were thin and fragile, and widely spaced. The mandibular bone was powerful, with a well-developed angular process. An image of an *M. gregalis* cranium is shown in Figure 3.



Figure 3. Cranium of *M. gregalis* from North-Eastern Kazakhstan

The cranium of the narrow-headed vole is well adapted to the burrowing lifestyle of populations in North-Eastern Kazakhstan. The narrow skull and powerful mandibular bone allow the animal to dig soil effectively. Large teeth with high crowns are used to chew coarse plant foods such as plant roots and stems (Kropacheva, 2021).

During the research period, measurements were made for 12 craniometric signs in *M. gregalis* individuals. 58 skulls from technogenic zones and 102 skulls from the control area have been studied. The average values of the craniometric signs of the narrow-headed vole in technogenic and control zones are shown in the Table 1.

An analysis of the data in the sexual section found no significant ($p \leq 0.05$) difference between females and males in terms of craniometric parameters. Therefore,

the sex factor will not be considered while analyzing the variability of the linear lengths of the skull. There is evidence in the literature of a significant difference in craniometric characteristics in males and females of high-altitude biotopes. Usually, the measurements of males are larger than those of females in high-altitude areas of the Central Caucasus (Balciuskas et al., 2020).

Table 1. Craniometric characteristics of narrow-headed voles from technogenic and control zones, mm

Attribute, mm	Impact zone	Buffer zone	Background zone	Control zones
Condylobasal length	22.58 ± 1.13	21.71 ± 1.08	23.11 ± 1.15	23.43 ± 1.17
Rostrum width	3.32 ± 0.17	3.17 ± 0.16	3.26 ± 0.16	3.01 ± 0.15
Rostrum length	4.71 ± 0.23	3.99 ± 0.2	4.97 ± 0.25	4.71 ± 0.23
Facial length	12.65 ± 0.63	12.36 ± 0.62	12.76 ± 0.64	13.1 ± 0.66
Cerebral length	10.96 ± 0.55	10.35 ± 0.52	10.26 ± 0.51	11.44 ± 0.57
Largest width of the skull	8.81 ± 0.44	9.03 ± 0.45	8.71 ± 0.43	8.96 ± 0.45
Height of cerebral part	8.18 ± 0.41	7.77 ± 0.39	8.05 ± 0.4	7.83 ± 0.39
Suborbital length	3.26 ± 0.16	3.02 ± 0.15	3.51 ± 0.17	2.8 ± 0.14
Interocular width	2.6 ± 0.13	2.56 ± 0.13	2.43 ± 0.12	2.52 ± 0.13
Occipital width	8.88 ± 0.44	8.62 ± 0.43	9.11 ± 0.45	9.79 ± 0.49
Length of molars	4.84 ± 0.24	5.02 ± 0.25	5.33 ± 0.27	5.23 ± 0.26
Length of nasal bones	5.75 ± 0.29	5.57 ± 0.28	5.93 ± 0.3	6.1 ± 0.3

To compare the average values between all groups and to check the equality of the average values, the ANOVA test (analysis of variance) have been used. This test allows us to determine whether there are statistically significant differences in the average values of features between groups. The variable factor in our study was the different distance from the source of the anthropogenic load. As they approached the plant, the anthropogenic impact on the environment increased. We hypothesized that craniometric measurements in animals of impact and buffer regions would have the greatest statistically significant differences from the control group.

The condylobasal length in *M. gregalis* was the distance from the occipital condyle to the anterior alveoli of the upper incisors (Fig. 4). An examination of the condylobasal length for four groups of animals revealed statistically significant differences in the average values of the trait ($p = 0.0072$). With a value of $p \leq 0.05$, we can reject the null hypothesis that the average values of the trait in all four groups are equal. This means that there is a statistically significant difference between at least one pair of groups.

The Mann-Whitney test was used to compare the impact and control zones. The results showed statistically significant differences in the medians between the groups ($p = 0.0232$). The results of the Mann-Whitney test show that the median of the impact group is significantly lower than the median of the control group. The average rank value of the impact group is 5.6207, and the average rank value of the control group is 52.879. The differences between the buffer group and the control group had statistically significant values in the medians ($p = 0.0002$). The Mann-Whitney test showed no statistically significant differences in the medians between the background and control groups ($p = 0.1912$).

The interocular width in *M. gregalis* is the distance between the inner edges of the eye sockets (Fig. 5). This is an important morphometric feature that is used to identify and study variations in the size and shape of the skull in these rodents. The measurement was carried out in millimeters and calculated as the distance from the inside of one eye socket to the inside of the other eye socket, parallel to the nasal bone.

The ANOVA test illustrated statistically significant differences in the average values of the interocular width between the groups of narrow-headed voles. Since the p value is less than 0.05 ($p = 0.0003$), we reject the null hypothesis that the average values of the interocular width in the groups are equal. The Mann-Whitney test showed statistically significant differences in the medians between the impact and control groups ($p = 0.0201$).

