

# Cytogenetic damage in gill and gonad cells of bivalve molluscs

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Cytogenetic effects were examined in gill and gonad cells of bivalves *Anodonta cygnea*, *Unio tumidus* and *Dreissena polymorpha* inhabiting the Lithuanian lakes Birva, Skaistis, Drūkšiai, Verkiai and the artificial Elektrėnai and Kauno marios water reservoirs. The highest level of aneugenic effects (cells with hypodiploid, hyperdiploid and polyploidy chromosome sets) was found in molluscs from contaminated artificial water reservoirs, and significantly fewer injuries were detected in bivalves from the uncontaminated lakes Verkiai and Skaistis.

**Key words:** aneugenic effects, chromosomes, *Unio*, *Anodonta*, *Dreissena*

## INTRODUCTION

A wide variety of industrial, domestic and agricultural wastes is discharged into aquatic systems, and a large number of waste compounds can induce hazardous effects in aquatic organisms. Aquatic organisms are able to accumulate harmful substances up to high concentrations in their tissues (Jha, 2004). It is known that certain contaminants exert their effects via genotoxic and metabolically toxic mechanisms, simultaneously causing carcinogenesis, embryotoxicity and a long-term damage in organisms (Jha et al., 2000). Pollutants of hydrosystems exist in complex mixtures where they can undergo a wide variety of interactions and biotransformations. During such processes, the additive, synergistic or antagonistic effects may be observed. On the other hand, so-called “natural pesticides”, which are more carcinogenic and mutagenic than “man-made” compounds, are synthesized in natural ecosystems (De Flora et al., 1991). Therefore, a clear understanding of cumulative effects of environmental pollution on natural systems is needed.

Increased awareness at industrial and municipal contamination sites throughout Lithuania has led to consider areas that are ecologically stressful for aquatic organisms. Therefore, there is a necessity for the selection of target indicator species and sensitive biomarkers able to encourage a comprehensive assessment of antropogenic impacts *in situ*.

Molluscs appear to be one of the most suitable groups of bioindicators and are often used in ecotoxicological studies. They are sedentary long-living organisms resistant to contaminants and can survive in an environment where other organisms

are eliminated. Due to filter, or detritus, feeding style they can accumulate toxic substances and achieve tissue concentrations 100 to 1000 times higher than the concentrations that usually occur in water (Naimo, 1995).

The objective of this study was to examine aneugenic effects on freshwater bivalve molluscs inhabiting different water bodies in Lithuania, differing in their ecotoxic exposure. Indigenous bivalve molluscs were collected from heavily polluted sites of Elektrėnai and Kaunas water reservoirs and from four lakes differing in contamination levels. At the subcellular level, the chromosome set was been identified as a target for the effects of aneugenic compounds.

## MATERIALS AND METHODS

Cytogenetic disturbances were assessed in gill and gonad cells of mollusks. Lakes Verkiai and Skaistis served as reference sites. The sampling place at the Kaunas reservoir (by Arlaviškės) is affected by pollution from the Nemunas river and the sampling location at the Elektrėnai reservoir (by the Strėva river inflow) is contaminated by pollutants from the thermal electrical power plant. Lake Birva receives local household wastewaters and Lake Drūkšiai sewages from the Ignalina NPP (Fig. 1).

Blocking bivalve cell divisions at metaphase was achieved by injecting a 0.1–0.2% aqueous solution of colchicine into adult molluscs 4–10 hours before dissection. Pieces of sample tissues were dissected from molluscs and prepared according to modified methods previously used in karyological studies of trematodes (Baršienė, Grabda-Kazubskas, 1988) and molluscs (Baršienė et al., 1996). Hypotonization of mollusc tissues was performed in distilled water at room temperature for 40–90 min.

