



УРАЛЬСКИЙ НАУЧНО-ПРАКТИЧЕСКИЙ
ЦЕНТР РАДИАЦИОННОЙ МЕДИЦИНЫ
Urals research Center for Radiation Medicine



Statens strålevern
Norwegian Radiation Protection Authority

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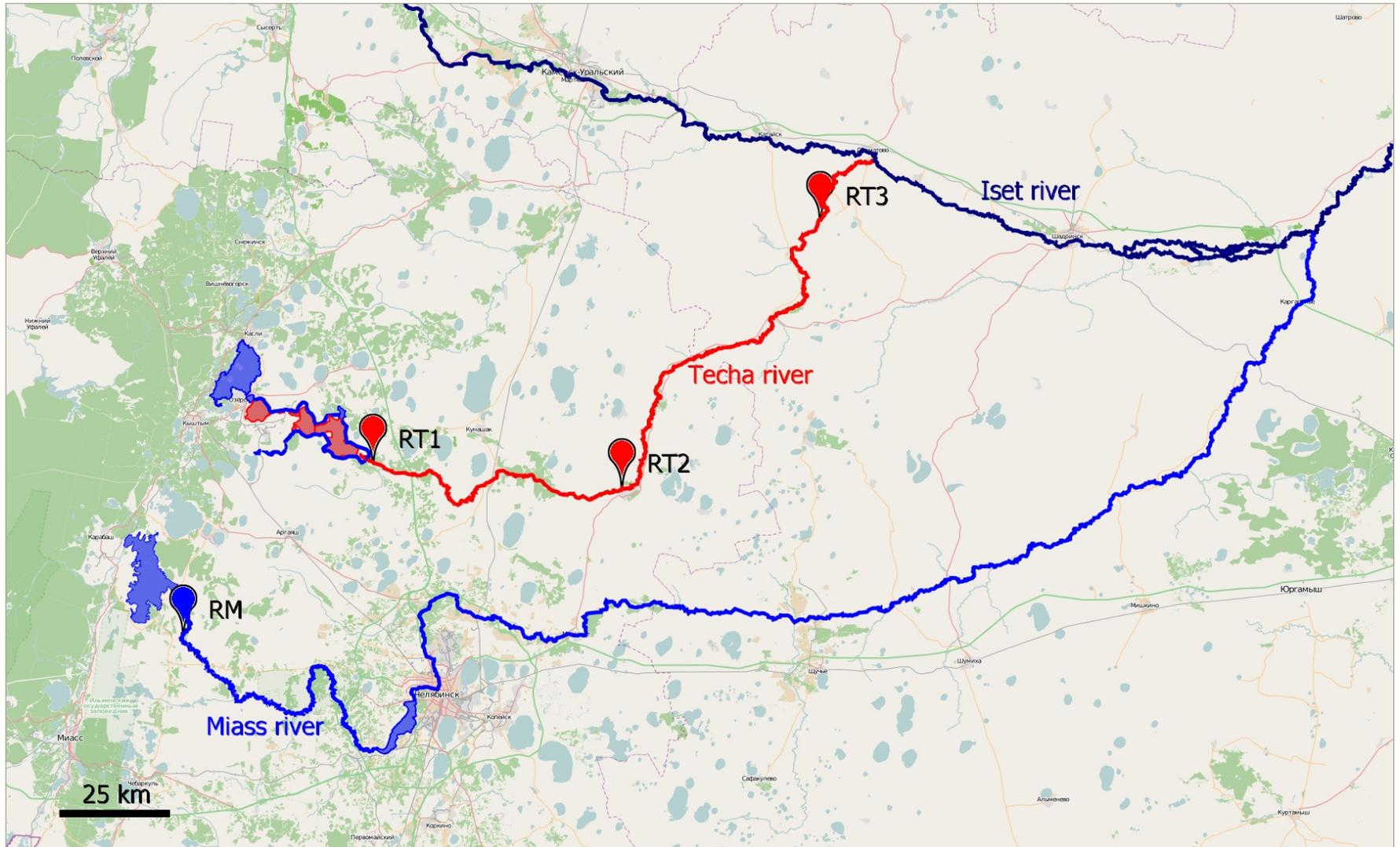
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ВЛИЯНИЕ РАДИАЦИОННОГО ВОЗДЕЙСТВИЯ НА ИХТИОФАУНУ р. ТЕЧА

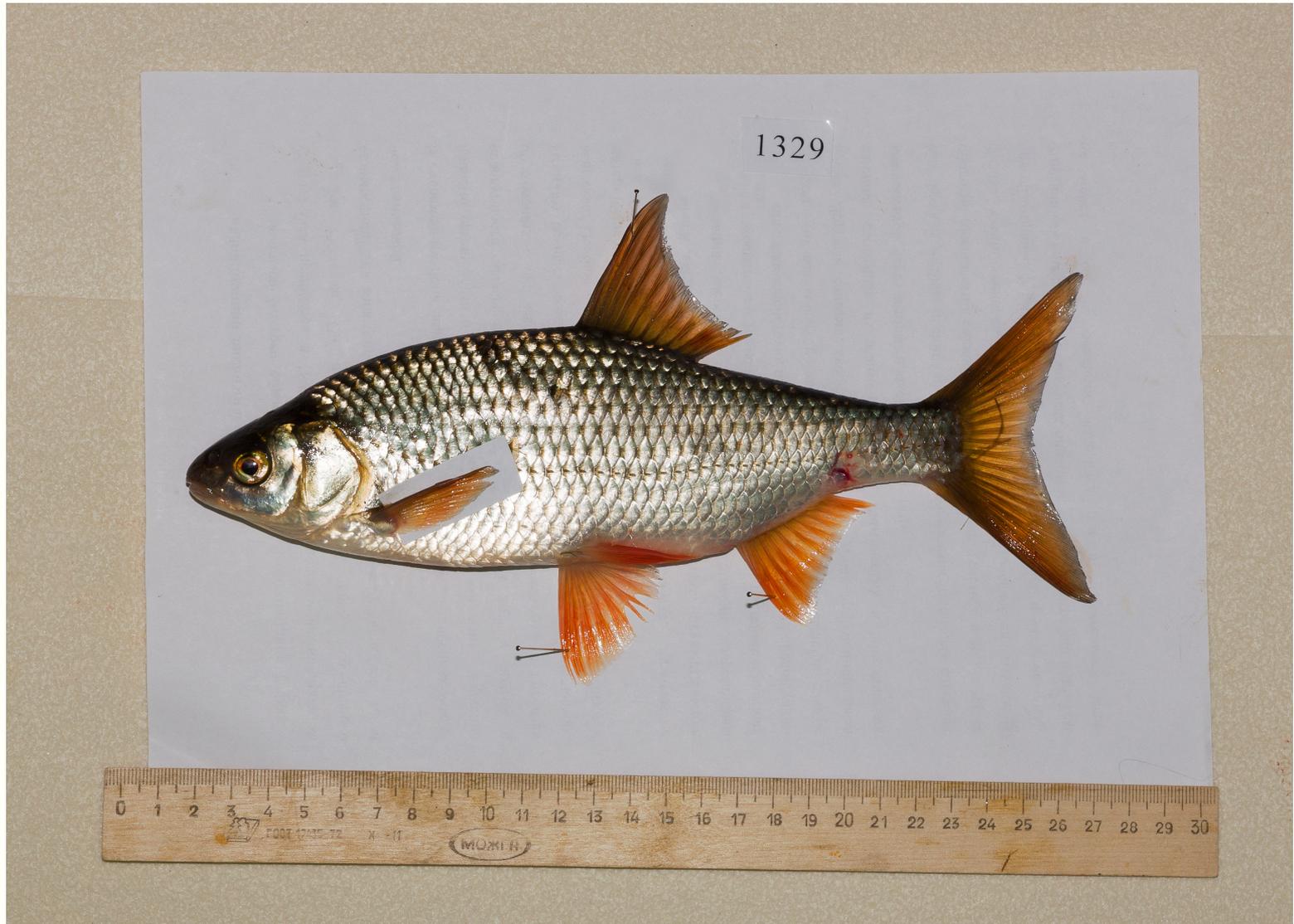


BASIC RESULTS

Research area. Location of sampling stations at the Techa River and the Miass River



Rouch (*Rutilus rutilus*)



Perch (*Perca fluviatilis*)



Pike (*Esox lucius*)



Mean concentrations of anthropogenic radionuclides in water of the Techa River and the Miass River

Sampling Stations	Water
^{137}Cs , Bq/l	
RM	$0.03 \pm 0.03^*$
RT1	0.35 ± 0.01
RT2	0.087 ± 0.01
RT3	$0.03 \pm 0.03^*$
^{90}Sr , Bq/l	
RM	0.04 ± 0.04
RT1	29 ± 1
RT2	14 ± 1
RT3	9.5 ± 0.5

