Micronucleus occurrence in diploid and triploid rainbow trout (*Oncorhynchus mykiss* Walbaum)

I. Strunjak-Perovic, R. Coz-Rakovac, N. Topic Popovic

Division of Materials Chemistry, Rudjer Boskovic Institute, Zagreb, Croatia

ABSTRACT: The aim of the study was to observe the influence of different ploidy levels in fish on micronucleus occurrence. Twenty minutes after fertilization, one group of rainbow trout eggs was exposed to water temperatures of 26°C in duration of 20 minutes to induce triploidy. Second group was kept in water temperature of 10°C, which is optimal for development of rainbow trout. The frequency of micronucleated erythrocytes was determined in the peripheral circulation of rainbow trout 67 days (following absorption of the yolk – swim-up stage) and 128 days (fry stage) post fertilization. There was a significant difference ($P < 0.001$) between frequency of micronucleated erythrocytes of diploid (1.10 ± 0.96‰) and triploid (2.41 ± 1.28‰) fish at swim-up stage. Increased mean values of micronucleus in diploid (1.80 ± 1.57‰) and triploid (5.92 ± 3.80‰) fry were also recorded.

Keywords: rainbow trout; diploid; triploid; micronucleus

Gain and loss of whole chromosomes leads to aneuploidy in which the chromosome number differs from the normal haploid (*n*) or diploid (2*n*) chromosome number. Changes to an exact multiple of the haploid number (e.g. 3*n*, 4*n*) is termed polyploidy, triploidy and tetraploidy, respectively. In distinction from mammals, where triploidy is usually associated with lethal to sublethal conditions (Gold and Avise, 1976; Aardema *et al*., 1998), in lower vertebrates, triploids are more viable and have been observed in hybrids between unisexual and bisexual species of fish, amphibians and reptiles (Thorgaard and Gall, 1979). Interest in the potential use of triploids in fish culture has been based on the assumption that these fish are in general sterile. Namely, suppressed gonadal development in triploid individual may allow metabolic energy and nutritional resources, normally used for development of sexual characteristics and reproduction, to be directed into faster somatic growth (Thorgaard, 1983). Experimentally, triploids have been induced by different treatments (chemical and mechanical) (Purdom, 1983; Thorgaard, 1983; Teskeredzic *et al*., 1993). The most common method for inducing triploidy is thermal shock. This procedure, when applied shortly after normal fertilization, prevent block extrusion of the second polar body resulting in incorporation of a third chromosome set into the genome (Purdom *et al*., 1985).

Besides the inducing of polyploidy, heat shock has genotoxic effects (Anitha *et al*., 2000). Genotoxic effects can result by micronuclei (MN) induction. Micronuclei are cytoplasmic chromatin-containing bodies formed during the metaphase/anaphase transition of mitosis (cell division) either from a whole lagging chromosome (aneugenic event) or lagging acentric chromosome fragments caused by chromosomal breakage (clastogenic event) which do not integrate in the daughter cell nuclei following anaphase (Heddle, 1973; Schmidt, 1975; Miller *et al*., 1998).

As different investigators relate MN appearance with polyploidy and aneuploidy in mammalian cells caused by different genotoxic compounds or treatment (Ciranni *et al*., 1995; Uryvaeva and Delone, 1995; Elhajouji *et al*., 1998; Miller *et al*., 1998; Gonzalez-Cid *et al*., 1999), the aim of our study was to establish MN appearance in triploid rainbow trout. To the best of the author's knowledge this is the first study of this kind conducted on triploid fish.

MATERIAL AND METHODS

The experiment was performed at fish farm Gracani (Croatia) with the constant influx of spring water. Rainbow trout (*Oncorhynchus mykiss*

Walbaum) eggs and milt were collected by stripping ripe spawners (one female – body weight 3.850 g, and two males – body weights 2.320 g and 1.939 g, respectively). Afterwards, eggs were divided into two groups 312 and 313 eggs/group). All eggs were fertilized by the semidry method as described by Bojcic *et al*. (1982) and maintained in circulatory spring water of 10°C. Twenty minutes post fertilization, one group of eggs was immersed in a thermoregulated bath at water temperature of 26°C for 20 minutes (temperature was maintained by Techne-Tempette TE-8D). Second group was kept at water temperature of 10°C. Following treatment, the eggs were placed in an incubator supplied with spring water and reared at ambient temperature until the yolk sac was absorbed (67 days). Dead eggs were removed once a week and survival was calculated as the percentage of the eggs that hatched. At swim-up stage, fry were transferred to tank divided into two compartments, also supplied with spring water. The fry were fed once a day with commercial fish food. Water temperature was measured daily. Dissolved oxygen, carbon dioxide, pH, organic particulates, ammonium and hardness were measured once every two weeks.

