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DNA damage, acetylcholinesterase activity and lysosomal stability in native and transplanted mussels (*Mytilus edulis*) in areas close to coastal chemical dumping sites in Denmark

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Abstract

Biomarkers of genotoxicity (DNA damage, measured as tail moment in the Comet assay), neurotoxicity (acetylcholinesterase inhibition, AChE) and general stress (lysosomal membrane stability, LMS) were studied in native and transplanted blue mussels (*Mytilus edulis*) in coastal areas of western Denmark potentially affected by anthropogenic pollution originating from chemical dumping sites. The results indicate responses to pollution in all the biomarkers applied at the suspected areas, but the results were not consistent. Seasonal fluctuations in exposure situations at the study sites make interpretation of chemical pollution complex, as seen especially in the variability in results on DNA damage, and also in regard to AChE activity. These investigations further stress the importance of understanding the effects of natural factors (salinity, temperature, water levels, rain and storm events) in correct interpretation of the biomarker data obtained. In addition, adaptation of populations to local contamination may play a role in some of the response patterns observed.

Keywords: DNA damage; Comet assay; Acetylcholinesterase activity; Lysosomal membrane stability; Biomarker; Pollution monitoring; Blue mussel; *Mytilus edulis*

1. Introduction

Pollution from discharge of industrial wastewater and leakage from coastal waste sites poses a threat to marine ecosystems. Chemical monitoring of water, sediment and biota is a common measure to describe the degree of contamination. However, information on the concentrations of chemicals in the environment or in biota does not necessarily lead to reliable assessments on environmental impacts or human health problems. A complex chemical environment together with the inadequate prediction power of biological effects of contaminants available from chemical monitoring data have resulted in the search for appropriate so-called biomarkers of pollution effects (McCarthy and Shugart, 1990; Huggett et al., 1992; Lam and Gray, 2003; Handy et al., 2003). During the last decades a large number of biomarkers have been tested and validated for their applicability to detect biological effects as indicators of chemical stress at different levels of biological organisation (e.g. Wedderburn et al., 1998; De Lafontaine et al., 2000; Katsiadaki et al., 2002; Rodriguez-Cea et al., 2003; van der Oost et al., 2003; Bolognesi et al., 2004; Quinn et al., 2005; Amiard et al., 2006; Magni et al., 2006; Moore et al., 2006; Nigro et al., 2006).

In the present study, the blue mussel (*Mytilus edulis*) was used as the biomonitoring organism and three biomarkers, DNA damage (Comet assay), acetylcholinesterase (AChE) activity, and lysosomal membrane stability (LMS) were used to indicate effects of exposure to pollution at molecular and cellular levels. One of the main advantages of using mussels is that the observed adverse effects in sessile organisms can be assumed to be caused by local pollution.

DNA damage observed in aquatic species can indicate exposure to genotoxic chemicals, which is believed to cause disturbances in ecosystems due to mutations in germ line cells and interference with reproduction (Würgler and Kramers, 1992), and, as the worst case scenario, can lead to an elevated extinction risk of sensitive species (Diekmann et al., 2004a,b). As an

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example, a monitoring study on DNA damage in caged mussels transplanted from clean sites to polluted sites (Steinert et al., 1998a) showed positive correlations between DNA damage in haemocytes and sperm nuclei (tail length in Comet assay) and the level of hazardous chemicals in sediment and mussels. The same study also showed a positive correlation between DNA damage and the time of exposure. In a recent monitoring study examining the consequences of the *Prestige* oil spill DNA damage was observed in oil-exposed *Mytilus galloprovincialis* (Laffon et al., 2006). In Køge Bay (south of Copenhagen, Denmark) DNA damage detected by the Comet assay in *M. edulis* sampled from polluted sites along the coastline showed a positive correlation with heavy metal (Cr, Ni and Cd) levels in the sediment (Rank et al., 2005). Also micronucleus (MN) frequency in mussels has been observed to be a very sensitive biomarker of exposure to various types of pollution (Baršienė et al., 2004, 2006a,b; Venier and Zamperion, 2005; Kopecka et al., 2006; Schiedek et al., 2006). The biological endpoint of the Comet assay, and partly of the MN test, is DNA strand breaks, and the studies mentioned above have shown that it is possible to use these genotoxic biomarkers as a measure of environmental pollution.

