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Genotoxicant accumulation and cellular defence activation in bivalves chronically exposed to waterborne contaminants from the Seine River

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Abstract

The aim of the present work was to investigate genotoxicant accumulation and biological responses of zebra mussels and blue mussels collected along a pollution gradient in the Seine estuary and in the Seine Bay. The sampling area included three contaminated and one reference sites for each species. The study focused on polyaromatic hydrocarbons (PAH), lindane, polychlorobiphenyls (PCB) and metals known to be potential genotoxicants and/or reactive oxygen species (ROS) inducers. Enzymatic activities related to cellular defence systems including the phase II enzyme glutathione S-transferase (GST) and three antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathion peroxydase (GPx) were measured in gills. DNA adducts and DNA strand breaks (Comet assay) were measured in digestive gland and hemocytes, respectively. Species differences were observed in metal accumulation (As and Pb), GPx activity and DNA adduct formation. A marked upstream–downstream gradient was reported for PAH body burden and to a lesser extent for PCB and metals with the highest values measured just downstream the industrialized area of Rouen. GST and SOD activities in gills of bivalves were positively related to PAH and metals body burden, respectively. Activation of those cellular defences may prevent accumulation of electrophilic metabolites and free radicals and thus may protect DNA and others macromolecules against oxidation and adduction. Although DNA strand breaks and bulky adducts were detected in both species, levels were relatively low and no significant site differences were observed in June 2003. Our results indicate a clear relationship between genotoxicant accumulation and positive activation of detoxification and antioxidant systems but poor consequences in term of DNA damage for wild population of mussels inhabiting the Seine estuary.

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Keywords: Blue mussel; Zebra mussel; Metals; PAH; PCB; Bulky DNA adducts; Comet assay; Cu/Zn superoxide dismutase; Glutathione peroxydase; Catalase; Glutathione S-transferase

1. Introduction

For decades, numerous field studies including data from the Réseau National d'Observation de la qualité du mileu marin, RNO (Claisse et al., 1992) and from the NST Mussel Watch Project (O'Connor, 1998) have demonstrated the utility of using mussels for pollution monitoring of coastal marine areas. Indeed, mussels are sedentary filter-feeder known to accumulate high levels of certain metals and organic compounds and to exhibit cellular and physiological responses providing a time-integrated measurement of environmental contamination as well as induced biological effects (Solé et al., 1995). However most of field studies focused on marine mussels or oysters or a few fresh water

Abbreviations: CAT, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; dAMP, 2′-deoxyadenosine 3′-monophosphate; DRZ, diagonal radioactive zone; dw, dry weight; EDTA, ethylene-diamine tetra-acetic acid; GPx, glutathione peroxidase; GRd, glutathione reductase; GST, glutathione S-transferase; GSH, reduced glutathione form; GSSG, oxidized glutathione form; IEF, isoelectric focusing; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PAH, polyaromatic hydrocarbons; PCB, polychlorobiphenyls; POP, persistent organic pollutants; RALs, relative adduct levels; RNO, Réseau National d'Observation du milieu marin; ROS, reactive oxygen species; SOD, superoxide dismutase; TBT, tri-butyltin; TLC, thin layer chromatography; SEVAG, solution of chloroform and isoamyl alcohol 24/1 (v/v)

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bivalve species but little information is available on bivalves living in estuaries. In fact, it is of great concern since (i) most of pollutant originated from human activity are transported to the sea via rivers, (ii) a large part of contaminants adsorbed to particulates is trapped in the estuarine area and (iii) highly fluctuating environmental conditions might represent an additional stress factor for estuarine species.

Molluscs, and bivalves in particular, possess a wide range of defences to prevent toxic effects of chemicals at the cellular level: (i) metallothioneins and multi xenobiotic resistance proteins that actively reduce cellular entrance of toxicants, (ii) detoxification and antioxidant systems allowing neutralization and elimination of parent compounds, metabolites and by-products and finally (iii) DNA repair and protein and lipid turnover which maintain cell integrity. Most of those processes are driven by inducible proteins whose expression and/or activity levels have been shown to be related to the cellular content of certain toxicants. For instance, numerous components of the detoxification and antioxidant systems in mollusc species have been shown to be specifically induced by metals or PAH in controlled laboratory conditions (Livingstone, 1994; Regoli and Principato, 1995; Boutet et al., 2004) or by complex mixture of pollutants in the field (Cossu et al., 1997; Nasci et al., 1998; Gowland et al., 2002) and hence are thought to be particularly suitable for pollution biomonitoring.

