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Risk of environmental genotoxicity in the Baltic Sea over the period of 2009–2011 assessed by micronuclei frequencies in blood erythrocytes of flounder (*Platichthys flesus*), herring (*Clupea harengus*) and eelpout (*Zoarces viviparus*)

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A B S T R A C T

Environmental genotoxicity was investigated at 82 locations encompassing different regions of the Baltic Sea. Micronuclei (MN) analysis was performed in erythrocytes of 1892 specimens of flounder *Platichthys flesus*, herring *Clupea harengus* and eelpout *Zoarces viviparus*, three of the most common native fish species of the Baltic Sea collected in 2009–2011. MN background levels in fish were determined using data obtained in 2001–2011 from 107 Baltic sites. Extremely high genotoxicity risk zones were found for flounder at 11 stations out of 16 in 2009 and 33 stations of 41 in 2010–2011, for herring, at 5 of 18 stations in 2009 and 20 of 43 stations in 2010–2011, in eelpout only at one out of 29 stations. The sampling stations were restricted mainly to the southern and eastern Baltic Sea offshore zones and in most of them, MN frequencies in flounder and herring significantly exceeded the reference and background levels of micronuclei. This is a first attempt to evaluate the background MN responses, as well as low, high and extremely high genotoxicity risk levels for native fish species.

1. Introduction

The Baltic Sea is one of the most contaminated marine ecosystems. Summarizing results of field studies carried out within the framework of the EU funded pan-European BEEP project on biological effects of contaminants in organisms inhabiting the Baltic Sea, Lehtonen et al., 2006 concluded that, although the loads of some classical chemical toxic substances (e.g., PCBs, DDTs) have been reduced over the last decades, chemical pollution by a wide spectrum of hazardous substances may be assumed to be higher nowadays than ever before. According to the results of the integrated HELCOM CHASE assessment (HELCOM, 2010) based on data from the period 1999–2007 for hazardous substances and selected biological effects in the Baltic Sea, 137 out of the 144 areas assessed were classified as being “disturbed by hazardous substances”, including all open-sea areas of the Baltic Sea analyzed. In the southern Baltic Sea, the Kiel and Mecklenburg Bights were classified as most polluted and ecologically worst areas (HELCOM, 2010).

In the HELCOM assessment, (HELCOM, 2010) it was pointed out that a large number of different substances exceeded the threshold levels in the different Baltic Sea sub-basins. In fish, mussels and bird tissues, PCBs, dioxins, heavy metals, organometals, alkylphenols, phthalates, brominated compounds, polycyclic aromatic hydrocarbons (PAHs), DDTs and chlorinated pesticides, and caesium-137 were found at the highest concentrations in relation to target levels. The southern region of the Baltic Sea is polluted by all of the above-mentioned substances (HELCOM, 2010). Many of these substances inherently are genotoxic compounds and may exert genotoxicity effects via direct action or the activation of toxic metabolic mechanisms and are, thus, of concern regarding their potential impact on aquatic organisms and human health. Chemical substances with genotoxicity potential can be sub-divided into four groups: (1) substances directly inducing DNA damage; (2) substances the metabolites of which cause DNA damage; (3)

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substances that increase the production of reactive oxygen species (ROS) and free radicals, which can subsequently damage both DNA bases and the deoxyribose backbone; (4) substances that inhibit DNA synthesis and repair (Lee and Steinert, 2003). Genotoxic compounds can bind to DNA causing the formation of DNA adducts, single and double strand breakages, modifications in DNA repair and crosslink consistent pattern, as well as alterations of cell functions, reproduction disturbances, growth inhibition, or even carcinogenesis (Ohe et al., 2004). As a consequence, further generations of the organisms can suffer from reduced fitness, fertility or embryonic viability (Russo et al., 2004). Non-repaired genetic damage is considered important since it provides a fundamental early warning sign of adverse long-term effects of contaminants at population and, furthermore, ecosystem levels (Rybakovas et al., 2009). Furthermore, contaminants, usually discharged in complex mixtures, can provoke interactions between unknown substances and lead to the unpredictability in genotoxic responses to pollution (Jha, 2008).

Environmental genotoxicity in the Baltic Sea was earlier assessed in the Swedish part of the Gulf of Bothnia (Al-Sabti and Härdig, 1990) and in Danish waters in Køge Bay, Little Belt, Store Belt and Kattegat (Wrisberg et al., 1992). Later studies, carried out by the Institute of Ecology (Lithuania), covered the Lithuanian economic zone (Baršienė and Baršytė Lovejoy, 2000; Baršienė, 2002; Baršienė and Rybakovas, 2006; Baršienė et al., 2004, 2005a, 2006a, 2006b, 2008, in press), the Gulf of Gdansk (Baršienė et al., 2006b; Kopecka et al., 2006; Napierska et al., 2009), Swedish coastal sites Kvädöfjärden and those in the Stockholm archipelago (Baršienė et al., 2006b; unpublished data), the Wismar Bay (Baršienė et al., 2006b; Schiedek et al., 2006) and 12 offshore areas of the Baltic Sea (Rybakovas et al., 2009).

