Issues concerning the measurement of Radioadaptation of small mammals in the East Urals Radioactive Trace Zone (EURT)

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WORKSHOP ON UNCERTAINTIES IN FIELD STUDIES ON CHRONIC LOW LEVEL EFFECTS DUE TO RADIATION

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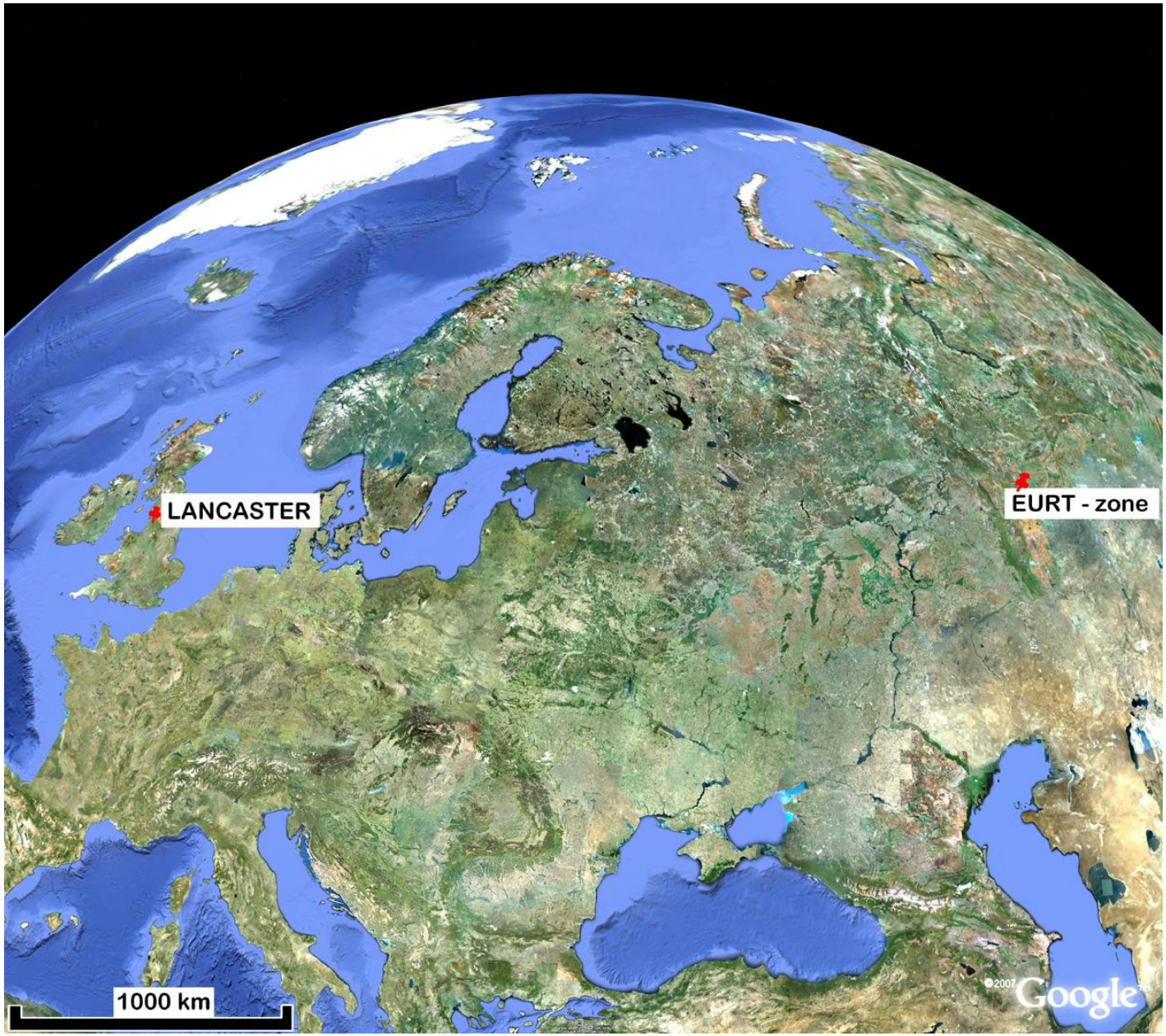
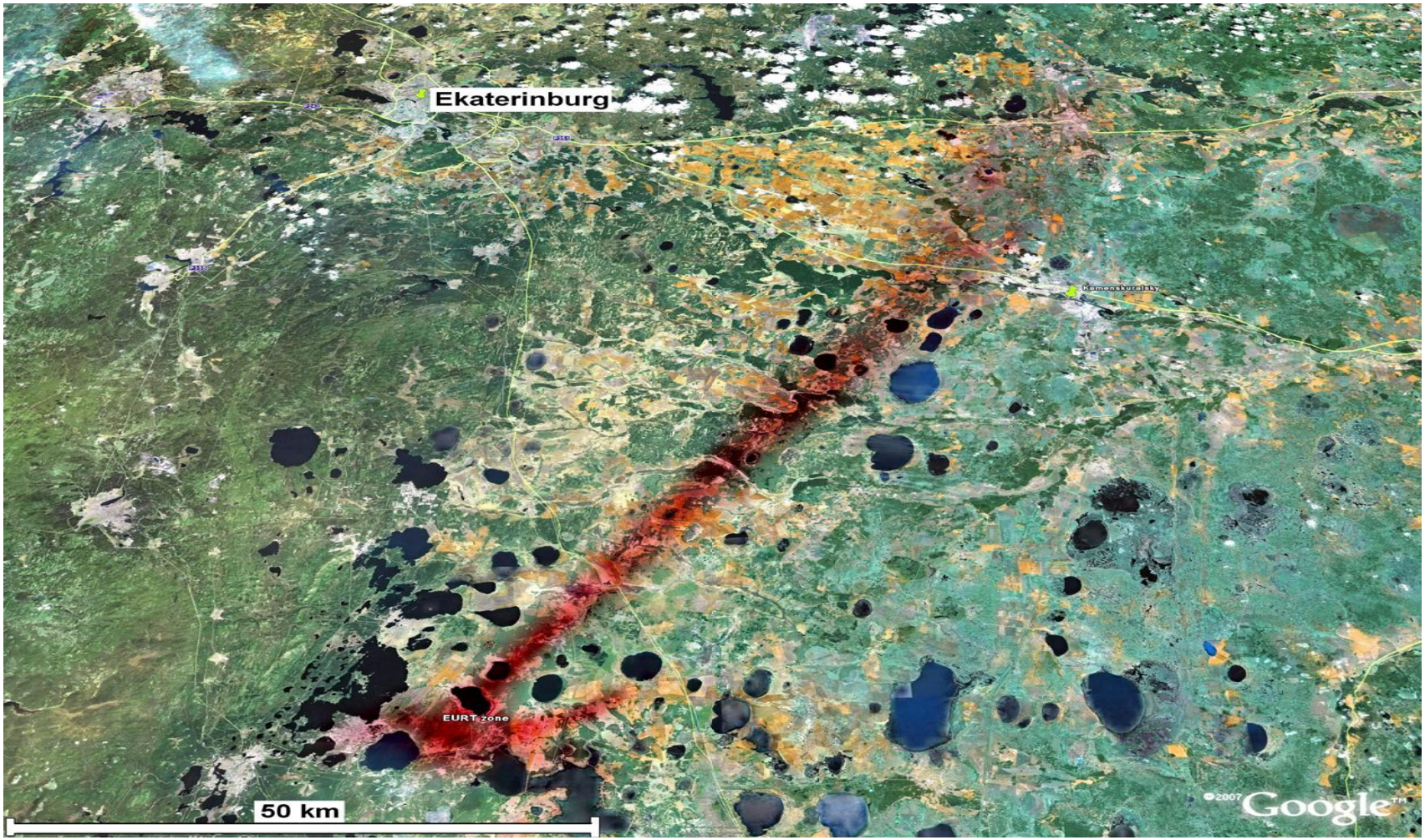
Localization of the East Urals Radioactive Trace (EURT) Zone



Localization of the East Urals Radioactive Trace (EURT) Zone

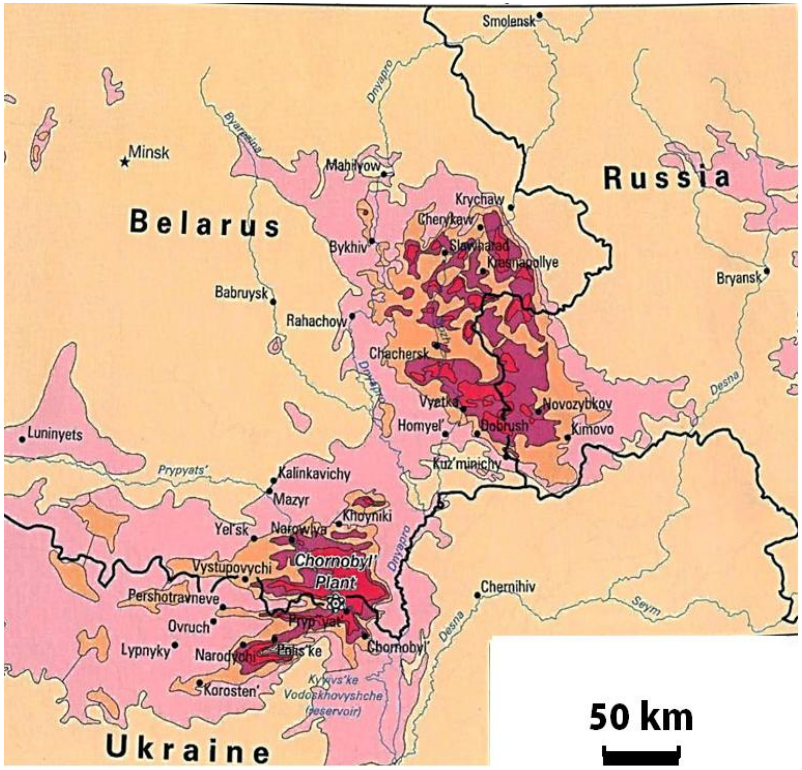
The Uniqueness of the East Ural Radioactive Trace (EURT) Zone

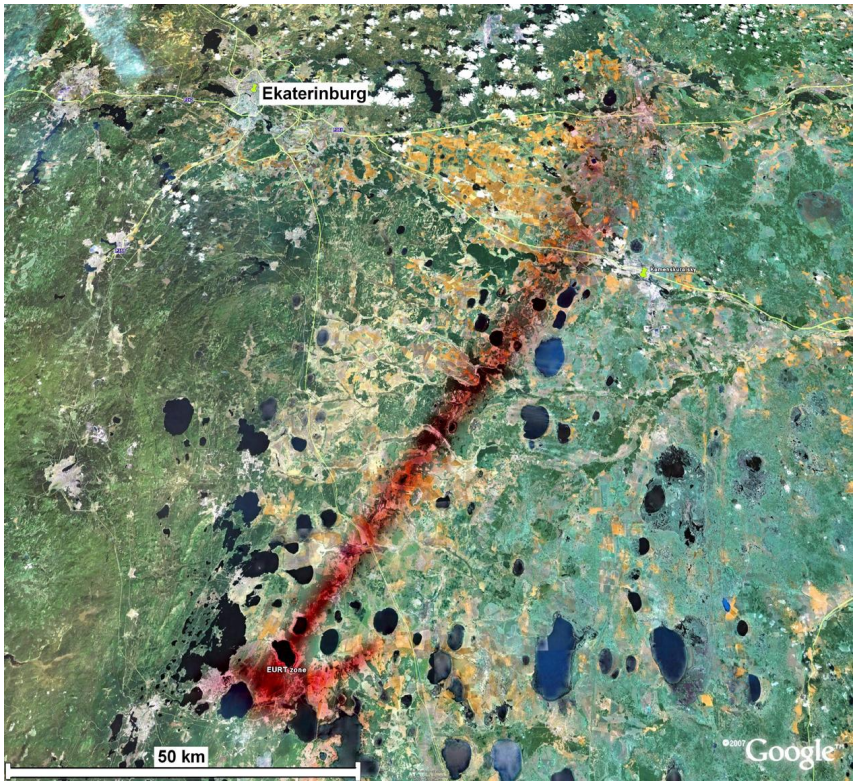


The EURT zone is unique so it is characterized by specific configuration, being an extended (about 300 km) and narrow territory with a rapidly falling pollution gradient. Due to the small transversal size of the radioactive cloud, nuclear fallout was concentrated along the axis of its movement.

Transversal sizes of radiocantamination zones

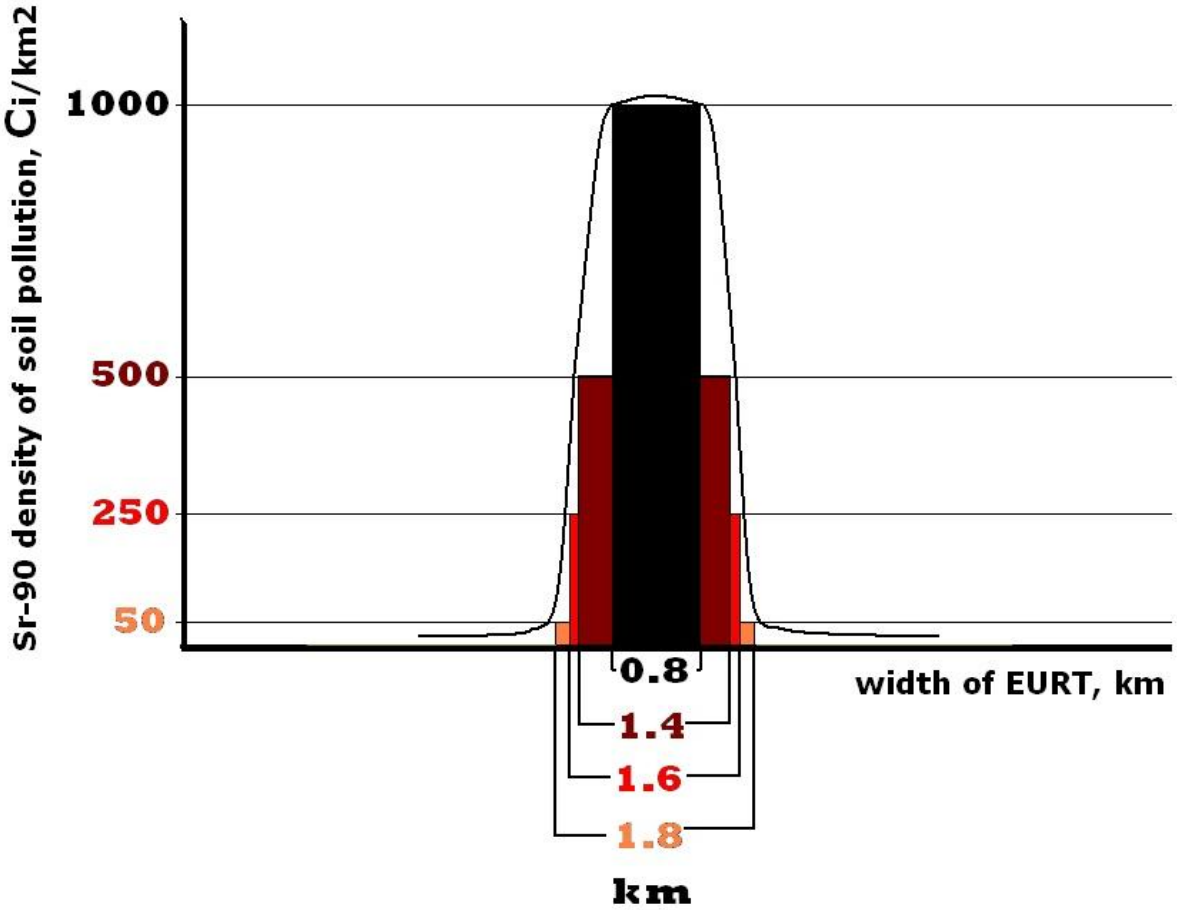
(Chernobyl > 100 km, EURT < 2 km)





CHERNOBYL

EURT



Transversal cut of the EURT zone, gradients

In the studied area, the width of the zone with the density of 90Sr soil contamination equal to

37,0 MBq/m2 (1000 Ci/km2) is 800 m;
18.5 MBq/m2 (500 Ci/km2) - 1400 m;
9.25 MBq/m2 (250 Ci/km2) - 1580 m;
1.85 MBq/m2 (50 Ci/km2) - 1800 m.

