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MICRONUCLEATED ERYTHROCYTES IN FISH FROM SEVERAL ESTONIAN WATERBODIES

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Abstract. A micronucleus test was used to determine the usual background level of genotoxic damages in fish for the first time in Estonia in 1990—93. Altogether 233 fish (18 species) from 15 waterbodies were analysed. The numbers of micronucleated erythrocytes (ME) ranged individually from 0.05 up to 7.82‰. Industrial Northeast Estonia and Lake Peipsi (Peipus) revealed an increased genotoxic effect on fish. Fish species containing the largest numbers of ME under the same conditions were perch (*Perca fluviatilis* L., Percidae), ruff (*Gymnocephalus cernua* (L.), Percidae), and stickleback (*Gasterosteus aculeatus* L., Gasterosteidae). A comparison of ME levels in two common freshwater fish species, perch and roach (*Rutilus rutilus* (L.), Cyprinidae), showed a clear difference at a significance level $P<0.01$.

Key words: freshwater fish, micronucleated erythrocytes, monitoring fish species.

INTRODUCTION

In aquatic toxicology the components of peripheral blood of fish have been found to reflect environmental pollution (Nikinmaa, 1992). The micronucleus test is regarded as one of the most promising, inexpensive, and rapid screening techniques suitable for evaluating exposure to contaminants in the case of marine and freshwater fishes (Landolt & Kockan, 1983).

Micronuclei are smaller secondary nuclei formed following chromosomal breakage (Schmid, 1976). Their level is expressed as an index of cytogenetic damage (Heddle et al., 1991). Aneuploidy-inducing agents and spindle poisons induce micronuclei with centromeres, while clastogens induce micronuclei without centromeres (Gudi et al., 1992).

Most studies on the micronucleus test are made in vitro to testify the genotoxicity of certain chemicals. The highest values of micronuclei in the circulating erythrocytes of benzene-treated mice were found 21 days after the treatment (Barale et al., 1985). Similar results were observed in the case of single X-ray-radiated rainbow trouts (Schultz et al., 1993). Micronucleus levels may remain elevated up to two years if the toxic exposure is repeated (Wehr et al., 1987).

Spontaneous levels of circulating erythrocytic micronuclei in fishes are lower, averaging from 0.6 to 0.8‰ (Hose et al., 1987), as compared to the spontaneous levels of 1 to 3‰ reported for mammal (mice) polychromatic erythrocytes. The frequencies of erythrocytic micronuclei in fishes from contaminated sites are as high as 3 to 7‰ (Hose et al., 1987).

The micronucleus test appears to be insensitive in the case of liver neoplasia (Smith, 1990) and does not correlate with the concentration of toxic chemicals in the spleen and in the liver (Hose et al., 1987; Carrasco et al., 1990). In spite of these facts experiments of induced spawning have shown maternal micronucleus frequencies in white croaker (*Genyonemus lineatus*, Sciaenidae) to be predictive of reproductive success with eggs from fish with elevated micronucleus counts having lower fertilization rates.

The range of fish species used for the micronucleus test is broad including rainbow trout (Palm et al., 1992; Schultz et al., 1993) and perch (Al-Sabti & Härdig, 1990) from those inhabiting Estonia. Possible differences between species in sensitivity for detecting the genotoxic effect been less studied. The main difference is recognized as being due to the stage of the trophic food web (Hose et al., 1987).

In the present study micronucleated erythrocyte (ME) frequencies were measured in the peripheral blood of several fish species common in different types of waterbodies in Estonia. The goal of the study was to determine the levels of ME in waterbodies of different locations and compare them to those found in rainbow trouts reared in oil-shale drainage water (Palm et al., 1992). We attempted to find out the best indicator species with the comparatively highest ME values under the same conditions.

MATERIAL AND METHODS

To get an overview of the background levels of ME frequencies, 233 individual fish were captured from 15 different waterbodies in Estonia (Fig. 1, Table). Special section nets were used in lakes and a special electric device in brooks and rivers to collect fish species of different size.