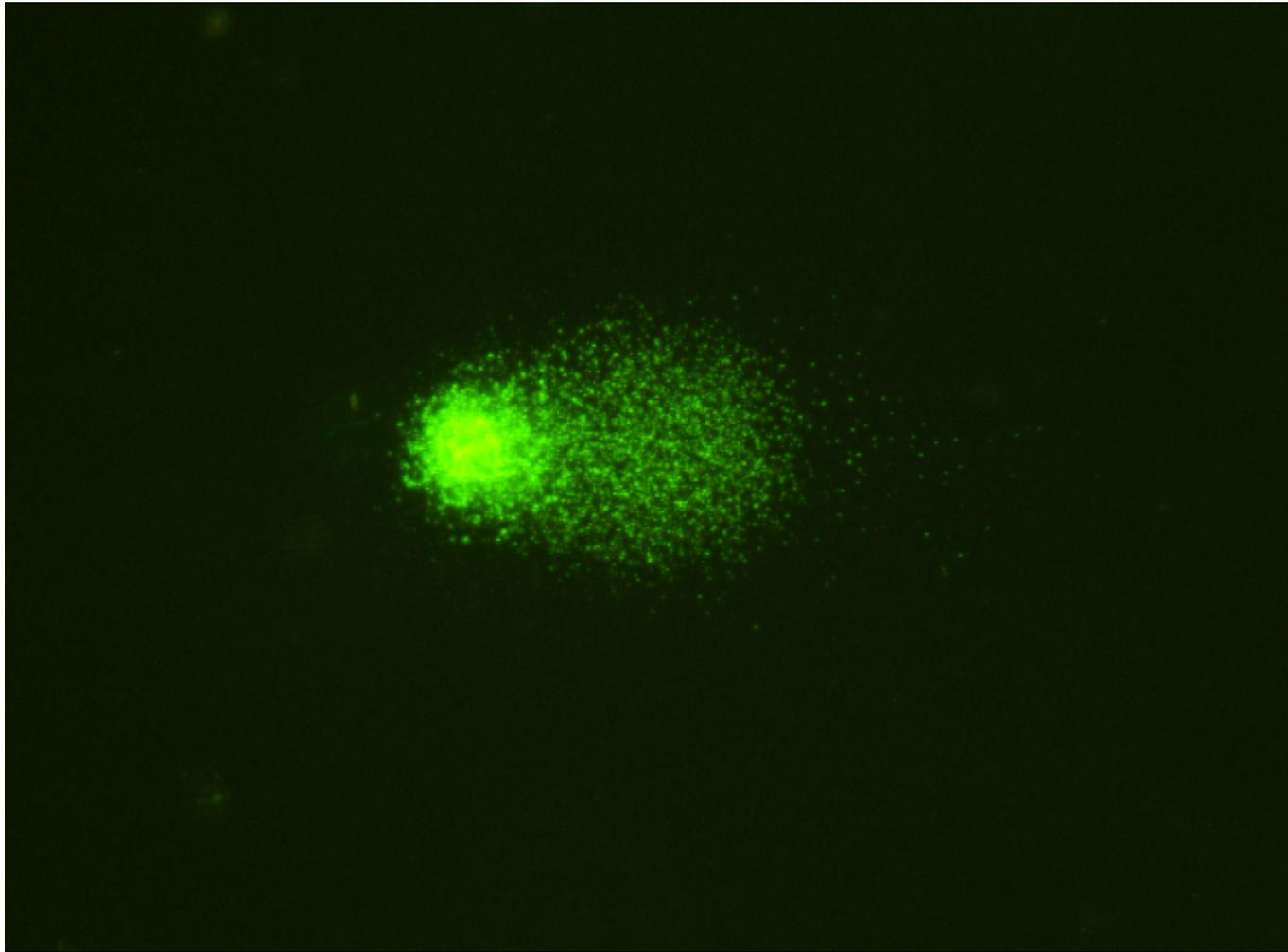
Average above-background dose rates of external and internal exposure, as well as cumulative dose rates for fish inhabiting the Techa River

Dose	RT1	RT2	RT3
	Mean (CV), $\mu\text{Gy/day}$ (a.u.)	Mean (CV), $\mu\text{Gy/day}$ (a.u.)	Mean (CV), $\mu\text{Gy/day}$ (a.u.)
Perch	N=58	N=43	N=55
Internal exposure	14 (0.5)	8 (0.4)	4 (0.8)
External exposure	110 (1.5)	3 (1)	0.5 (1.6)
Total	124 (1.2)	11 (0.4)	4 (0.8)
Roach	N=55	N=64	N=56
Internal exposure	19 (0.5)	13 (0.7)	8 (0.6)
External exposure	89 (1.6)	4 (0.5)	0.7 (1.1)
Total	108 (1.4)	16 (0.6)	9 (0.6)
Pike	N=38	N=32	N=15
Internal exposure	8 (0.4)	5 (0.4)	2 (0.5)
External exposure	140 (1.1)	4 (0.8)	0.8 (1.1)
Total	150 (1.1)	9 (0.4)	3 (0.7)

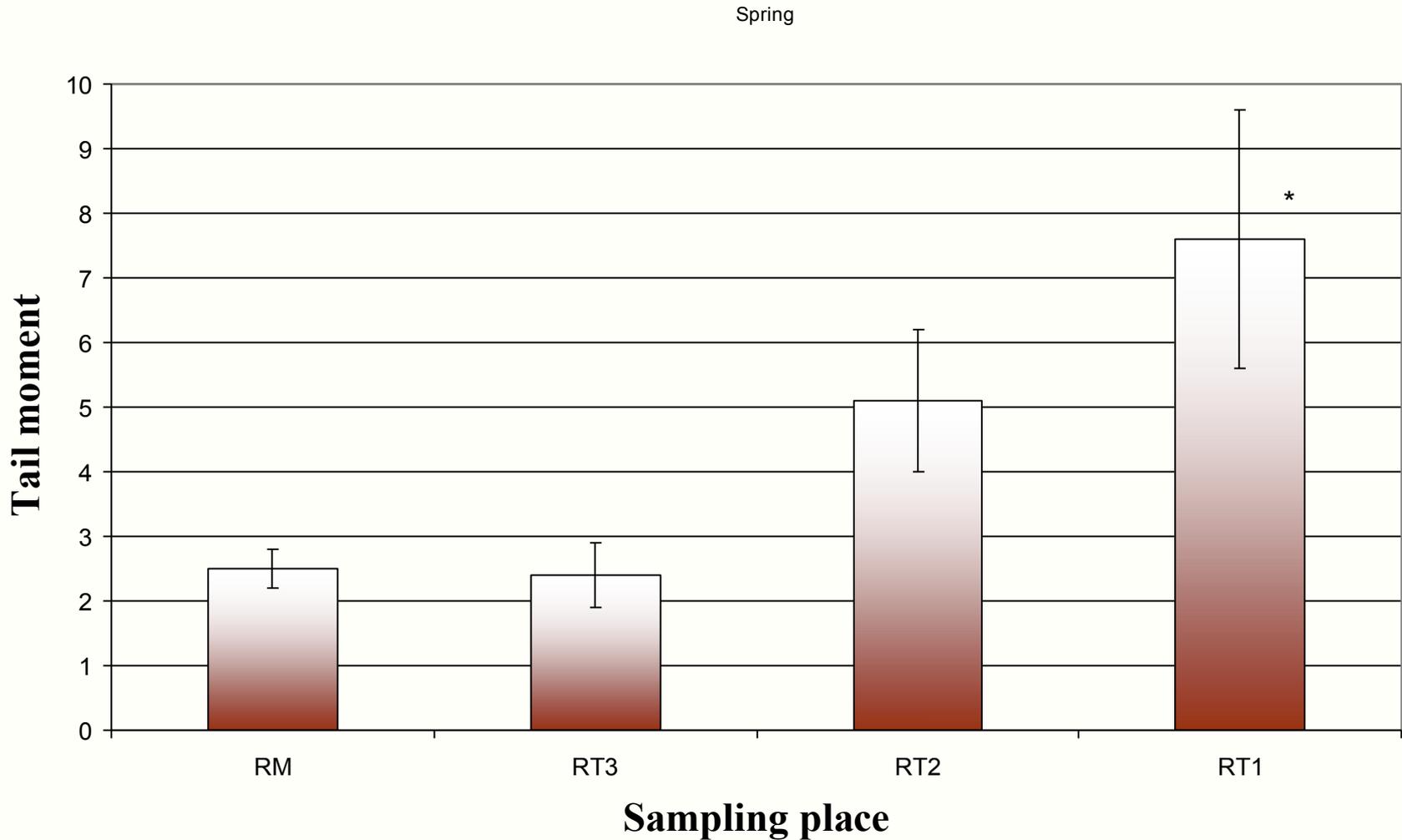
Ichthyological research

- Determination of the level of nuclear DNA damages in peripheral blood cells (comet assay) in fish.
- Determination of the cytogenetic damages and morphological anomalies of erythrocytes in peripheral blood of fish.
- Hematological research.
- Biochemical research.
- Assessment of fish sperm motility.
- Study of fin coloration.
- Determination of demographic and morphological parameters in fish.
- Species composition of ichthyofauna in fish catches.

Assessment of the condition of the nuclear DNA in cells of peripheral blood (Comet assay) in fish