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Fig. 1. Study areas; (1) Lake Drūkšiai, (2) Lake Verkiai, (3) Lake Skaistis, (4) Lake Birva, (5) Elektrėnai reservoir, (6) Kaunas reservoir

Table. Material for cytogenetical studies of bivalve molluscs from the lakes Verkiai, Skaistis, Drūkšiai and from the Elektrėnai and Kaunas water reservoirs

Mollusc species	Verkiai	Skaistis	Birva	Drūkšiai	Elektrėnai reservoir	Kaunas reservoir
<i>A. cygnea</i>	18	–	21	12	–	15
<i>U. tumidus</i>	10	–	10	–	20	20
<i>Dreissena polymorpha</i>	–	20	20	15	35	15

The material was fixed with a 3 : 1 ethanol acetic acid solution which was changed three times: after 30 min after 1 h and after 24 h. Tissues were dissociated in 45% acetic acid, and cells were smeared on slides and slightly heated up to human body temperature on a flame. The slides were stained with 4% Giemsa for 30–50 min using phosphate buffer solution, pH = 6.8. The mitotic metaphase and meiotic stages were examined with a Jena Med cytology microscope (Germany) at a final magnification of 1000×. Numerical changes of chromosomes in mitotic and meiotic nuclei, such as cell hypo-, hyperploidy and polyploidy, were determined.

The chromosome number variability  $p$  was counted as a percentage according to the formula:

$$p = (\Sigma a / \Sigma x) 100\%$$

where  $a$  is an abnormal or normal cell number and  $x$  stands for all examined cells.

## RESULTS

In gill and gonad cells of *Anodonta cygnea*, the diploid chromosome set was equal to 38. Such number of chromosomes was

found in 72.2–91.8% of cells. The highest level of cytogenetic damage was detected in cells of *A. cygnea* inhabiting the Kauno marios reservoir, and the lowest – in those collected from Lake Verkiai ( $P < 0.05$ ; Fig. 2). Comparatively frequent polyploid cells (17.7%) were registered in clams from the Kauno marios reservoir and in clams from Lake Birva (14.8%). In clams from Lake Drūkšiai we found an elevated level of hypodiploid cells (13.9%; cells containing one or more chromosomes less than in diploid sets).

The diploid chromosome number in *Unio tumidus* was also equal to 38. The amount of diploid cells varied in a range from 50.0% in clams sampled from the Elektrėnai water reservoir to 89.4% in molluscs from Lake Verkiai. A high level of aneugenic effects (34.6%) was found in clams from the Kauno marios reservoir ( $P < 0.05$ ). Polyploid cells up to 24.0% were registered in tissues of clams from the Elektrėnai water reservoir (Fig. 2).

In gills and gonads of zebra mussel *Dreissena polymorpha* from Lake Skaistis, 85.2% of cells possessed 32 chromosomes in their diploid set. A significantly higher level of cytogenetic damage was detected in zebra mussels inhabiting the Kauno marios reservoir and Lake Drūkšiai ( $P < 0.05$ ). In mussels from the Kauno marios reservoir, 61.3% of gill cells possessed a diploid set. A comparatively high amount of polyploid cells (20.5%) was found in mussels from this reservoir (Fig. 2).