Pygmy wood mouse *(Sylvaemus uralensis,* Pall, 1811)

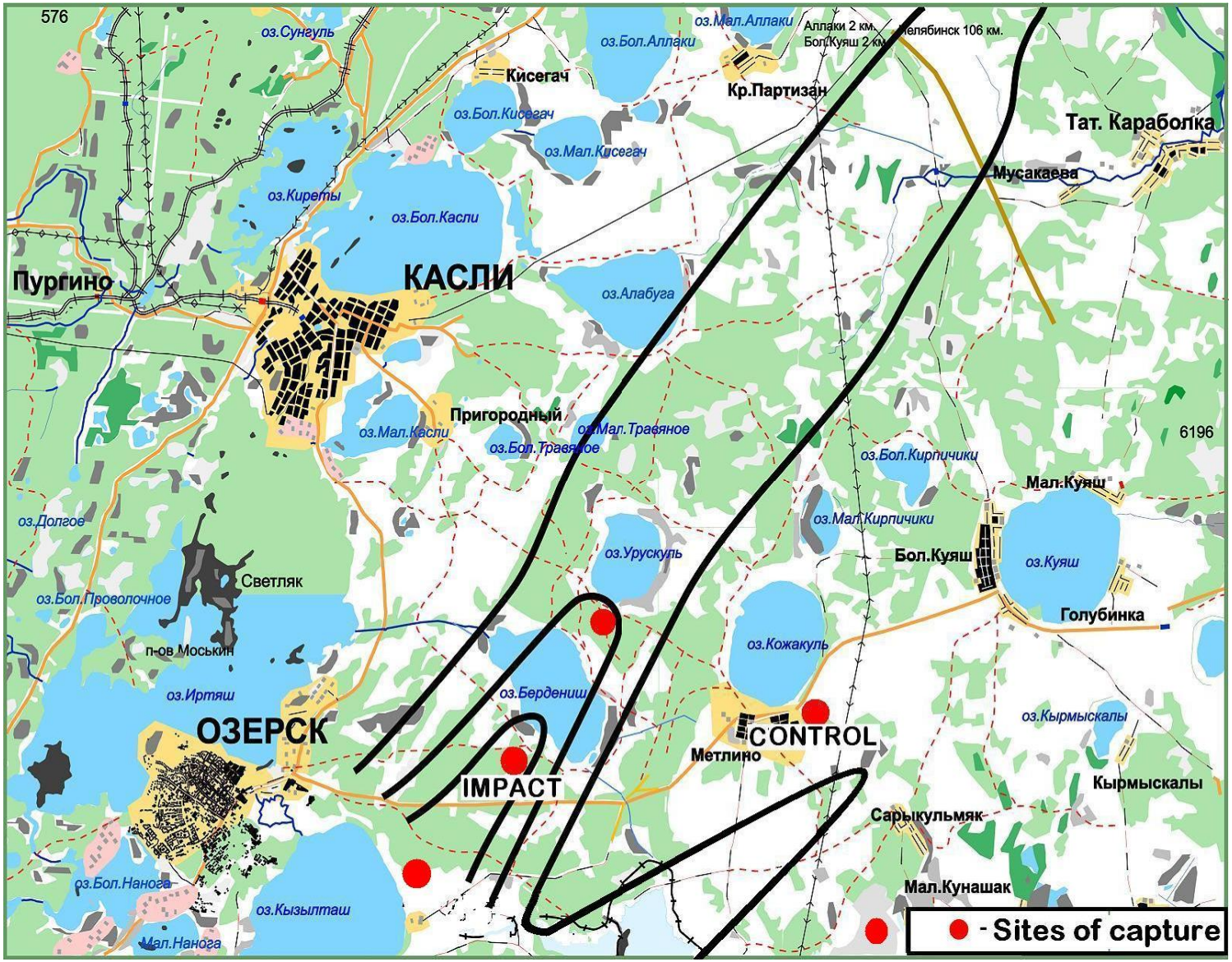
Field mouse (*Apodemus agrarius* Pall, 1771**)**

Mole-vole *(Ellobius talpinus* Pall, 1770**)**

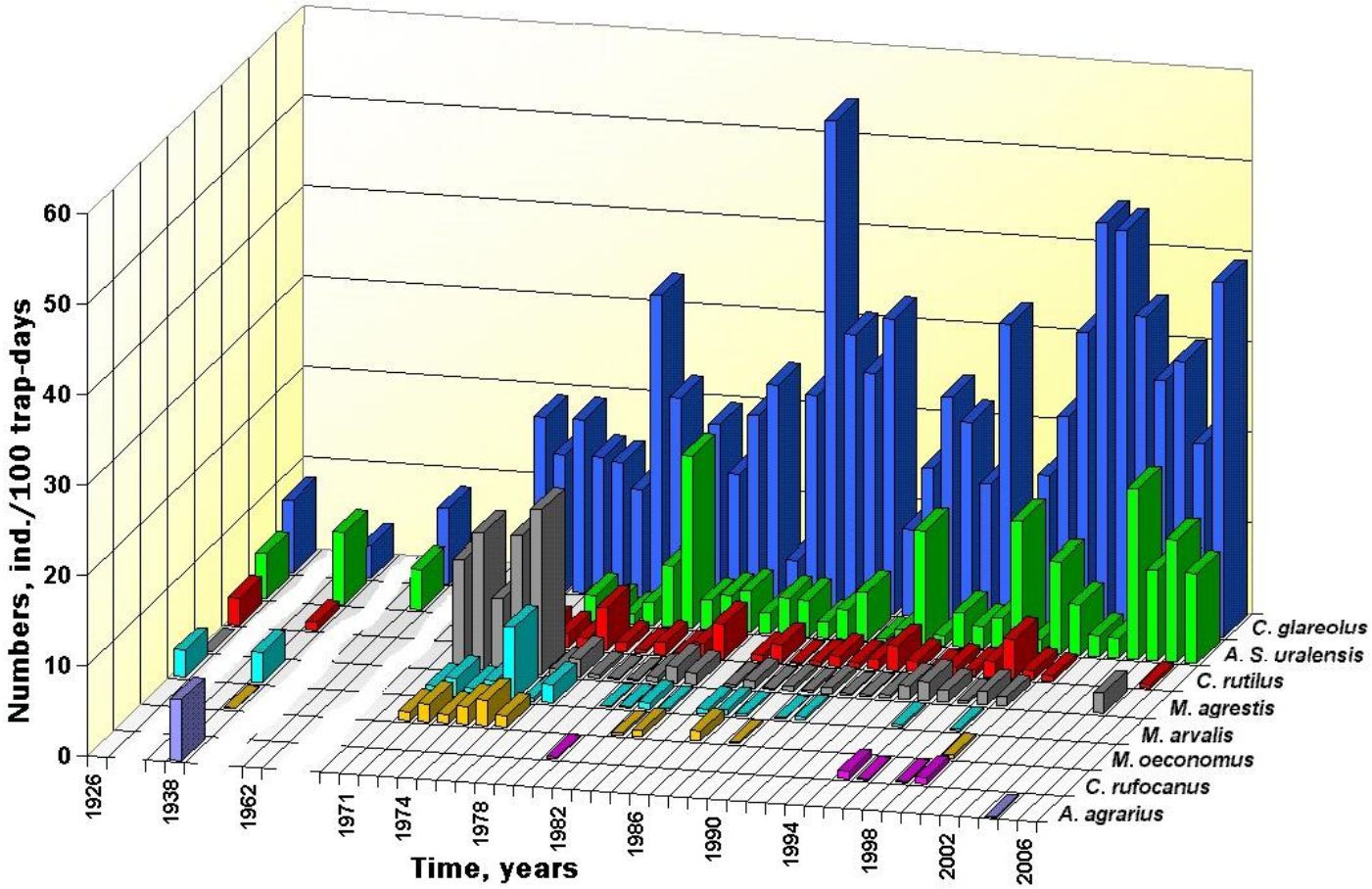
Northern red-backed vole (*Clethrionomys rutilus Pall*, 1779)

Objects of investigations

Main characteristics	Mice and voles	Mole-voles
Way of life and Organization of settlement	Terrestrial rodents, Single way of life	Underground digger, winters' hibernation in Urals, Live by families
Migration	Active during the all year round	Quite low migratory activity
Average lifespan	From 3-4 months to 1,5 years	Up 6 years
Density of Soil contamination in EURT-zone by 90Sr	18.5 MBq/m^2 (451 Ci/km^2)	37,0 MBq/m^2 (1000 Ci/km^2)



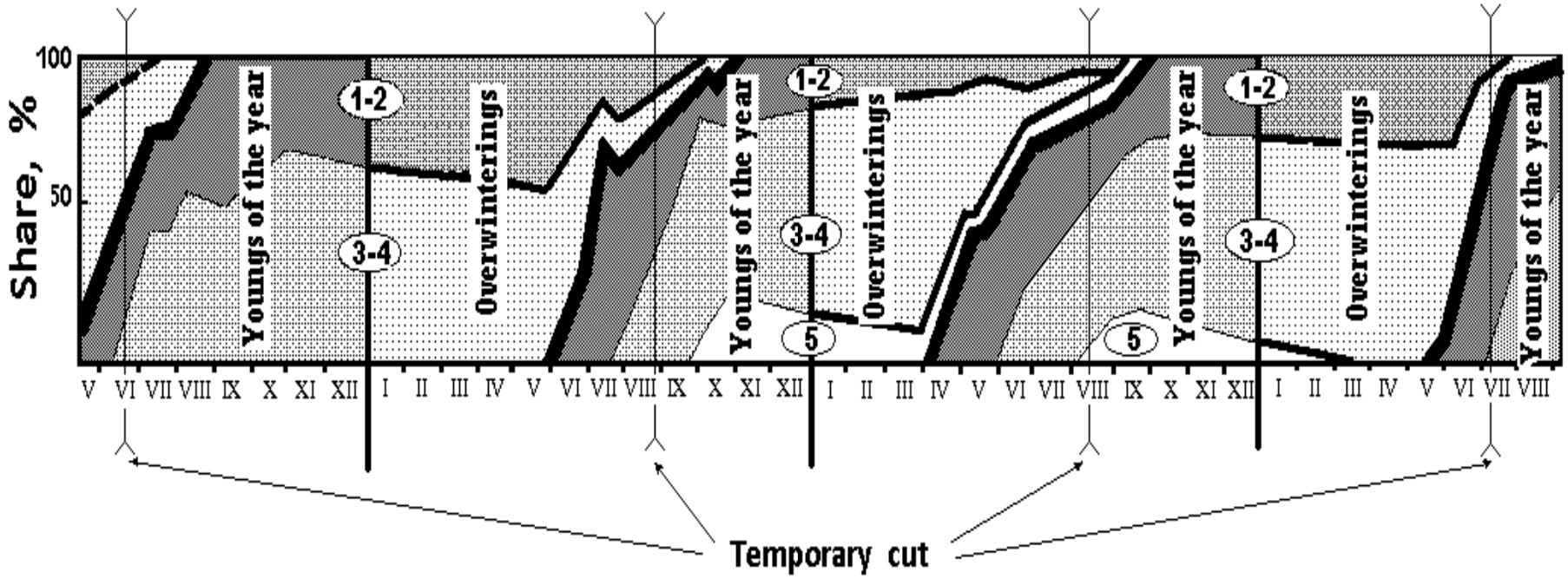
The sketch-map of the EURT zone



Population dynamics of eight rodent' species (annual average values)

Olenev. Russian Journal of Ecology. 2002. V. 33. No. 5. 321–330

Rodents have the most liability and the most complex structure from all mammals everywhere. It provides them high ecological plasticity. The constancy of a population as single unit is provided by migrations. Here it is the general pattern of population size in eight rodent species. Population changes in different species occur asynchronously. Apparently, specific influences of the same environmental factors are differently perceived by cohabitant species and evoke different adaptive responses and, hence, in population dynamics.

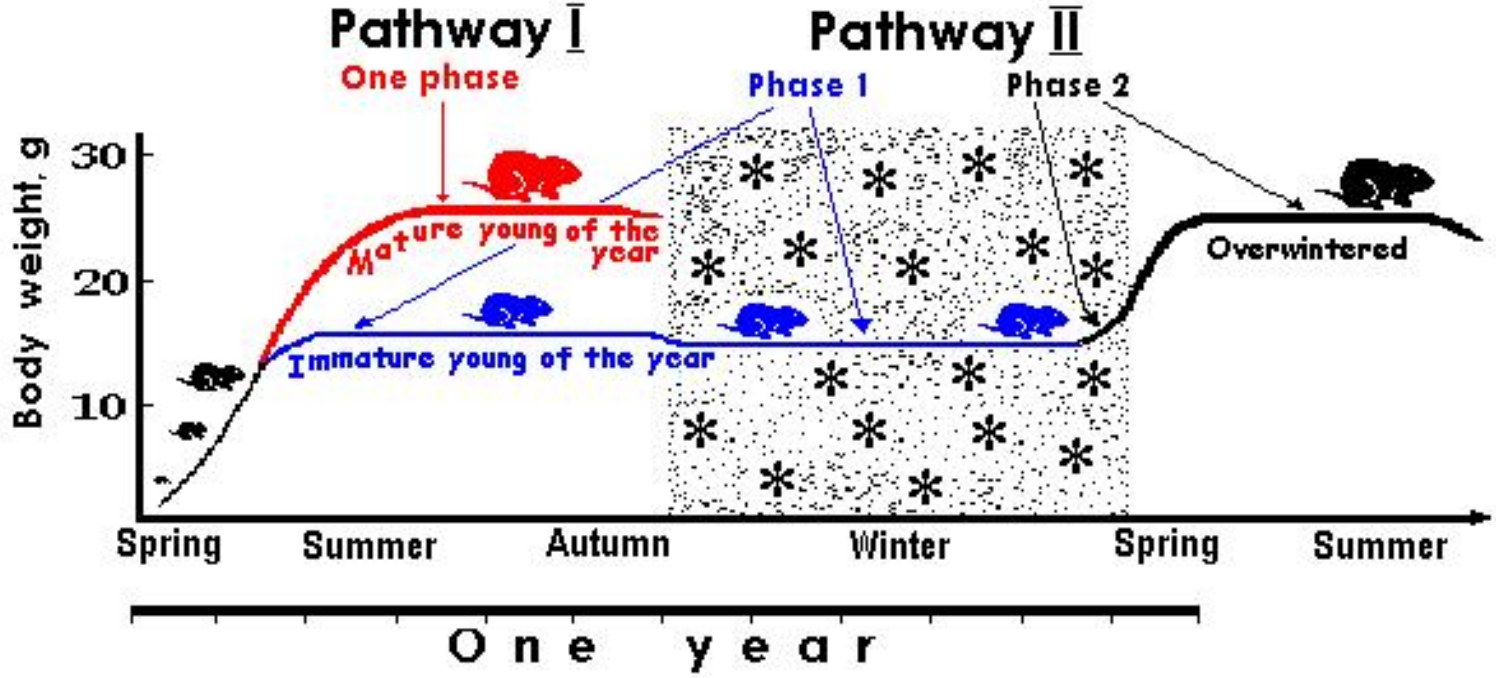


Age structure' dynamics of *S. uralensis* from EURT zone: share of individuals and life span of different cohorts (examples of four years)

Grigorkina, Olenev. ***Doklady Biological Sciences. 2012. V. 443. 91–93***

On temporary cut you can see the complex intrapopulational structure of rodents. It is submitted by a series of cohorts (1-5) - elementary structural age units of population.