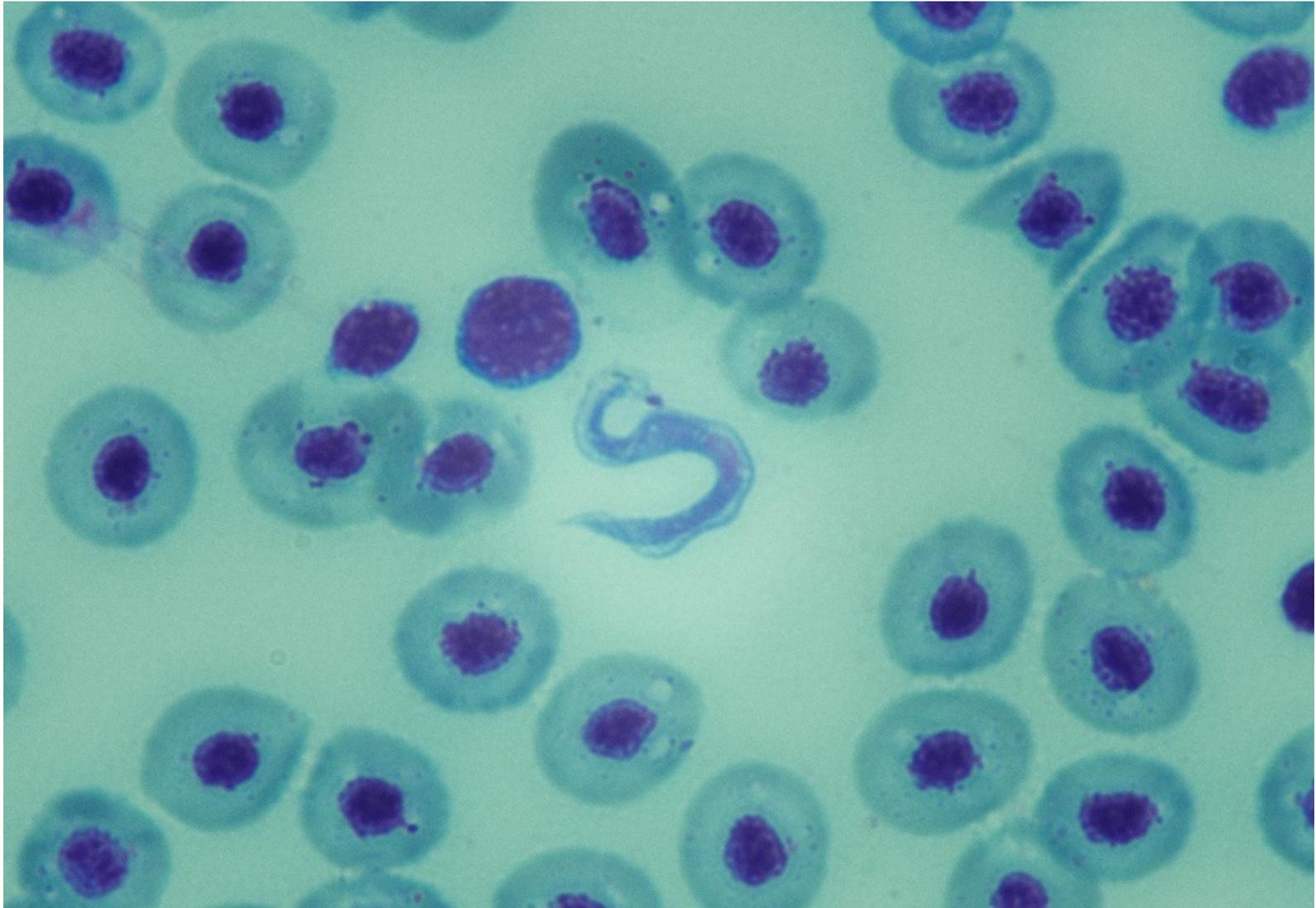


Comet assay results. Roach, spring 2013

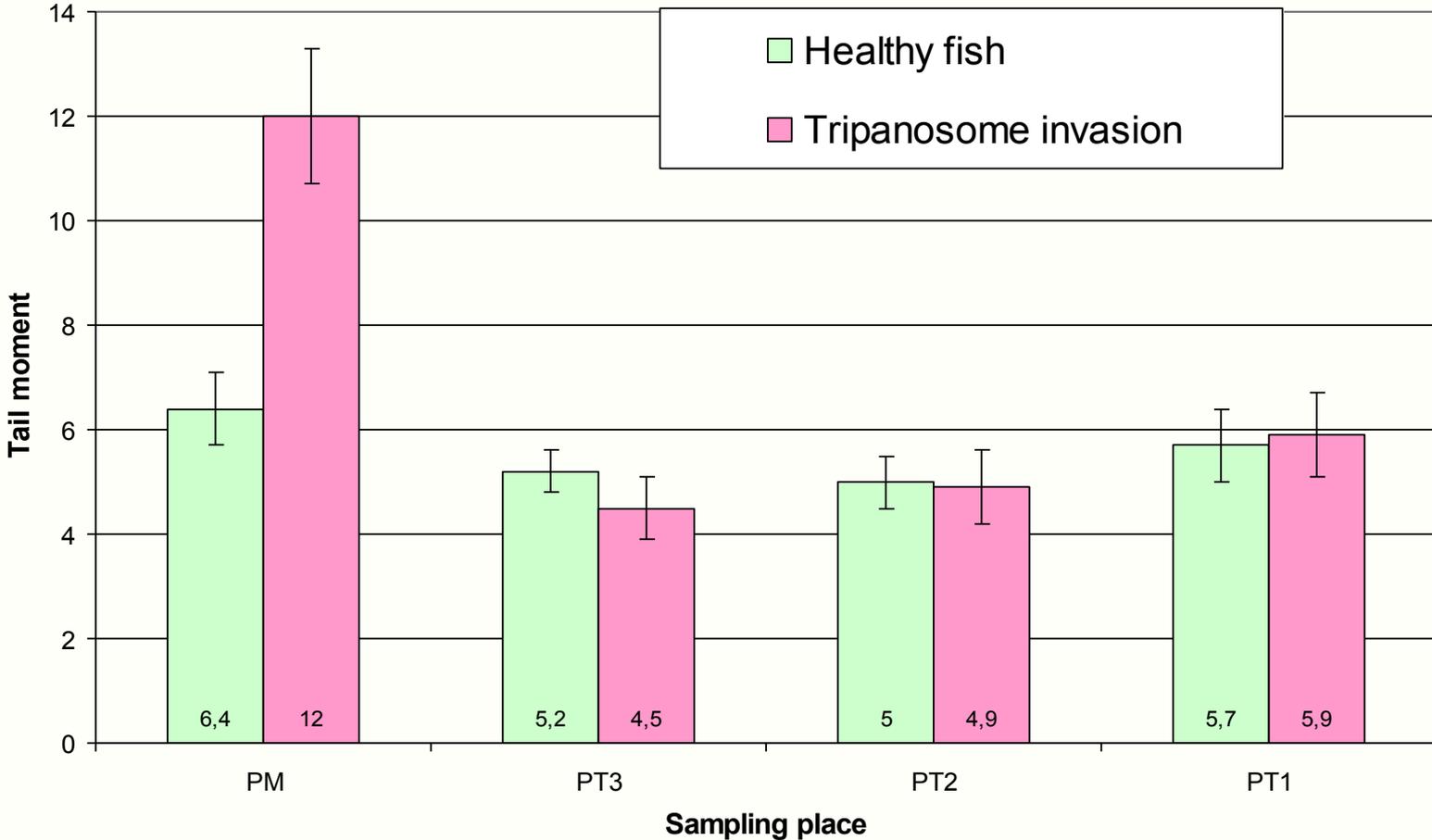


There is a significant dependency of Comet assay tail moment on dose rate for roach, caught in spring 2013 ($t_{31} = 3.03$; $p = 0.0049$)

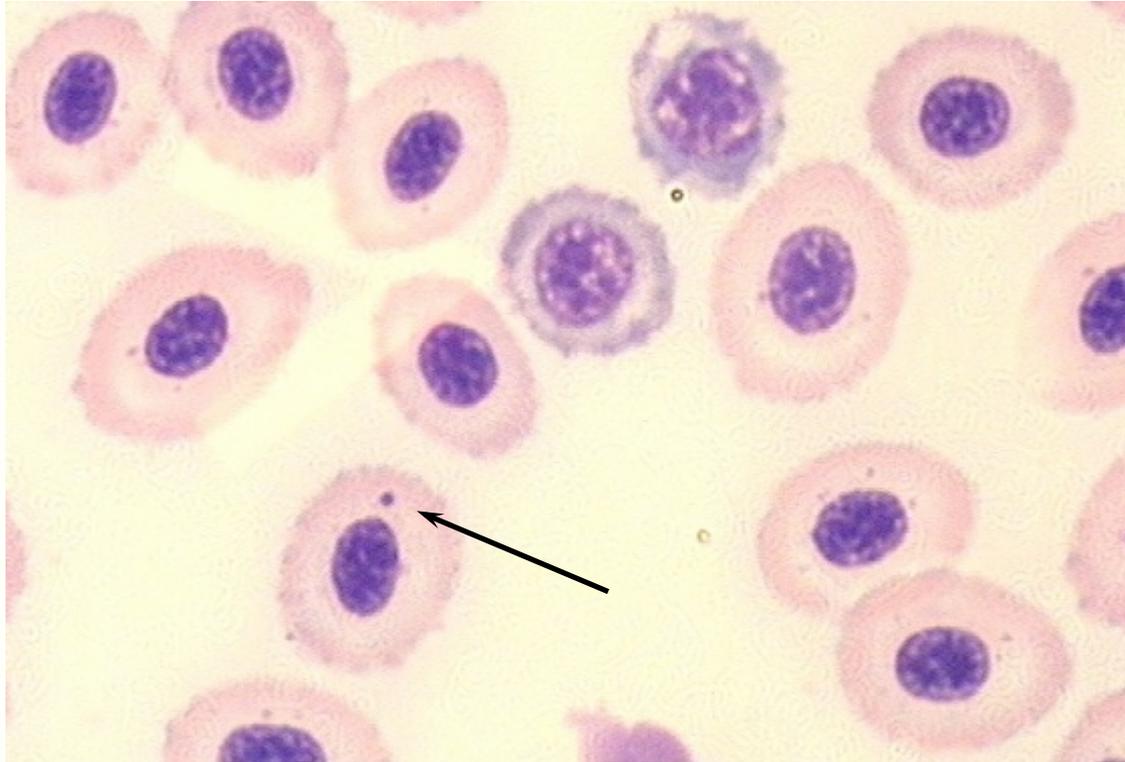
Frequency of trypanosomes invasion in fish caught at various sampling stations at Techa River and at Miass River



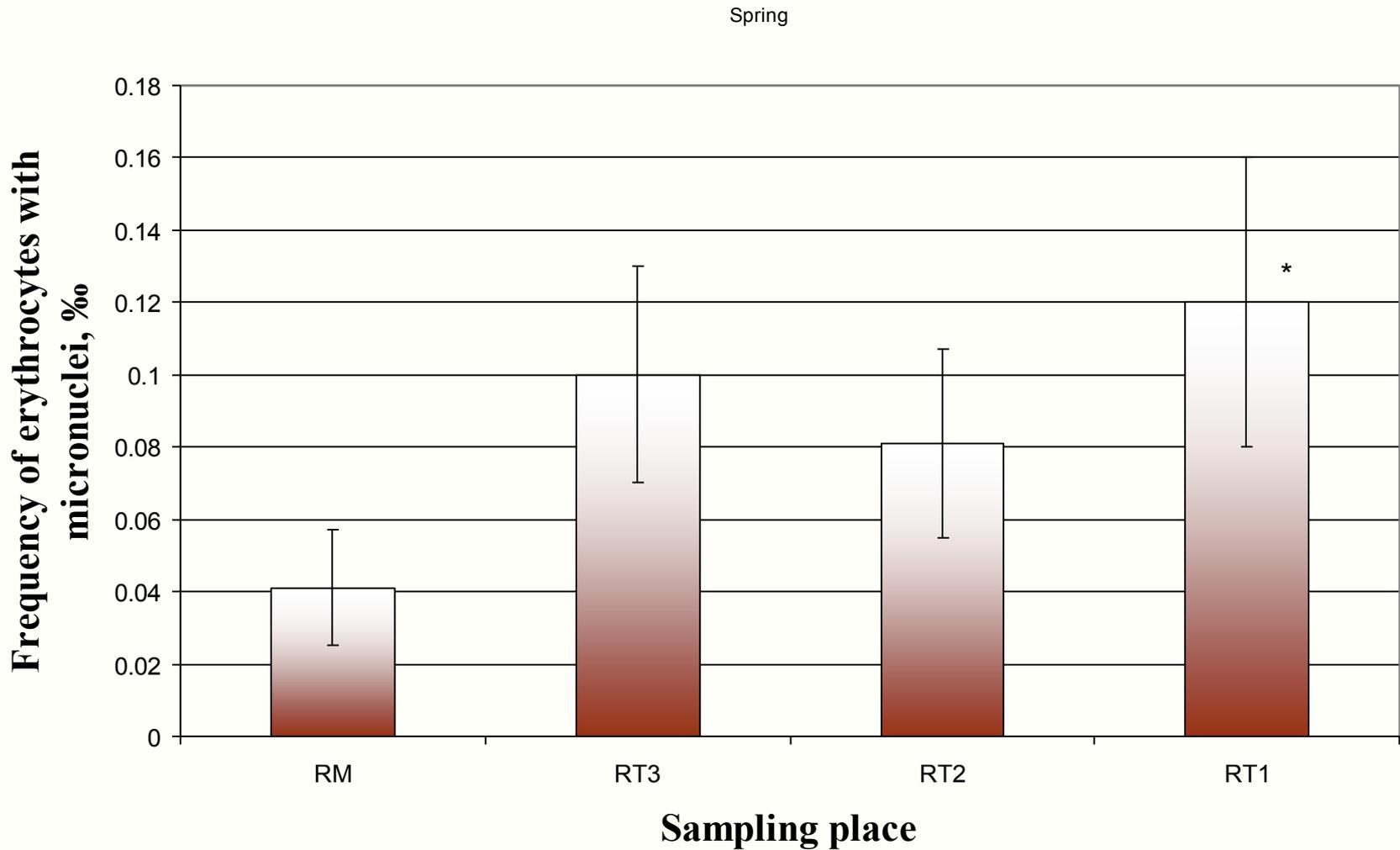
The status of the nuclear DNA in the peripheral blood cells in all fish species of interest from the Techa River and the Miass River, pooled data for the years 2012-2013