Blood samples for the smears were taken from the caudal vessels of 80 fish from each group 67 (following absorption of the yolk) and 128 days (fry stage) post fertilization, fixed in 95% methanol for 3 minutes, left to air dry and stained with 20% Giemsa solution for 15 minutes. The scoring was performed with Opton (Germany) microscope at 1 600× magnification. A thousand erythrocytes were scored per slide to determine the frequency of cells with micronucleus and 25 erythrocytes per slide were analyzed to detect triploidy. Criteria for MN were performed according to Tates *et al*. (1980) defining diameter smaller than one third of the nucleus; non-refractibility of the MN to exclude small particles; color the same as/or lighter than nucleus. The presence of triploidy was determined from the volume of erythrocyte nuclei as described by Stanley and Allen (1979). Shortly, triploid fish were identified by measurement of erythrocyte nuclei length and width with ocular micrometer and calculation of nuclear volume ($V = 4/3\pi ab^2$; a = major semiaxis, b = minor semiaxis).

Statistical evaluation was carried out using Mann-Whitney Rank Sum Test (Sigma Statistical Software, Version 1.0).

RESULTS AND DISCUSSION

During the experimental period ambient water temperature ranged 7.5–10.1°C, pH 7.43–7.58, concentration of dissolved oxygen 9.9–12.7 mg/l, dissolved carbon dioxide 6.3–13.2 mg/l, organic particulates 4.11–6.30 mg/l, concentration of ammonia <0.10 mg/l and total hardness 84.54–103.9 mg/l as $CaCO_3$. These values were optimal for rainbow trout development.

Analysis of blood smears showed that all rainbow trout exposed to thermal shock soon after fertilization had significantly higher ($P < 0.05$) erythrocyte nuclei volume (116.5 ± 12.81 μm^3) (Figure 1) than diploid (untreated) individuals (69.9 ± 14.257 μm^3) (Figure 2). The heat shock of 26°C for 20 minutes duration, administered 20 minutes after insemination, gave 100% induction of triploidy. Chorrout and Quillet (1982) succeeded in inducing 100% triploidy in rainbow trout by applying 26°C heat shock last-

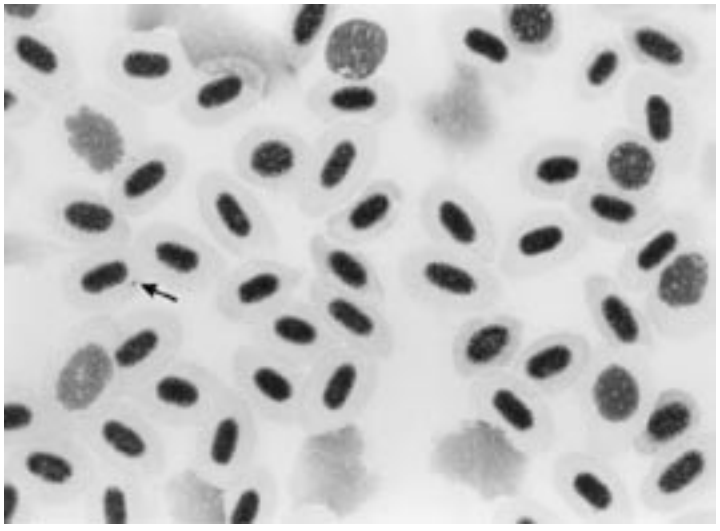

Figure 1. Erythrocytes of triploid rainbow trout Arrow: a micronucleated erythrocyte

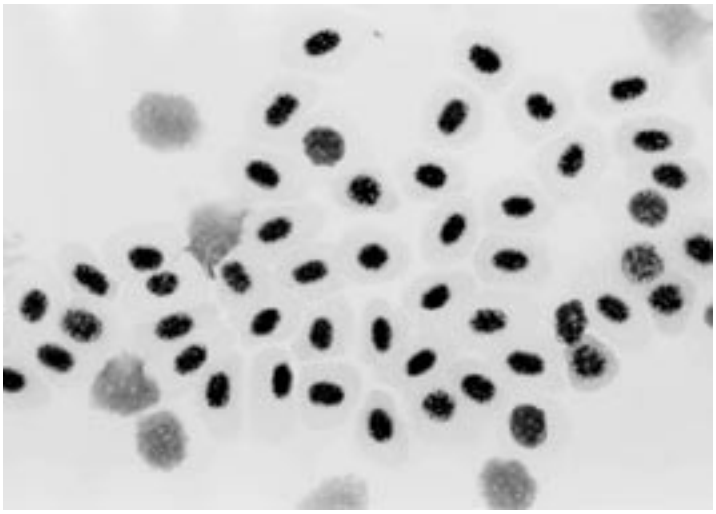

Figure 2. Erythrocytes of diploid rainbow trout

ing 20 minutes but commencing 25 minutes after insemination. Survival in control group was 81.09% but survival of triploids was low (46.97%). It has often been reported and possibly relates to "egg quality" or to the susceptibility of eggs of different origin to triploidising treatments (Lou and Purdom, 1984; Johnstone, 1985).