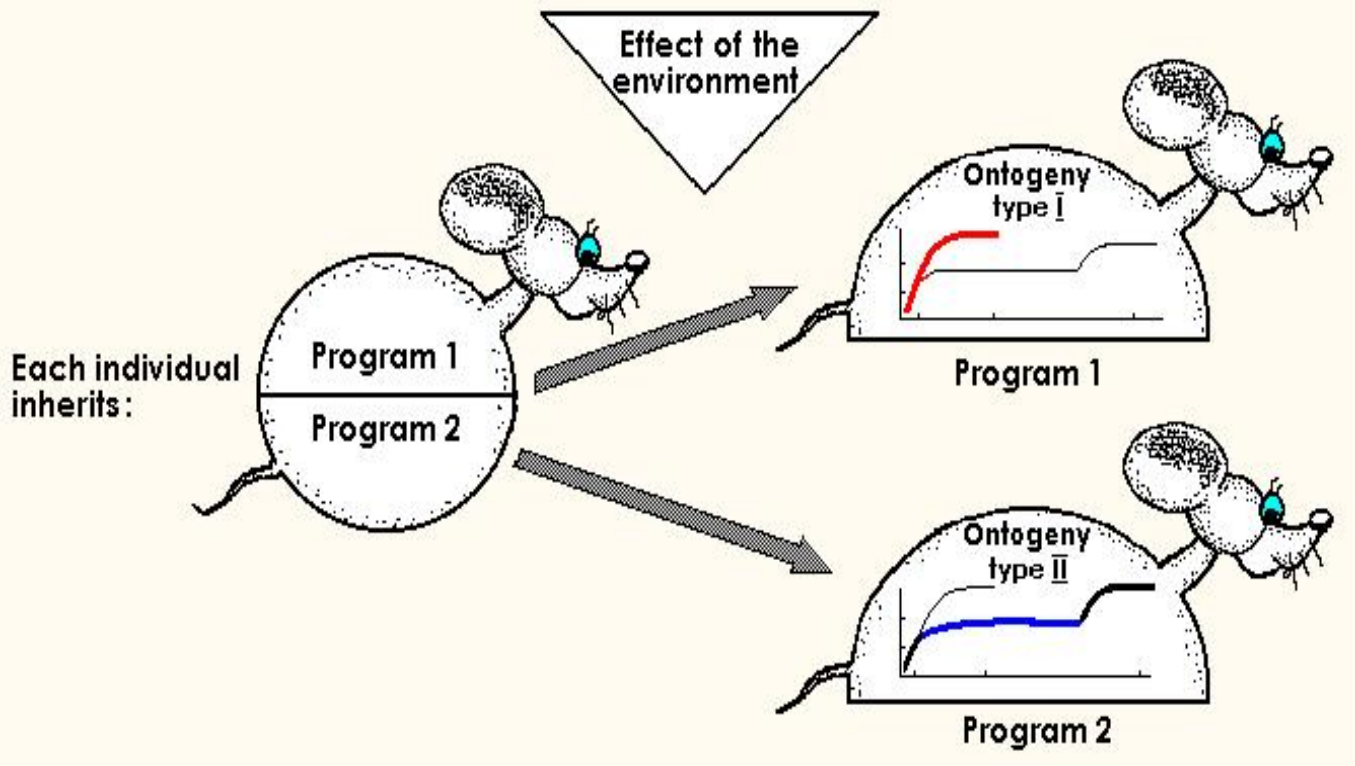
Cohorts are a regular mass offspring starting from the beginning of spring reproduction.



Scheme of Two Alternative Pathways of Mouse-like Rodents Ontogeny

Olenev. Russian Journal of Ecology. 2002. V. 33. No. 5. 321–330

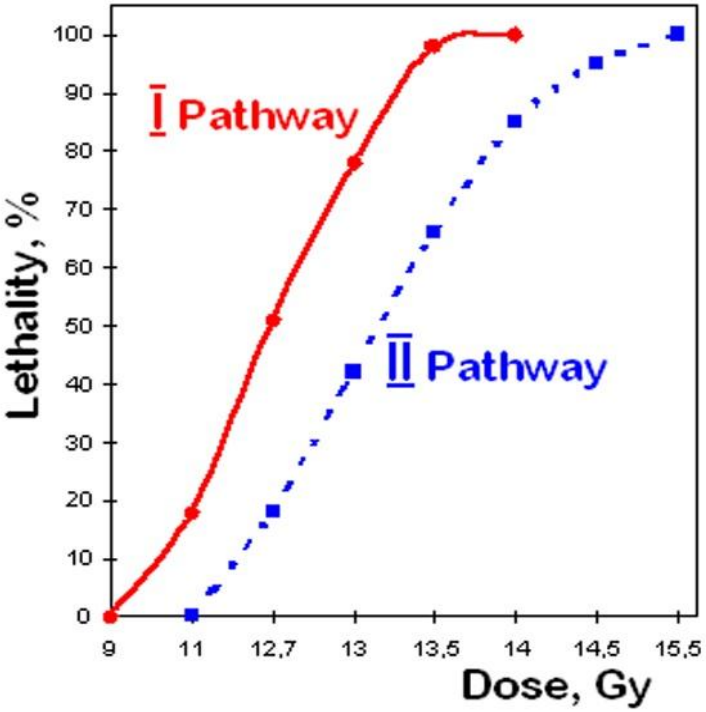
Mature young of the year (*the only phase of ontogeny*), body mass – 25 g, take part in **reproduction**, **high metabolic processes** and **rapid aging**. Function is increase in population numbers.
Immature young of the year (*pathway II of ontogeny, the first phase*), body mass 16-18 g, **do not mature**, **low metabolism and aging** (2 times). Function is to preserve the population until the next spring with the smallest possible losses and in the status of
Overwintered (*pathway II of ontogeny, the second phase*), to begin the cycle of population renewal.
Juveniles - young animals (age no more than 30–45 days, body mass no more than 12 g) that have not yet diverged with respect to the type of ontogeny, **high metabolic rate**.
The incompleteness of growth processes.

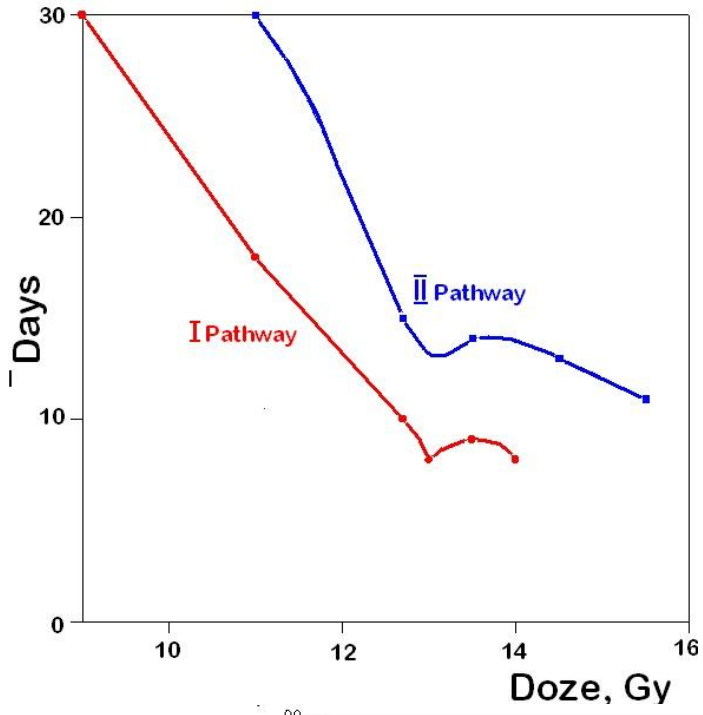


Scheme of Nonspecific Trigger Mechanism of Two Types of Growth and Development of Rodents

Olenev. Russian Journal of Ecology. 2002. V. 33. No. 5. 321–330

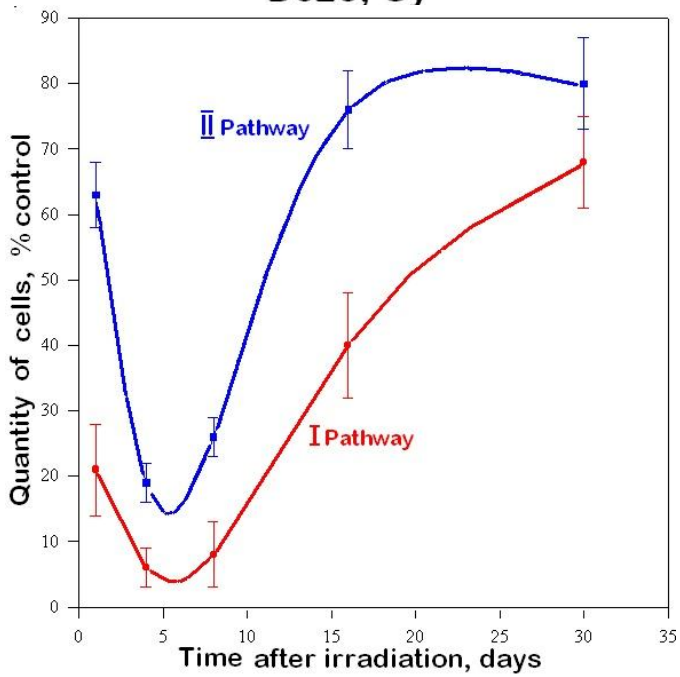
Two alternative pathways of ontogeny are manifestation of polyvatiant development of animals. Each specimen inherits two programs of ontogenetic development on the base of own genotype, but environment is a trigger, which switch on one of these program.





Lethality (LD50/30), average life span, kinetics of leucocytes in rodents of different pathways, subjected by acute gamma-irradiation

Olenev, Grigorkina. Russian Journal of Ecology. 1998. V. 29. No. 6. 401–405.

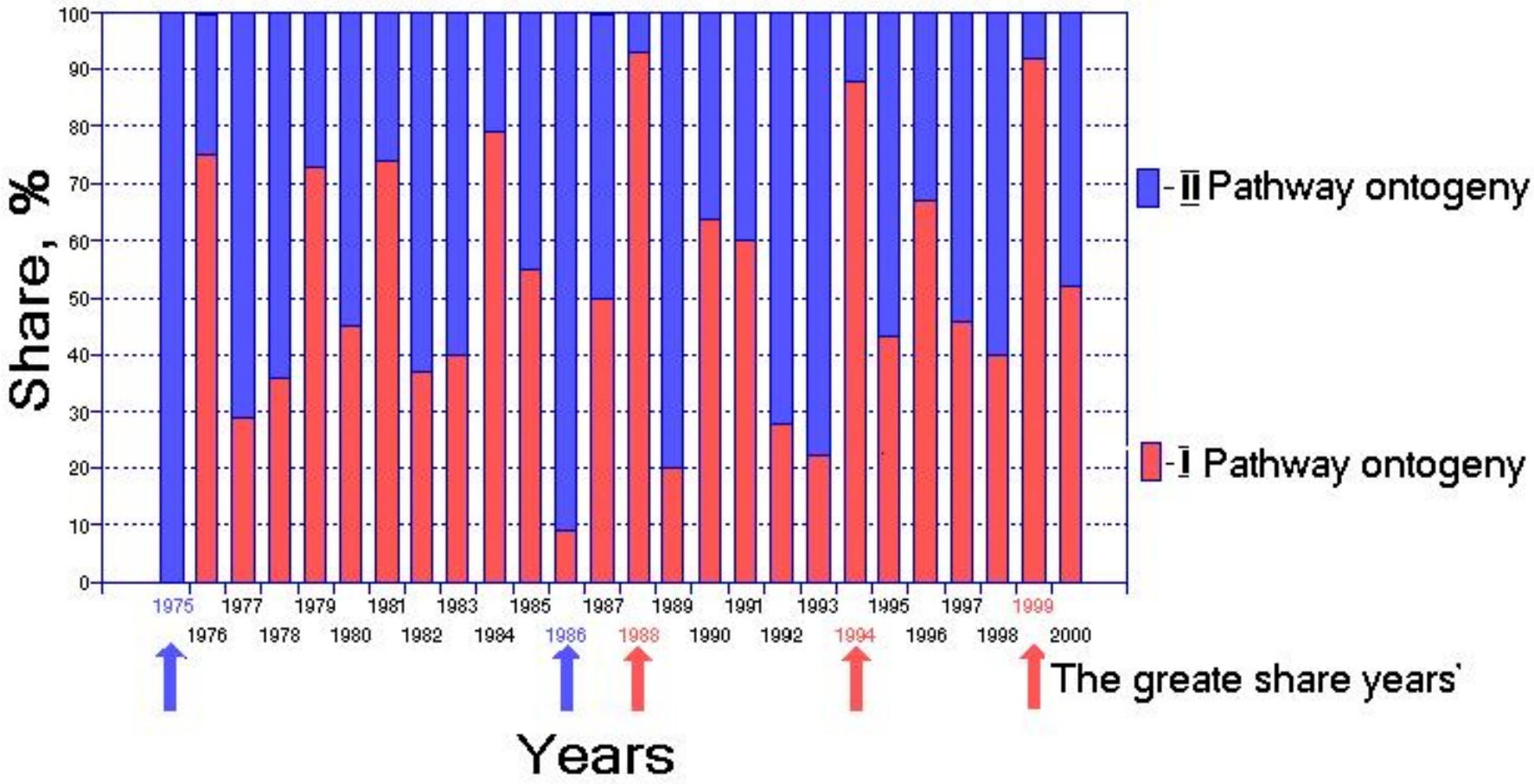


Thus we studied the radiobiological aspect of the functional population structure and found that individuals of two alternative Pathways of Mouse-like Rodents Ontogeny differ essentially by radioresistance.

Animals of the same individual age but different functional status significantly differ by average semilethal dose (LD50/30), mortality after irradiation at the same dose - 53.6% (I pathway) and 17.6% (II pathway) and in the response of the hemopoietic system.

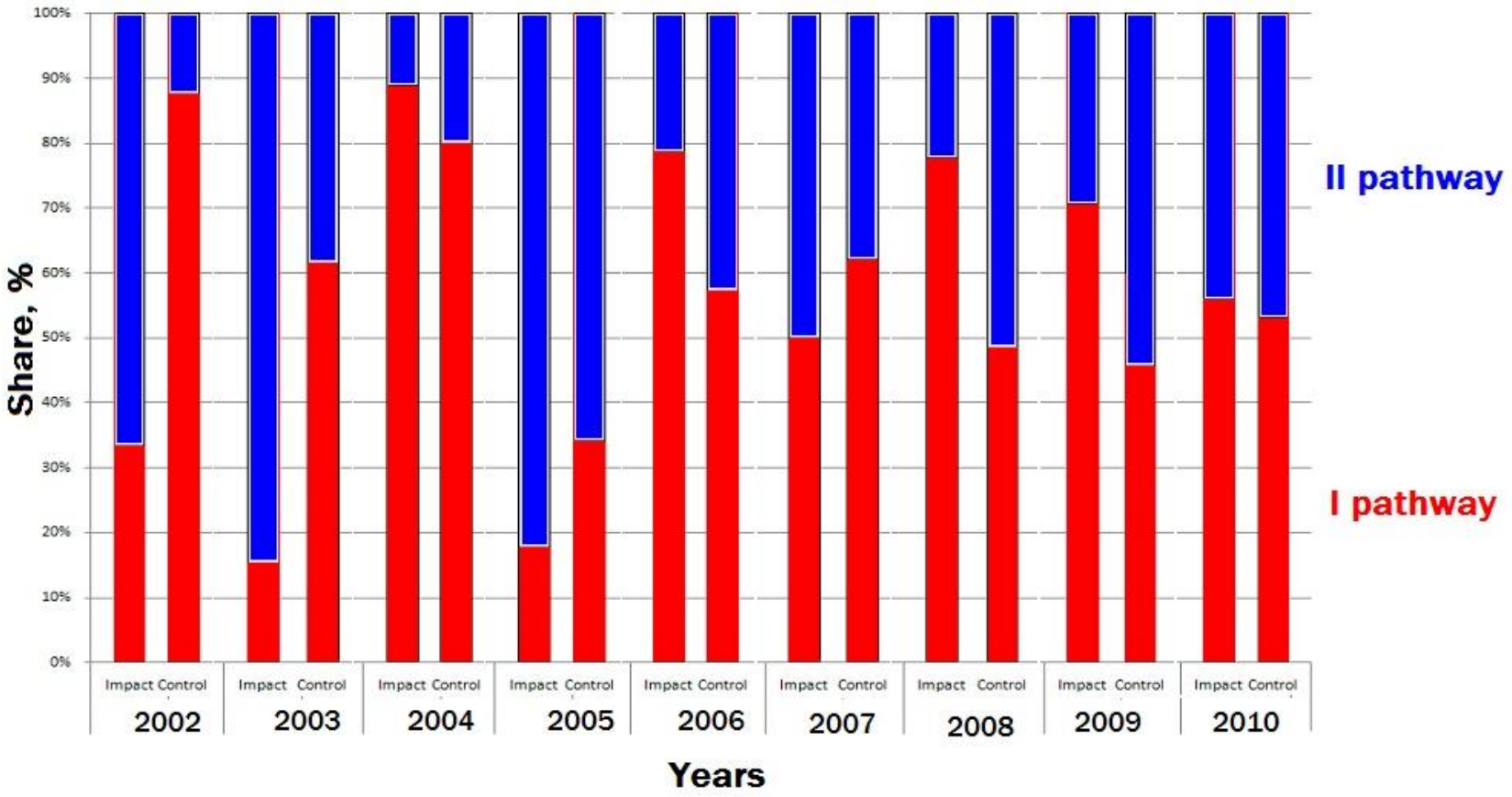
In natural populations this can influence the ratio of reproductive and nonreproductive animals and, consequently, the population size.