Based on these data, a large database on environmental genotoxicity in the Baltic Sea was established. In fish, the data collected in the period of 2001–2011 were available for flounder (*Platichthys flesus*) from 75 stations, for herring (*Clupea harengus*) from 59 stations and for eelpout (*Zoarces viviparus*) from 35 stations. Data for cod (*Gadus morhua*), plaice (*Pleuronectes platessa*) and turbot (*Psetta maxima*) were collected from 25 stations. Environmental genotoxicity was also evaluated in bivalve mollusks *Mytilus edulis* (45 stations), *Mytilus trossulus* (6 stations) and *Macoma balthica* (28 stations). A validation of the micronucleus test was performed repeatedly in a variety of laboratory exposure studies using contaminants from different chemical groups (Baršienė et al., 2004, 2005b, 2006a, 2006b, 2010a, 2010b; Bagni et al., 2005; Baršienė and Andreikėnaitė, 2007; unpublished data).

The existing large database allows defining the reference and background levels of genotoxicity responses in different marine fish and molluscs, i.e., the formation of micronuclei (MN), nuclear buds (NB) and bi-nucleated cells with nucleoplasmic bridges (BNb). These endpoints reflect the action of aneugenic and clastogenic substances in different species inhabiting various regions of the Baltic Sea and other marine ecosystems.

The main goal of the present study was to assess environmental genotoxicity levels in blood erythrocytes of three of the most common native fish species of the Baltic Sea and to map genotoxicity risk levels in different zones of the Baltic Sea in 2009–2011. As indicator of genotoxicity, the formation of micronuclei in blood erythrocytes, as a large lesion at a sub-cellular level, was evaluated. The selection of fish blood erythrocytes as a target cell to investigate genetic damage was based on the important role of blood in the transfer of hazardous substances absorbed through skin, gill and other tissues of the aquatic organisms.

2. Materials and methods

Material for the micronuclei (MN) analysis in flounder, herring and eelpout was collected from June 2009 to March 2011 at a total of 82 study stations located in different regions of the Baltic Sea. The locations of the fish sampling stations are presented in Figs. 1 and 2. The list of the fish sampling stations and their

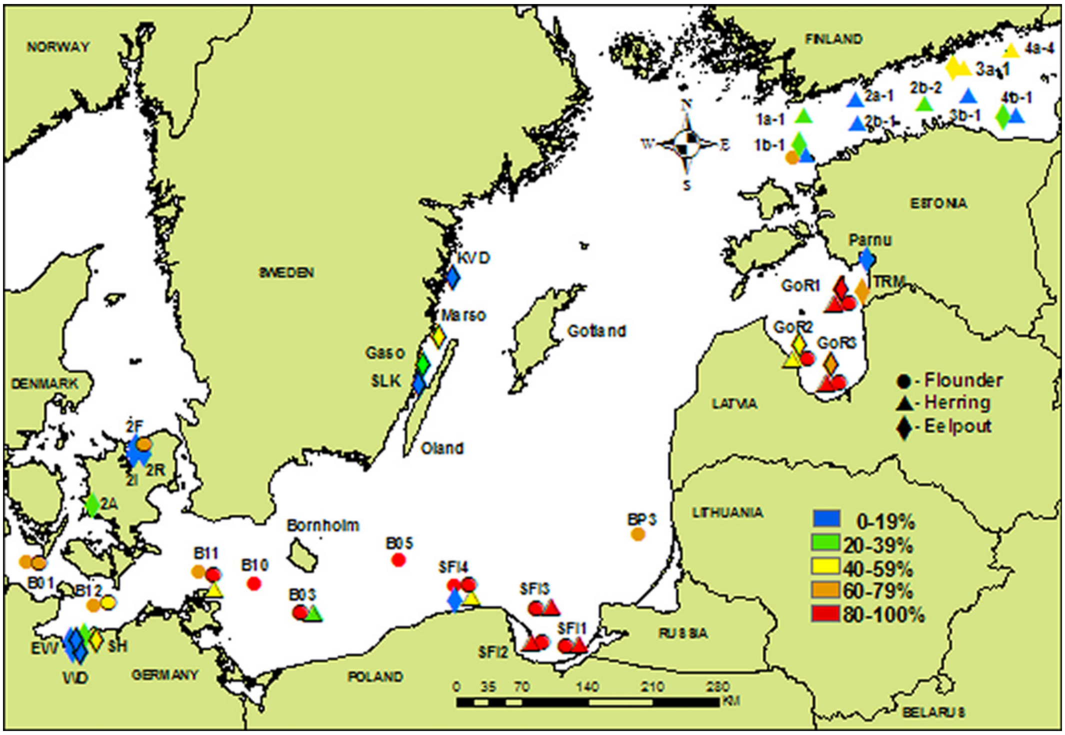

Fig. 1. Results of environmental genotoxicity risk assessment in flounder (*Platichthys flesus*), herring (*Clupea harengus*) and eelpout (*Zoarces viviparus*) collected from different regions of the Baltic Sea in 2009.