As MN can occur as a consequence of both structural and numerical chromosomal aberrations (Miller *et al*., 1998), in this study, the frequencies of micronucleated erythrocytes in a control and heat shocked population of rainbow trout were analysed. Micronucleus induction was observed in both fish groups and results are presented in Table 1. Data showed significantly ($P < 0.001$) higher number of micronucleated erythrocytes in triploid than in diploid individuals at both sampling periods. The MN in diploids we attributed to spontaneous occurrence.

The average MN frequency in the triploid fish collected 67 days after insemination was approximately 2 fold higher than in diploid fish. A clear increase in MN frequencies was found even 128 days after temperature shock and the difference between triploid and diploid group increased. The level of micronucleated erythrocytes was 3 fold higher in triploids than in diploids.

Table 1. The average micronucleated erythrocyte frequency between diploid and triploid fish at swim-up and fry stage

Rainbow trout	MN/1 000 erythrocytes (mean ± SD)	
	67 days post fertilization	128 day post fertilization
Diploid	1.10 ± 0.96	1.80 ± 1.57
Triploid	2.41 ± 1.28	5.92 ± 3.80

From the present study it is evident that MN formation was directly linked to the polyploidy. Our findings are in good agreement with other investigations conducted on mammals, which noted that the MN frequency correlates with increased variability in chromosome number. Uryvaeva and Delone (1995) showed that liver cells from mouse carrying MN were the higher ploides rather than the population in general and Ciranni *et al*. (1995) also found a significant presence of types of MN that are probably aneuploid related. Elhajouji *et al*. (1998) reported about increasing frequency of MN in tetraploid lymphocytes. Since triploid red blood cells are polyploid cells, they could contain multiple molecular targets for genetic damage in comparison with diploid cells during cell division. During division these cells could likely form micronuclei. Yamazaki and Goodier (1993) in the chromosome preparations from polyploid fish embryos noted fragments that remained isolated between the two daughter nuclei at telophase. Fragmented chromosomes were found in cytoplasmic regions far from the spindle or nuclear areas but authors did not correlate it with micronucleus.

Anitha *et al*. (2000) found MN in adult gold fish subjected to heat shock. They noted that the effect of temperature on the induction of micronuclei was not dose dependent but the frequency of MN was correlated with the number of polyploid set of chromosomes In distinction from Anitha *et al*. (2000) who recorded nuclei deviation from their regular structure when subjected to heat shock, in

this experiment erythrocyte nuclei did not exhibit such deviation.

Yamada *et al*. (1989) also reported that polyploidy increased when cells were cultured at over 40.3°C compared with cells cultured at 37°C and structural chromosome aberrations (breaks, stickiness, fragmentation) were observed at 41.5°C.

Asanami and Shimono (1997) suggested that hyperthermia disturbs the mitotic apparatus in mammalian cells *in vivo* and induces micronuclei formation.

Heat shock in our study primarily induced increasing chromosome number and the consequence of this event with time distance was formation of MN.

In summary, our results confirmed previous investigation, which indicated that the micronuclei are consequence of numerical chromosomal aberrations but we showed that an early application of heat shock on fish eggs has indirect consequence on latter occurrence of micronucleus.

Acknowledgments

The authors thank Mr. Mirko Goricar for supplying rainbow trout eggs and milt, and dr. Emin Teskeredzic for the use of tanks on fish farm in Gracani.

REFERENCES

Aardema M.J., Albertini S., Arni P., Henderson L.M., Kirsch-Volders M., Mackay J.M., Sarrif A.M., Stringer D.A., Taalman R.D.F. (1998): Aneuploidy: A report of an ECETOC task force. Mutat. Res., *410*, 3–79.

Anitha B., Chandra N., Gopinath P.M., Durairaj G. (2000): Genotoxicity evaluation of heat shock in gold fish (*Carassius auratus*). Mutat. Res., *469*, 1–8.

Asanami S., Shimono K. (1997): High body temperatures induces micronuclei in mouse bone marrow. Mutat. Res.: Genetic Toxicology and Environmental Mutagenesis, *390*, 79–83.