Olenev, Grigorkina. Russian Journal of Ecology. 1998. V. 29. No. 6. 401–405.



Dynamics of the ratio between the types of ontogeny in murine rodents (en example of bank vole (*Clethrionomys glareolus)*, 25 years monitoring)

Olenev. Russian Journal of Ecology. 2002. V. 33. No. 5. 321–330

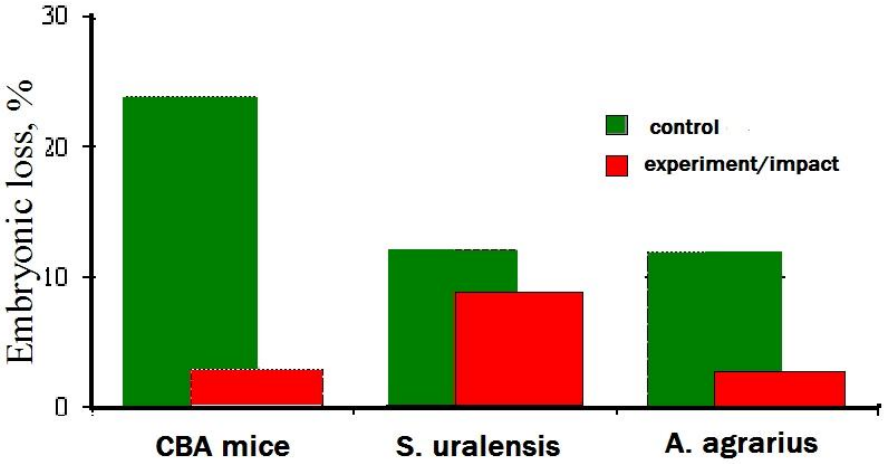


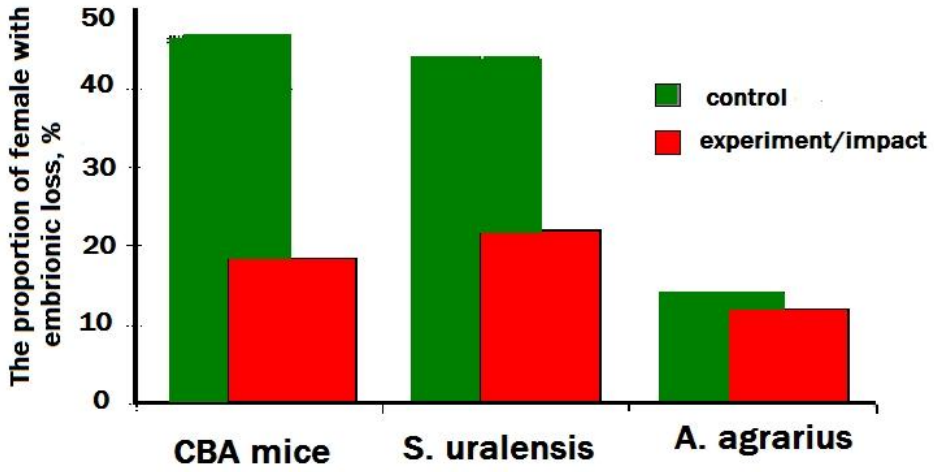
Dynamics of the ratio between the types of ontogeny in *S. uralensis* from EURT zone and control plots (2002-2010)

Grigorkina, Olenev. Radioprotection. 2011. V. 46. No 6. 437–443

The of types ontogeny ratio is varied in broad range from 15 to 90%.

Individuals of the **first type of ontogeny** - the mature children of overwintered animals, which form the second generation, dominate in the impact population.



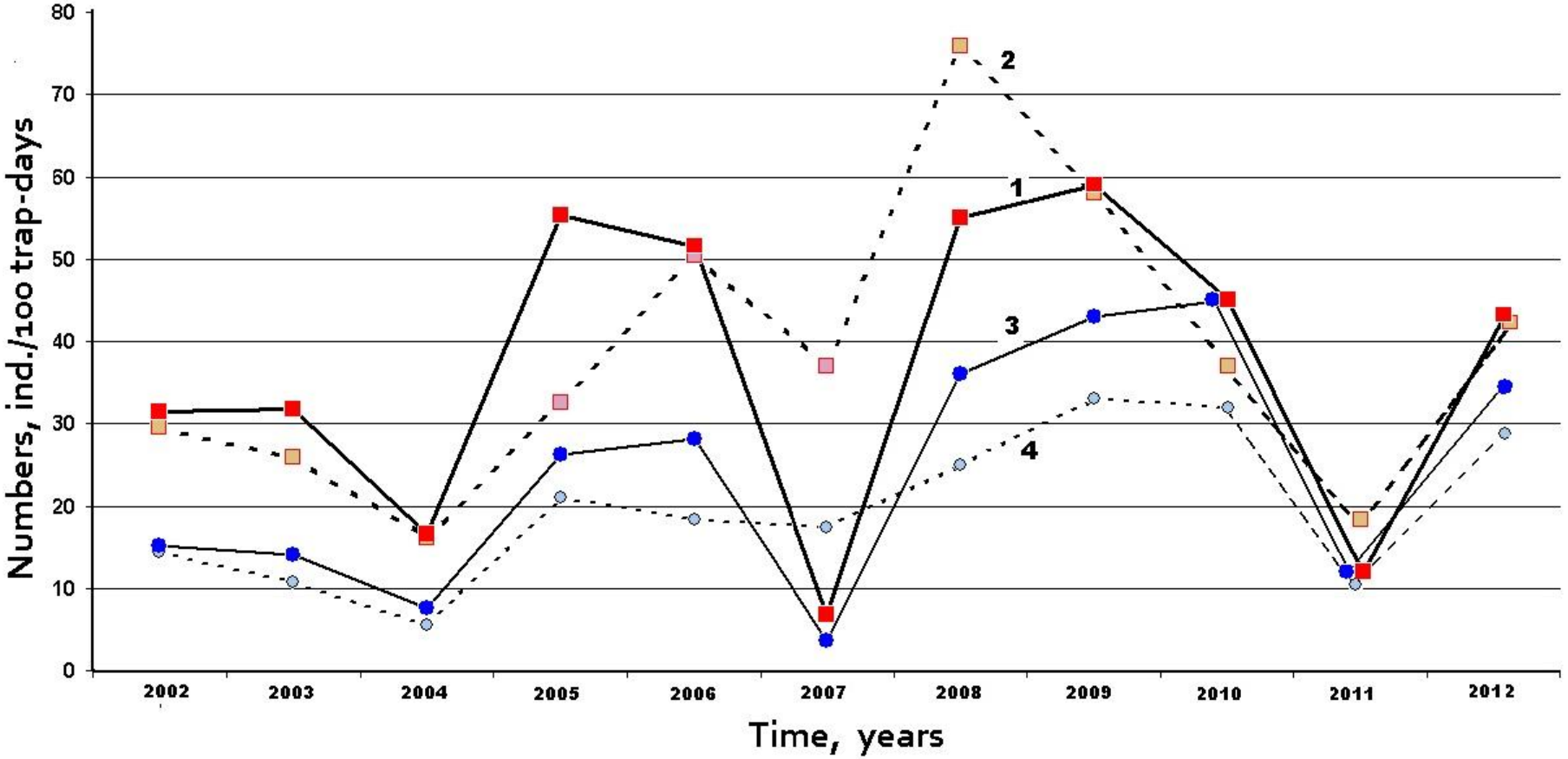


Embryonic loss and the proportion of females with embryonic loss in CBA mice in a model experiment and in rodents from the EURT zone and contral area

***Grigorkina*. Doklady Biological Sciences, *2007*. V. 412. 27–29**

Reproductive characteristics of EURT mice: actual fecundity was higher, whereas embryonic mortality and the proportion of females with embryonic losses were significantly lower compared with neighboring areas.

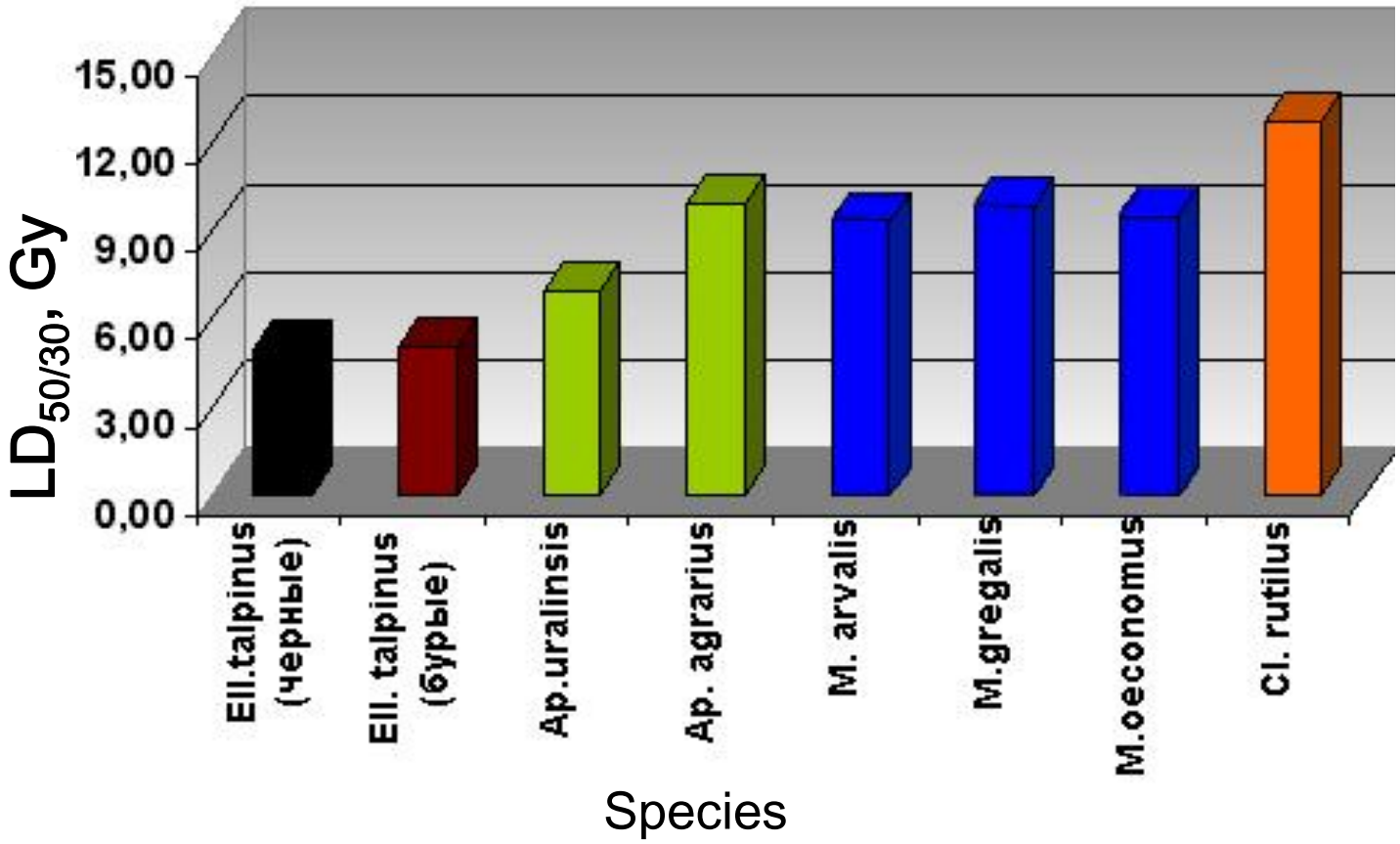
Thus, stability of a population, as homeostatic system, is reached due to intensification of metabolism and population reproduction i.e. increase of proportion of first type' of ontogeny individuals (mature and breeding young of the year).



Numbers of rodents (1, 2) and *S. uralensis* (3, 4) in the impact and background plots (2002-2012, annual average values)

Grigorkina, Olenev. Radioprotection. 2011. V. 46. No 6. 437–443

All these mechanisms: complex age structure, variability in dynamics of types ontogeny ratio, intensification of reproduction, reduced embryonic loss and proportion females with embryonic losses, age cross, and also high heterogeneity of overwintered provide high number of a population in a zone of radioactive pollution. Dynamics of numbers are resulting of all population processes.

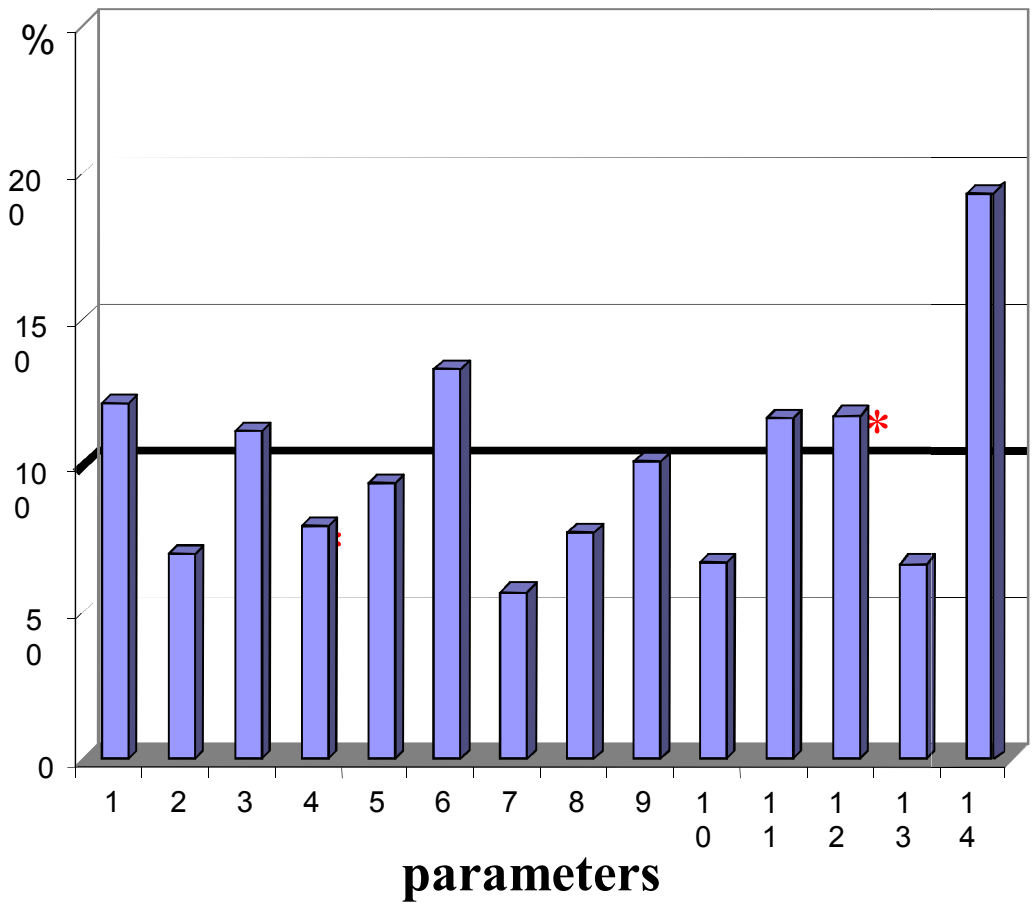


Radioresistance of rodents inhabiting EURT zone (Southern Urals)

