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Environmental genotoxicity and cytotoxicity in flounder (*Platichthys flesus*), herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) from chemical munitions dumping zones in the southern Baltic Sea

Janina Baršienė[a,*], Laura Butrimavičienė[a], Wlodzimierz Grygiel[b], Thomas Lang[c], Aleksandras Michailovas[a], Tomas Jackūnas[a]

[a] Nature Research Centre, Institute of Ecology, Akademijos Str. 2, 08412 Vilnius, Lithuania
[b] National Marine Fisheries Research Institute in Gdynia, 1 Kollataja Street, 81-332 Gdynia, Poland
[c] Thünen Institute of Fisheries Ecology, Deichstraße 12, 27472 Cuxhaven, Germany

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ABSTRACT

The data on environmental genotoxicity and cytotoxicity levels as well as on genotoxicity risk in flounder (*Platichthys flesus*), herring (*Clupea harengus*) and cod (*Gadus morhua*) collected in 2010–2012 at 42 stations located in chemical munitions dumping areas of the southern Baltic Sea are presented. The frequency of micronuclei, nuclear buds and nucleoplasmic bridges in erythrocytes was used as genotoxicity endpoint and the induction of fragmented-apoptotic, bi-nucleated and 8-shaped erythrocytes as cytotoxicity endpoint. The most significantly increased geno-cytotoxicity levels were determined in fish collected near known chemical munitions dumpsites. Extremely high genotoxicity risk for flounder were identified at 21 out of 24 stations, for herring at 29 out of 31 and for cod at 5 out of 10 stations studied. The reference level of genotoxicity was not recorded at any of the stations revealing that in the sampling area fish were affected generally.

1. Introduction

The marine environment is increasingly contaminated with xenobiotic compounds, including hydrocarbons such as petroleum and its derivates, heavy metals, chlorinated pesticides, radionuclides and many other substances discharged from industries, agriculture or municipal activities (Korpinen et al., 2012). International attention over the last few decades has been attracted by the problem of sea-dumped chemical warfare agents (CWA), which after World Wars I and II were accumulated and dumped into the seas in hundreds of thousands tons (Kaffka, 1995). For a long time, the problem was concealed and politically sensitive, as the munitions dumping operations were secret with undefined responsibility. The full extent of munitions dumping operations has remained uncleared to date and the real picture of dumping locations is still incomplete. Consequently, there is considerable uncertainty as to ecological risks associated with CWA in the marine environment due to the lack of information on the location, amount and subsequent distribution of chemical warfare agents (Missiaen et al., 2010) as well as their biological effects on marine organisms.

About thirteen thousand of tons of chemical warfare agents have been dumped at different sites of the Baltic Sea, mainly in the Bornholm and Gotland basins. The main CWAs were sulphur mustard (63%), arsenic-containing compounds such as Clark and arsine oils, Adamsite (31%) and α-chloroacetophenone (5%) (HELCOM, 1994). Due to unvalued, imperfect and indecorous dumping operations and later relocation by bottom fishing gears, warfare agents appeared in over a much wider territory than the one registered in operation documents (HELCOM, 1993; Astot et al., 2007). Chemical munitions east of the Bornholm Island were dumped in a considerably large area. Warfare agents were mainly dumped in munitions, mostly in bombs, shells and containers. Munitions were also packed into wooden boxes, which could drift before sinking. Later these wooden boxes with warfare agents were reported to have drifted to Bornholm or the Swedish coastline (HELCOM, 1996). Munitions that have been retrieved since 1992 proved to be heavily corroded or empty (Sanderson and Fauser, 2008).

* Corresponding author. Tel.: +370 6 8260979; fax: +370 5 2729257.
E-mail addresses: janina.barsiene@ekoi.lt, janbar@ekoi.lt (J. Baršienė).

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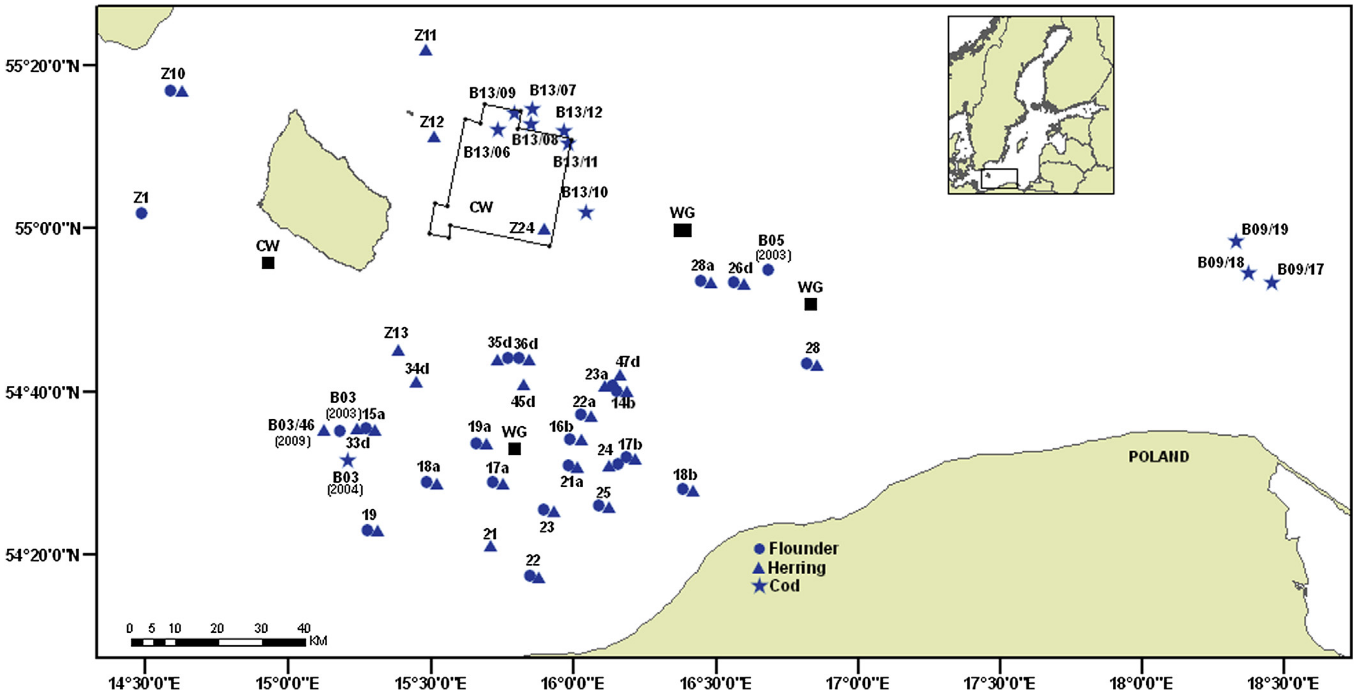