Based on the results of the mean equality test ($p = 0.0602$), the t-test with unequal variances ($p = 0.0492$), and the nonparametric test, we can conclude that there is a statistically significant difference in the mean values of the trait between the buffer and control groups ($p = 0.0493$). However, the value of $p = 0.0651$ of the Mann-Whitney test does not allow us to assert that there are differences in medians between the buffer and control groups.

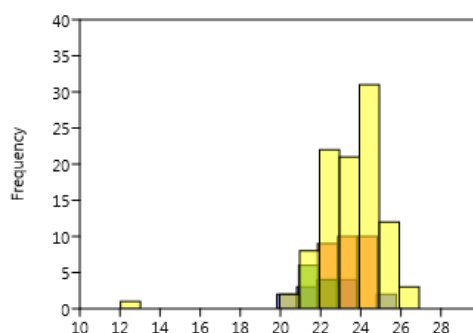


Figure 4. Frequency distribution of condylobasal length. Blue – impact area, yellow – control, pink – buffer, green – background area

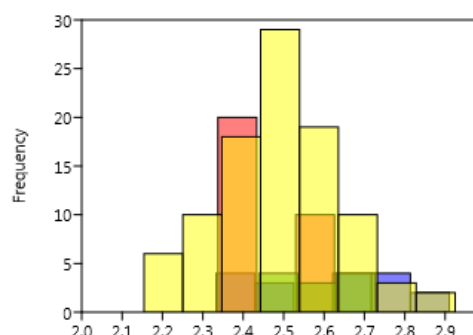


Figure 5. Frequency distribution of interocular width. Blue – impact area, yellow – control, pink – buffer, green – background area

The suborbital length of the skull in a narrow-headed vole was measured as the distance between the anterior edge of the mandible (symphysis) and the posterior edge of the suborbital opening (Fig. 6). The mean equality test showed that there are

statistically significant differences in the mean values of the trait between the four groups. Since the value of p is less than 0.05, we reject the null hypothesis that the average values of the trait in all four groups are equal. The differences between the groups are average in size and may be related to various factors such as genetic differences, environmental conditions, or diet.

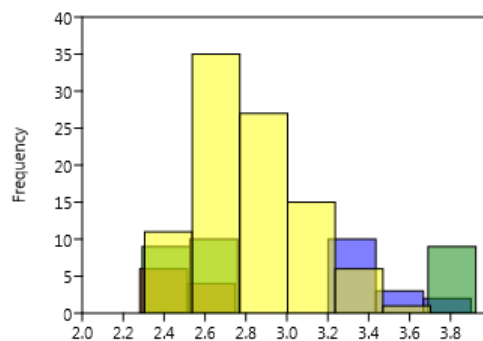


Figure 6. Frequency distribution of suborbital length. Blue – impact area, yellow – control, pink – buffer, green – background area

The median equality test demonstrated that there is a statistically significant difference in medians between the impact and control groups. The test results show that the median of impact group A (about 13.682) is significantly lower than the median of the control group (about 41.818). This means that there are statistically significant differences in the medians between the two groups ($p \leq 0.05$). The Mann-Whitney test showed that there is a difference ($p \leq 0.05$) between the suborbital length of the buffer and control groups. The test results show that the median of the background group (about 1.2381) is significantly lower than the median of the control group (about 51.762).

When studying the values of the suborbital length of the background group, the median equality test did not show statistically significant differences in the medians ($p = 0.8092$). Since the p value is greater than 0.05, we cannot reject the null hypothesis, so there is not enough evidence to support differences between these groups.

The rodent rostrum is a specialized structure that is adapted to their gnawing mode of nutrition and other sensory needs (Klingenberg, 2003). The width of the rostrum was measured between the most lateral point of the left maxillary margin and the most lateral point of the right maxillary margin, parallel to the sagittal plane of the skull of *M. Gregalis* (Fig. 7). A comparison of the width of the rostrums of the animals revealed a statistically significant difference between the groups ($p \leq 0.05$). The value of the variance components shows that the variance between the groups is greater than the variance within the groups, indicating that there are differences between the groups. The results of the ANOVA test indicate that the groups differ in the studied feature.

Since the assumption of equal variance has been violated, the Welch test was also calculated to check for differences in averages in the case of unequal variances. The Welch test results confirm the presence of statistically significant differences between the groups ($F = 11.39$, $df = 35.19$). An examination of the values of the impact and control groups showed a statistically significant difference in the medians ($p = 0.0001$). Based on the results of the median equality test and the nonparametric test, we can conclude that there is a statistically significant difference in medians between the buffer

and control groups since the p value is less than 0.05. We also recorded data on a significant difference in the width of the rostrum in the background zone and the control zone. Since the p value is less than 0.05, we reject the null hypothesis.