Grigorkina. Doklady Biological Sciences. 2002. V. 385. 371–373

We studied next characteristics in animals of differein ecological specialization from EUTR zone and control site:

- ✓ **Haematological**
- ✓ **Immunological**
- ✓ **cytogenetic**
- ✓ **micronuclea test**
- ✓ **adaptive response**
- ✓ **rates of 90Sr accumulation in the bone tissue of rodents**



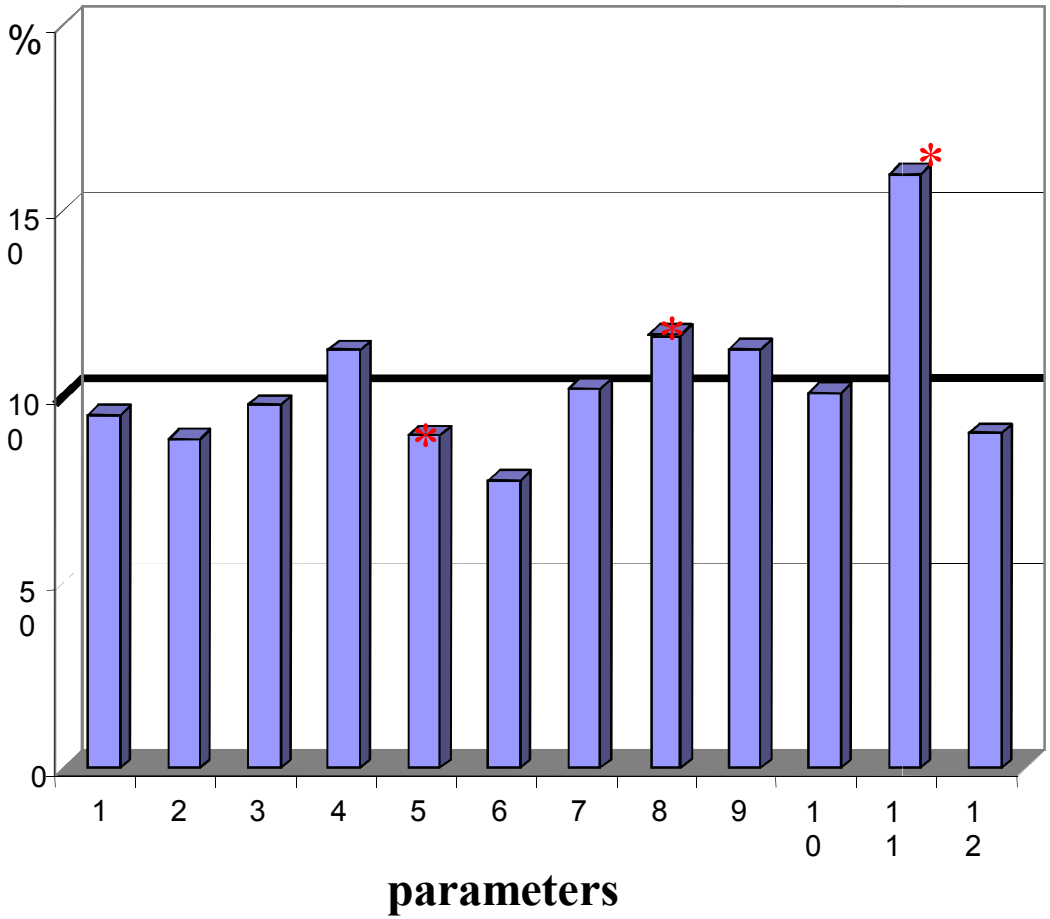
1.Splenocytes, *106/mcl
2. Thymocytes, *106/ mcl
3. Mielocariocytes, *106/ mcl
4. Leucocytes, *103/ mcl
5.Erythrocytes, *106/mcl
6.Reticulocytes, %
7. Eosiniphyles, %
8.St-neutrophyles, %
9.S-neutrophyles, %
10. Monocytes, %
11. Lymphocytes, %
12. Small lymphocytes, %
13. Plasmocytes, %
14. Abnormal leucocytes, %

* - significant differences compared to the control, $p \leq 0,05$

Hematological parameters in *Ellobius talpinus* from the EURT zone (1000 Ci/km2), % from the control

Grigorkina, Pashnina. Radiat. biology. Radioecology. 2007. V. 47. No. 3. 389–396 (in Russian)

We revealed that mole-voles from the EURT zone did not display any signs of damage of hemapoiesis while inhabiting the more radioactively contaminated plot than mice and voles did.



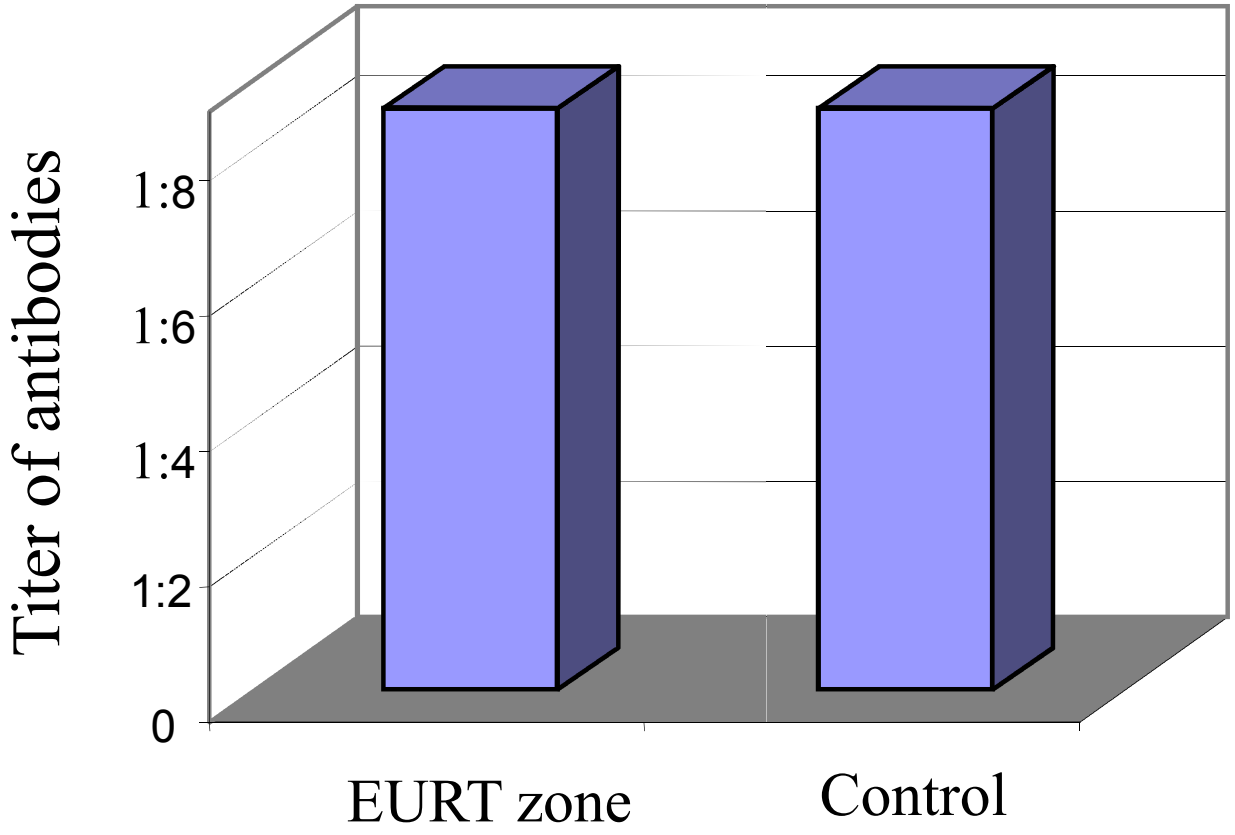
1. T- lymphocytes, %
2. B- lymphocytes, %
3. T-helpers, %
4. T-cytotoxical, %
5. Regulatory index (Th/Tc)
6. % of phagocyting neutrophyles (% PhN), %
7. Phagocyte number (PhN)
8. Index of stimulation of %PhN
9. Index of stimulation of PhN
10. Activity of the peroxidase of the blood, mM/min/l
11. Specific activity of the peroxidase of the blood, mM/min/l*106
12. Activity of the complement, u.e.

* - significant differences compared to the control, $p \leq 0,05$

Immunological parameters in *Ellobius talpinus* from the EURT zone (1000 Ci/km2), % from the control

Grigorkina, Pashnina. Radiat. biology. Radioecology. 2007. V. 47. No. 3. 389–396 (in Russian)

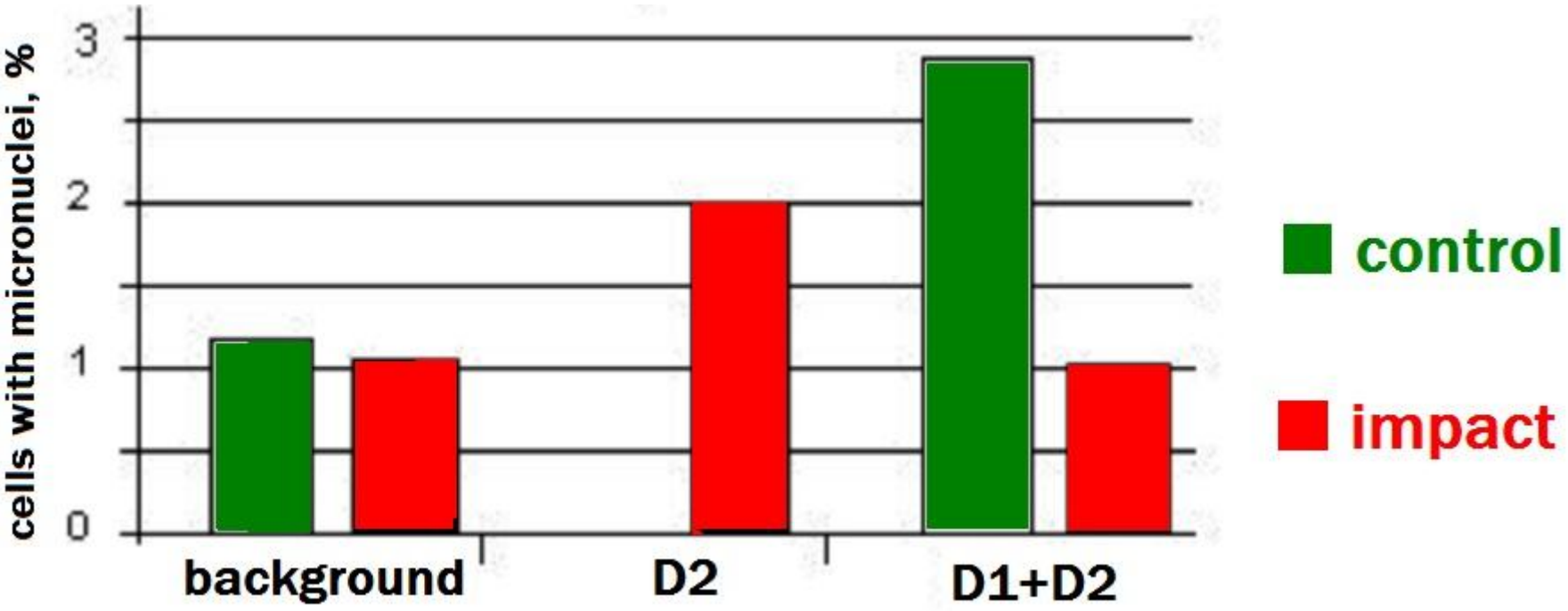
Some signs of activation of the immune system of mole-voles were recorded: increase of the ratio of small lympholeucocytes, higher values of stimulation indices (percent of phagocyting neutrophyles, phagocytes number and specific activity of peroxidase of the blood) in comparison with a control group.



Titer of antibodies to the vaccine "Trivac" in *Ellobius talpinus* Pall. from the EURT zone (1000 Ci/km2) and control site

Grigorkina, Pashnina. Radiat. biology. Radioecology. 2007. V. 47. No. 3. 389–396 (in Russian)

Vaccination of animals showed the absence of differences between mole-voles from the impact and the control plots in titers of antibody to viruses of polyvalent dry vaccine. Consequently, the reserved abilities and the functional activity of immune systems in mole-voles from the epicenter of EURT were higher than in these animals from the control site.

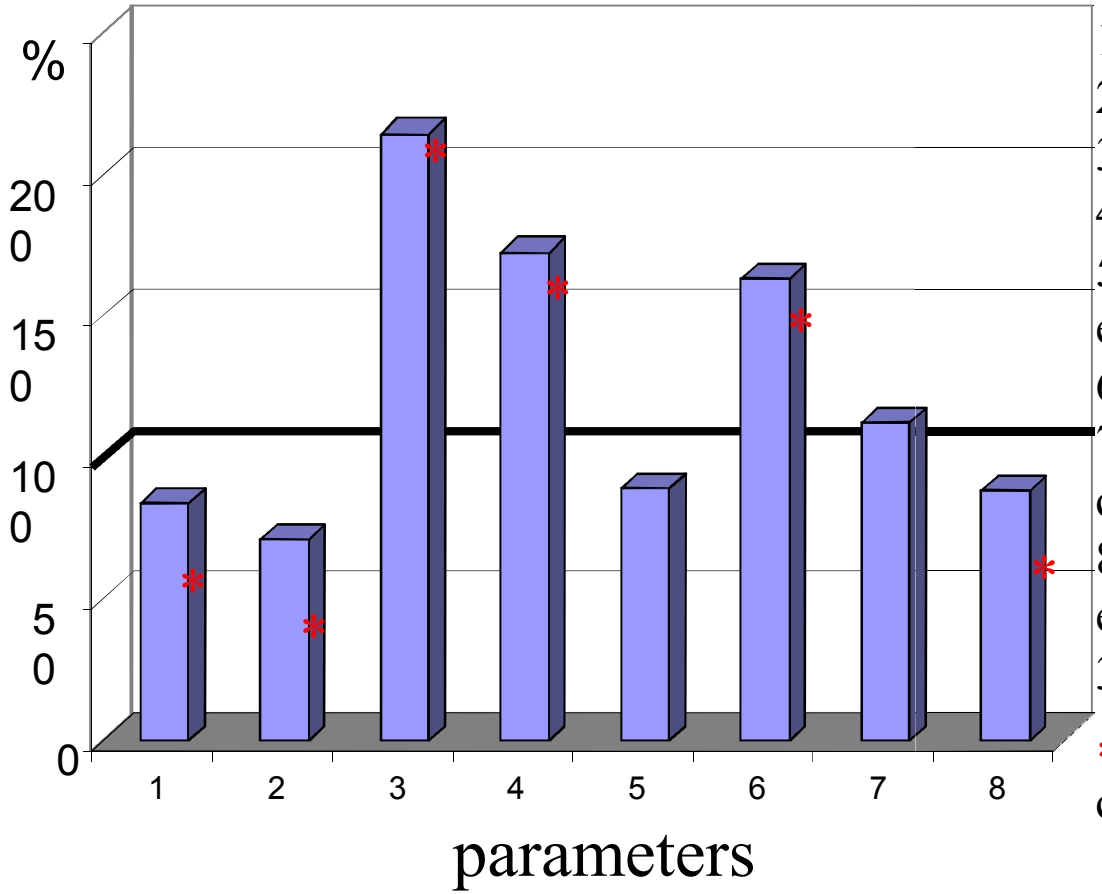


Adaptive Response in *Ellobius talpinus* from Radiocontaminated Environment

Grigorkina. Doklady Biological Sciences. 2010. V. 430. 42–44.

The frequency of occurrence of cells with numerous micronuclei of various shape (spheroid, tubule-like, and comma shapes) in the impact group was **four times higher** than in the control. Irradiation with the damaging dose (*D*2=2Gy) resulted in a two fold increase in the number of cells with micronuclei in the impact animals. However, in impact group *D*1+*D*2 (**0.02 Gy+2.0 Gy)**, their number was as lower than in spontaneous test. Notably in control group *D*1+*D*2, this value was greater by a factor of 2,7.