Peripheral blood was taken from the heart by means of a heparinized syringe. In the case of small individuals the blood was sampled after opening the abdominal cavity. The slides were prepared and examined using the method described by Hose and co-authors (1987) by staining in 2% Giemsa solution in posphate buffer (pH=6.8) for 20 min to 1.5 h. Depending on the quality of a slide, 5000 to 10 000 erythrocytes per fish were counted manually, by one person, from coded slides using an oil immersion objective (×900).

Waterbodies in the succession of micronucleated erythrocyte (ME) levels

ME, ‰ pooled	ME, ‰	Species	*n*	Date and place of capture
1	2	3	4	5
3.56	3.80	*Gymnocephalus cernua* (L.)	9	Nov. 1992, Lake Peipsi,
	1.40	*Stizostedion lucioperca* (L.)	1	central part
2.27	2.27	*Oncorhynchus mykiss* (Walb.)	6	April 1991, Narva Fishery Farm
2.01	2.40	*Gasterosteus aculeatus* L.	4	June 1992, Matsalu Bay
	2.10	*Perca fluviatilis* L.	13	
	1.50	*Rutilus rutilus* (L.)	1	
	1.20	*Esox lucius* L.	1	
	1.10	*Leuciscus idus* (L.)	1	

1	2	3	4	5
2.00	2.50	*Rutilus rutilus* (L.)	2	Nov. 1992, Lake Peipsi,
	1.50	*Perca fluviatilis* L.	2	near Kallaste
1.78	1.93	*Salmo trutta* L.	3	Sept. 1991, Loode Brook
	1.63	*Lampetra planeri* (Bloch)	3	
1.61	2.05	*Perca fluviatilis* L.	4	Aug. 1991, Lake Uljaste
	1.40	*Coregonus peled* (Gmelin)	6	
1.58	1.58	*Coregonus peled* (Gmelin)	13	Nov. 1991, Lake Uljaste
1.45	1.49*	*Oncorhynchus mykiss* (Walb.)	10	Nov. 1990, Jõuga Brook
	1.40	*Cyprinus carpio* L.	10	
1.38	1.42	*Perca fluviatilis* L.	8	Oct. 1993, Lake Ahnejärv
	1.36	*Leucaspius delineatus* (Heckel)	5	
	1.34	*Rutilus rutilus* (L.)	7	
1.30	1.30	*Nemacheilus barbatulus* (L.)	2	Sept. 1991, Timmkanal Brook
1.25	1.75	*Perca fluviatilis* L.	17	May 1993, Lake Uljaste
	1.08	*Coregonus peled* (Gmelin)	8	
	0.75	*Rutilus rutilus* (L.)	17	
1.24	1.24	*Carassius carassius* (L.)	5	Oct. 1993, Lake Uljaste Soojärv
1.10	2.30	*Scardinius erythrophthalmus*	1	June 1991, lakes Kalli
	1.10	*Leuciscus idus* (L.)	1	and Leegu
	1.00	*Abramis brama* (L.)	3	
	0.85	*Blicca bjoerkna* (L.)	2	
	0.70	*Rutilus rutilus* (L.)	1	
0.75	0.75	*Salmo trutta* L.	22	June 1992, Prandi River
0.47	0.60	*Gobio gobio* (L.)	1	Sept. 1992, Laeva River
	0.50	*Nemacheilus barbatulus* (L.)	2	
	0.30	*Esox lucius* L.	1	
0.30**	0.30	*Cyprinus carpio* L.	6	April 1991, Lake Võrtsjärv
0.28	0.45	*Esox lucius* L.	2	June 1992, Lake Võrts-
	0.45	*Abramis brama* (L.)	2	järv
	0.30	*Perca fluviatilis* L.	9	
	0.30	*Stizostedion lucioperca* (L.)	2	
0.13	0.13	*Abramis brama* L.	9	June 1991, Lake Korijärv
0.05	0.05*	*Oncorhynchus mykiss* (Walb.)	12	Nov. 1990, Põlva Fishery Farm
		Total	233	

n — number of individuals;
* — published earlier (Palm et al., 1992);
** — kept for 4 months in the freshwater basin of Lake Võrtsjärv after rearing for 6 months in the Jõuga Brook where the ME level was measured to be 1.45‰;
— · — · — is the line between spontaneous and induced levels of ME (Hose et al., 1987).