Bojcic C., Debeljak Lj., Vukovic T., Jovanovic-Krsjanin B., Apostolski K., Rzanicanin B., Turk M., Volk S., Drecun Dj., Habekovic D., Hristic Dj., Fijan N., Pazur K., Bunjevac I., Marosevic Dj. (1982): Slatkovodno ribarstvo (in Croatian). Ribozajednica Zagreb, Jugoslavenska medicinska naklada, Zagreb. 606 pp.

Chourrout D., Qillet E. (1982): Induced gynogenesis in the rainbow trout: sex and survival of progenies. Production of all triploid populations. Theor. Appl. Genet., *63*, 201–205.

Ciranni R., Antonetti M., Migliore L. (1995): Vanadium salts induce cytogenetic effects *in vivo* treated mice. Mutat. Res.: Genetic Toxicology, *343*, 53–60.

Elhajouji A., Cunha M., Kirsch-Volders M. (1998): Spindle poisons can induce polyploidy by mitotic slippage and micronucleate mononucleates in the cytokinesis-block assay. Mutagenesis, *13*, 193–198.

Gold J.R., Avise J.C. (1976): Spontaneous triploidy in the California roach *Hesperoleucus symmetricus* (Pisces: Cyprinidae). Cytogenet. Cell Genet., *17*, 144–-149.

Gonzalez-Cid M., Cuello M.T., Larripa I. (1999): Comparison of the aneugenic effects of vinorelbine and vincristine in cultured human lymphocytes. Mutagenesis, *14*, 63–66.

Heddle J.A. (1973): A rapid *in vitro* test for chromosomal damage. Mutat. Res., *18*, 187–190.

Johnstone R. (1985): Induction of triploidy in Atlantic salmon by heat shock. Aquaculture, *49*, 133–139.

Lou Y.D., Purdom C.E. (1984): Polyploidy induced by hydrostatic pressure in rainbow trout *Salmo gairdneri* Richardson. J. Fish. Biol., *25*, 345–351.

Miller B., Pötter-Locher F., Seelbach A., Stopper H., Utesch D., Madle S. (1998): Evaluation of the *in vitro* micronucleus test as an alternative to the *in vitro* chromosomal aberration assay: position of the GUM working group on the *in vitro* micronucleus test. Mutat. Res., *410*, 81–116.

Purdom C.E. (1983): Genetic engineering by the manipulation of chromosomes. Aquaculture, *33*, 287–300.

Purdom C.E., Thompson D., Lou Y.D. (1985): Genetic engineering in rainbow trout, *Salmo gairdneri* Richardson, by the suppression of meiotic and mitotic metaphase. J. Fish. Biol., *27*, 73–79.

Schmidt W. (1975): The micronucleus test. Mutat. Res., *31*, 9–15.

Stanley J.G., Allen S.K. Jr. (1979): Polyploid mosaics induced by cytochalasin B in landlocked Atlantic salmon (*Salmo salar*). Trans. Am. Fish. Soc., *108*, 462–466.

Tates A.D., Neuteboom I., Hofker M., Den Engelse L. (1980): A micronucleus technique for detecting clastogenic effects of mutagens/carcinogens (DEN, DMN) in hepatocytes of rat liver *in vivo*. Mutat. Res., *74*, 11–20.

Teskeredzic E., Teskeredzic Z., Donaldson E.M., McLean E., Solar I. (1993): Triploidization of coho salmon following application of heat and electric shocks. Aquaculture, *116*, 287–294.

Thorgaard G.H. (1983): Chromosome set manipulation and sex control in fish. In: Hoar W.S., Randall D.J., Donaldson E.M. (eds.): Fish Physiology. Vol. 9, Part B. Academic Press, New York. 405–434.

Thorgaard G.H., Gall G.A.E. (1979): Adult triploids in a rainbow trout family. Genetics, *93*, 961–973.

Uryvaeva I.V., Delone G.V. (1995): An improved method of mouse liver micronucleus analysis – An application to age-related genetic alteration and polyploidy study. Mutat. Res.: Environmental Mutagenesis & Related Subjects, *334*, 71–80.

Yamada K., Ichikawa Y., Okumura H. (1989): Effects of high temperatures on chromosomes of normal and transformed human cell. Human Cell, *2*, 80–85.

Yamazaki F., Goodier J. (1993): Cytogenetic effects of hydrostatic pressure treatment to suppress the first cleavage of salmon embryos. Aquaculture, *110*, 51–59.

Received: 02–12–31
Accepted after corrections: 03–06–13

Corresponding Author

Ivancica Strunjak-Perovic, Division of Materials Chemistry, Rudjer Boskovic Institute, Bijenicka 54, 10000 Zagreb, Croatia
E-mail: strunjak@irb.hr