Thus it is the first case was detected the adaptive response in mole-voles dwelling in contaminated zone**, which can be regarded as convincing evidence for the development of**

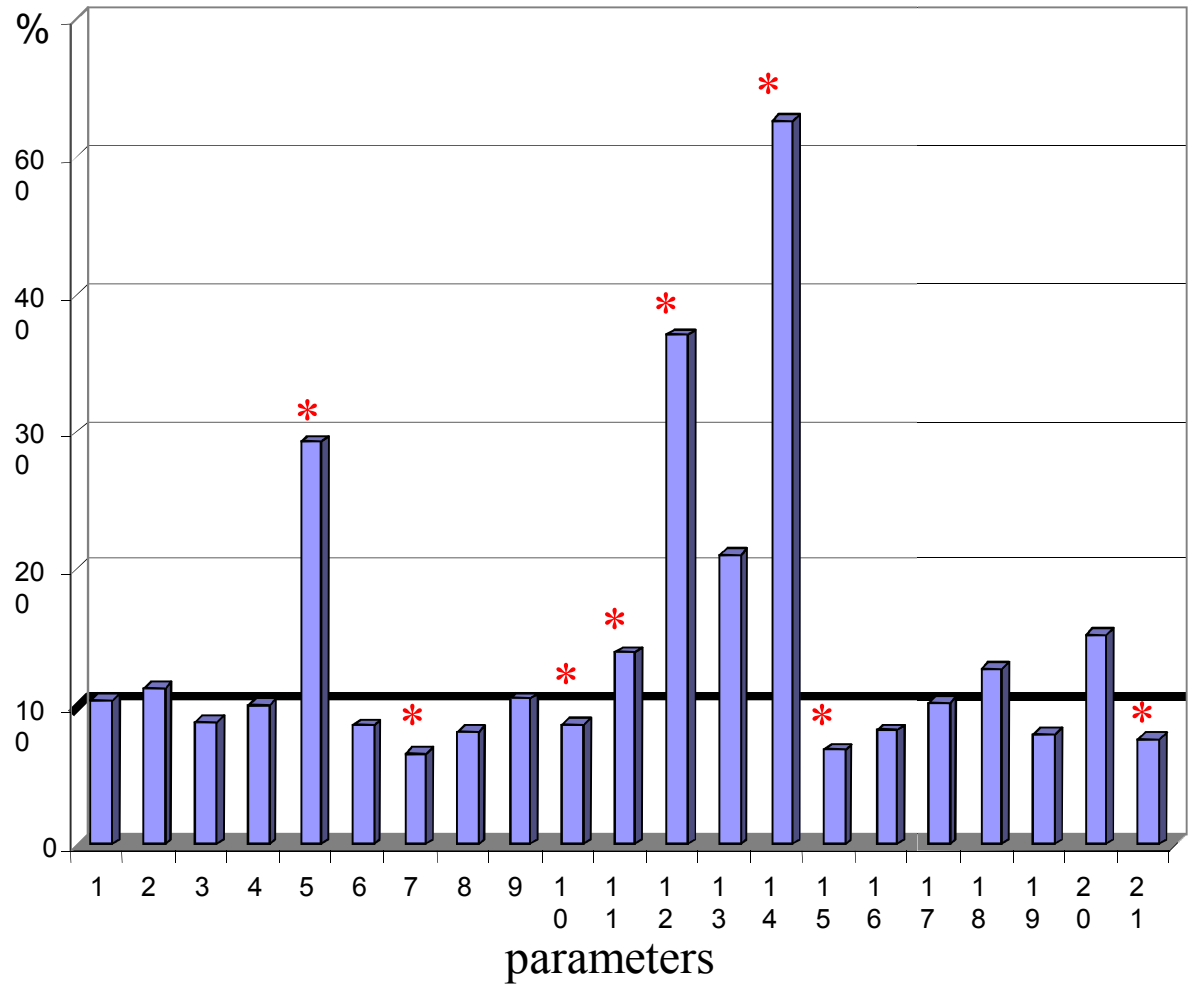


1.Erythrocytes, *10^6/mcl
2.Reticulocytes, %
3. Erythroblastes, %
4. Basophylic erythroblastes, %
5. Polychromatophylic erythroblastes, %
6. Oxiphylic erythroblastes, %
7. Total number of erythropoietic cells, %
8. Index of maturing of erythropoietic cells
3-8 – parameters of mielogram
* - significant differences compared to the control, $p \leq 0,05$

Erythropoietic parameters in *S. uralensis* Pall. from the EURT zone (451 Ci/km^2), % from the control

Grigorkina, Pashnina. Radiat. biology. Radioecology. 2007. V. 47. No. 3. 389–396 (in Russian)

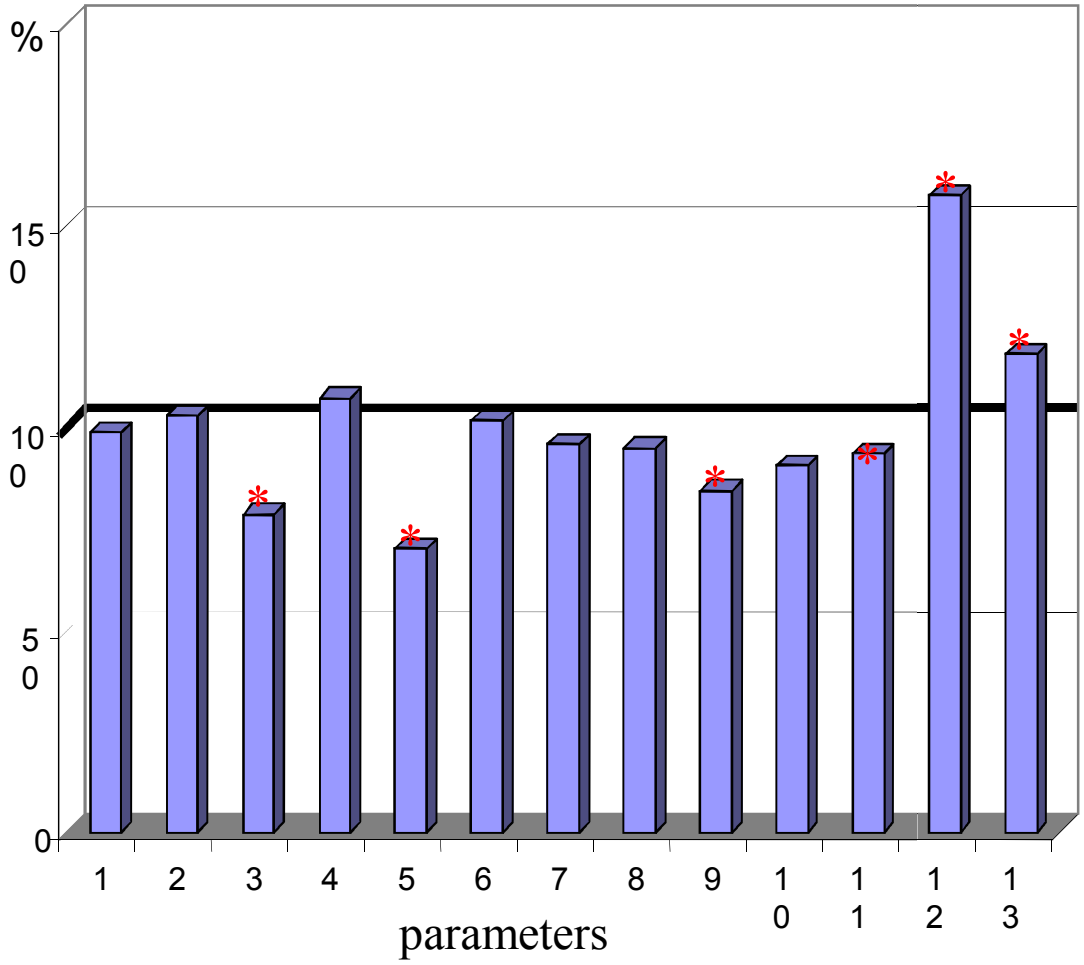
On the contrary, in mice and red-backed voles from the EURT zone displayed multiple alterations in the hemopoietic system, delay in the maturation of erythropoietic cells in the bone marrow.



1.Splenocytes, *10^6/mcl
2. Thymocytes, *10^6/ mcl
3. Mielocariocytes, *10^6/ mcl
4. Leucocytes, *10^3/ mcl
5. Eosinophyles, %
6.St-neutrophyles, %
7.S-neutrophyles, %
8. Monocytes, %
9. Lymphocytes, %
10. Small lymphocytes, %
11. Plasmocytes, %
12. Abnormal leucocytes, %
13. Blastes, %
14. Mieloblastes, %
15. Promielocytes, %
16. Mielocytes, %
17. Metamielocytes, %
18. Eosinophyles, %
19. Lymphocytes, %
20. Monocytes, %
21. Index of maturing of neutroph. cells
13-21 – parameters of mielogram
* - signifnt differences compared to the control, $p \leq 0{,}05$

Leucopoietic parameters in *S. uralensis* from the EURT zone (451 Ci/km2), % from the control

Grigorkina, Pashnina. Radiat. biology. Radioecology. 2007. V. 47. No. 3. 389–396 (in Russian)

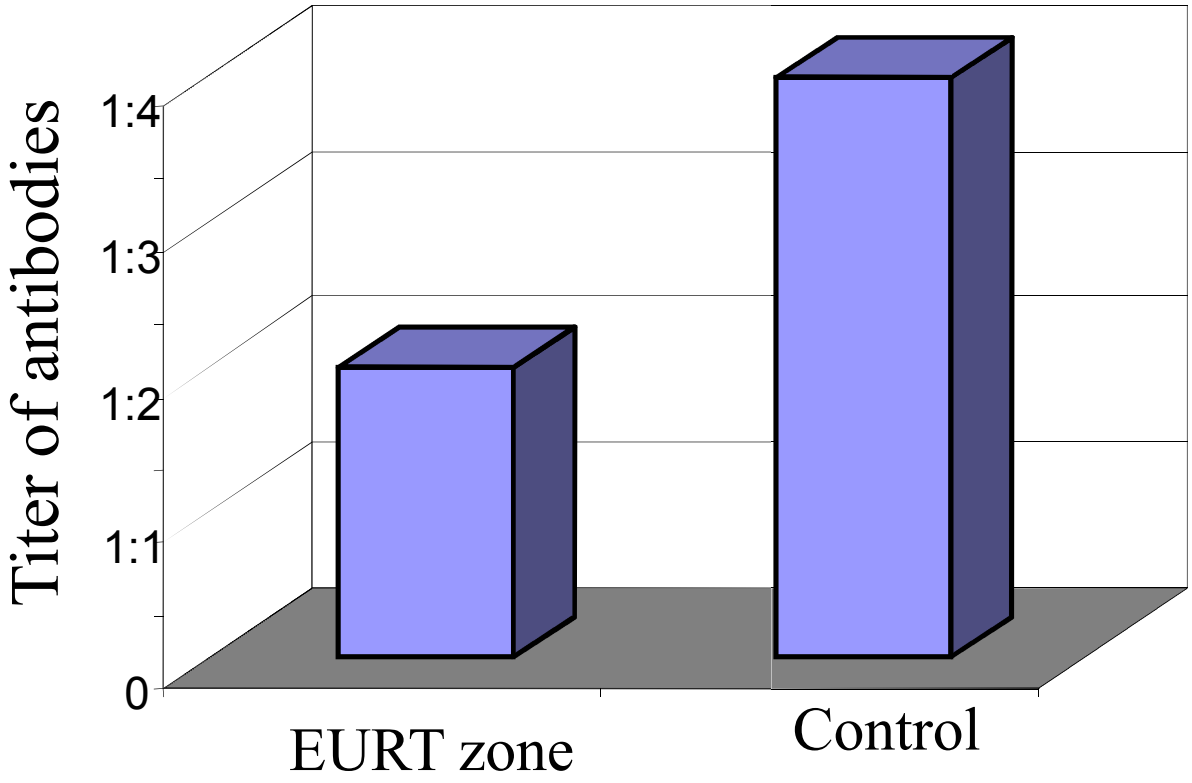


1. T- lymphocytes, %
2. B- lymphocytes, %
3. T-helpers, %
4. T-cytotoxical, %
5. Regulatory index (Th/Tc)
6. % of phagocyting neutrophyles (%PhN), %
7. Phagocyte number (PhN)
8. Index of stimulation of %PhN
9. Index of stimulation of PhN
10. Activity of the peroxidase of the blood, mM/min/l
11. Specific activity of the peroxidase of the blood, mM/min/l*10^6
12. Circulating immune complexes, u. e.
13. Activity of the complement, u.e.

* - significant differences compared to the control, $p \leq 0,05$

Immunological parameters in *S. uralensis* from the EURT zone (451 Ci/km2), % from the control
Grigorkina, Pashnina. Radiation biology. Radioecology. 2007. V. 47. No. 3. 389–396 (in Russian)

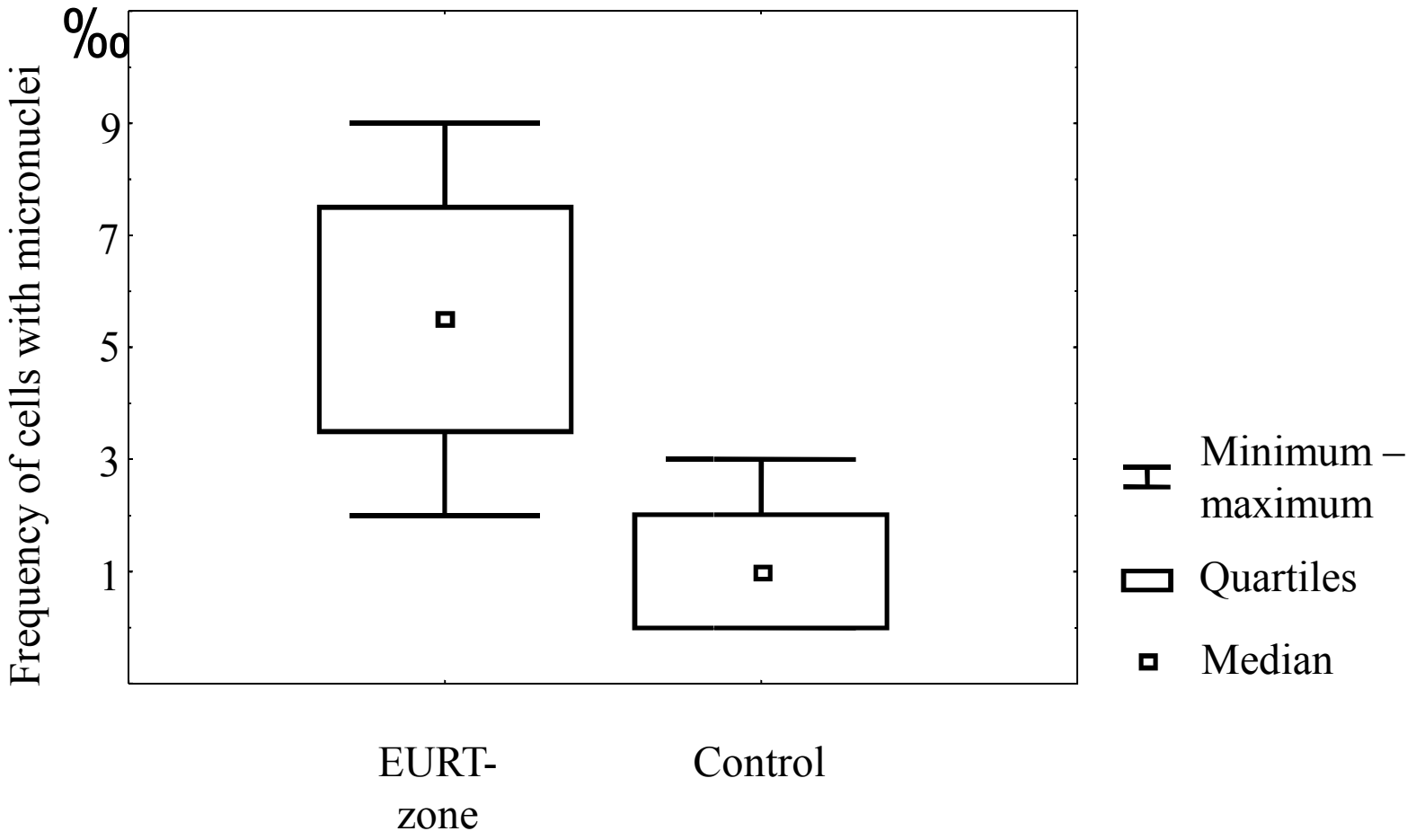
Structural violations of cells of the lymphopoietic group would inevitably change their functional activity and reserved possibilities. Lowering in the index of stimulation of phagocyte numbers as well as lowered relative activity of peroxidase might serve as an evidence of this.
Significant increase in circulating of immune complexes was also revealed in mice.