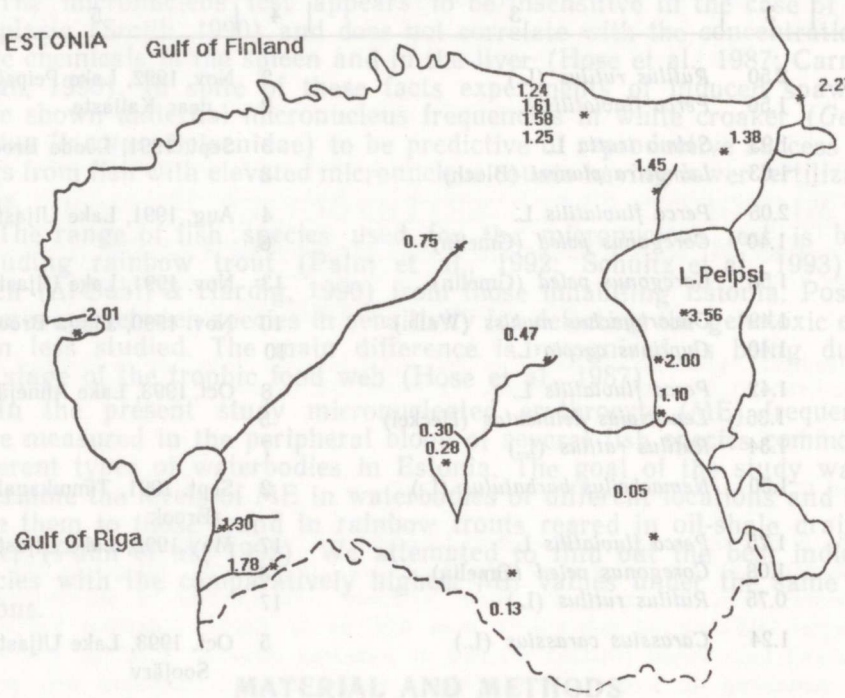


Fig. 1. Mean values of micronucleated erythrocytes (ME, ‰) in the peripheral blood of fish and their place of capture. More details are available in the Table.