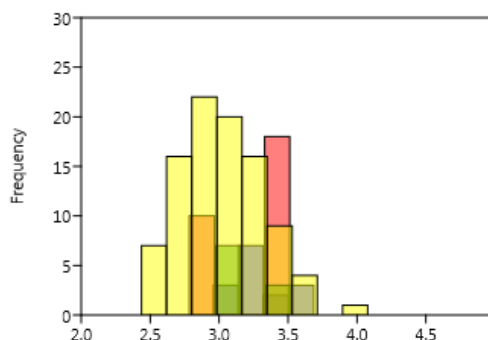


Figure 7. Frequency distribution of rostrum width. Blue – impact area, yellow – control, pink – buffer, green – background area

The rostrum of the narrow-headed vole is relatively long and narrow, which is an adaptation to its gnawing way of feeding. The narrow and elongated rostrum allows the vole to place its front incisors at an angle, providing maximum mechanical force for gnawing on hard objects such as nuts, seeds, and twigs. The length of the rostrum can also be used to distinguish the narrow-headed vole from other vole species (Fig. 8). For example, the gray vole has a shorter and wider rostrum than the narrow-headed vole, and the red vole has an even shorter and wider rostrum (Samuels, 2009).

An analysis of variance (ANOVA) has been carried out to check for differences in the mean values between the four groups. The ANOVA results demonstrate that there are statistically significant differences in the mean values. The value of the coefficient of variation (ω^2) indicates that the effect size of the differences between the groups is average. The ICC value indicates that the intra-group correlation is positive, which indicates that there are differences between the groups.

Comparing the impact group and the control group, we observe the absence of significant differences in the studied trait ($p = 0.6983$). In the buffer zone, we also do not get statistically significant differences ($p = 0.0613$). However, it should be noted that the value of p (0.061309) is close to the significance level of 0.05. This means that there is some evidence for differences in medians between the groups, but this evidence is not statistically significant.

The samples from the background area revealed that there are statistically significant differences in terms of the length of the rostrum ($p \leq 0.05$) from the samples of the control zone. Thus, the comparison of anthropogenic zones with the control zone indicates the absence of possible anthropogenic effects on the length of the rostrum.

The length of the facial part of the narrow-headed vole is from 12 to 16 mm in the Northeastern range (Fig. 9). The ANOVA results show that there are no statistically significant differences in the mean values between the four groups. This indicates that the groups do not differ based on the length of the facial part ($p = 0.0759$). The value of the coefficient of variation (ω^2) indicates that the effect size of the differences between the groups is small. The ICC value indicates that the intra-group correlation is positive but low, which indicates that the differences between the groups are minimal.

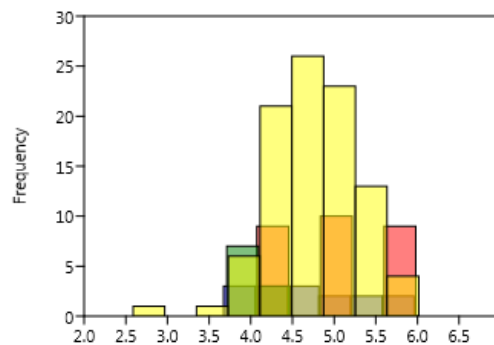


Figure 8. Frequency distribution of rostrum length. Blue – impact area, yellow – control, pink – buffer, green – background area

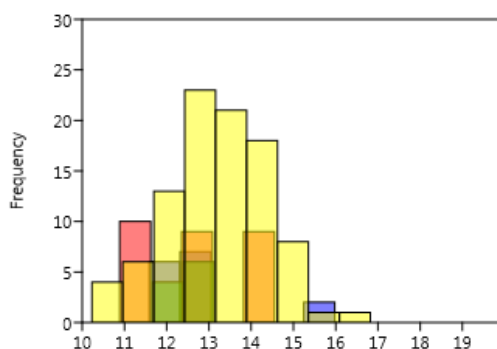


Figure 9. Frequency distribution of facial length. Blue – impact area, yellow – control, pink – buffer, green – background area

The length of the brain part (Fig. 10) is relatively large compared to other species of voles in the region. This is due to the peculiarities of the structure of the nervous system and brain, as well as lifestyle, the need to navigate difficult passages to find food and habitat on the ground. The results of calculations of the differences between the studied groups on this basis demonstrate significant differences. The value of the coefficient of variation (ω^2) indicates that the effect size of the differences between the groups is large. The ICC value indicates that the intra-group correlation is positive and moderate, which indicates that there are differences between the groups. The results of ANOVA and additional indicators demonstrate that there are statistically significant differences in the mean values of the trait between the four groups ($p \leq 0.05$).

An analysis of the results of the median equality test has shown no statistically significant differences in the medians between the impact and control groups ($p = 0.1125$). For the group of buffer and control samples, a result was obtained on a statistically correct difference in the parameter of the length of the cerebral region ($p \leq 0.05$). For the background and control zones, a result was also obtained on a significant difference in the studied trait.

By studying the height of the cerebral region in a narrow-headed vole of technogenic zones, a significant difference was determined with the control group ($p \leq 0.05$). For the impact zone, results were obtained on a significant increase in the height of the cerebral region of the narrow-headed vole (Fig. 11). The results of the Mann-Whitney (U) test illustrate statistically significant differences in medians between the impact and control

groups ($p \leq 0.05$), as well as between the buffer and control zones. An analysis of variance (ANOVA) has been also performed to check for differences in mean values between the background and control groups. Based on the results of the parametric t-test ($p = 0.9505$), the t-test for unequal variances ($p = 0.919$), and the nonparametric test ($p = 0.9405$), we can conclude that there are no statistically significant differences in the mean values of the cerebral height of the narrow-headed vole of the background and control groups.