Titer of antibodies to the vaccine "Trivac" in *A. agrarius* from the EURT zone (451 Ci/km2) and control site

Grigorkina, Pashnina. Radiation biology. Radioecology. 2007. V. 47. No. 3. 389–396 (in Russian)

In mice from the EURT zone displayed lesser antibody titer than animals from the control site and increase in the frequency of cells with micronuclei.



Frequency of cells with micronuclei in mice and voles

Grigorkina, Pashnina. Radiat. biology. Radioecology. 2007. V. 47. No. 3. 389–396 (in Russian)

Different indices of rodents from the EURT zone and undisturbed areas

Grigorkina, Olenev. ***Radioprotection. 2009. V. 44. No 5. 129–134***

Species	Frequency of cells with micronuclei and chromosome lesions	Hematological indices	Immunological indices
Wood mice	+*	+*	+*
Field mice	+*	+*	+*
Northern red-backed voles	+*	+*	No data
Mole voles	–	–	–

Note: * significant differences in the complex of indices of rodents from EURT zone compared to the control

Adaptive response in Northern mole voles (*Ellobius talpinus*) which inhabited a radioactively contaminated area in series of generations can be regarded as convincing evidence for development of genetic radioadaptation.

Specific activity of 90 Sr in bone tissue and frequency of chromosomal lesions in *S. uralensis* from the EURT zone and background sites

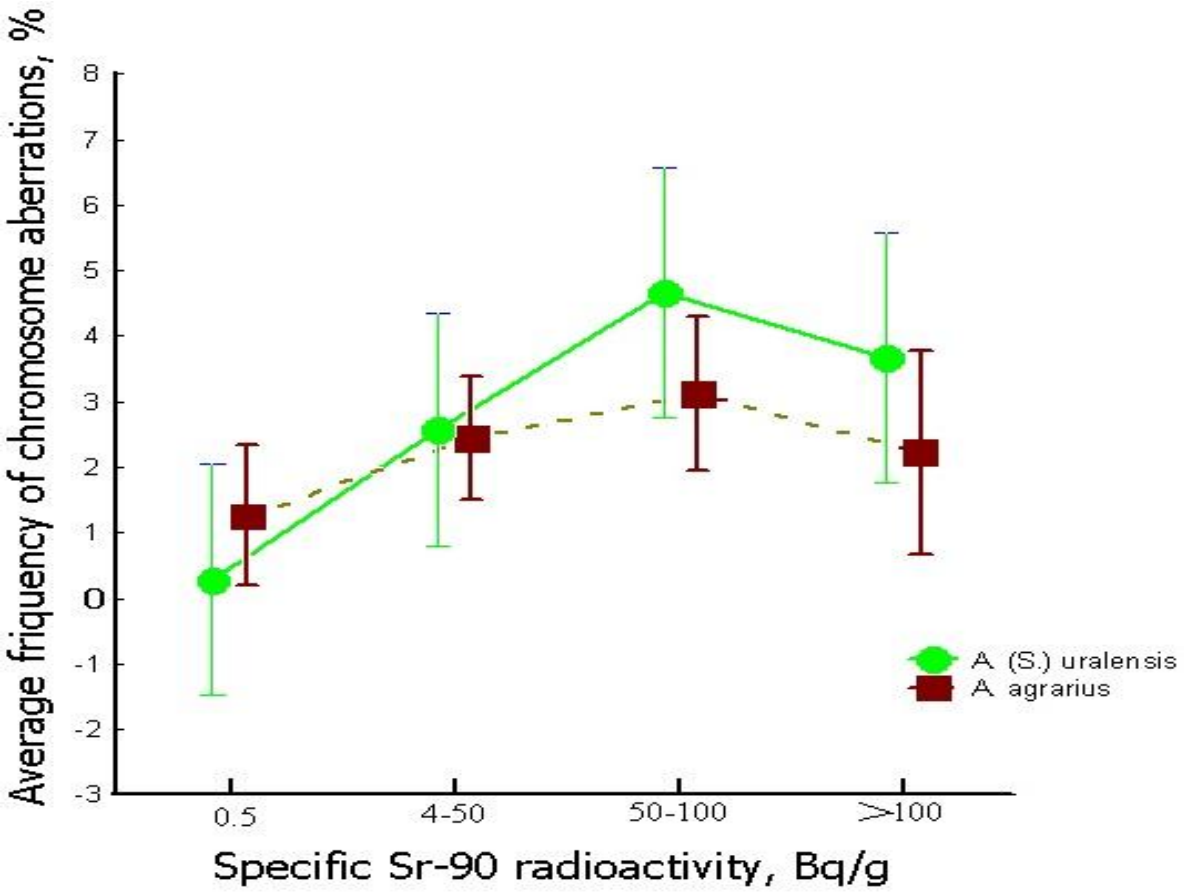
Yalkovskaya, Grigorkina, Tarasov. Biophysics. 2011. V. 56. No. 1. 140–144

Sites of capture	Number of animals (cells)	90Sr activity Bq/g,	Share of cells (%)		
			with chromosome aberrations	aneuploid and poliploid	With gapes
Berdenish (EURT)	10 (500)	75.7±15.9	4.20±1.25	0.60±0.31	5.00±1.20
Uruskul (EURT)	7 (350)	88.8±27.3	3.14±1.14	1.14±0.59	2.86±0.86
Metlino (control)	11 (550)	1.7±0.8	0.55±0.28	0.36±0.24	1.09±0.41
Uetskoe (control)	18 (900)	-	0.33±0.18	1.11±0.33	0.44±0.20
H (Kruskal-Walles test); *p*			χ^2; *p*		
		17.40 0.0002	38.541; <0.0001	3.069; 0.358	38.499; <0.0001

Specific activity of 90 Sr in bone tissue and frequency of chromosomal lesions in *A. agrarius* from the EURT zone and background sites

Yalkovskaya, Grigorkina, Tarasov. **Biophysics. 2011. V. 56. No. 1.140–144.**

Sites of capture	Number of animals (cells)	90Sr activity Bq/g,	Share of cells (%)		
			with chromosome aberrations	aneuploid and poliploid	With gapes
Berdenish (EURT)	39 (1950)	69.6±9.7	2.87±0.37	0.62±0.18	2.67±0.33
Uruskul (EURT)	12 (600)	12.0±5.6	2.17±0.63	0.33±0.22	2.67±0.51
Metlino (control)	18 (900)	2.62±1.1	1.33±0.39	0.33±0.18	2.88±0.54
Uetskoe (control)	10 (500)	-	1.40±0.67	0.40±0.26	1.80±0.47
H (Kruskal-Walles test); *p*			χ^2; *p*		
		36.046 < 0.0001	8.602; 0.035	1.487; 0.685	1.600; 0.660

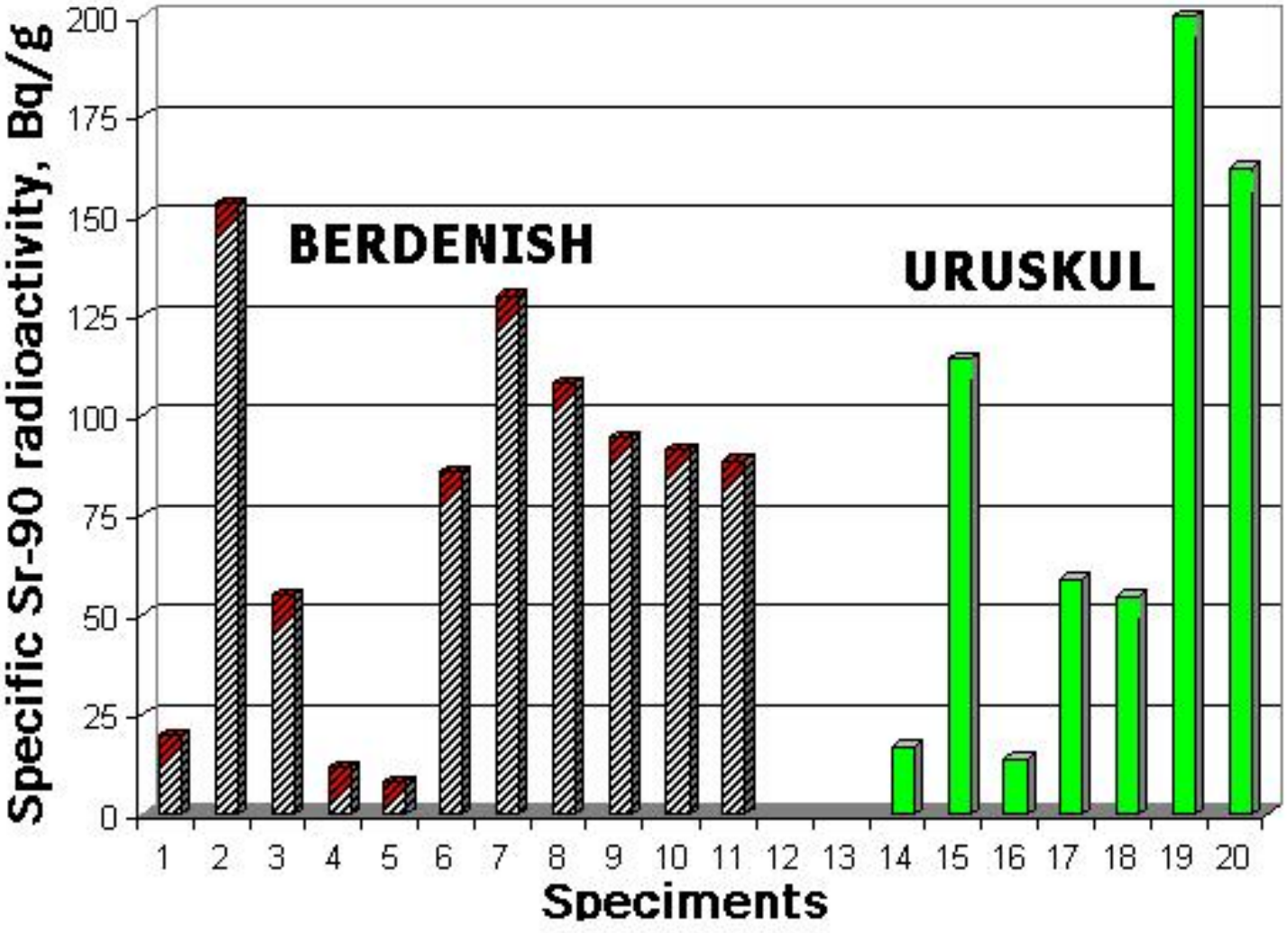


Frequency of chromosomal aberrations in *S. uralensis* and *A. agrarius* depending on specific activity of 90Sr in bone tissue

Yalkovskaya, Grigorkina, Tarasov. Biophysics. 2011. V. 56. No. 1. 140–144.

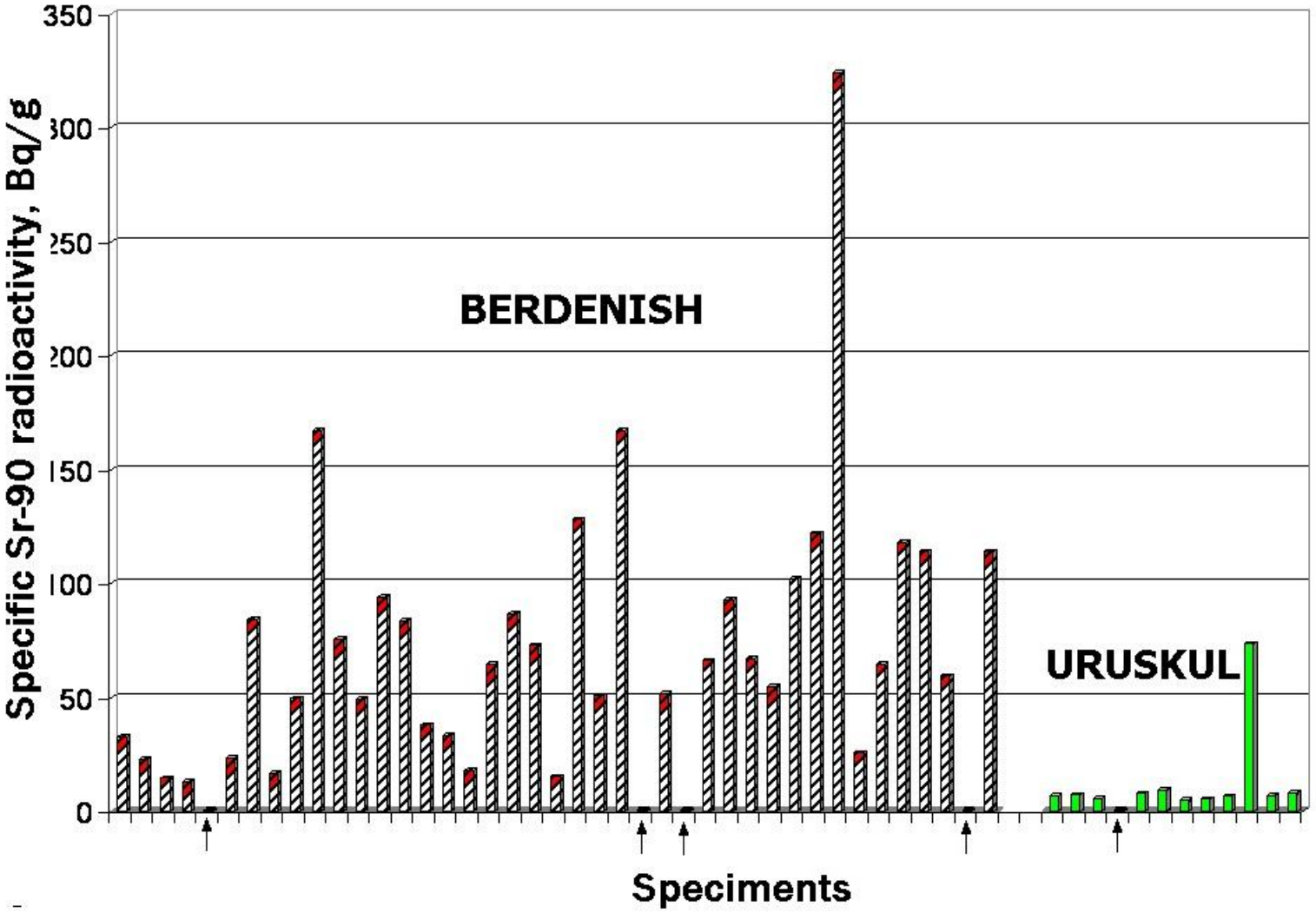
Significant positive correlation of the frequency of aberrant cells and the concentration of ^{90}Sr (Rs = 0.514, p = 0.007) (Rs = 0.514, p = 0.007).

An excess over background values of radionuclide accumulation by eight or more times leads to a significant increase in chromosomal instability at the population level.