Frequency of erythrocytes with micronuclei in peripheral blood of fish, ‰

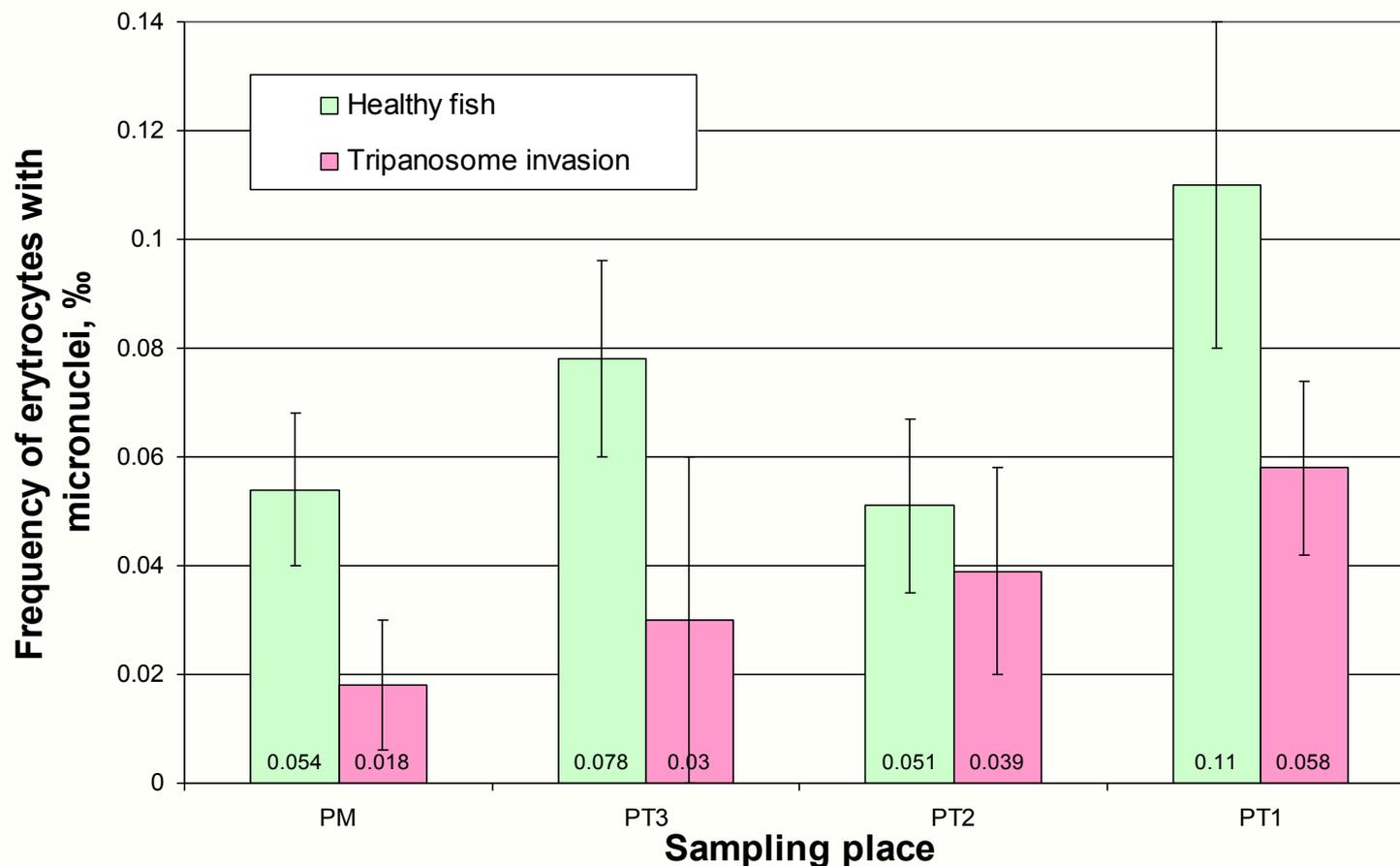


Frequency of erythrocytes with micronuclei. Roach, spring 2012, 2013

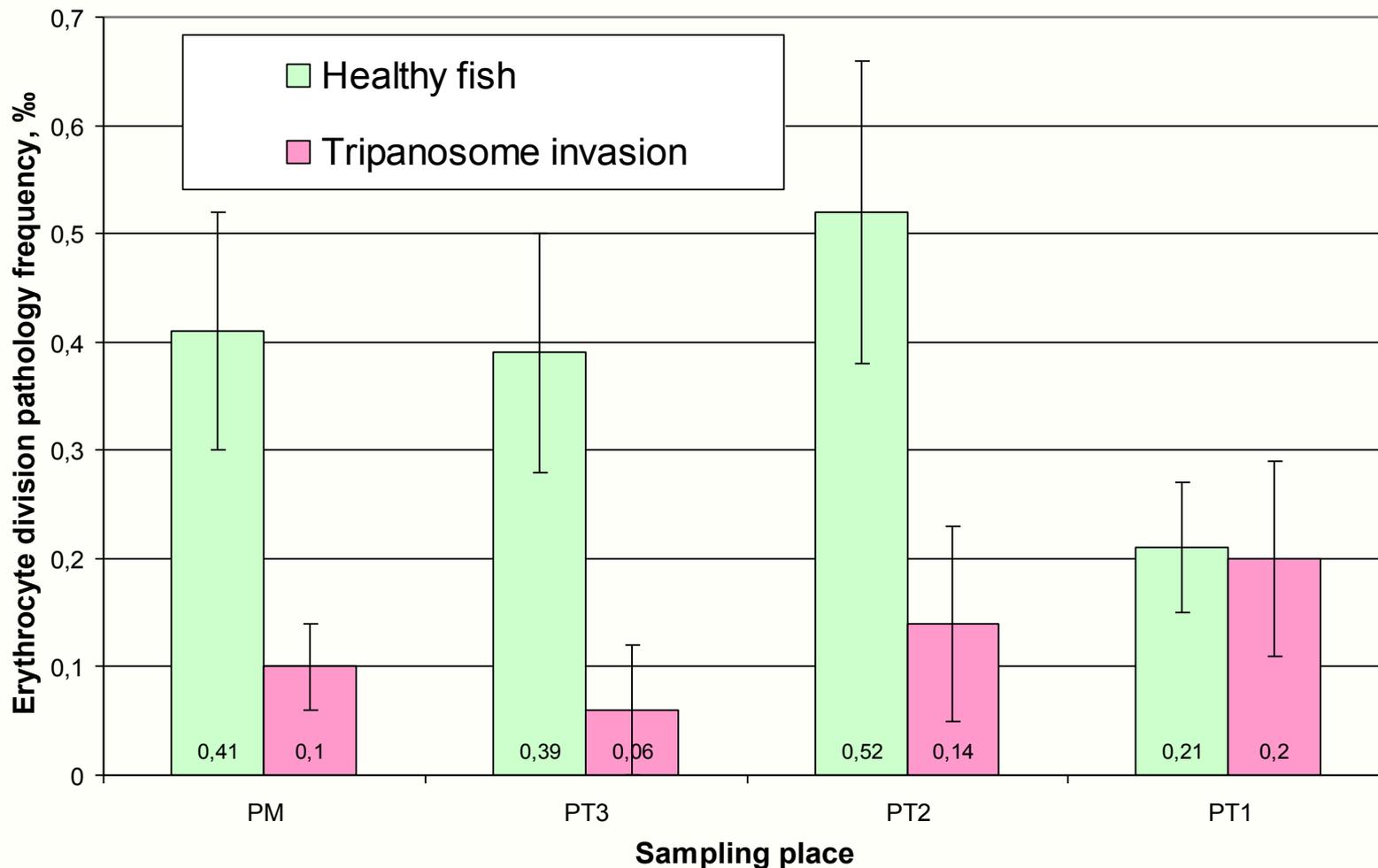


A significant dependency of frequency of erythrocytes with micronuclei on dose rate for roach, caught in spring 2013 ($t_{31} = 2.4$; $p = 0.022$), was revealed