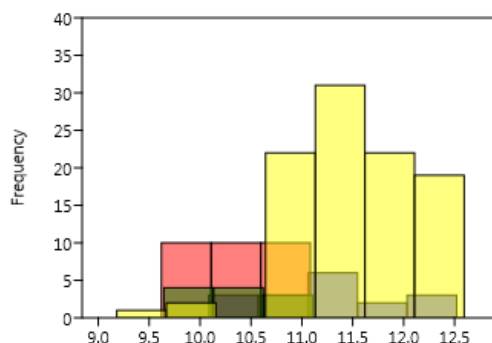


Figure 10. Frequency distribution of cerebral length. Blue – impact area, yellow – control, pink – buffer, green – background area

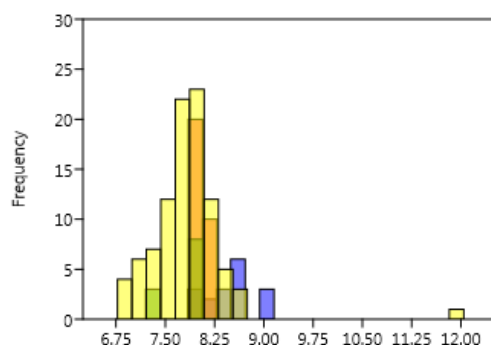


Figure 11. Frequency distribution of cerebral length. Blue – impact area, yellow – control, pink – buffer, green – background area

We associate the phenomenon of brain enlargement in narrow-headed voles living in impact and buffer territories with the intense brain activity of the animals. The proximity of roads, outbuildings, fences, and the airport makes it difficult for animals to move, as well as reduces the food supply. To survive, the animals have to improve the processing of sensory information. The elongated brain region provides more space for the olfactory bulbs and auditory nuclei, which improves their ability to detect and interpret sensory signals (Mucignat-Caretta, 2010). In addition, the enlarged brain region allows rodents to develop complex behavioral strategies for finding, obtaining, and processing food (Whishaw and Kolb, 2015). In general, we conclude that the increase in the length of the cerebral region in narrow-headed voles is the result of adaptations that allowed them to succeed in an anthropogenic habitat and become one of the dominant species of small mammals in the study area. It is assumed that these changes are non-directional variability in environmental impacts.

The largest width of the skull in a narrow-headed vole is measured as the distance between the outer edges of the zygomatic arches at the widest point (*Fig. 12*). This measurement is known as the greatest zygomatic width. It is a measurement that can provide information about the body size, skull shape, eating habits, behavior, and evolutionary relationships of an animal. For instance, animals with wide skulls, such as rodents, have a wider field of vision than animals with narrow skulls (Dent, 2018).

The analysis of variance to verify the equality of the average values illustrated a statistically significant difference ($p = 0.0367$) between the technogenic and control groups on this basis. This difference has been confirmed by the Welch test and analysis of the components of variance. Analysis of the variance components shows that a relatively small part of the total variance is due to differences between groups. The intra-class correlation coefficient and the omega-squared coefficient indicate that the effect of belonging to a group is small. Thus, we can conclude that although there are statistically significant differences between the groups, these differences are relatively small and account for only a small part of the overall variance.

The median equality test was conducted to test the hypothesis that the medians of two independent groups: impact and control. The Mann-Whitney test ($p = 0.9104$) and the Monte Carlo permutation test ($p = 0.9118$) show that there is no statistically significant difference between the medians of the groups.

Examining the results of the buffer and control groups, we observe a statistically significant difference ($p = 0.0107$). This result is confirmed by the Monte Carlo permutation test ($p = 0.012$). We attribute significant differences between the medians to several factors. First, perhaps the groups represent different populations with different medians. Secondly, the groups were exposed to various anthropogenic influences, which increased their influence as the distance from the plant decreased.

The value of the U-statistic indicates the degree of difference between the medians of the background and control groups. The greater the difference between the medians, the greater the value of U. The difference between the results of the two groups was not statistically significant ($p = 0.0975$). The Monte Carlo permutation test gives a similar result: $p = 0.0912$. Both the Mann-Whitney test and the Monte Carlo permutation test show that there is no statistically significant difference between the medians of the groups. This indicates that the medians of the two groups do not differ. However, it should be noted that the p-value of the Mann-Whitney test is close to the level of statistical significance ($p = 0.05$). This means that with a larger sample size, a statistically significant difference between the medians of the two groups could be found.

Occipital width is measured as the distance between the most prominent points of the occipital condyles, which are located on the occipital bone at the back of the skull (*Fig. 13*). The narrow-headed vole is characterized by a narrow skull, which is reflected in the small length of the occipital width. The length of the occipital width can be used to estimate the age of the vole, since the length of the occipital width increases with the age of the animal.

