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Environmental genotoxicity and cytotoxicity levels in fish from the North Sea offshore region and Atlantic coastal waters

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ABSTRACT

In the framework of the ICON project, environmental genotoxicity and cytotoxicity levels were assessed in blood erythrocytes of dab (*Limanda limanda*) and haddock (*Melanogrammus aeglefinus*) collected at 25 stations in the North Sea and near the coast of Iceland in August–October 2008. Micronuclei, nuclear buds and bi-nucleated cells with nucleoplasmic bridges were assessed as environmental genotoxicity biomarkers, and the frequency of fragmented-apoptotic and bi-nucleated erythrocytes were assessed as environmental cytotoxicity biomarkers. The lowest frequencies of genotoxic and cytotoxic abnormalities were detected in fish from the Icelandic study stations. The highest frequencies of abnormalities were recorded in dab from the Dogger Bank and the German Bight, in haddock from the Egersund Bank and from an area off the Firth of Forth (North Sea). In fish from the Icelandic reference area, frequencies of genotoxicity and cytotoxicity responses were significantly lower than in fish from most areas of the North Sea.

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1. Introduction

Thousands of new chemicals annually enter the European market, which in total constitutes approximately 100,000 substances (OSPAR, 2010). A large proportion of anthropogenic chemicals are hazardous to aquatic organisms. They are persistent, able to accumulate in living organisms, toxic, able to mimic hormones or interfere with enzyme-controlled biological processes and are capable of modifying genetic material. In total, approximately 300 substances are considered to be of possible concern for the marine environment, of which 40 substances and groups of substances were identified as chemicals requiring priority action (OSPAR, 2010) aimed at preventing and reducing pollution. However, these substances often occur below the detection limit but may still act as genotoxins or carcinogens, even at very low concentrations. Furthermore, contaminants are usually discharged in complex mixtures, and interactions between unknown compounds can lead to unpredictable genotoxicity responses to pollution (Jha, 2008). Genotoxic compounds or their metabolites can bind to DNA molecules and trigger a damaging chain of biological changes, such as impaired enzyme function or general metabolism, cytotoxicity, immunotoxicity, reproduction disturbances, growth inhibition or carcinogenesis (Ohe et al., 2004). Such changes can be passed on to ensuing generations and, in some cases, may lead to a loss of genetic diversity (Dixon et al., 1999; Jha, 2004).

Environmental genotoxicity studies enable the description of the particular impacts of pollution in areas of concern, and genotoxicity biomarkers can serve as early warning signals of environmental pollution by chemicals able to modify the DNA of organisms. The implementation of the EU Marine Strategy Framework Directive (EC, 2008/56/EC) delineates the development of common criteria for the assessment of Good Environmental Status (GES) and environmental monitoring across Europe. The joint ICES/OSPAR Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC) developed an integrated approach to the monitoring of chemical contaminants and biological effects and assessment criteria for Descriptor 8 of Good Environmental Status under the Marine Strategy Framework Directive. The micronucleus (MN) assay was included in a core set of measurements of GES and the background document on the MN assay as a tool for assessing cytogenetic/DNA damage in marine organisms is presented in the ICES SGIMC report (ICES, 2011).

The micronucleus (MN) test is one of the most frequently used assays and has served as an indicator of cytogenetic damage for over 30 years (Fenech et al., 2003). It has proven to be a sensitive and rapid technique to detect structural and numerical chromosomal alterations induced by clastogenic and aneugenic agents (Heddle et al., 1991). The formation of nuclear buds (NB) reflects the unequal capacity of organisms to expel from the nucleus

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damaged, amplified, replication-failed or improperly condensed DNA and chromosome fragments without telomeres or centromeres (Lindberg et al., 2007). Bi-nucleated cells with nucleoplasmic bridges (BNb) are formed from dicentric chromosomes in which centromeres have been dragged to the opposite poles of the dividing cell at the anaphase, followed by formation of a nuclear membrane around the chromosomes. BNb formation is a marker of DNA damage (Fenech and Crott, 2002) that has been applied to genotoxicity studies in fish (Summak et al., 2010). In addition, environmental cytotoxicity endpoints reflecting nuclear abnormalities related to cell death (fragmented-apoptotic cells, FA) and defects in cytokinesis (bi-nucleated cells, BN) were analyzed in the present study. Integrated analysis of micronuclei and other nuclear abnormalities is a widely applied method for the *in situ* assessment of marine environmental genotoxicity (Carrasco et al., 1990; Dolcetti and Venier, 2002; Pacheco and Santos, 2002; Çavaş and Ergene-Gozukara, 2005; Frenzilli et al., 2004; Napierska et al., 2009; Baršienė and Andreikėnaitė, 2007; Rybakovas et al., 2009).

Information on environmental genotoxicity and cytotoxicity in coastal and offshore areas of the North Sea is limited. In a study by Bresler et al. (1999), frequencies of MN as well as alkaline and acidic DNA unwinding were evaluated in blue mussels (*Mytilus edulis*) from the German Bight. The highest genotoxicity levels were found in mussels collected in areas impacted by the waters of the Elbe River, and the lowest were found in mussels collected near the shore of the island of Helgoland. In order to assess environmental genotoxicity levels along a pollution gradient in the Firth of Forth region of the North Sea, the genotoxicity effects MN, other nuclear abnormalities and DNA strand breaks (Comet assay) were evaluated in blood erythrocytes of butterfish (*Pholis gunnellus*). Elevated frequencies of MN as well as lobed and blebbed erythrocytes were detected in fish from industrially contaminated areas (Bombail et al., 2001). Levels of environmental contamination with PCBs, PAHs and metals correlated with formation of DNA strand breaks in erythrocytes and liver cells of dab (*Limanda limanda*) from the eastern English Channel, in the areas of the Seine and Somme Bays. Higher levels of genotoxicity biomarkers were observed in adult dab compared to juvenile fishes, with females being more sensitive than males (Akcha et al., 2003).