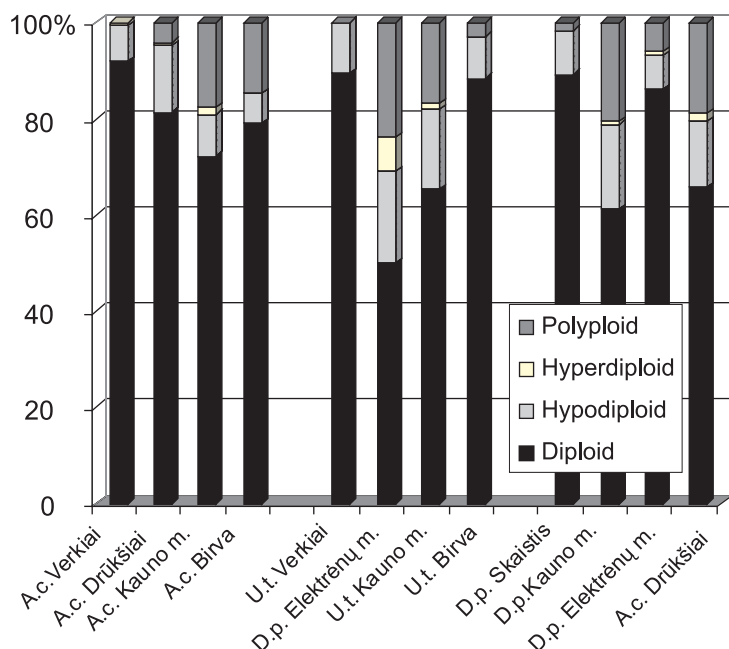


Fig. 2. Aneugenic effects in gill and gonad cells of bivalve molluscs collected from different lakes and water reservoirs

## DISCUSSION

It is known that the effects induced by the environmental contaminants are initially displayed at the molecular (biochemical, genetic) level; as a consequence of such disturbances, various cytological, morphological, physiological and other changes occur. At the later stages, changes may occur at higher biological levels and affect the ability of organisms to grow, to reproduce or to survive. Cytogenetic damage in cells can initiate cancerogenesis in various tissues and organs. Agents that cause genetic effects may be present at very low, sublethal concentrations (Anderson et al., 1994).

*In vivo* studies of cytogenetic damage in indigenous organisms represent a direct testing of environmental sites, and such factors as bioavailability and metabolic transformation could be integrated directly in the response of organisms to the action of chemical stressors. Thus, cumulative effects of complex mixtures of pollutants could be evaluated *in situ* (Hose, 1994).

In this study, numerical changes of chromosomes (aneugenic effects) were investigated in gill and gonad cells of three bivalve mollusc species. Aneugenic effects were observed in tissues of molluscs inhabiting different freshwater bodies in Lithuania, with the lowest levels in bivalves from the reference lakes Skaistis and Verkiiai. It is noteworthy that the highest levels of aneugenic effects were observed in bivalve molluscs sampled from the artificial water reservoirs Elektrėnai and Kauno marios. The study site in the Elektrėnai water reservoir suffers the polluting influence of the Lithuanian thermal power plant, and the site in Kauno marios are polluted by the inflow from the Nemunas river. Cell hypodiploidy (cells consisting of one or more chromosomes less than in diploid sets) was the main type of aneugenic effects in bivalve gills. Polyploid cells were most frequent in gonads of bivalves from the artificial water reservoirs.

Radionuclides, aromatic and alkyl hydrocarbons and other hazardous organic compounds were found in the Nemunas river and in Kauno marios reservoir (Ellington et al., 1994; Butkus

et al., 1997). Relatively high concentrations of heavy metals – Cr (up to 45 mg/kg), Pb (up to 32 mg/kg), Ni (up to 28 mg/kg) were detected in sediments of the Kauno marios reservoir (Kruopienė, 1997).

Production of electricity at the Elektrėnai thermal power station depends on the production rate of the Ignalina nuclear power plant. Since 1992, it has subsequently been operated at 5% of installed capacity, but until 1992 it annually produced approximately 10 TWh / year. At the Elektrėnai thermal power station, on average 150–200 mill. t of heavy fuel – oil (sulphur content up to 3.5%) and up to 100 mill. m<sup>3</sup> of natural gas are burned every year. As a fuel, orimulsion (sulphur content up to 3.0%) also can be used. The emission of hazardous organic compounds, heavy metals (mainly Mg, Ni, V, Fe, Ca and Na) and sulfur oxides occurs mostly through the air. Studies performed by Čeburnis with colleagues (2002) demonstrate that at a one-kilometer distance about 6% of combustion metals are trapped from the air column, and within 30 km about 15% out of the total emitted metals are washed out by rain and snow events. The industrial sewage system of the Elektrėnai power station is connected to an oil separation plant. From the outlet of the oil separation plant, water is directed to a municipal sewage purification plant. The rain water system is discharged to the recipient via a series of ponds. The recipient for all water – the Elektrėnai reservoir – is connected to the Strėva river.