The results of the ANOVA and Welch test reveal that there is a statistically significant difference between the averages of the groups. Despite the fact that the variances between the groups are heterogeneous, the Welch test confirms ANOVA's conclusion about a statistically significant difference. Analysis of the variance components shows that a relatively small part of the total variance is due to differences between groups. The intra-class correlation coefficient and the omega-squared coefficient indicate that the effect of

belonging to a group is moderate. Thus, we can conclude that although there are statistically significant differences between the four groups, these differences are moderate and account for only a fraction of the overall variance.

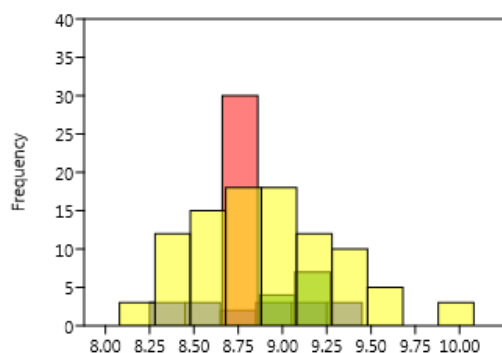


Figure 12. Frequency distribution of largest width of the skull. Blue – impact area, yellow – control, pink – buffer, green – background area

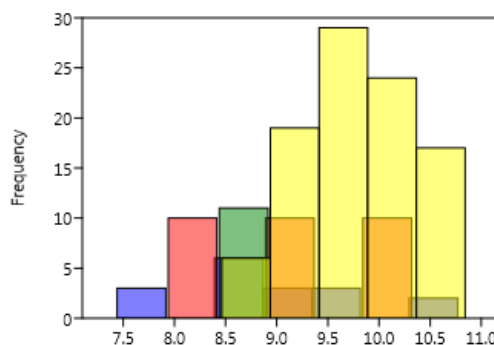


Figure 13. Frequency distribution of occipital width. Blue – impact area, yellow – control, pink – buffer, green – background area

Comparing the value of the occipital width of voles only in the impact and control zones, the Mann-Whitney test showed a statistically significant difference between the medians ($p \leq 0.05$). The Monte Carlo permutation test confirms the conclusion of the Mann-Whitney test, showing that the probability of obtaining the same or more extreme z-statistic value provided that the medians of the groups are the same, is only 0.0001. We can conclude that there is a statistically significant difference between the medians of the impact and control groups, and that this difference is vital.

The Mann-Whitney test and the Monte Carlo permutation test, when calculating the lengths of the occipital width of the buffer and control territories, show that there is a statistically significant difference between the medians ($p \leq 0.05$). This indicates that the medians of the two groups are significantly different.

We observe similar results when calculating the Mann-Whitney test of the background and control territories. Both the Mann-Whitney test and the Monte Carlo permutation test show that there is a statistically significant difference between the medians of the groups ($p \leq 0.05$).

The length of the dentition have been measured from the front of the incisors to the back of the molars (Fig. 14). Rodents have constantly growing teeth, so the length of

the tooth varied in animals of different age groups. Factors that can affect the length of the dentition include nutrition, age, and genetics. The dentition of rodents plays an important role in their nutrition. The length of the dentition can affect the ability of rodents to eat and receive the necessary nutrients. The dentition of rodents can also be an indicator of their overall health. Rodents with a shorter dentition length may have health problems such as malnutrition or dental disease. Thus, the length of the dentition in rodents can provide information about the effects of toxic substances, environmental stress, and the general state of animal health (Gdula-Argasińska et al., 2004).

The results of the p-values of the ANOVA of the four studied groups are statistically significant ($p \leq 0.05$). The permutation test also showed a statistically significant difference between the average values of the groups ($p \leq 0.05$). The Welch F test confirmed a statistically significant difference between the mean values of the groups, even under the condition of heterogeneous variances. This means that the average values of the groups are not the same.

The results of the Mann-Whitney test ($p \leq 0.05$) and the p-value of Monte Carlo permutation ($p = 0.0001$) confirm a statistically significant difference between the medians of the impact and control groups. Based on the results of the Mann-Whitney test ($p = 0.0652$) and the permutation test ($p = 0.065$), it can be concluded that there is insufficient evidence to assert that the medians of the buffer and control groups differ. The absence of a significant difference is also demonstrated by the background and control groups.

The length of the nasal bones in the narrow-headed vole is of great importance for understanding its ecology, behavior, and evolution (Fig. 15). The length of the nasal bones affects the olfactory abilities of the vole. Longer nasal bones provide a larger surface area for the olfactory epithelium, which allows the vole to better detect and distinguish odors (Tai et al., 2009). The length of the nasal bones is also associated with the social behavior of the narrow-headed vole. Researches have shown that voles with longer nasal bones exhibit more aggressive behavior towards unfamiliar individuals. This may be due to the fact that longer nasal bones allow the vole to better recognize and identify unfamiliar individuals (Zheng et al., 2013).