Fig. 1. Location of sampling stations in the Baltic Sea (CW and WG – known warfare dumpsites).

Studies of the Bornholm chemical munitions dumpsite showed that contamination of bottom sediments with CWA was patchy and was recorded up to about 100 m away from the shipwrecks (Missiaen et al., 2010). Several authors reported significant concentrations of As in sediments from dumping sites, suggesting that leakage of CWAs contributed to arsenic contamination (Munro et al., 1999; Tørnes et al., 2002; Garnaga and Stankevičius, 2005). Mustard and organoarsenic warfare agents were also detected in sediments from CWA dumping areas located in the North Sea (Granbom, 1996; Tørnes et al., 2006) and the Southern Adriatic Sea (Amato et al., 2006). Attributed to the leakage of yperite from rusted bomb shells dumped in the Southern Adriatic Sea, 3–4 times higher levels of As and Hg were recorded in fillets of two benthic fish species and genotoxicity effects of CWA were described. The suitability of fish for the environmental quality assessment was emphasised (Della Torre et al., 2010, 2013).

The main objective of the present study was to evaluate the level of environmental genotoxicity and cytotoxicity in three native fish species inhabiting the chemical munitions dumping zones located in the Bornholm Basin of the Baltic Sea. Furthermore, environmental genotoxicity risk was assessed at each of the study stations. This basin is regarded as the most important spawning area for commercially important fish species in the Baltic Sea (Köster et al., 2003). A number of studies have indicated that fish respond to low concentrations of genotoxic substances that may be present in chronically polluted areas, and, therefore fishes are regarded as appropriate environmental genotoxicity bioindicators (Baršienė et al., 2006; Schiedek et al., 2006; Kopecka et al., 2006; Yadav and Trivedi, 2009; Nahrgang et al., 2010). Furthermore, considering a continuous intake of contaminants through water (respiration) and food, fish may be particularly sensitive to arsenic compounds released to the environment (Bears et al., 2006).

In the present study, the induction of micronuclei (MN), nuclear buds (NB), nuclear buds on filament (NBf) and bi-nucleated cells with nucleoplasmic bridges (BNb) were used as genotoxicity endpoints and the induction of fragmented-apoptotic (FA), bi-nucleated (BN) and 8-shaped nucleus cells were assessed as cytotoxicity endpoints in erythrocytes of three fish species. The micronucleus (MN) test is a sensitive and fast approach to detect structural and numerical chromosomal alterations induced by clastogenic and aneugenic agents (Heddle et al., 1991). The formation of nuclear buds may reflect an unequal capacity of organisms to expel damaged, amplified, failed replication or condensed improperly DNA, chromosome fragments without telomeres and centromeres from the nucleus (Lindberg et al., 2007). Induction of bi-nucleated cells with nucleoplasmic bridges has been used as a marker of dicentric chromosomes, and thereby served as an index of cytogenetic damage in fish (Summak et al., 2010). The investigated cytotoxicity endpoint, fragmented-apoptotic erythrocytes, is a form of regulated cell death. A higher rate of cytokinesis failure can result in an increase of bi-nucleated and 8-shaped nucleus cells.

2. Materials and methods

2.1. Sampling of fish

For the environmental genotoxicity and cytotoxicity assessment, flounder (*Platichthys flesus*), herring (*Clupea harengus*) and cod (*Gadus morhua*) specimens were collected at 42 study stations located in the Bornholm Basin from November 2010 to February 2012 (Fig. 1). Samples were obtained from research catches carried out by the RVs "Baltica" and "Walther Herwig III" using standard bottom or pelagic trawls. The list of study stations, geographic coordinates of trawling stations, depth of trawling (m) and hydrological parameters such as water temperature (°C), salinity and O_2 concentration (mg/l) are presented in Table 1. The dates of sampling surveys and numbers of collected fish specimens are presented in Table 2.

Blood samples were collected from 188 flounder (23 stations), 307 herring (31 stations) and 127 cod specimens (10 stations). Most study stations for flounder and herring were located along the chemical munitions transportation (to the Bornholm and Gotland