Radioecological studies in the Ignalina region demonstrate that the surrounding areas near the nuclear power plant are being constantly polluted with <sup>137</sup>Cs, <sup>134</sup>Cs, <sup>89</sup>Sr, <sup>90</sup>Sr, <sup>60</sup>Co, <sup>54</sup>Mn, <sup>95</sup>Nb, <sup>59</sup>Fe and by other technogenic radionuclides (Marčiulionienė et al., 1992; Mažeika, Motiejūnas, 2001). Studies performed by Marčiulionienė and colleagues (2001) demonstrate that in the water of Lake Drūkšiai and in its coastal areas, the concentrations of <sup>60</sup>Co and <sup>54</sup>Mn can reach up to 200 and 90 Bq/kg, respectively, and in algae and bottom sediments up to 180 and 204 Bq/kg, respectively. The Ignalina nuclear power plant also contaminates Lake Drūkšiai with thermal pollution. In the summer

time, in the water discharge place, the water temperature can reach 30–31 °C. Different organic compounds, heavy metals, and radionuclides enter Lake Drūkšiai with cooling water (Marčiulionienė, 2003). Treated municipal water from the Visaginas town is disposed into Lake Drūkšiai through the Gulbinėlė stream. About 5.5–8.5 mill. m<sup>3</sup> of pretreated water gets into the lake every year with the mean annual concentrations of nitrogen 37.7 mg N/l and phosphorus 3.5 mg P/l (Mažeika et al., 2006).

In our earlier studies, we found that hypodiploid and polyploid cells were usually more frequent in molluscs inhabiting areas highly polluted with different contaminants (Baršienė, 1994). In aquatic habitats of Lithuania polluted with heavy metals, PAHs, PCBs, and in the areas of the Chernobyl fallout spots cytogenetic damage in molluscs was higher than in molluscs from unpolluted areas. The frequency of polyploid cells, chromosome set instability and neoplastic lesions were higher in molluscs inhabiting areas polluted by both chemicals and radionuclides, which shows the synergistic effects of these pollutants (Baršienė, 1994, 1997, 2002; Baršienė et al., 1994, 1996; Baršytė, 1997). Aneugenic effects in molluscs from different locations of the Klaipėda port area revealed the highest cytogenetic damage (up to 46.2% of studied cells) in molluscs from Malkū Bay in 1995 and 1996 and a significant decrease (to 27.2% in 1999) after dredging contaminated sediments from the bay (Baršienė, 2002). Assessment of cytogenetic damage in *Mytilus edulis* (MN test) and the crustacean *Balanus improvisus* (aneugenic effects) inhabiting the Baltic Sea at the Būtinge oil terminal has shown the highest genotoxicity level in the zone of sewage effluents from Palanga town and the Mažeikiai oil refinery (Baršienė, 2002).

Aneuploidy can result from cell cycle checkpoint errors, spindle, and/or kinetochore defects as a consequence of exposure to aneugenic agents. The causal factors of somatic aneuploidy and the mechanisms of action of aneugenic chemicals have been described (Parry et al., 2002). Herbicides (Bouilly et al., 2004), aromatic hydrocarbons (Dixon, 1982) and heavy metals (Bouilly et al., 2006) have a potential to induce aneugenic effects. Various aneugens are able to influence cell division and to induce structural chromosomal aberrations. The well-known aneugens colcemid, colchicine and vincristine have been investigated for the induction of structural chromosomal aberrations, polyploid cells and alterations in the mitotic index. The results of a study performed by Arni and Hertner (1997) showed that at low and intermediate concentrations, aneugens induced polyploidy and an increase in mitotic index, but no chromosomal aberrations.