The ANOVA test showed that there is a statistically significant difference between the averages of the four groups ($F(3, 144) = 2.189$, $p = 0.09191$). To further study the differences between the groups, the Welch F test was performed, which confirmed a statistically significant difference between the average values of the groups even under the condition of heterogeneous variances ($F(34.31) = 6.812$, $p = 0.001006$). Based on the results of the Mann-Whitney test and the permutation test, it can be concluded that there is insufficient evidence to assert that the medians of the two impact and control groups differ. There is also no significant difference between the buffer and control groups.

The results of the U test ($p = 0.0067$) and the permutation test ($p = 0.0053$) show that there is a statistically significant difference between the medians of the two groups. Thus, the length of the nasal bones in animals living near the plant cannot be a feature used in bioindicators studies.

Thus, we recorded a significant increase in the cerebral part of the craniums of the narrow-headed vole and a significant decrease in the facial part and general parameters of the skull size, such as condylobasal length, in animals living in the areas closest to the plant (impact and buffer areas). There is an increase in the size of the skull relative to the sagittal plane.

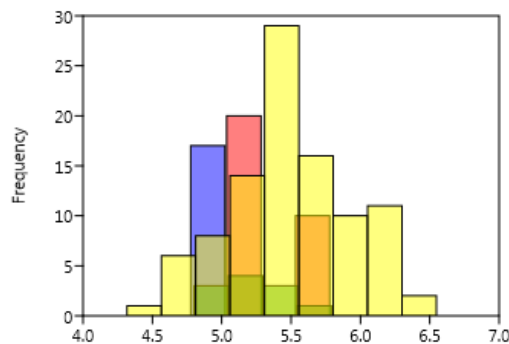


Figure 14. Frequency distribution of dentition length. Blue – impact area, yellow – control, pink – buffer, green – background area

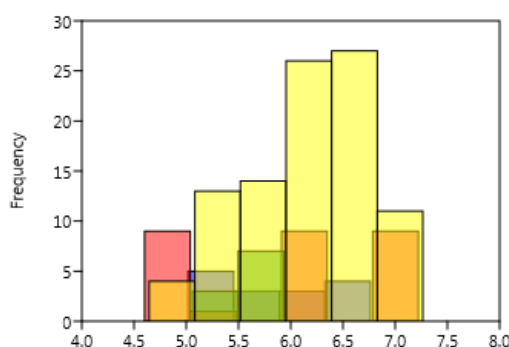


Figure 15. Frequency distribution of nasal bones length. Blue – impact area, yellow – control, pink – buffer, green – background area

We come to conclusion that rodents living in anthropogenic conditions are smaller in size compared to control individuals, as indicated by the study (Martiniakova et al., 2012). This means that when adapting to life next to a person, there is a decrease in craniometric parameters, but at the same time, there is an increase in the relative size of the cerebral part of the skull. This change is probably due to the adaptation associated with accelerated growth, as described by Schwartz (1980). He noted that in fast-growing animals, skulls are usually taller and wider, with a less developed facial part, which is typical for young individuals, while in slow-growing animals, the proportions of the skull are flattened and more elongated, which is typical for adults.

Comparing populations living in anthropogenic area and in the national park (control group), we can observe features consistent with the morphological changes that can occur during domestication. However, there is an increase in the size of the cerebral part of the skull. We can attribute the differences to the habitat, biomechanical requirements of feeding (Wroe et al., 2007), or cognitive processes (e.g., increased danger when encountering humans). The environment directly affects diets and their mechanical chewing requirements.

The impact of the urban environment on rapidly reproducing species, including Rodents, is manifested in the development of cognitive abilities (Snell-Rood and Wick, 2013). The authors conclude that species capable of intensive reproduction may exhibit a more pronounced evolutionary response to urban habitats. The more generations that have appeared after urbanization, the better they are adapted to their habitat (Møller,

2008). Selection occurs in which organisms with a relatively large number of neural connections are predisposed to survive in an urban environment because they can learn to cope with new conditions (Carrete and Tella, 2011).

The conditions of the anthropogenic habitat favor the manifestation of changes in populations. We observe the manifestation of the properties of species plasticity, flexibility in development and functioning, which allows the species to survive and reproduce in different conditions. These changes may reflect the process of phenotypic plasticity in modern evolution (Uller et al., 2018; Wilkins, 2014).

Craniometric features, such as the size and proportions of the skull, can be used in bioindicator studies to assess the impact of anthropogenic activities on rodents. Changes in craniometric features may indicate various environmental stresses caused by anthropogenic influences, such as pollution, habitat loss, and climate change. Monitoring of craniometric features in rodent populations over time can help assess the impact of long-term impacts of anthropogenic activities. Changes in these characteristics may indicate the adaptation of the population to new conditions.

Conclusion

The decrease in the size of the skull in small mammals in anthropogenic area may occur due to several factors. The anthropogenic area of the plant have limited dimensions and fences, which can lead to limited food and shelter availability for the animals. This deficiency results in a deterioration in the mammal's nutrition and development, including the size and skull development. However, an increase in the brain part indicates active thought processes associated with overcoming obstacles and searching for food. The studied territories are accompanied by industry, noise, environmental pollution, and other changes in habitat conditions. These factors have a negative impact on the body and development of mammals, which can affect the size and shape of the skull (Kupriyanova et al., 2003). Animals of the technogenic zone may have a genetic predisposition to a reduced skull size under the influence of environmental conditions. This may be the result of selection, when individuals with a smaller skull adapt more successfully to new living conditions and leave the same offspring. In anthropogenic territories, there is increased competition (Hein and Jacob, 2015) between animals for limited resources, which can lead to a change in their skulls. Mammals with smaller skulls may have an advantage in access to food and shelter.