AChE is an enzyme involved in the synaptic transmission of nerve impulses and is strongly and directly inhibited by neurotoxic compounds like organophosphate and carbamate pesticides targeted to cause this mode of toxicity (e.g. Bocquene and Galgani, 1998). More recently, the responsiveness of AChE to many other chemical groups, e.g. heavy metals, hydrocarbons and detergents (Zinkl et al., 1991; Payne et al., 1996; Guilhermino et al., 1998), and algal toxins (Lehtonen et al., 2003) has also been acknowledged. AChE may thus prove to be a useful biomarker for detecting general physiological stress in aquatic organisms caused by exposure to contaminants. Recent studies in mussels from the Baltic Sea have shown that AChE is a very useful biomarker of pollution stress under a variety of environmental factors and chemical mixtures in different geographical regions (Baršienė et al., 2006a; Kopecka et al., 2006; Schiedek et al., 2006). However, AChE activity in bivalves is modified also by abiotic factors such as salinity and temperature (Leiniö and Lehtonen, 2005; Pfeifer et al., 2005).

A change in LMS of living haemolymph cells using the Neutral Red retention (NRR) assay reflects the health status of mussels, indicating physiological stress caused by pollutants (Moore et al., 2004a,b). The value of this method as a biomarker of general pollution has been shown in various studies (Lowe et al., 1995; Wedderburn et al., 1998; Regoli et al., 2004; Mamaca et al., 2005; Moore et al., 2006; Nigro et al., 2006).

Many biomarkers are affected, to some degree, by abiotic factors (Steinert et al., 1998b; Buschini et al., 2003; Bolognesi et al., 2004; Leiniö and Lehtonen, 2005; Pfeifer et al., 2005), while also different populations/genotypes may show different responses (Palmqvist et al., 2003). Also adaptation to chronic pollution may results in low or no response situations (Shaw et al., 2002; Wu et al., 2005). Therefore, direct comparisons between native populations collected from different areas must always be made with care. To overcome this problem, the approach of transplantation (caging) of target species collected from the same population and deployed in the study area has been used to yield comparable information in regard to prevailing environmental conditions including chemical pollution (e.g. Salazar and Salazar, 1997; Ericson et al., 2002; Bolognesi et al., 2004; Nigro et al., 2006; Damiens et al., 2007).

The present biomarker study focuses on aquatic pollution from an agrochemical plant established in 1953 in northwest Denmark. During the period from 1957 to 1962, organophosphorous pesticide waste from the factory as well as chemical waste from other parts of Denmark (parathion, DDT, nicotine, mercury (Hg), arsenic (As), copper (Cu)) was dumped in the dunes at the North Sea western coast at the groyne called Høfde 42 (Fig. 1). Ever since then there has been public concern about the hazard of leakage of chemicals from this dumping site (Elkjær and Petersen, 2004). Further on, when the original plant situated at the "Old factory ground" (Fig. 1) was moved to Rønland, a lot of chemical waste was left in the soil. Already in the early 1980s high levels of mercury were measured in mussels collected close to the groyne in the North Sea and close to the old factory at Rønland in 1984 (Riisgård, 1984). Some of the chemical waste from Rønland was removed in the 1980s but it is still necessary to pump (26 drillings so far) and clean the waste to avoid discharges of chemicals into the aquatic environment. In the new factory ground on Rønland the chemical waste was placed in pits located at the eastern part of the peninsula. In 1977 the pits were found to be leaky and drillings were established to pump away some of the hazardous leakage. Remedial pumping is still going on and the wastewater is pumped to the treatment plant at the new factory.

The purpose of the present study was to investigate the applicability of three different biomarkers in *M. edulis* for the monitoring and assessment of biological effects in indigenous and caged animals deployed at stations in the aquatic environment around the agrochemical industry and near the coastal waste sites. The three biomarkers were chosen because they represent different endpoints of general interest and were expected to show different effects in accordance with chemical pollution. The Comet assay was used because some heavy metals with genotoxic potential were known to leach from the dumping sites. AChE activity was naturally of interest because organophosphates were the main group of contaminants of concern in this area, and, finally, LMS is able to show a more general toxic response to the chemicals.

2. Material and methods

2.1. Study design

The study was carried out in co-operation with the County of Ringkøbing, which for many years has been the responsible authority for pollution monitoring in the study area. The main purpose was to investigate if mussels transplanted from a clean area to a polluted area are affected by leakage from the coastal dumping sites. With the intention to optimise the detection of effects we hypothesised that the rate of leaking of chemicals would be highest just after a storm event when the water level changed from high to low, hereby "sucking" the chemicals out