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Seasonal and intertidal impact on DNA adduct levels in gills of blue mussels (*Mytilus edulis* L.)

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Gill DNA adduct and total PAH tissue levels were higher in mussels in the intertidal than subtidal zone, and higher in winter than summer.

Abstract

The aim of this study was to elucidate possible seasonal variation in DNA adduct levels in blue mussels (*Mytilus edulis*), and to investigate the impact of intertidal exposure on the DNA adduct levels, i.e. to explore if DNA adduct levels in mussels in the intertidal zone differ from those in the subtidal zone. Blue mussels were deployed separately in the intertidal and subtidal zone at a contaminated and a reference site in Iceland, and sampled regularly during one year. Gill DNA adduct levels were found to be higher in mussels in the intertidal zone compared to the subtidal zone at the contaminated site, the difference being largest in winter. Total PAH tissue levels were also higher in mussels in the intertidal zone. Seasonal variation was observed in both DNA adduct and PAH tissue levels in mussels at the contaminated site, with lower levels from the time of transplantation in summer to autumn, maximum levels in winter, which decreased to lower levels again in spring and summer the following year. DNA adducts and PAH levels were low or below the detection limits in mussels at the reference site at all times, both in the intertidal and subtidal zone.

Keywords: DNA adducts; *Mytilus edulis*; Intertidal impact; Seasonal variation; Iceland

1. Introduction

Little is known about the impact of contaminant exposure on intertidal vs. subtidal coastal areas. The intertidal area is a sensitive ecosystem, alternately exposed to air or submerged in seawater due to the tidal cycle. Various abiotic components in the intertidal zone such as temperature and oxygen availability fluctuate widely with differences in duration of exposure controlled by tidal frequency. Intertidal organisms are therefore subject to extreme environmental factors, which possibly makes them more vulnerable to pollutant exposure than subtidal organisms.

Contaminants are constantly being released to the environment, many of them being genotoxic. Genotoxic pollutants are a cause of special concern, since they are capable of interacting with the genetic material of organisms, thereby affecting its structural integrity, and the fidelity of its biological expression (Wogan and Gorelick, 1985). Damaged DNA not repaired in time, may have serious consequences such as cell death, tumour initiation, embryo toxicity, mutations or other dysfunctions (Shugart, 1995; Kurelec, 1993). Mutations

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in somatic cells may possibly be detrimental to the exposed individual, whereas mutational events in germ cells may be passed on to the offspring, thereby affecting subsequent generations, and the genetic structure of the population (Bickham et al., 2000; Mulvey et al., 2003).

Polycyclic aromatic hydrocarbons (PAHs) are a large group of widespread environmental contaminants, some of which are known to be genotoxic by binding to the DNA after biotransformation. They are released into the environment by incomplete combustion of fossil fuels, creosote production, discharges from the electrochemical industry such as aluminium smelters, oil spills and water produced from oil production (Lorenz and Glovik, 1972; Neff, 1979; Brendehaug et al., 1992). DNA adducts are a sensitive and reliable biomarker for PAH exposure and genotoxic effects (e.g. Varanasi et al., 1989; Stein et al., 1993; Kurelec and Gupta, 1993; Ericson et al., 1999), and have also been used to assess genotoxic exposure in the marine environment. The ^{32}P postlabelling technique which is used in this work to detect DNA adducts has been under development for the past 25 years (Randerath et al., 1981; Gupta et al., 1982; Reddy and Randerath, 1986; Reichert et al., 1992; Reichert and French, 1994), and is currently the most sensitive method for analysing large hydrophobic DNA adducts, as it detects one DNA adduct in 10^9–10^{10} nucleotides.

Teleosts have frequently been used as bioindicators of genotoxic exposure in aquatic environments, but DNA adduct formation and analysis in mytilid mussels has received increasing attention (e.g. Venier and Canova, 1996; Harvey and Parry, 1997; Ericson et al., 2002). Mussels have a less active enzyme system for biotransformation of PAHs than fish, but are frequently used as indicator organisms to monitor coastal pollution, since they have many advantages as such. What especially makes mussels favourable as bioindicators is that they are widespread, easily accessible, often available in high numbers and sessile. They are also easily transplanted and kept in cages, and are important components of many coastal ecosystems. The blue mussel (*Mytilus edulis*) is widely used as a bioindicator, mainly for monitoring pollutant tissue levels, but increasingly also for biomarker studies. The ability of *Mytilus edulis* to form adducts has been demonstrated in both field studies (e.g. Harvey et al., 1997; Ericson et al., 2002), and laboratory experiments (e.g. Harvey and Parry, 1997; Shaw et al., 2000). DNA adducts have been detected mainly in gills rather than hepatopancreas, and large adducts persist for at least 2 weeks under experimental conditions (Ericson et al., 2002; Skarphéðinsdóttir et al., 2003). In order to understand and apply DNA adduct levels in mussels as a biomarker to monitor genotoxic pollution, it is of fundamental importance to investigate which factors affect the DNA adduct formation. In our previous studies on DNA adducts in blue mussels in the field, we found indications of a seasonal variation in adduct levels, as well as differences between intertidal and subtidal mussels (Ericson et al., 2002). This type of information is very valuable when designing, interpreting and comparing contaminant monitoring studies.

The aim of this study was twofold; to elucidate possible seasonal variation in DNA adduct levels in the mussels, and to investigate the impact of intertidal exposure on the DNA adduct levels, i.e. to explore if DNA adduct levels in mussels in the intertidal zone differ from those in the subtidal zone. This was tested by transplanting mussels from a clean reference site to the contaminated Reykjavík harbour in net cages situated in the subtidal and the intertidal zone for 1 year. Mussels were also deployed at the same depths at the clean reference site in Hvalfjörður.

2. Materials and methods

2.1. Chemicals

Salmon sperm DNA (S-1626), spermidine (S-2626), dithiothreitol (D-9779), RNase A (R-4642), micrococcal endonuclease (N-3755), spleen phosphodiesterase (P-9041) and polyethylenimine (P-3143) were obtained from Sigma-Aldrich Sweden AB, Stockholm, Sweden. Proteinase K (1000144), RNase T_1 (109 193), nuclease P1 (236 225), T4 polynucleotide kinase (3′-phosphatase-free, 838 292) and phenol (1814303) were supplied by Roche Diagnostics Scandinavia AB, Sweden. [γ-^{32}P]ATP was obtained from Amersham Biosciences, Uppsala, Sweden, and cellulose (MN 301) from Tamro MedLab AB, Mölndal, Sweden. The standard adduct 7*R*,8*S*,9*S*-trihydroxy-10*R*-(N^2-deoxyguanosyl-3′-phosphate)-7,8,9,10-tetrahydro-benzo[*a*]pyrene (B[a]PDE-dG-3′p) was obtained from NCI Chemical Repository, Midwest Research Institute, Kansas City, Missouri, USA. All other chemicals used were of analytical grade or higher.

2.2. Experimental setup and study sites

Blue mussels (*Mytilus edulis*) were sampled in June 2001 at the clean site Fossá in Hvalfjörður, SW Iceland, and deployed at two different depths in the contaminated Reykjavík harbour, as well as in Hvítanes in Hvalfjörður for reference (see Fig. 1 for site locations). The mussels were deployed: (1) in the mid intertidal zone at 1.9 m depth, and (2) in the subtidal zone at 4.9 m depth. The intertidal range in the area is 3.8 m, so mussels deployed in the intertidal zone were exposed to tidal water; alternately exposed to air or submerged in water, while mussels deployed in the subtidal zone were submerged in water at all times. The mussels were deployed in net bags, three replicates at each depth.