The influence degree of each of these factors may vary depending on the specific situation and the mammals' species.

Conflict of interests. All authors declare that they have no conflict of interest in publishing this manuscript.

REFERENCES

- [1] Balčiauskas, L., Amshokova, A., Balčiauskienė, L., Benedek, A. M., Cichocki, J., Csanady, A., Gil De Mendonça, P., Nistoreanu, V. (2020): Geographical clines in the size of the herb field mouse (*Apodemus uralensis*). – *Integrative Zoology* 15(1): 55-68.
- [2] Bárdos, B., Kövér, G., Szabó, A., Gerencsér, Z., Nagy, I. (2022): Feed preference and feeding behavior of different mouse species in laboratory housing. – *Acta Agraria Kaposvariensis* 26(2): 17-26.

- [3] Ben Saleh, A. R., Annabi, A., Said, K. (2012): Morphometric variation in black rat *Rattus rattus* (Rodentia: Muridae): from Tunisia. – *Acta Zoologica Bulgarica* 64: 381-387.
- [4] Carrete, M., Tella, J. L. (2011): Inter-individual variability in fear of humans and relative brain size of the species are related to contemporary urban invasion in birds. – *PloS One* 6(4): e18859.
- [5] Cuevas, A., Febrero, M., Fraiman, R. (2004): An ANOVA test for functional data. – *Computational Statistics & Data Analysis* 47(1): 111-122.
- [6] Cui, Z., Liu, X., Song, S., Yang, M. (2019): The characteristics of metabolism and thermoregulation of *Microtus gregalis*. – *Acta Theriologica Sinica* 39(3): 295.
- [7] d'Havé, H., Scheirs, J., Mubiana, V. K., Verhagen, R., Blust, R., De Coen, W. (2005): Nondestructive pollution exposure assessment in the European hedgehog (*Erinaceus europaeus*): relationships between concentrations of metals and arsenic in hair, spines, and soil. – *Environmental Toxicology and Chemistry* 24(9): 2356-2364.
- [8] Dai, M., Shen, W., Stern, H. S. (2022): Sensitivity analysis for the adjusted Mann-Whitney test with observational studies. – *Observational Studies* 8(1): 1-29.
- [9] Dent, M. L., Screven, L. A., Kobrina, A. (2018): Hearing in Rodents. – In: Dent, M. L., Fay, R. R., Popper, A. N. (eds.) *Rodent Bioacoustics*. Springer, Cham, pp. 71-105.
- [10] Fraser, E. D. G. (2020): The challenge of feeding a diverse and growing population. – *Physiology & Behavior* 221: 112908.
- [11] Gdula-Argasińska, J., Appleton, J., Sawicka-Kapusta, K., Spence, B. (2004): Further investigation of the heavy metal content of the teeth of the bank vole as an exposure indicator of environmental pollution in Poland. – *Environmental Pollution* 131(1): 71-79.
- [12] Gryseels, S., Goüy de Bellocq, J., Makundi, R., Vanmechelen, K., Broeckhove, J., Mazoch, V., Šumbera, R., Zima, J., Leirs, Jr., H., Baird, S. J. E. (2016): Genetic distinction between contiguous urban and rural multimammate mice in Tanzania despite gene flow. – *Journal of Evolutionary Biology* 29(10): 1952-1967.
- [13] Gureeva, A. V., Lebedev, V. S., Feoktistova, N. Y., Surov, A. V. (2021): Geographical variability of the craniological characters in Eversmann's hamsters and the taxonomic structure of the genus *Allocricetulus* (Cricetidae). – *Biology Bulletin* 48: 1380-1388.
- [14] Hamers, T., Van den Berg, J. H., Van Gestel, C. A., Van Schooten, F. J., Murk, A. J. (2006): Risk assessment of metals and organic pollutants for herbivorous and carnivorous small mammal food chains in a polluted floodplain (Biesbosch, The Netherlands). – *Environmental Pollution* 144(2): 581-595.
- [15] Hammer, Ø., Harper, D. A. T., Ryan, P. D. (2001): PAST: paleontological statistics software package for education and data analysis. – *Palaeontologia Electronica* 4(1).
- [16] Ikbal, N. H. M., Pathmanathan, D., Bhassu, S., Simarani, K., Omar, H. (2019): Morphometric analysis of craniodental characters of the House Rat, *Rattus rattus* (Rodentia: Muridae) in Peninsular Malaysia. – *Sains Malaysiana* 48(10): 2103-2111.
- [17] Kataev, G. D. (2005): The state of the mammal community of boreal forest ecosystems in the vicinity of a nickel-smelting plant. – *Russian Journal of Ecology* 36(6): 421-426.
- [18] Klingenberg, C. P., Mebus, K., Auffray, J. C. (2003): Developmental integration in a complex morphological structure: how distinct are the modules in the mouse mandible? – *Evolution & Development* 5(5): 522-531.
- [19] Kropacheva, Y. E., Smirnov, N. G., Zykov, S. V. (2021): Growth rate of cheek teeth in narrow-skulled vole (*Lasiopodomys gregalis*) depending on food abrasiveness. – *Russian Journal of Ecology* 52: 496-503.
- [20] Kulczycka, J., Lelek, Ł., Lewandowska, A., Wirth, H., Bergesen, J. D. (2016): Environmental impacts of energy-efficient pyrometallurgical copper smelting technologies: the consequences of technological changes from 2010 to 2050. – *Journal of Industrial Ecology* 20(2): 304-316.
- [21] Martiniakova, M., Omelka, R., Jancova, A., Formicki, G., Stawarz, R., Bauerova, M. (2012): Accumulation of risk elements in kidney, liver, testis, uterus and bone of free-