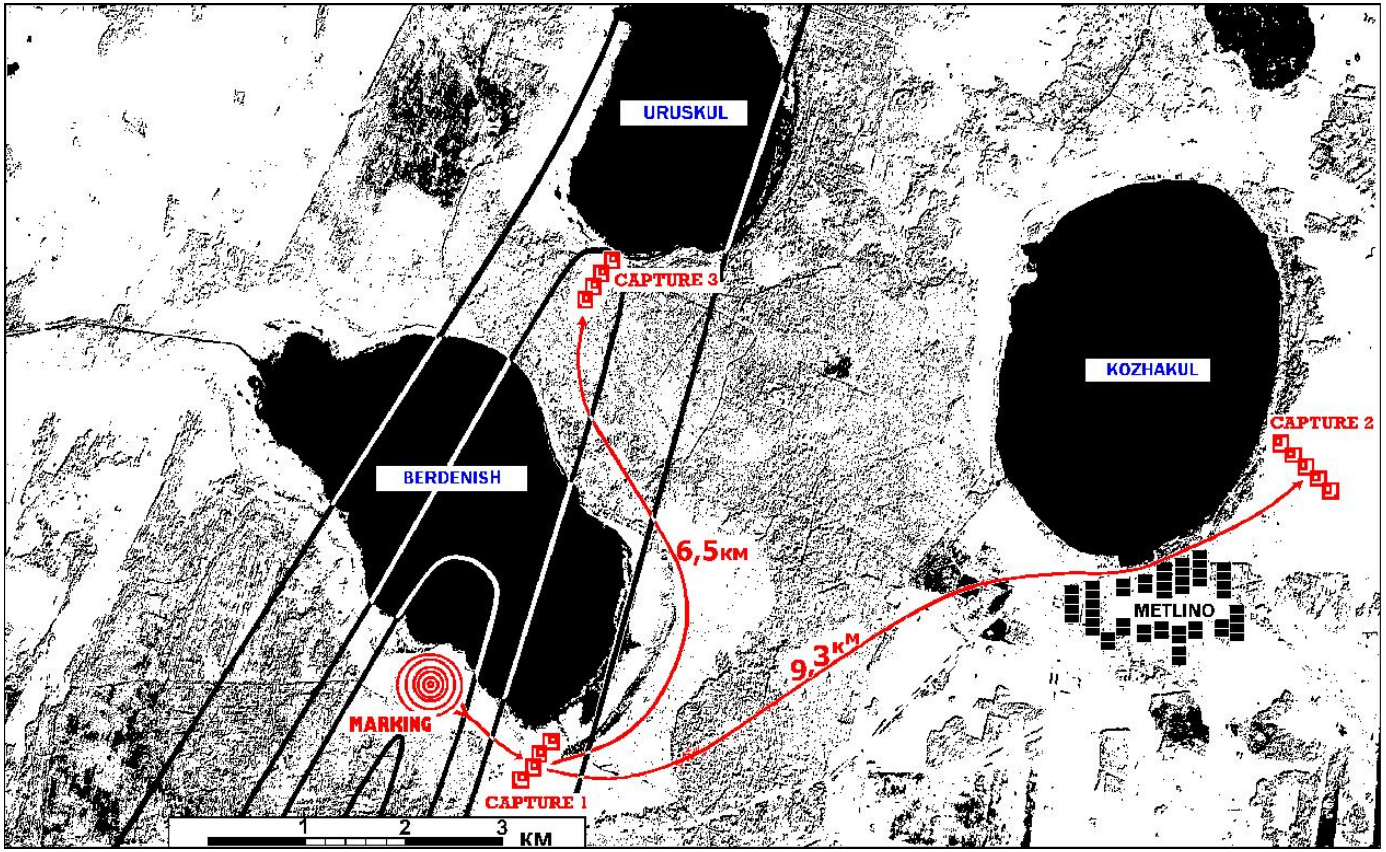
Specific activity of Sr-90 in bone tissue of *S.uralensis* from the EURT zone (Berdenish - 451 Ci/km2, Uruskul - 62 Ci/km2; individual data)

***Yalkovskaya, Grigorkina, Tarasov*. Biophysics. 2011. V. 56. No. 1. 140–144.**



Specific activity of Sr-90 in bone tissue of A. agrarius from the EURT zone (Berdenish - 451 Ci/km2, Uruskul - 62 Ci/km2; individual data)

Yalkovskaya, Grigorkina, Tarasov. ***Biophysics. 2011. V. 56. No. 1.140–144.***



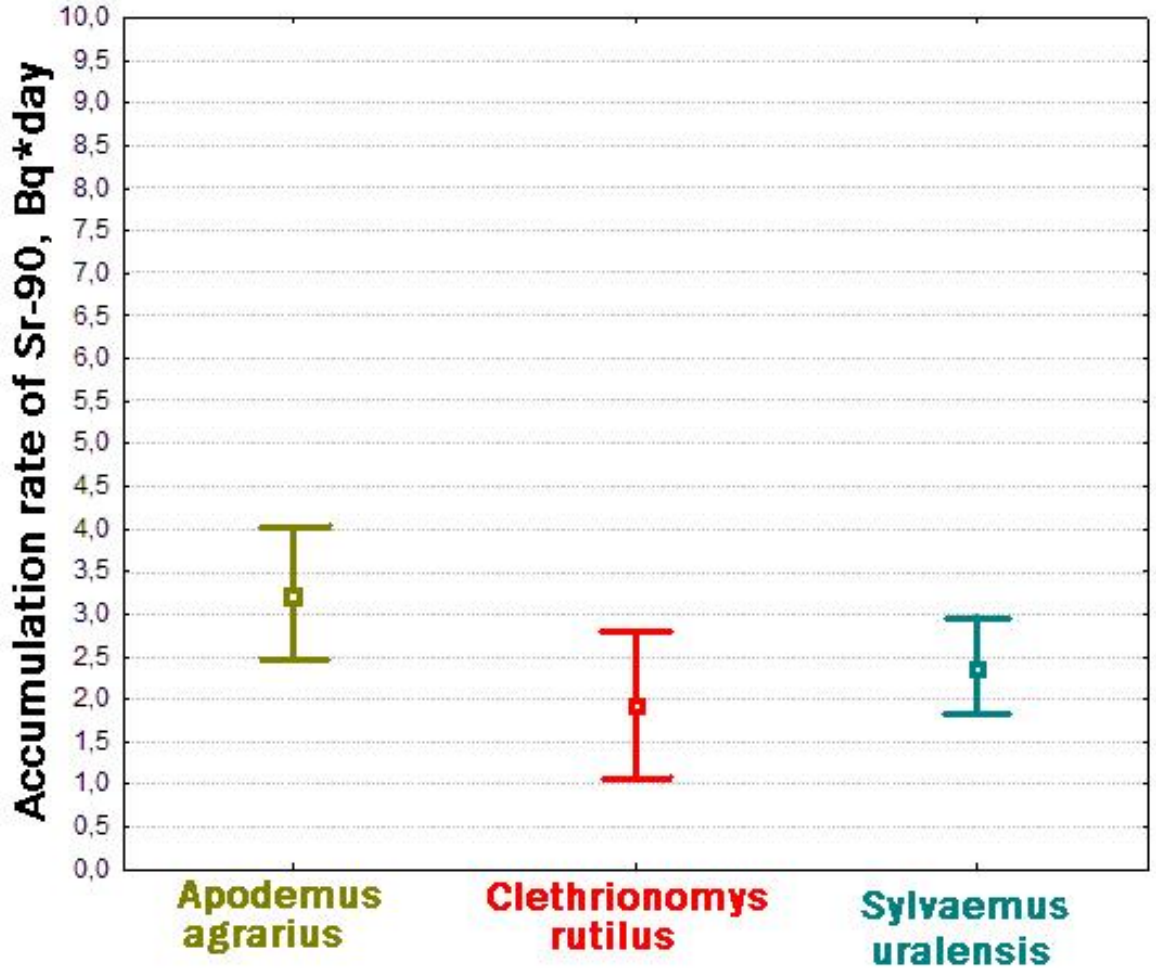
Scheme of marking of rodents by tetracycline in the central part of EURT zone and capture ones at adjacent areas

Grigorkina, Olenev. Radiat. Biology. Radioecology. 2013. V. 53, No 1.76–83 (in Russian)

The ratio of rodent's migrants is varied up to 40% in the narrow and extended EURT zone:

(1) Migrations are considerably decrease the probability that certain adaptive changes will be fixed and inherited in a series of generations;

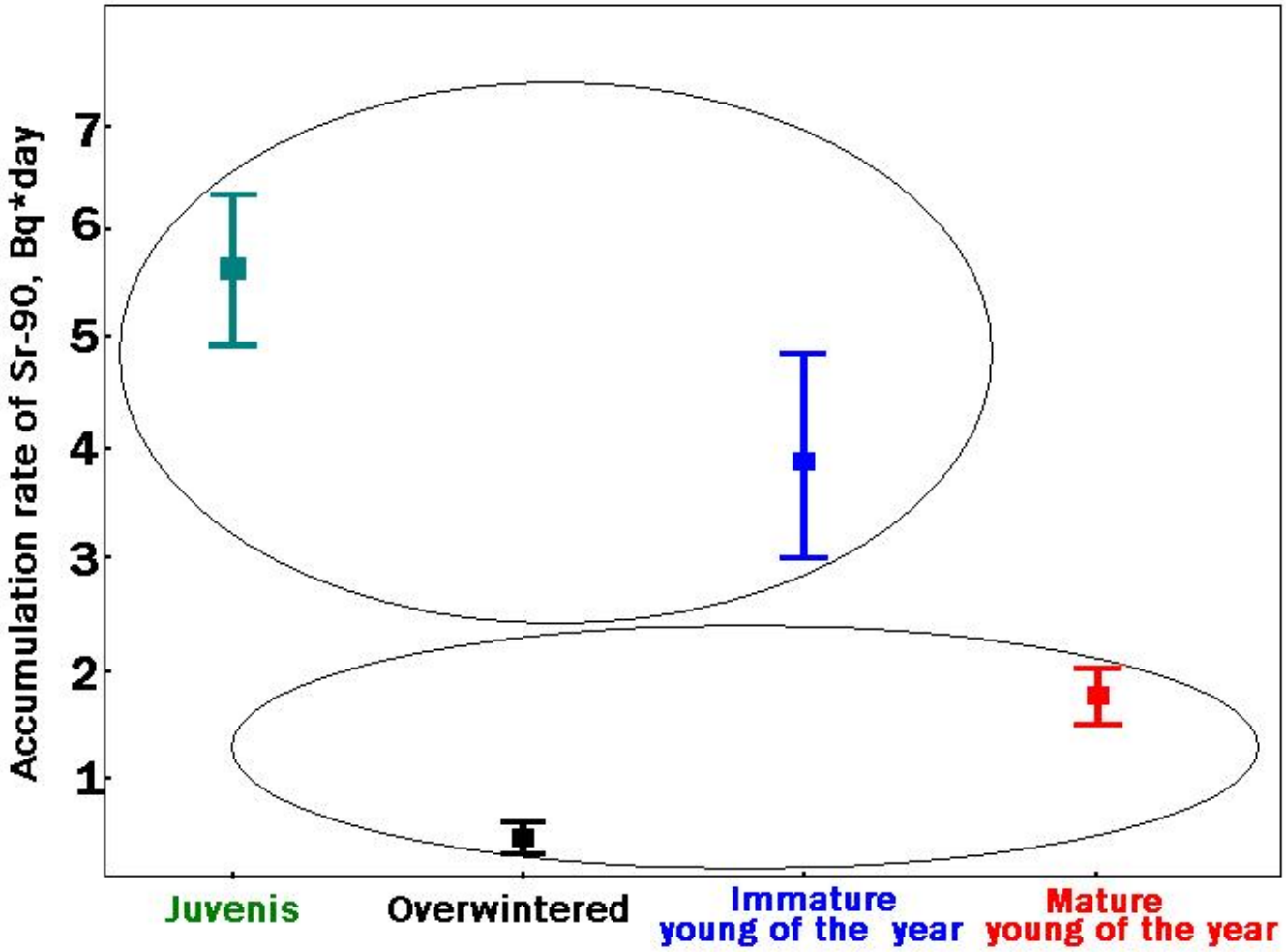
(2) Migrations are the base for transmission of radioinduced effects in adjacent areas. The fact of a flowing population should be taken into account in a wide spectrum of investigations at the analysis of the remote consequences of chronic radiation.



Interspecies comparison of accumulation rate of 90Sr in modal rodents' groups

Grigorkina et al. Vestnik Ural. Med. Acad. Nauki. 2009. No2 (25). 135-136 (in Russian)

Accumulation rate of Sr-90 in bone tissue of both different species and functional status calculated under the formula: 90Srv = [90Sr] /age = [Bq/g*day].



Accumulation rate of 90Sr in animals of different functional status

Grigorkina et al. Vestnik Ural. Med. Acad. Nauki. 2009. No 2 (25). 135-136 (in Russian)

At the intrapopulational level (for S. uralensis), we can see effect significant in Sr90 accumulation rate (**Kruskal-Walles test -** 30.8; $p < 0.001$). **Thus, the results of the analysis testify, that the specific rate of 90Sr accumulation is determined by the functional status of animals.**

Conclusions

Thus, we illustrate the ambivalent nature of radioresistance of rodents of different ecological specialization.

There are no pathological shifts in more radiosensitive mole-voles from the more radioactively contaminated site (90Sr - 37 MBq/m2 = 1000 Ci/km2).

One might conclude that long-term inhabitance of mole-voles in the epicenter of the EURT zone (ca. 50 generations from the moment of Kyshtym Accident) lead to compelled adaptation of animals to the radiocontaminated biocoenosis.

Mole-voles are subterranean borrowing-dwellers with a low ability for dispersal.

So their ecophysiological features and the territorial insulation of their settlement in the EURT zone for a half-century history of the Kyshtym Accident promoted the development of radioadaptation in this specie.

On the contrary, numerous immunological, hematological and cytogenetic disturbances were found in more radioresistant wood and field mice and northern red-backed voles inhabiting the EURT zone (451 Ci/km2) as compared with the reference samples. Mice and voles form of a flowing population due to their active migrations, so they slip off the prolonged influence of a damaging factor. This circumstance prevents the development of radioadaptation. Such a feature is favored by the EURT zone configuration that looks like a prolonged and narrow territory having rapidly decreasing gradient of contamination.

Hence, for the first time some convincing evidence of genetic radioadaptation of animals inhabiting the radiocontaminated zone during a long period and in the course of changing generations was obtained at the example of mole-voles (radiosensitive specie). The leading role in the development of radioadaptation is played by ecophysiological traits as well as by the main habits of a species.

Besides, the configuration of contaminated zone is maybe not less important ecological factor.

The key uncertainties in our field studies

The main **uncertainties** of radioecological investigations in rodents inhabiting the zone of local radioactive contamination – Eastern Urals Radioactive Trace (EURT), which influence our ability to estimate both the radionuclide accumulation and absorbed dose assessment:

- configuration and size of the contaminated zone;

- complex intrapopulational structure of mouse-likes rodents – alternative types of ontogeny development;

- migration of rodents;

- spatial and temporal heterogeneity of zone' contamination;

- non radiation ecological factors, which modify the effects of radiation exposure

Ways of overcoming uncertainties in the EURT zone

1) Usage the functional - ontogeny approach (Olenev, 2002) in practice of radioecological investigations, so it allows to work with homogeneous samples of rodents from natural environment;

2) To take into account both a role of a configuration of any technogenic polluted zone and rodents' migrations in adaptation.

3) To know the real accumulation rate of Sr-90 in individuals of different functional status and for study the role of migrations in Sr-90 accumulation in rodents' skeleton we decided to carry out the experiment with marking animals from clean area (including their offsprings which are reproduced in the experimental plot) in natural radioactive contaminated environment.

We built the enclosed experimental plot in the central part of the EURT zone - the most contaminated area (density of soil pollution by ^{90}Sr –16690 kBq/m^2 = 451 Ci/km^2). This experiment is going on now

What this experiment will give us?

(1) to receive the basic data on specific rate of ^{90}Sr accumulation in a skeleton of rodents of different age and functional status in respect to their exposition in the radioactive environment (experimental plot);

(2) by usage the radioactive label (^{90}Sr) one could retrospectively estimate a share of migrants from adjacent areas in materials of long-term monitoring;

(3) to come nearer to definition of dose loads from internal irradiation (^{90}Sr) - a key question of viability estimation of populations vertebrate animals;

(4) to clear mechanisms and strategy of small mammals adaptation in radioactive environment.


Thank you for attention