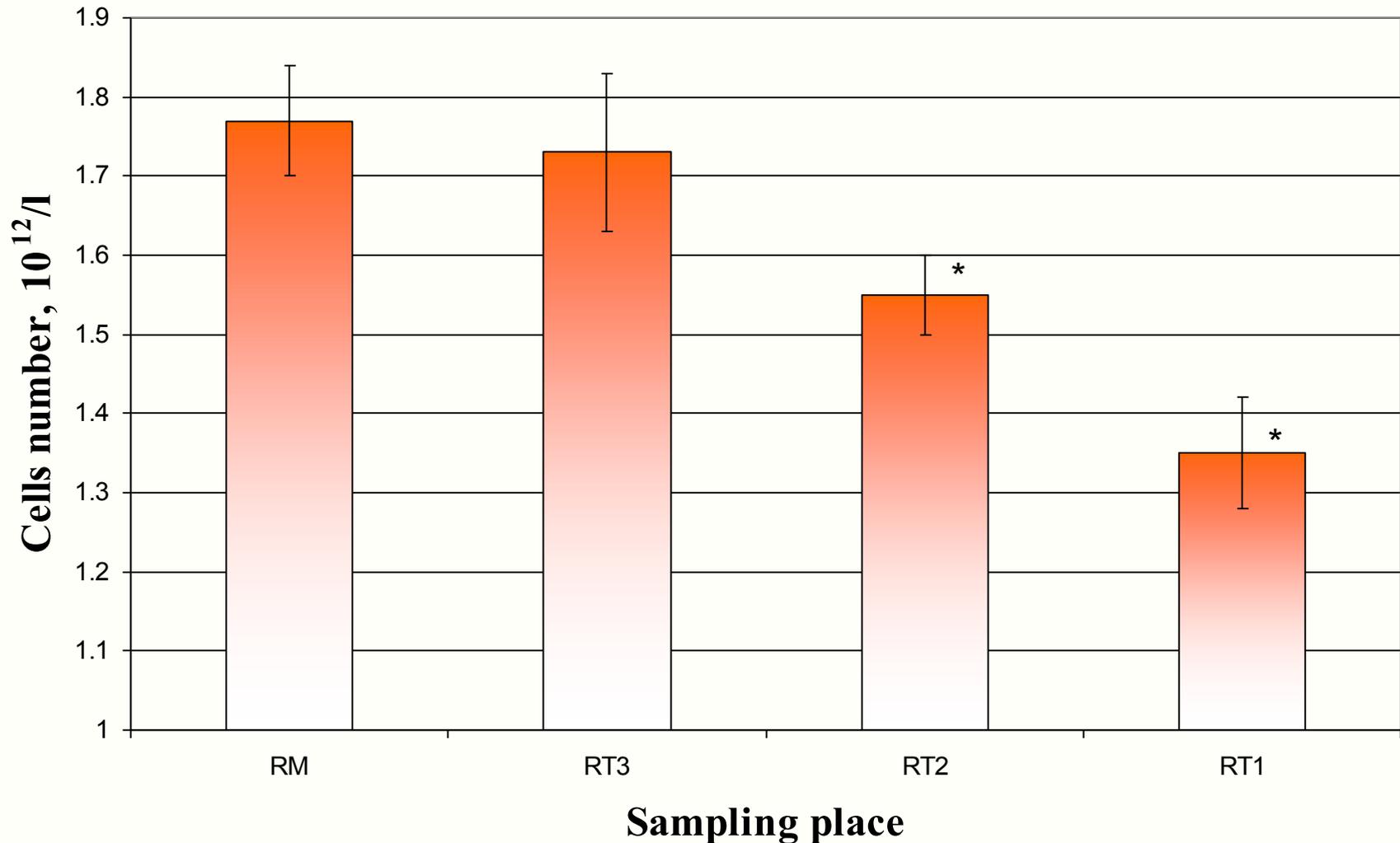
Frequency of erythrocytes with micronuclei in all fish species of interest from the Techa River and the Miass River, pooled data for the years 2012-2013



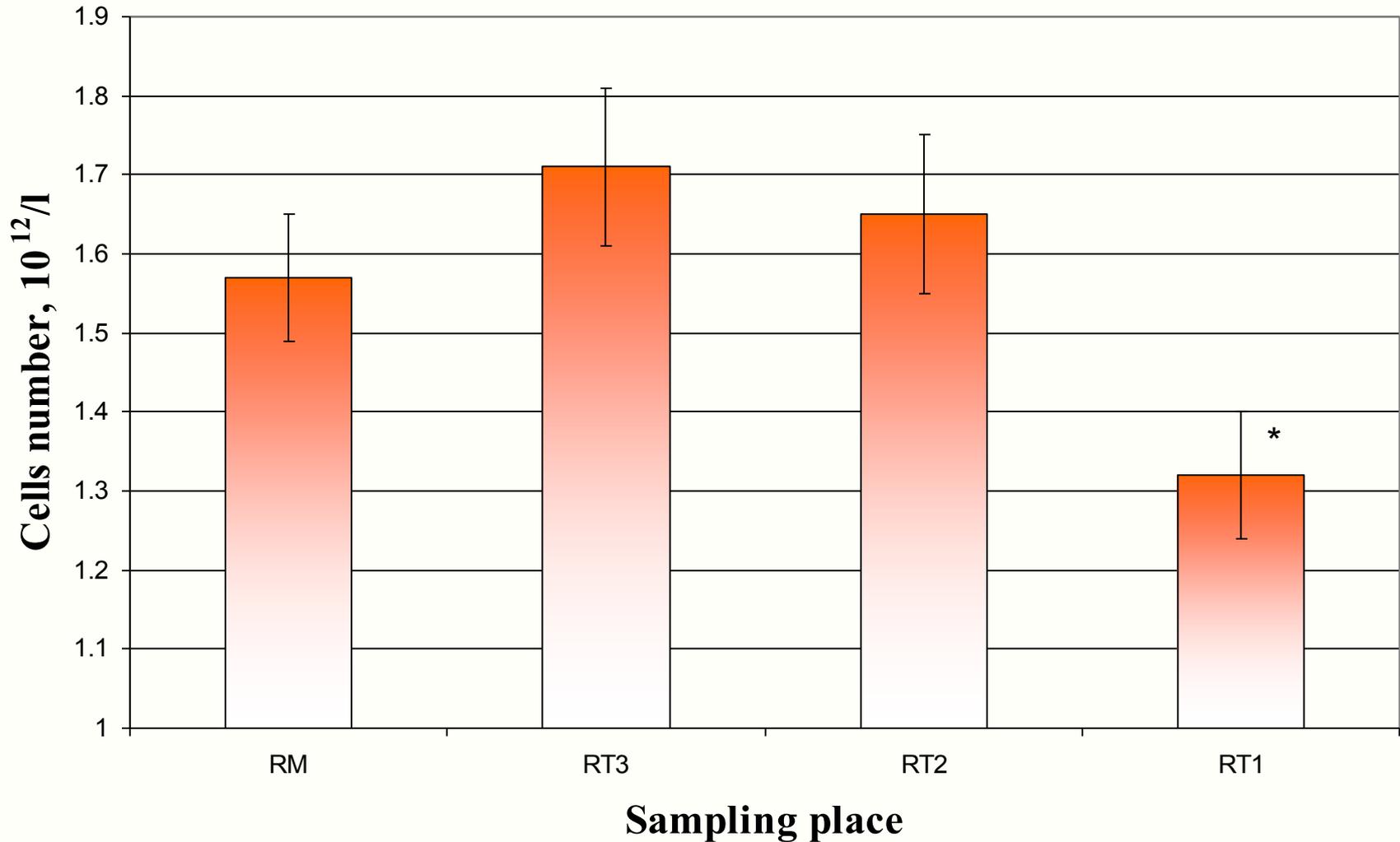
Frequency of erythrocyte with division pathology in all fish species of interest from the Tеча River and the Miass River, pooled data for the years 2012-2013



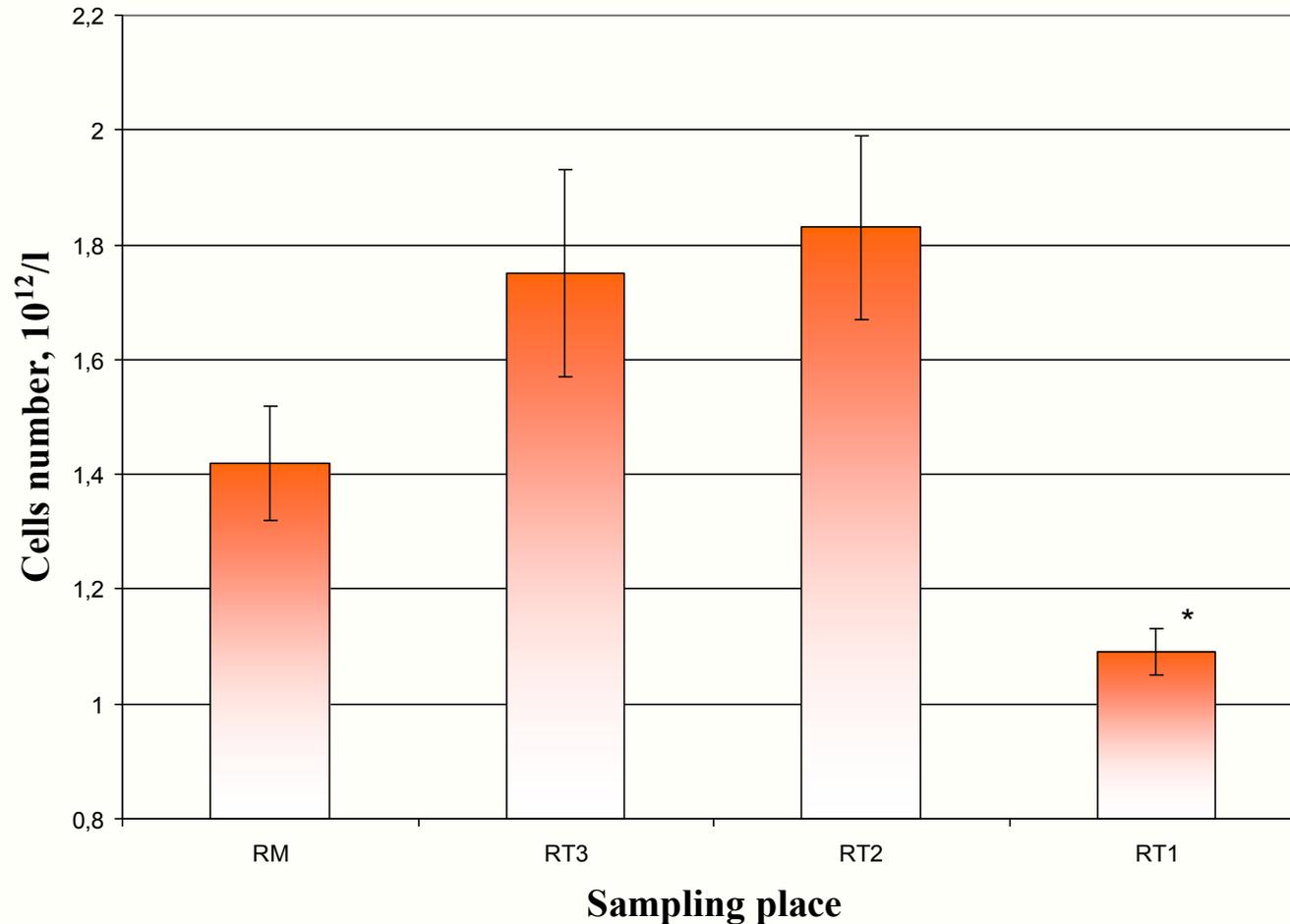
Number of erythrocytes in peripheral blood of roach, 2012 - 2013



