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Environmental genotoxicity and cytotoxicity in the offshore zones of the Baltic and the North Seas

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ABSTRACT

Micronuclei (MN), nuclear buds (NB) and fragmented-apoptotic cells (FA) were analyzed in mature peripheral blood and immature cephalic kidney erythrocytes of flounder (*Platichthys flesus*), dab (*Limanda limanda*) and cod (*Gadus morhua*) from 12 offshore sites in the Baltic Sea (479 specimens) and 11 sites in the North Sea (291 specimens), which were collected during three research vessel cruises in December 2002, 2003 and in September 2004. The highest levels of environmental genotoxicity (frequencies of MN up to 0.5‰, NB – up to 0.75‰) and cytotoxicity (FA – up to 0.53‰) were observed in flatfishes from areas close to oil and gas platforms in the North Sea and in zones related to the extensive shipping and potentially influenced by contamination from large European Rivers (Elbe, Vistula, Oder). In dab from the offshore zones of the North Sea, the levels of nuclear abnormalities were higher as compared to those in dab from the Baltic Sea. Responses in immature kidney erythrocytes were higher than in mature erythrocytes from peripheral blood. MN frequency lower than 0.05‰ (the Baltic Sea) and lower than 0.1‰ (the North Sea) could be suspected as a reference level in the peripheral blood erythrocytes of flatfish.

1. Introduction

Surrounded by European countries, the Baltic and the North Seas are constantly exposed to anthropogenic pressure. With pollution from land-based discharges, coastal industries, municipal outflows, agricultural activities, oil and gas industry (drilling and transportation) and transport activities, thousands of chemicals are entering the marine environment. A large proportion of these compounds are potentially toxic, genotoxic and carcinogenic to aquatic organisms (Jha, 2004, 2008). The interaction of genotoxic contaminants with DNA causes various genetic disturbances, which often are irreversible and can be transmitted to the next generations (Depledge and Fossi, 1994; Dixon et al., 1999; Jha, 2004). The analysis of environmental genotoxicity provides early warning signs of adverse long-term effects of contamination. A number of biomarkers have been applied for assessment of environmental genotoxicity and the micronuclei (MN) test, as one of the most popular approaches, has served as an index of cytogenetic damage for more than 30 years (Boller and Schmid, 1970; Schmid, 1975; Heddle et al., 1991). The assay is well elaborated and allows to evaluate the genotoxicity of compounds at low concentrations and to assess dose–response relationships of both DNA reactive and non-DNA reactive genotoxins (e.g. aneugens) (Al-Sabti et al., 1994; Palhares and Grisolia, 2002; Pacheco and Santos, 2002; Gravato and Santos, 2002, 2003; Rodriguez-Cea et al., 2003; Teles et al., 2003). Micronuclei arise from chromosome fragments or whole chromosomes that lag at cell division due to lack of centromere, damage in centromere region, or defect in cytokinesis (Fenech, 2000). MN are small, secondary structures of chromatin, are surrounded by membranes, located in the cytoplasm and have no detectable link to the cell nucleus (Boller and Schmid, 1970; Heddle, 1973; Heddle et al., 1991; Mac Gregor, 1991).

The MN test has successfully been applied in fish for the assessment of marine genotoxicity *in situ* (Hayashi et al., 1998; Al-Sabti and Metcalfe, 1995; Bolognesi et al., 1996, 2006a; Pietrapiana et al., 2002; Çavaş and Ergene-Gozukara, 2003, 2005a; Baršienė et al., 2004, 2005, 2006a,b,c; Köhler and Ellesat, 2008). In recent years, growing attention has been paid to nuclear abnormalities other than MN and it was demonstrated that these nuclear abnormalities can serve as an index of genotoxic and cytotoxic damage (Carrasco et al., 1990; Dolcetti and Venier, 2002; Ateeq et al., 2002; Pacheco and Santos, 2002; Gravato and Santos, 2002, 2003; Çavaş and Ergene-Gozukara, 2003, 2005b; Frenzilli et al, 2004; Bolognesi et al., 2006b; Baršienė et al., 2006a,c; Baršienė and Andreikėnaitė, 2007). Nuclear buds are morphologically similar to micronuclei with the exception that they are joined to the nucleus by a thin nucleoplasmic connection. It is suggested that nuclear buds arise from the elimination of amplified DNA (Miele et al., 1989; Shimizu et al., 1998, 2000) and possibly of DNA-repair

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complexes (Haaf et al., 1999). Studies performed by Lindberg et al. (2007) reveal that in normal and folate-deprived human lymphocytes the majority of nuclear buds originate from interstitial acentric chromosomal fragments, some of them are formed from terminal chromosome fragments or whole chromosomes, possibly representing nuclear membrane entrapment of DNA that has been left in cytoplasm after nuclear division or excess DNA. Though, most of the nuclear buds have different origin than micronuclei, studies in human tumor cells have indicated, that in the S-phase of the cell cycle amplified chromosomal or double minute DNA may form buds, later disintegrate from the nucleus and become a micronucleus (Pedeutour et al., 1994; Shimizu et al., 1998, 2000; Yankiwski et al., 2000). Fragmentation of cells and apoptosis can be provoked by a variety of factors, including those with genotoxic potential (Steinert, 1996; Steinert et al., 1998; Mičić et al., 2001; Baršienė et al., 2005, 2006a,d), or directly associated to cytotoxic events (Bolognesi et al., 2006b). Data on other nuclear abnormalities presents a wider view on cellular processes following the exposure and permits the measurement of important complementary genotoxicity and cytotoxicity events.