A positive relationship between the herbicide atrazine and aneuploidy has been demonstrated in bivalves *Crassostrea gigas* adults, and an interesting phenomenon was disclosed in their offspring which exhibited a significantly higher aneuploidy levels when their parents had been exposed to atrazine. In depuration, aneugenic effects remain during 2.5 months (Bouilly et al., 2004).

Relations between gill neoplasia in the bivalve *Macoma balthica* from the polluted Gulf of Gdansk (Poland) and high levels of aneugenic effects have been observed (Thiriou-Quievreux, Wolowicz, 2001). It is known that heavy metals can cause an oxidative DNA damage (Hartwig, 1995). The formation of reactive oxygen species leads to oxidative DNA damage and induction of DNA strand breaks, DNA–protein cross-links, and therefore

chromosomal aberrations are formed. On the other hand, heavy metals As and Hg are mitotic spindle poisons and can induce aneugenic effects as a result of chromosome desegregation (Kirsch-Volders, 1986).

Polycyclic aromatic hydrocarbons are well-known genotoxins and may cause genetic damage in aquatic organisms (Venier, Canova, 1996). There are some studies that describe an increase of environmental genotoxicity in mussels inhabiting zones affected by oil spill (Baršienė et al., 2004, 2006; Laffon et al., 2005; Gorbi et al., 2008). Treatment with crude oil provoked a significant increase of micronuclei and other nuclear abnormalities in mussel gills (Baršienė, Andreikėnaitė, 2007). A field study on PAH records and genotoxic effects in inter-tidal bivalves *Perna perna* have demonstrated that the frequency of micronuclei are significantly related to PAH concentrations (Francioni et al., 2007). Increased levels of DNA damage in bivalves *Mytilus galloprovincialis* caged for 4 weeks in the area of an offshore platform in the Adriatic Sea have been demonstrated using the Comet assay and micronucleus test (Gorbi et al., 2008). Exposure of zebra mussels *Dreissena polymorpha* to benzo(a)pyrene induced DNA and cytogenetic damage (Binelli et al., 2008).

Taking into consideration the complexity of environmental contamination and induced biological effects, a conceptual basis for ecological risk assessment, which would encompass a broad scope of the mechanisms, consequences and the fate of some heavily contaminated ecosystems, is needed. Future studies of environmental pollution impacts on freshwater systems should concentrate on relationships among chemical stressors and aneugenic as well as clastogenic impacts. One of the most promising tools is transfer and caging of target organisms into heavily industrialized sites of aquatic systems. This has implications for the construction of an active monitoring system which will provide for non-destructive techniques in the ecotoxicological approach. Application of modern cytogenetic approaches and the scoring of cytogenetic damage in somatic and gonadal cells will allow assessing a correlation between eco-genotoxicity effects *in situ* and environmental contamination.

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#### CITOGENETINĖS PAŽAIDOS DVIGELDŽIŲ MOLIUSKŲ ŽIAUNŲ LĄSTELĖSE

##### Santrauka

Citogenetinių pažeidimų dažnis buvo įvertintas Birvos, Skaisčio, Drūkšių, Verkių ežeruose, Elektrėnų vandens saugykloje bei Kauno mariose gyvenančių dvigeldžių moliuskų žiaunų ir gonadų ląstelėse. Tyrimams buvo pasirinktos *Anodonta cygnea*, *Unio tumidus* ir *Dreissena polymorpha* rūšys. Aukščiausias aneugeninių pažeidimų dažnis (ląstelės su hipodiploidiniu, hiperdiploidiniu ir poliploidiniu chromosomų rinkiniu) buvo nustatytas moliuskuose, kurie gyveno Elektrėnų vandens saugykloje bei Kauno mariose, santykinai užterštuose dirbtiniuose vandens telkiniuose. Patikimai mažesnis pažeidimų dažnis buvo Verkių ir Skaisčio ežeruose gyvenusių dvigeldžių moliuskų ląstelėse.

**Raktažodžiai:** aneugeninis poveikis, chromosomos, *Unio*, *Anodonta*, *Dreissena*