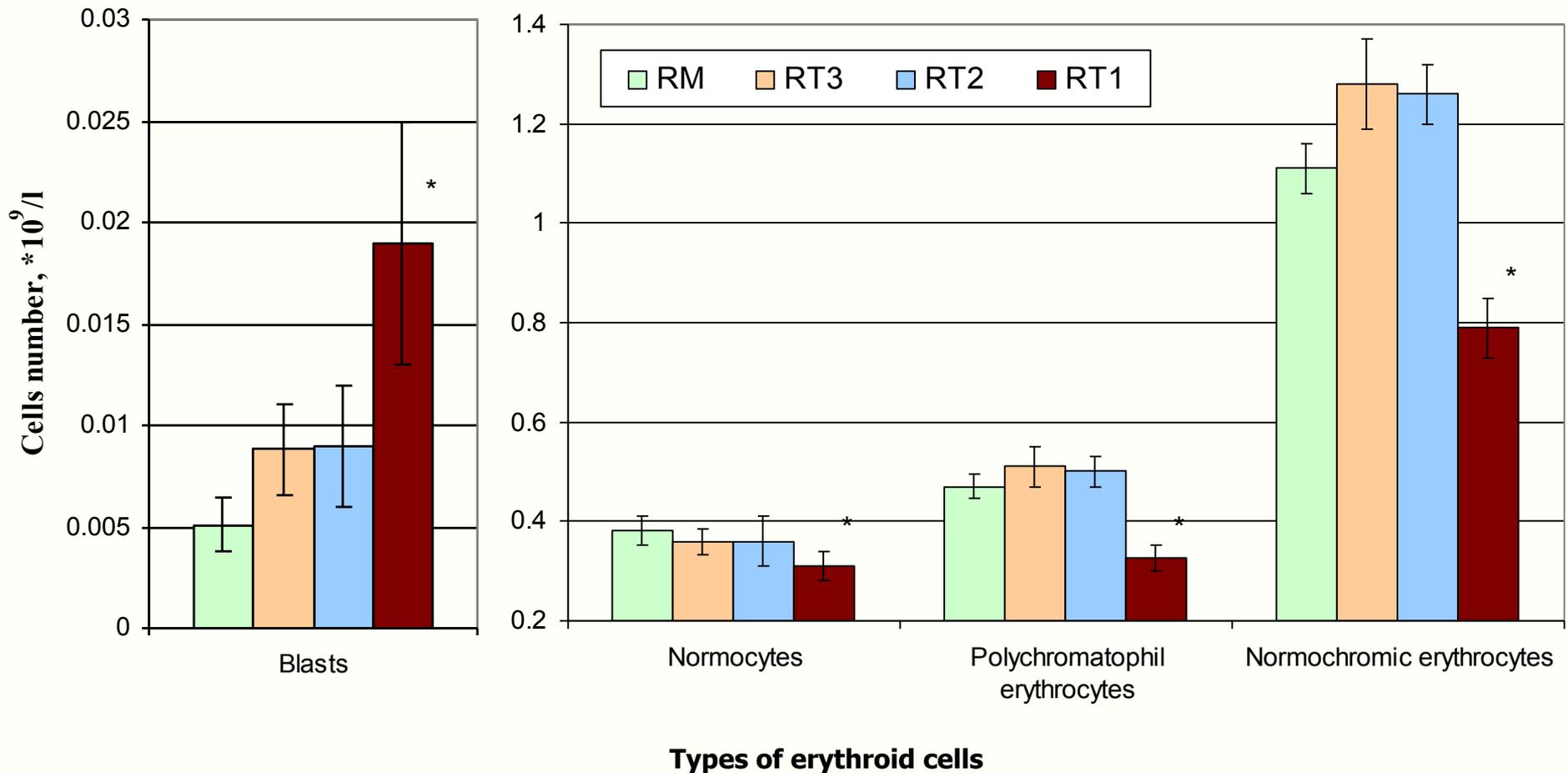
Number of erythrocytes in peripheral blood of perch, 2012 - 2013



Number of erythrocytes in peripheral blood of pike, 2012 - 2013



Absolute number of cells of erythroid lineage in blood of fish (summarized data for roach, perch, and pike), Spring 2012, 2013



Cells losses at different stages of erythropoiesis in fish, cumulative data for all studied fish species (RT1, 2012-2013)

Factors	Erythroblasts → Normocytes	Normocytes → Erythrocytes
Trypanosome	16%	20%
Radiation	65%	6%
Trypanosome + Radiation	32%	32%

Evaluation of the effect of the investigated factors (radiation exposure, fish species, sex, age, season, trypanosome invasion) on hematological parameters was performed using MANOVA in the main linear model. The best model was obtained for the parameter of the total number of cells in the peripheral blood

$$y = 0,353 - 0,00122 * TDR - 0.236 * fattening + 0.00097 * TDR * fattening , \\ (R^2 = 0.51; F_{3, 484} = 165.4; p < 2.2e- 16)$$

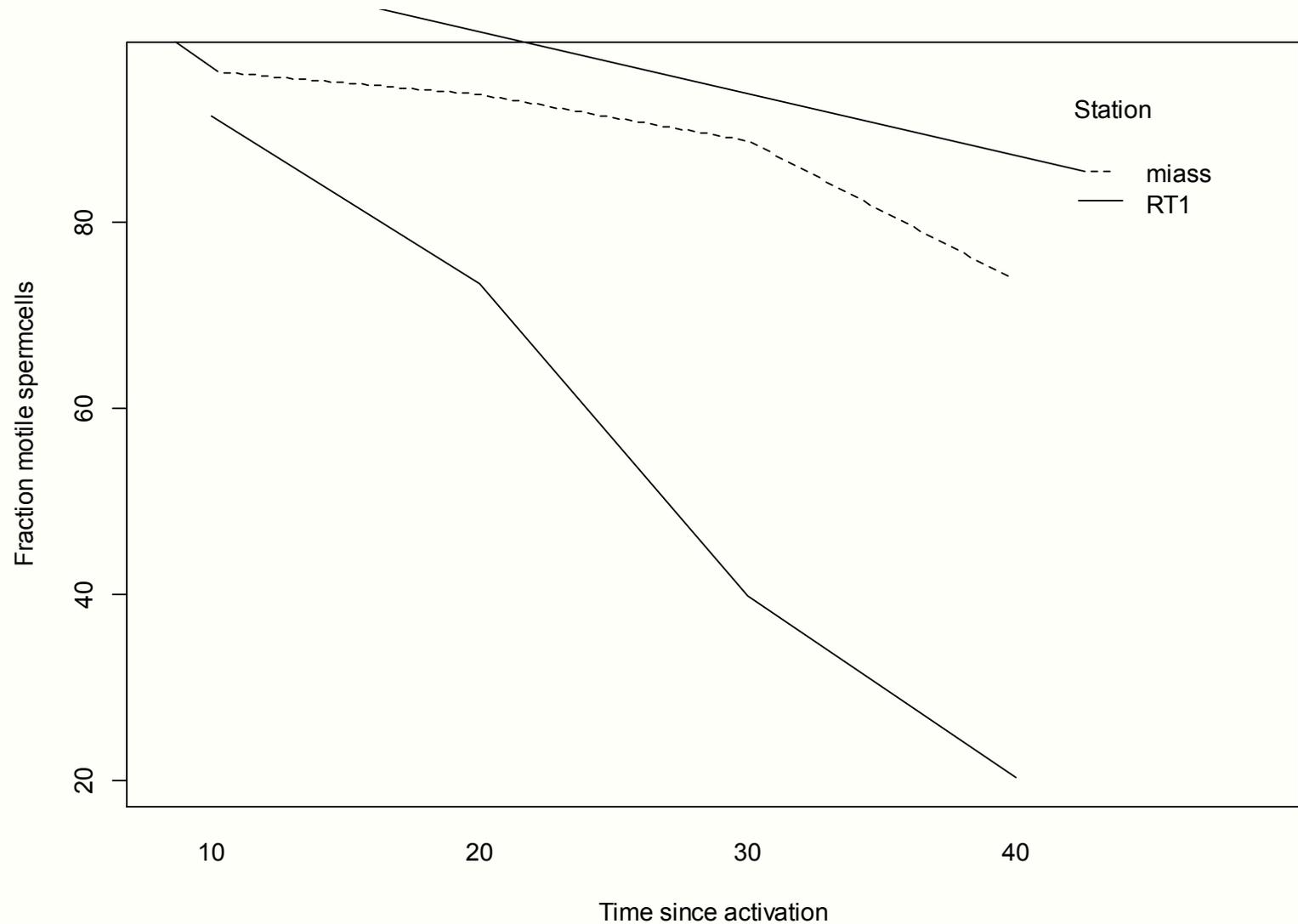
where y - lg number of peripheral blood cells , $\times 10^{12}$ cell/l ; TDR - the total dose , $\mu\text{Gy}/\text{day}$; fattening - Fattening period (index value = 0 or 1)

As effect of the season was revealed, the influence of various factors on the analyzed parameters only for fish during spawning was analyzed:

$$y = 0.352 - 0.00138 * TDR + 0.00063 * TDR * sick, \\ (R^2 = 0.34; F_{2, 253} = 64.7; p < 2.2e- 16)$$

where y - lg number of peripheral blood cells , $\times 10^{12}$ cell/l ; TDR - the total dose , $\mu\text{Gy}/\text{day}$; sick - the presence (1) or absence (0) of trypanosome invasion

The change in fraction of motile sperm cells in Perch during the first 40 sec. in the two stations



Fin coloration

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After Fin selection on the picture the average colour of fin was determined. The value channels a^* and b^* of the colour space $L^*a^*b^*$ were analyzed.