- living wild rodents from a polluted area in Slovakia. – Journal of Environmental Science and Health, Part A 47(9): 1202-1206.
- [22] Masindi, V., Muedi, K. L. (2018): Environmental contamination by heavy metals. – Heavy Metals 10: 115-132.
- [23] Møller, A. P. (2008): Flight distance of urban birds, predation, and selection for urban life. – Behavioral Ecology and Sociobiology 63: 63-75.
- [24] Mucignat-Caretta, C. (2010): The rodent accessory olfactory system. – Journal of Comparative Physiology A 196: 767-777.
- [25] Parsons, K. J., Rigg, A., Conith, A. J., Kitchener, A. C., Harris, S., Zhu, H. (2020): Skull morphology diverges between urban and rural populations of red foxes mirroring patterns of domestication and macroevolution. – Proceedings of the Royal Society B 287(1928): 20200763.
- [26] Pearson, O. P. (1948): Metabolism of small mammals, with remarks on the lower limit of mammalian size. – Science 108(2793): 44-44.
- [27] Russo, D., Ancillotto, L. (2015): Sensitivity of bats to urbanization: a review. – Mammalian Biology 80(3): 205-212.
- [28] Samuels, J. X. (2009): Cranial morphology and dietary habits of rodents. – Zoological Journal of the Linnean Society 156(4): 864-888.
- [29] Schwartz, S. S. (1980): Ekologicheskie Zakonomernosti Evolyutsii (Ecological Regularities at Evolution). – Nauka, Moscow.
- [30] Smirnov, N. G., Kuz'mina, E. A., Golovachev, I. B., Fadeeva, T. V. (2007): The narrow-skulled vole (*Microtus gregalis* Pall.) in the dynamics of zonal rodent communities of northern Eurasia. – Russian Journal of Ecology 38(2): 106-111.
- [31] Tai, F., Wang, W., Broders, H., Sun, R., Liu, L., Wang, H. (2009): Comparison of social interaction and neural activation in the main olfactory bulb and the accessory olfactory bulb between *Microtus mandarinus* and *Microtus fortis*. – Current Zoology 55(4): 279-287.
- [32] Uller, T., Moczek, A. P., Watson, R. A., Brakefield, P. M., Laland, K. N. (2018): Developmental bias and evolution: a regulatory network perspective. – Genetics 209(4): 949-966.
- [33] Whishaw, I. Q., Kolb, B. (2015): The Behavior of the Laboratory Rat: A Handbook with Tests. – Oxford University Press.
- [34] Wilkins, A. S., Wrangham, R. W., Fitch, W. T. (2014): The “domestication syndrome” in mammals: a unified explanation based on neural crest cell behavior and genetics. – Genetics 197(3): 795-808.
- [35] Wroe, S., Clausen, P., McHenry, C., Moreno, K., Cunningham, E. (2007): Computer simulation of feeding behaviour in the thylacine and dingo as a novel test for convergence and niche overlap. – Proceedings of the Royal Society B: Biological Sciences 274(1627): 2819-2828.
- [36] Zaporozhets, A., Babak, V., Isaienko, V., Babikova, K. (2020): Analysis of the Air Pollution Monitoring System in Ukraine. – In: Babak, V., Isaienko, V., Zaporozhets, A. (eds.) Systems, Decision and Control in Energy I. Studies in Systems, Decision and Control. Vol. 298. Springer, Cham, pp. 85-110.
- [37] Zhang, L., Li, J. (2018): Anthropogenic impacts on cranial morphometrics of *Cricetulus triton* near cities in China. – Mammal Study 43: 179-187.
- [38] Zhang, Q., Yang, L., Zhang, Z. (2008): Variations in skull morphology of *Apodemus chevrieri* in habitats disturbed by human activities in Kunming. – Acta Theriologica Sinica 28: 299-305.
- [39] Zheng, D. J., Foley, L., Rehman, A., Ophir, A. G. (2013): Social recognition is context dependent in single male prairie voles. – Animal Behaviour 86(5): 1085-1095.