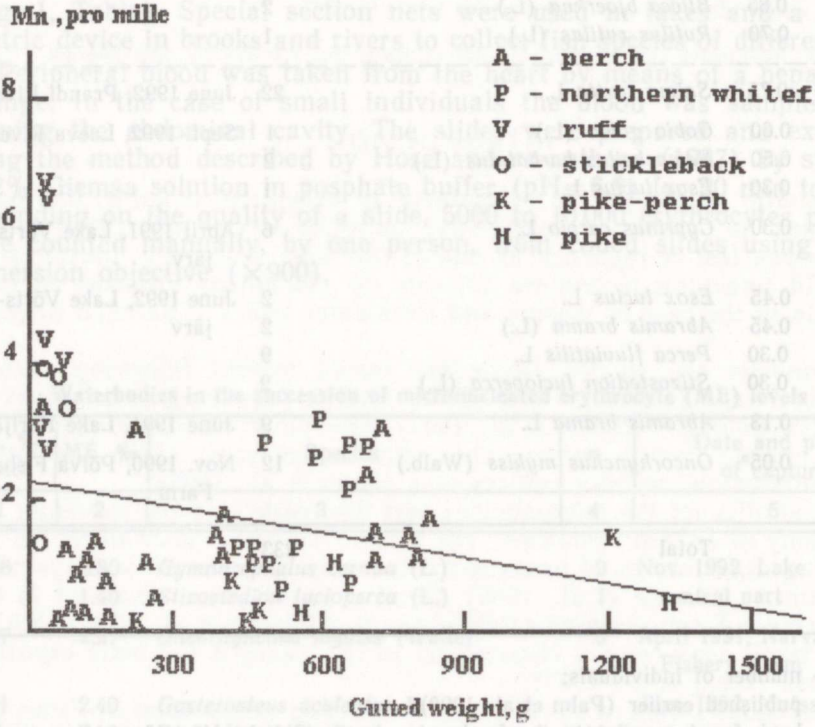


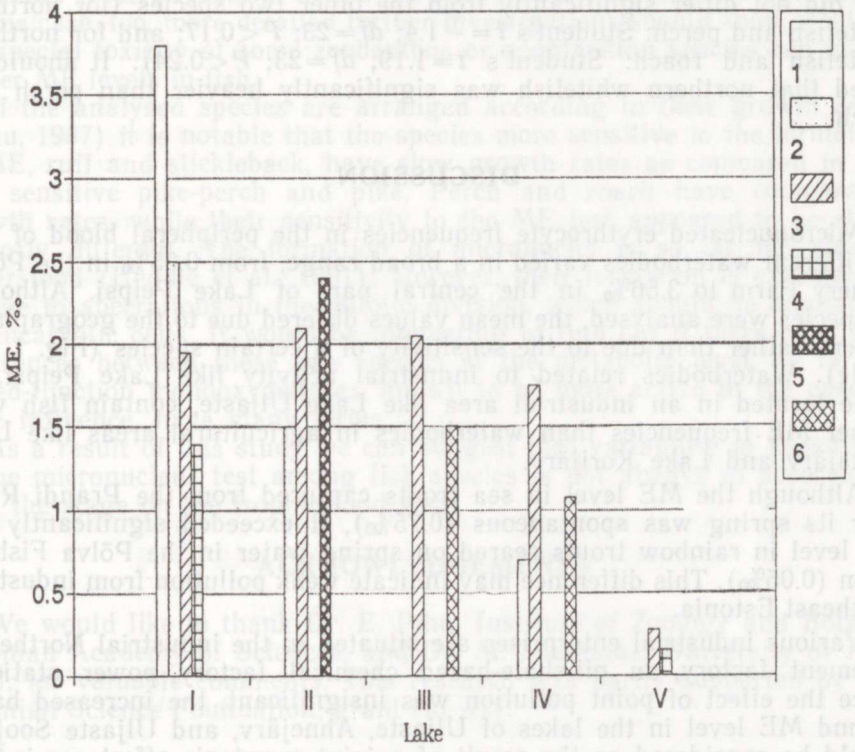
Fig. 2. Regression between micronucleated erythrocyte (ME, ‰) levels in the peripheral blood and the gutted weight of the analysed fish. The data on 54 fish from lakes Peipsi, Võrtsjärv, and Uljaste (1992) and from Matsalu Bay are used.

Fifty-four individual fish (out of 233) from three lakes (Uljaste, Peipsi, and Võrtsjärv) and from Matsalu Bay were weighed without internal organs (gutted weight) to avoid aberrations due to various occasional food conditions. For statistical analysis the correlation matrix calculation was used.

To determine the sensitivity of different fish species, 17 perches (*Perca fluviatilis* L., Percidae), 17 roaches (*Rutilus rutilus* (L.), Cyprinidae), and 8 northern whitefishes (*Coregonus peled* (Gemlin), Salmonidae) from Lake Uljaste were analysed using the methods described above. For statistical verification the two sample analysis was used.

RESULTS

The mean values of ME frequencies supplemented by data on the place and time of capture, species, and the number of individuals (n) are presented in the Table. Elevated levels of ME (higher than 0.8‰) were found in Lake Uljaste (1.61‰) located in industrial Northeast Estonia; in Lake Peipsi (3.56‰ in the central part, 2.0‰ near the town of Kallaste) with the industrial towns of Tartu and Pskov and oil-shale mines discharging their wastes into the lake; in the Narva Fishery Farm (2.27‰) where the cooling waters of the Narva thermal power plant are used; in Matsalu Bay (2.01‰), which collects its water from the Kasari River flowing mostly through agricultural lands.



Fgi. 3. Collation of micronucleated erythrocyte (ME) mean levels of different fish species in four waterbodies: I, Lake Peipsi (n=14; mean=3.11‰; S.D.=1.00); II, Matsalu Bay (n = 20; mean = 2.01‰; S.D. = 0.91); III, Lake Uljaste in November 1991 (n = 23; mean = 1.61‰; S.D.=0.28); IV, Lake Uljaste in May 1993 (n=42; mean =1.25‰; S.D.=0.98); V, Lake Võrtsjärv (n=20; mean =0.27‰; S.D.=0.17).