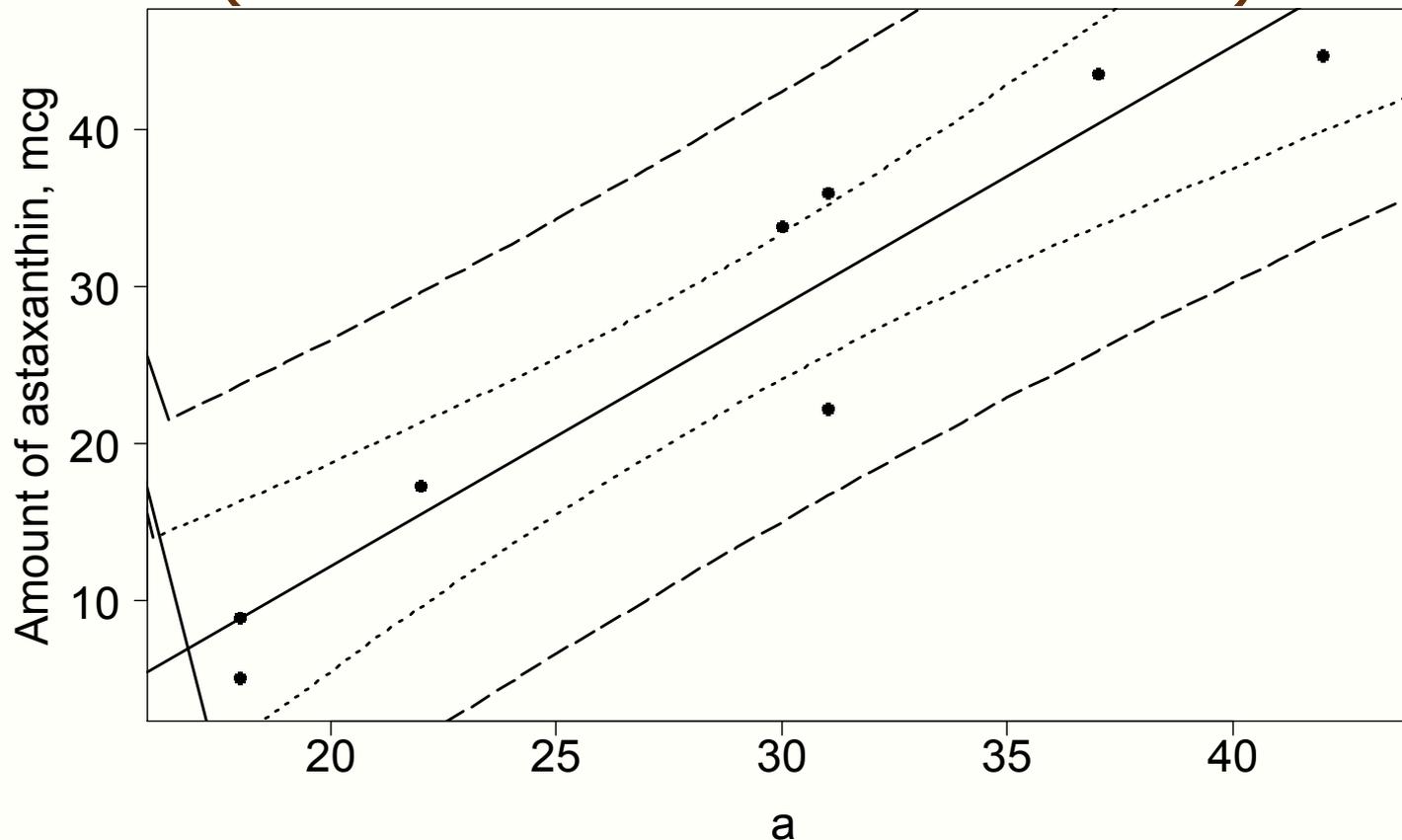
The most qualitative models describing the dependence of values of the a^* channel for the pectoral fins on the cumulative dose rate, age and trypanosome invasions were obtained for perch during Fattening:

$$y = - 3.0 + 0.063 \cdot \text{TDR} + 1.12 \cdot \text{Age} - 0.021 \cdot \text{TDR} \cdot \text{sick}$$

$(R^2 = 0.80; F_{3, 43} = 56.22; p = 6.3 \cdot 10^{-15})$

where, y - value of the a^* channel for pectoral fins, TDR - the total dose rate, $\mu\text{Gy/d}$, sick - the number of trypanosomes in the blood.

Dependency of amount of astaxantin in ventral fins on red color intensity of fins according to «a» channel value of «average» color of fin from the fish photo (color model CIE 1976 L*a*b*)



$$\text{Astaxantin amount, mcg} = (-21 \pm 7) + (1.66 \pm 0.23) * a$$

$$R^2 = 0.898, F_{1,6} = \text{statistic: } 52.81, p = 0.00035$$

A similar approach was used to determine pigment concentration in muscle of fish. (Are Folkestada et al. Rapid and non-invasive measurements of fat and pigment concentrations in live and slaughtered Atlantic salmon (*Salmo salar* L.) // *Aquaculture* 280 (2008) 129–135)

Demographic indexes of roach captured at sampling stations in 2012-2013

Parameter		Sampling station			
		RT1	RT2	RT3	RM
Quantity of the caught individuals		50	48	48	56
Sexual structure, %	females	70.00	50.00	56.25	60.00
	males	30.00	50.00	43.75	40.00
Age composition, %	1+	0	2.08	0	1.79
	2+	58.00	68.75	66.67	58.93
	3+	36.00	25.00	31.25	37.50
	4+	6.00	4.17	2.08	1.79

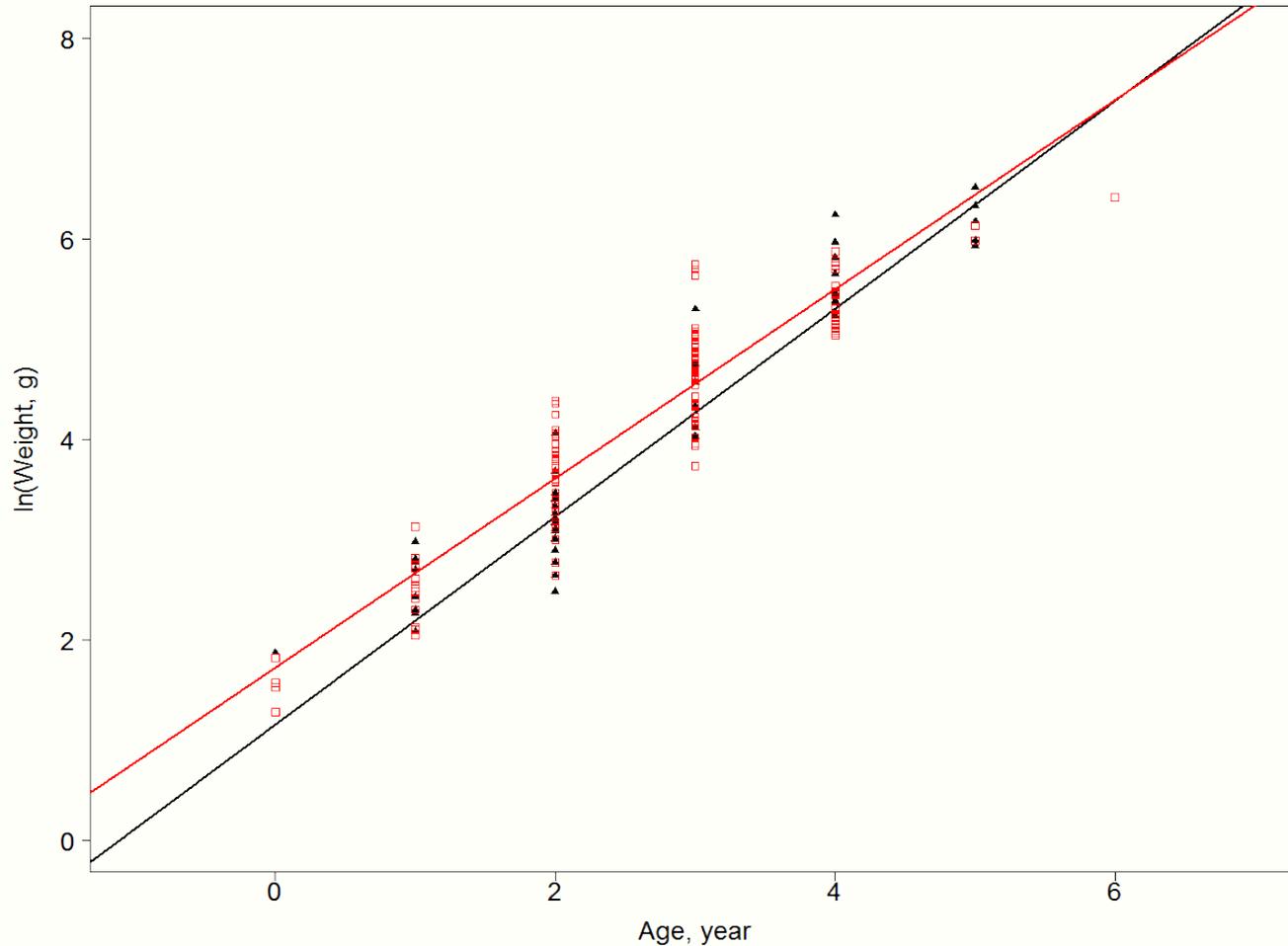
Demographic indexes of perch captured at sampling stations in 2012-2013

Parameter		Sampling station			
		RT1	RT2	RT3	RM
Quantity of the caught individuals		55	43	54	68
Sexual structure, %	females	50.91	44.19	60.38	51.52
	males	49.09	55.81	39.62	48.48
Age composition, %	0+	0	0	9.26	1.47
	1+	1.82	4.65	24.07	16.18
	2+	40.00	16.28	20.37	55.88
	3+	41.82	58.14	29.63	8.82
	4+	10.91	20.93	14.81	10.29
	5+	3.64	0	1.85	7.35
	6+	1.82	0	0	0

Demographic indexes of pike captured at sampling stations in 2012-2013

Parameter		Sampling station			
		RT1	RT2	RT3	RM
Quantity of the caught individuals		35	26	15	25
Sexual structure, %	females	45.45	61.54	53.33	21.74
	males	54.55	38.46	46.67	78.26
Age composition, %	0+	11.43	3.85	6.67	20.00
	1+	17.14	3.85	13.33	28.00
	2+	28.57	53.85	13.33	24.00
	3+	14.29	26.92	33.33	24.00
	4+	14.29	3.85	26.67	4.00
	5+	11.43	7.69	6.67	0
	6+	0	0	0	0
	7+	2.86	0	0	0