In the last decades, environmental genotoxicity in coastal areas of the Baltic and the North Seas was assessed in the number of studies (Al-Sabti and Hardig, 1990; Bresler et al., 1999; Baršiene and Baršyte Lovejoy, 2000; Baršiene et al., 2006b; Thiriot-Quiévreux and Wolowicz, 2001; Bombail et al., 2001; Frenzilli et al., 2004; Schiedek et al., 2006; Kopecka et al., 2006; Broeg and Lehtonen, 2006; Lehtonen et al., 2006; Stachnik and Wolowicz, 2007; Köhler and Ellesat, 2008). However, there is a lack of information on genotoxic and cytotoxic effects in the offshore areas of the Baltic and the North Seas. The aim of the current study was to evaluate genotoxic and cytotoxic effects of environmental pollution in selected offshore sites of the Baltic and the North Seas. The assessment of MN and nuclear buds (genotoxicity endpoints) and fragmented-apoptotic cells (cytotoxicity endpoint) was performed in mature peripheral blood erythrocytes of the flatfish species flounder (*Platichthys flesus*) and dab (*Limanda limanda*) as well as in Atlantic cod (*Gadus morhua*). Specific inter-tissue differences in induction of MN and the other nuclear abnormalities were observed during *in vivo* studies of the genotoxicity and cytotoxicity of endocrine disruptors and flame retardants (Baršienė et al., 2005) as well as crude oil and nonylphenol (Baršienė et al., 2006a) carried with Atlantic cod and turbot (*Scophthalmus maximus*). In order to assess tissues specific peculiarities of genotoxic and cytotoxic effects in offshore zones of the Baltic and the North Seas, in samples collected in September 2004 immature cephalic kidney erythrocytes were additionally analyzed.

2. Material and methods

2.1. Sampling

Samples were collected during three cruises with the German RV Walther Herwig III to the Baltic and the North Seas, organized by the the Institute of Fishery Ecology of the Federal Research Centre for Fishery. In December 2002, 2003 and September 2004, flounder (*P. flesus*), dab (*L. limanda*) and Atlantic cod (*G. morhua*) were collected in 12 sampling areas in the Baltic Sea and 11 areas in the North Sea (Fig. 1).

Brief description and details on geographical coordinates, sampling depth, water temperature and salinity of the sampling areas are presented in Tables 1 and 2. Data about studied organisms and number of specimens collected during each cruise in the different sampling areas are presented in Table 3. Fishes were collected by bottom trawling; using a 180 ft (1 foot = 0.3048 m) trawls, with a towing time of 1 h. Only live specimens, all strictly of the same size (total length (L) of flatfishes: 20–24 cm; cod: 28–32 cm) were processed for analysis.

2.2. Sample preparation and analysis

A drop of blood from caudal vessels was directly smeared on glass slides and air-dried. Smears of immature erythrocytes were prepared directly; using a small piece of cephalic kidney gently dragged along a clean slide and allowed to dry for 1–2 h (Baršienė, 1980). Smears were fixed in methanol for 10 min and were stained with 5% Giemsa solution for 8 min (Baršienė et al., 2004). The frequency of micronuclei, nuclear buds and fragmented-apoptotic cells was evaluated by scoring at 1000× magnification, using an Olympus BX51 microscope. A total of 5000 erythrocytes from peripheral blood were examined for each fish specimen. In flatfishes, which were sampled in September 2004, additionally 5000 immature erythrocytes from the cephalic kidney were analyzed.

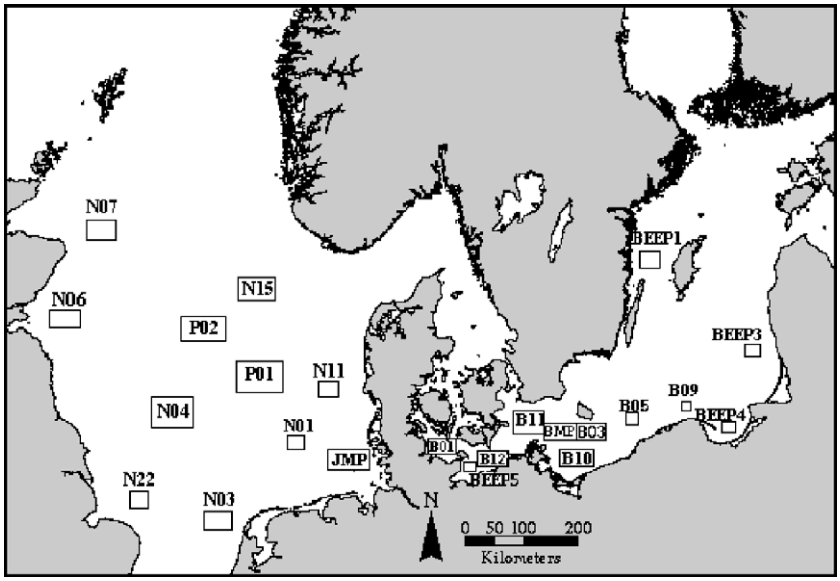

Fig. 1. Location of sampling areas in the Baltic and the North Sea offshore zones.