1, ruff; *2*, roach; *3*, perch; *4*, pike-perch; *5*, stickleback; *6*, northern whitefish.

Similar ME levels were found in three lakes located in industrial Northeast Estonia: 1.38‰ in Lake Ahnejärv, 1.25‰ in Lake Uljaste (1993), and 1.24‰ in Lake Uljaste Soojärv.

Repeated analyses in Lake Uljaste (August 1991, 1.61‰; November 1991, 1.58‰; May 1993, 1.25‰) show a decrease in the background genotoxic level. This trend should be checked in the future, as the lower data of May 1993 were mainly caused by low ME levels in roach (0.8‰).

The frequencies of ME increased also in the peripheral blood of fish from the small brooks of Loode (1.78‰) near its fall into the Gulf of Riga and Timmkanal (1.30‰) located in southwestern Estonia, and in lakes Kalli and Leegu (1.1‰) connected with Lake Peipsi.

ME frequencies lower than 0.8‰ were found mainly in south and southeast Estonia except the Prandi River (near its spring) located in central Estonia.

The correlation between the gutted weight of fish and the level of ME in the peripheral blood is demonstrated in Fig. 2. The correlation matrix calculation revealed a negative correlation ($n=54$; $r=-0.29$; $P<0.03$) between these two characteristics. The collation of mean values of ME in different fish species captured from four main waterbodies is shown in Fig. 3.

Two sample analysis of perch, northern whitefish, and roach from Lake Uljaste (on 12 May 1993) showed a certain significant difference between roach and perch (Student's $t=2.86$; $df=32$; $P<0.01$). Northern whitefish appeared to have medium ME values (mean=1.08‰, S.D.=0.6) and did not differ significantly from the other two species (for northern whitefish and perch: Student's $t=-1.4$; $df=23$; $P<0.17$; and for northern whitefish and roach: Student's $t=1.19$; $df=23$; $P<0.24$). It should be noted that northern whitefish was significantly heavier than perch and roach.

DISCUSSION

Micronucleated erythrocyte frequencies in the peripheral blood of fish in different waterbodies varied in a broad range, from 0.05‰ in the Põlva Fishery Farm to 3.56‰ in the central part of Lake Peipsi. Although 18 species were analysed, the mean values differed due to the geographical pattern rather than due to the sensitivity of a certain species (Fig. 1 and Table). Waterbodies related to industrial activity like Lake Peipsi, or those located in an industrial area like Lake Uljaste, contain fish with higher ME frequencies than waterbodies in agricultural areas like Lake Võrtsjärv and Lake Korijärv.

Although the ME level in sea trouts captured from the Prandi River near its spring was spontaneous (0.75‰), it exceeded significantly the ME level in rainbow trouts reared on spring water in the Põlva Fishery Farm (0.05‰). This difference may indicate weak pollution from industrial Northeast Estonia.

Various industrial enterprises are situated in the industrial Northeast: a cement factory, an oil-shale-based chemical factory, power stations. Since the effect of point pollution was insignificant, the increased background ME level in the lakes of Uljaste, Ahnejärv, and Uljaste Soojärv should be considered as the result of a joint genotoxic effect carried by air rather than by ground water. Increased ME levels (mean=2.01‰) in Matsalu Bay seemed surprising because the Kasari River falling into the bay drains its water mainly from agricultural lands. Here again a more detailed study should be carried out to determine the source(s) of pollution.

The comparison of the gutted weights of the analysed fish showed that

smaller fish are more sensitive to genotoxic influence ($r = -0.29$; $df = 53$; $P < 0.03$). As Figs. 2 and 3 show, this difference results from the fact that different species were studied. A difference within a single species will arise if individuals with extremely different weights are analysed simultaneously. In our study, the perch captured from Matsalu Bay ranged in weight from 29 to 864 g (Fig. 2), while the correlation between the gutted weight and the ME level was $P < 0.06$.