Dependency of body weight on age in perch in semi-logarithmic coordinates

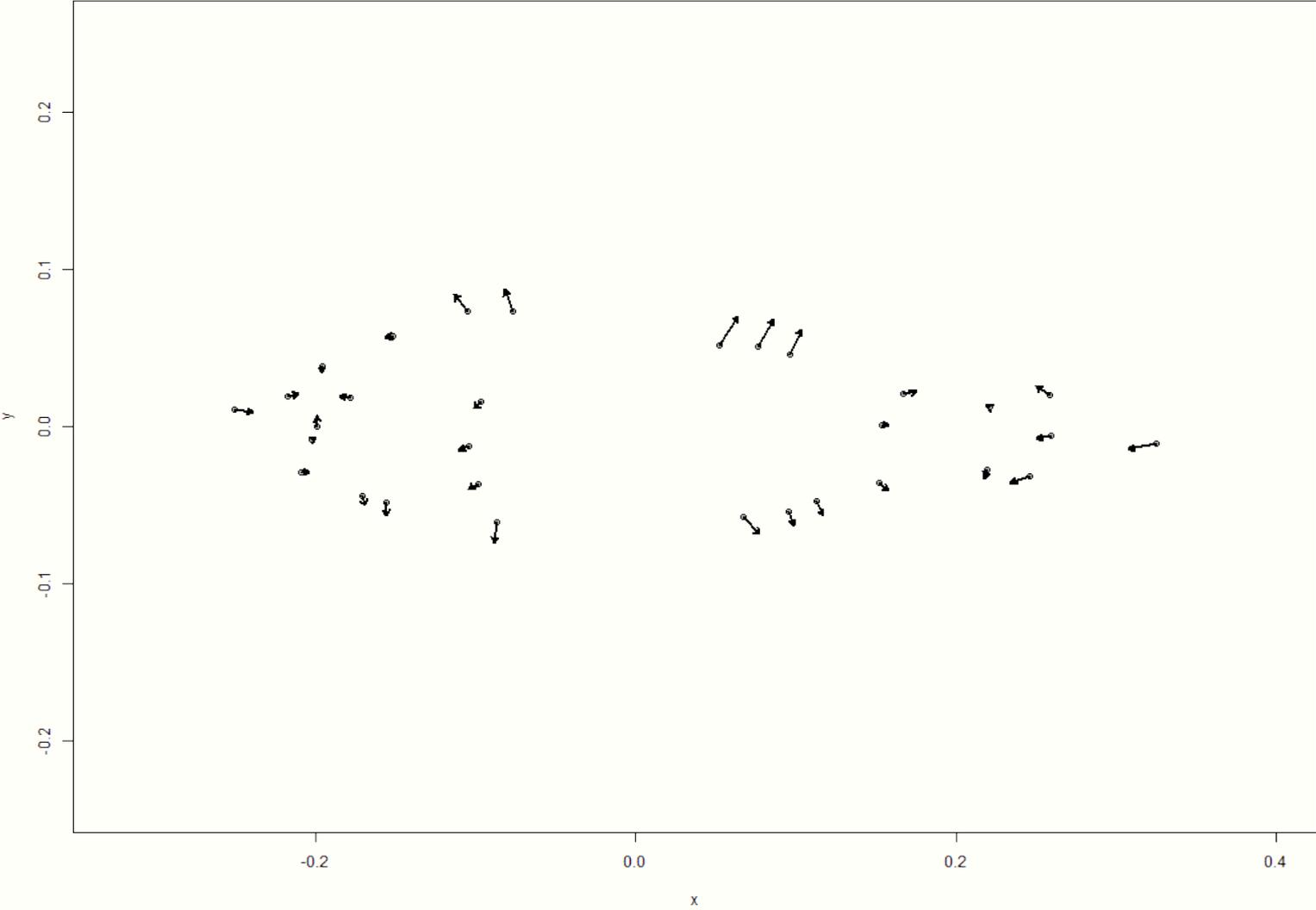


Red color – Techa river stations, black – Miass river stations.
 $F_{218, 216} = 18.96, p = 2.59 \text{ e-}08$

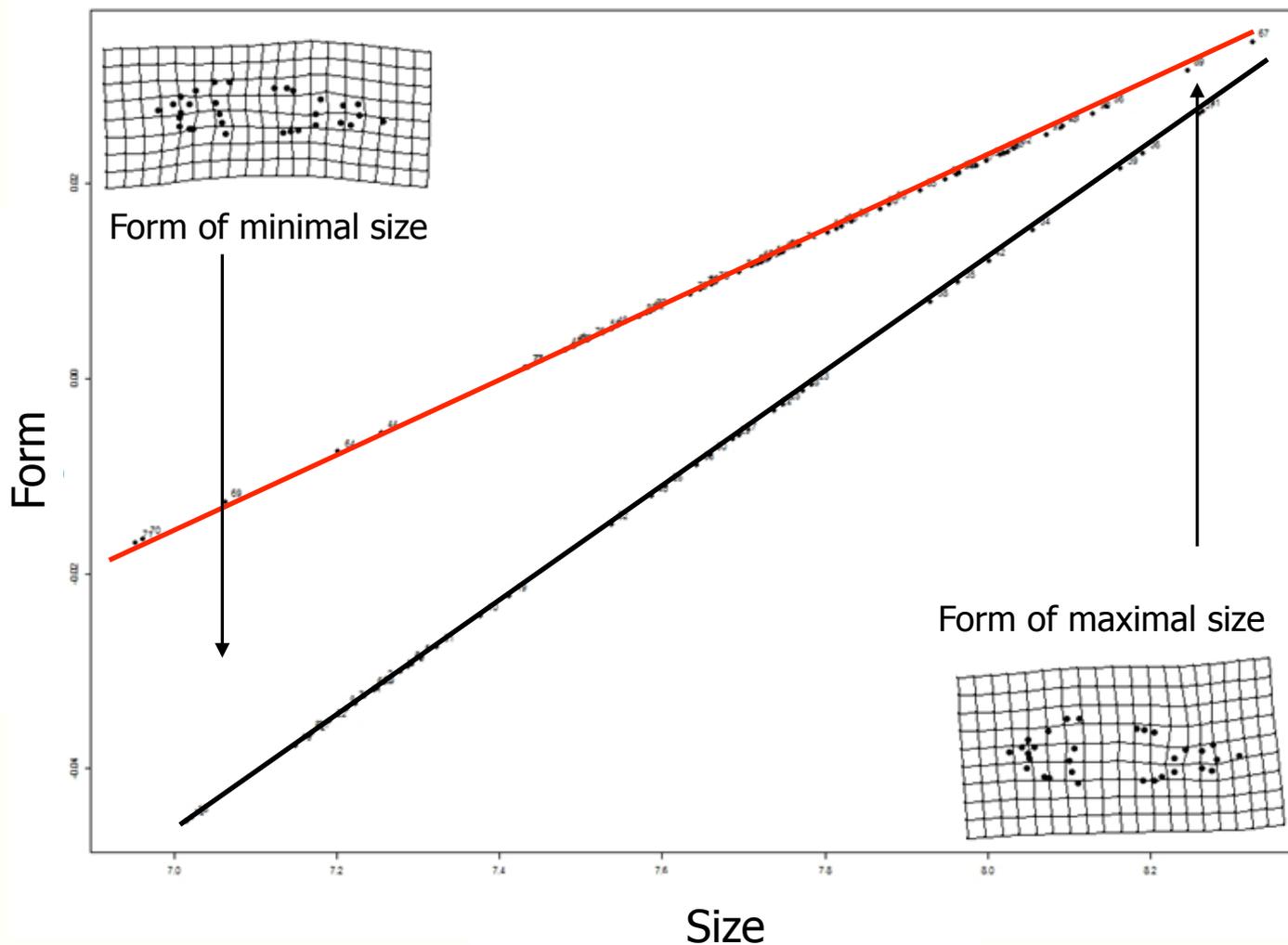
Geometric morphometry



Changes of perch form from Techa River to compare with perch from Miass River



Dependency of perch form on size for animals from Techa River and from Miass River



Red color – regression line for Techa river perch, black – regression line for Miass river perch.

Main conclusions:

1. Radiation exposure with a dose rate up to 220 $\mu\text{Gy}/\text{day}$ for freshwater fish, results in spawning period in dose-dependent reduction in the number of cells in the peripheral blood. Such changes are most pronounced in pike and roach. Using the proposed model of research (ecosystem of the Techa River, network of control stations, observation periods and analyzed critical parameters) allows to formulate and complete the task on determining the threshold levels of radiation exposure for fish provided the dosimetry system improvements and more detailed verification of the registered changes of the hematopoietic system. The result of such studies can serve as a basis for the development of regulatory measures for radiation protection of natural ecosystems.
2. Determination of reference species of the ichthyofauna in the radioactively contaminated watercourses is connected with critical endpoints, at the level of which these or those radiation-induced effects are occur: the reference species for evaluation of cytogenetic effects and evaluation of cellular DNA damage is roach; reference species for registration of radiation-induced reduction in the number of cells in the peripheral blood are pike and roach, the reference species for evaluation of radiation-induced physiological changes , manifested in the change of fin coloration is perch.
3. When organizing research or monitoring the status of the ichthyofauna of radioactively contaminated water bodies it is necessary to consider natural factors modifying the effect of radiation, such as spawning and fish trypanosome invasion in fish.

Thank you for your attention!



Results of the regression analysis of the dependence of the hematological parameters on the cumulative dose rate ($\mu\text{Gy}/\text{day}$) in a linear model ($Y = a + b \times x$) on the basis of data obtained in 2012 and 2013

Parameter	Slope	p value	R ²	F
Number of erythrocytes in blood, $\times 10^{12}$ cell/l	-0.0025	7.1E-11	0.11	45.1
Hematocrit, %	-0.03	6.1E-07	0.11	26.5
Number of non-dividing erythroid cells in blood, $\times 10^{12}$ cell/l	-0.0023	2.9E-10	0.1	42
Total number of cells in blood, $\times 10^{12}$ cell/l	-0.0027	1.4E-11	0.09	47.9
Number of normochromic erythrocytes in blood, $\times 10^{12}$ cell/l	-0.0017	1.1E-08	0.09	34.2
Relative number of leukocytes in the peripheral blood, %	0.013	1.3E-05	0.05	19.6
Relative number of erythrocytes in the peripheral blood, %	-0.013	2.0E-05	0.05	18.7
Relative number of lymphocytes in the peripheral blood, %	0.03	3.3E-05	0.05	17.7
Total percentage of erythroblasts and pronormocytes in all erythroid cells, %	0.0029	3.6E-05	0.05	17.5
Relative number of pronormocytes in all erythroid cells, %	0.0026	5.2E-05	0.04	16.8
NCE to the sum of erythroblasts + pronormocytes ratio	0.000059	8.9E-05	0.04	15.7
Relative number of neutrophils in all leukocytes in blood, %	-0.027	1.8E-04	0.04	14.4
Number of PCE in blood, $\times 10^{12}$ cell/l	-0.00048	1.8E-04	0.04	14.3
PCE to NCE ratio	-0.016	3.0E-03	0.02	8.93
Number of neutrophils in blood, $\times 10^{12}$ cell/l	-1.3E-05	5.6E-03	0.02	7.78
Number of pronormocytes, $\times 10^{12}$ cell/l	0.00002	2.0E-02	0.01	5.49
Relative number of NCE in all erythroid cells, %	-0.022	2.9E-02	0.01	4.8
Number of lymphocytes in blood, $\times 10^{12}$ cell/l	0.000098	3.7E-02	0.01	4.36
Cumulative number erythroblasts and pronormocytes in blood, $\times 10^{12}$ cell/l	0.000019	3.9E-02	0.01	4.29
Relative number of monocytes in the peripheral blood, %	-0.0035	4.5E-02	0.01	4.04