Increased levels of DNA strand breaks in blood erythrocytes and high concentrations of bile PAH metabolites were detected in eelpout (*Zoarces viviparus*) collected in Göteborg harbor 3 weeks after a bunker oil spill (10–100 tons). Significant recovery was observed in fish from the area 5 months after the accidental oil spill (Frenzilli et al., 2004). Levels of DNA adducts, up to 27 nmol/mol and up to 239 nmol/mol nucleotides, respectively, were observed in Atlantic cod (*Gadus morhua*) and corkwing wrasse (*Symphodus melops*) caught in the Karmsund area of the North Sea affected by pollution with combustion-related PAH compounds produced in connection with aluminum production (Aas et al., 2001). In blue mussel gill cells sampled in the harbor areas of Fiskaatangen (Norway) and Reykjavik (Iceland), levels of DNA adducts were up to 10 nmol/mol nucleotides. Levels of the DNA adducts correlated with PAH levels in the tissues and were higher in the gills than in digestive glands of the mussels (Skarphéðinsdóttir et al., 2007). A significantly elevated level of DNA damage (Comet assay) was observed in blue mussels collected in coastal areas of western Denmark potentially affected by anthropogenic pollution originating from chemical dumping sites (Rank et al., 2007). In the same study, the observed levels of genotoxicity effects were in accord with the results of heavy metal analysis, and caging of mussels in the contaminated area induced formation of DNA strand breaks after 64 days of exposure.

In comparison to a reference station, increased frequencies of MN in peripheral blood erythrocytes as well as higher levels of macroscopically visible neoplastic liver changes were observed in North Sea flounder (*Platichthys flesus*) from the German Bight influenced by contamination of the river Elbe. Increased MN formation was recorded in flounder with macroscopic liver neoplasms as well as in older individuals (Köhler and Ellesat, 2008). Frequencies of MN observed in liver erythrocytes of Atlantic cod and hematocytes of blue mussels caged in the vicinity of Norwegian oil platforms (Statfjord B/C and Troll B) correlated with distance to the platforms (Hylland et al., 2008). Produced water from the North Sea Ekofisk oil field induced genotoxic effects in blue mussels during laboratory exposure and caging *in situ* (Sundt et al., 2011). Increased levels of micronuclei, nuclear buds and fragmented apoptotic erythrocytes were observed in dab collected in North Sea areas close to oil and gas platforms, in zones with extensive shipping and in German Bight areas impacted by contaminants transported with waters of the rivers Elbe, Weser and Ems (Rybakovas et al., 2009).

A number of studies have stated that, because of the long-range transport of anthropogenic contaminants in marine areas around Europe, it is difficult to select a 'true' reference location with a pristine, uncontaminated environment (Baršienė et al., 2006a; Broeg and Lehtonen, 2006; Gercken et al., 2006; Kopecka et al., 2006; Lang et al., 2006; Schiedek et al., 2006). However, for the assessment of results from studies on biological effects of contaminants, it is very important to determine reference levels for the parameters studied. The main objective of the present study was, therefore, to evaluate the level of environmental genotoxicity and cytotoxicity in areas which are away from possible sources of pollution (Icelandic coastal sites) and to compare it with the levels determined in different areas of the North Sea considered to be impacted by contaminants. Additional tasks were to evaluate environmental geno-cytotoxicity differences observed in fish collected at different stations of the same study area and to compare the results of the present study with results of previous studies performed in 2004 (Rybakovas et al., 2009). During the implementation of the ICON project, a wide range of biomarkers, including liver histopathology, lysosomal membrane stability, DNA adducts, Comet assay, analysis of external fish diseases, PAH metabolites, EROD and acetylcholinesterase activity, were analyzed in the same specimens. In addition, chemical analysis of organic compounds, metals and methyl mercury was performed. Thus, the results of the present study will supplement the outcomes of the integrated assessment of these parameters. Analysis of biomarker responses will provide a link between environmental contamination and impact on aquatic organisms. The responses of five biomarkers were used for the assessment of geno-cytotoxicity at 25 sampling stations located in 11 areas: the frequency of micronuclei (MN), nuclear buds (NB) and bi-nucleated erythrocytes with nucleoplasmic bridges (BNb) in blood erythrocytes of two fish species (dab and haddock) were assessed as the endpoints of environmental genotoxicity, and the frequency of fragmented-apoptotic (FA) and bi-nucleated erythrocytes (BN) were measured as markers of environmental cytotoxicity. For environmental geno-cytotoxicity assessment in the North Sea and coastal areas of Iceland, haddock was used as a bioindicator species for the first time.

2. Materials and methods

2.1. Sampling and sample preparation

Samples were collected in August–September 2008 during cruise 315 of the RV Walther Herwig III, organized by the vTI Institute of Fisheries Ecology. Additional sampling in the Egersund Bank area was performed in October 2008 by the RV Scotia (organized by the Marine Laboratory, Marine Scotland). Dab (*L. limanda*) and haddock (*Melanogrammus aeglefinus*) were sampled in nine offshore areas (17 stations) of the North Sea and in two coastal

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