The comparison of the same fish species in different waterbodies (Fig. 3) and different species in the same waterbody shows that elevated ME frequencies are the highest in the peripheral blood of perch, ruff (*Gymnocephalus cernua* (L.), Percidae), and stickleback (*Gasterosteus aculeatus* L., Gasterosteidae). Enormously high numbers of ME (up to 13.1‰) were determined in perch off the coast of the Baltic Sea, Sweden, near a papermill (Al-Sabti & Härdig, 1990). The difference in the sensitivity of fish species to the ME test was previously demonstrated by Hose and co-authors (1987), who pointed out the significance of the stage on the food web: the more sensitive kelp bass (*Paralabrax clathratus*, Serranidae) feeds on small fish including white croaker (the less sensitive species).

In our study (Fig. 3) two more sensitive species, perch and ruff, feed on smaller fish, while stickleback feeds mainly on zooplankton. In Matsalu Bay, where both perch and stickleback were analysed, stickleback, although occupying a lower stage on the trophic food web, showed higher ME levels than perch.

Among less sensitive species pike-perch (*Stizostedion lucioperca* (L.), Percidae) feeds on smaller fish, and roach on zoobenthos and zooplankton. In this case, too, more detailed further investigations should show whether the special toxicity of some zoobenthos or zooplankton species can cause higher ME levels in fish.

If the analysed species are arranged according to their growth rates (Pihu, 1987) it is notable that the species more sensitive to the formation of ME, ruff and stickleback, have slow growth rates as compared to the less sensitive pike-perch and pike. Perch and roach have comparable growth rates, while their sensitivity to the ME test appeared to be significantly different. The formation of micronuclei is so far not clearly understood (Heddle et al., 1991); however, their formation is expected to be due to aberrations in the mitotic division, which should be similar in all eucaryotic cells. It would be interesting to find out if there exist any differences between these two species at the metabolic level, as far as mixed-function monooxygenases, known to be more active in the case of toxic influence, have many forms.

As a result of this study we can suggest that variability in sensitivity to the micronucleus test among fish species is not always in accordance with the stage on the trophic food web.

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MIKRONUKLEUSTEGA ERÜTROTSÜÜTIDE ESINEMISSAGEDUS MITMETE EESTI VEEKOGUDE KALADES

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Aastatel 1990—1993 kasutati Eesti kaladele avalduva genotoksilise taustmõju määramiseks esmakordselt mikronukleuse testi. 15 veekogust analüüsiti kokku 233 kala (18 liiki). Isenditi varieerus mikronukleuste esinemissagedus vahemikus 0,05—7,82‰. Tööstuslikus Kirde-Eestis ja Peipsi järves täheldati genotoksilise mõju suurenemist. Liigiti olid samades tingimustes tundlikuimad ahven (*Perca fluviatilis* L., Percidae), kiisk (*Gymnocephalus cernua* (L.), Percidae) ja ogalik (*Gasterosteus aculeatus* L., Gasterosteidae). Kahe tavalise mageveekala — ahvena ja särje (*Rutilus rutilus* (L.), Cyprinidae) — tundlikkuse võrdlus näitas selget liikidevahelist erinevust ($P<0,01$).

ЭРИТРОЦИТЫ С МИКРОНУКЛЕУСОМ В РЫБАХ ИЗ РАЗЛИЧНЫХ ВОДОЕМОВ ЭСТОНИИ

Ану ПАЛЬМ, Теэт КРАУЗЕ

В 1990—1993 гг. впервые в водоемах Эстонии была изучена частота встречаемости эритроцитов с микронуклеусом в крови рыб, чтобы уточнить генотоксичное влияние среды на рыб. Всего были проанализированы 233 индивида (18 видов) из 15 водоемов. Частота встречаемости эритроцитов с микронуклеусом в крови рыб варьировала от 0,05 до 7,82‰. Повышенная генотоксичность среды была обнаружена в регионе индустриальной Северо-Восточной Эстонии и в Чудском озере. Эритроциты с микронуклеусом чаще встречались у окуня (*Perca fluviatilis* L., Percidae), ерша (*Gymnocephalus cernua* (L.), Percidae) и трехиглой колюшки (*Gasterosteus aculeatus* L., Gasterosteidae). Сравнение двух обычных видов — окуня и плотвы (*Rutilus rutilus* (L.), Cyprinidae) прекрасно иллюстрирует различие в чувствительности этих двух видов по отношению к загрязнению на достоверном уровне ($P < 0{,}01$).