In the present study, biomarkers were selected according to the typology of the contamination in the Seine River. Indeed, several studies have documented the high contamination of sediments of the Seine River by a cocktail of pollutants including PAH and PCB and metal species, notably mercury and zinc and to a lesser extent copper and cadmium (Minier et al., 2006; Cachot et al., this issue). PAHs are well known damaging agents which are able to elicit DNA adduct formation (Venier and Canova, 1996; Akcha et al., 2000) but also to generate reactive oxygen species (Mitchelmore et al., 1998) in mussels. Metal species like copper have also been shown to generate oxyradicals and oxidative DNA damage in rat hepatocytes (Li et al., 2002). The glutathione S-transferases (GST) are quantitatively the most important phase II enzymes of the detoxification system (Fitzpatrick et al., 1997). They catalyse conjugation reaction of glutathione with various organic compounds including PAH. These enzymes also play a role in protection against oxidative stress by catalysing a selenium-independent glutathione-peroxidase activity (Prohaska, 1980). Three components of the antioxidant system were also investigated. The superoxide dismutases (SOD) are oxido-reductases which catalyse the dismutation of the superoxide anion into molecular oxygen and hydrogen peroxide. The cytosolic Cu/Zn SOD have previously been characterized in gills and digestive gland of the blue mussel and the acidic isoform was shown to be inducible by copper (Manduzio et al., 2003). Catalase (CAT) catalyses the reduction of hydrogen peroxide in water. Glutathione peroxidases (GPx) catalyse mainly the reduction of organic peroxides to alcohols using reduced glutathione. When the first lines of defences are overtaken, oxidative damage and adducts can occur at nucleophilic sites of cellular macromolecules. Dose-dependent increase of DNA strand breaks (Mitchelmore et al., 1998) but also oxidative DNA lesions (8-OHdG) and bulky DNA adducts (Canova et al., 1998) were reported in digestive gland of *Mytilus* sp. treated with benzo[a]pyrene. In addition, high levels of DNA adducts were measured in indigenous or transplanted mussels from industrial and urban areas (Solé et al., 1996; Ericson et al., 2002).

This study was conducted in the frame of an integrated and multimarker field study investigating the genotoxic risk in the Seine estuary. Data concerning contamination of sediments and their genotoxic and embryotoxic activities were reported in this issue (Cachot et al., this issue). The present paper focused on contamination and biological responses of two bivalve species, *Dreissena polymorpha* and *Mytilus edulis*, living in brackish or salt water areas of the Seine estuary. The first step aimed to determine PAH, PCB and metal contents of bivalves throughout the studied area in order to highlight the most impacted sites. In a second step, the levels of oxidative stress and DNA damage were evaluated in mussels in order to link effects to genotoxicant exposure. Finally, an attempt was made at comparing the levels of chemical accumulation and biological responses between the two bivalve species.

2. Materials and methods

2.1. Sampling of bivalves

Zebra mussels, *Dreissena polymorpha* Pallas are fresh water bivalves abundant and widely distributed all along the course of the Seine River (Akopian et al., 2001). Blue mussels, *Mytilus edulis*, are typically marine bivalves forming natural colonies on rocky shore of the Seine Bay. A preliminary sampling campaign was conduced in June 2002 focusing on two highly impacted sites, La Bouille and Oissel and one reference fresh water site, Yville-sur-Seine. A second campaign encompassing eight sites in the Seine estuary and in the Seine Bay was carried out in June 2003 (Table 1). Le Moulard was chosen as a marine reference site because of the very low levels of PAH, CB153, organochlorinated pesticides and metals such as Pb, Cd, Hg and Ni reported in blue mussels from this area (RNO database). Samplings were performed from the shore at low tide. Zebra mussels were collected on rocks and pon-

Table 1
Localization of sampling sites and characteristics of bivalves collected in the Seine estuary and in the Seine Bay in June 2003

Site	Species	Length (mm)[b]	Weight (g)[c]
Yville-sur-Seine[a]	*Dreissena polymorpha*	25 ± 1	1.7 ± 0.2
Oissel (left bank)	*Dreissena polymorpha*	24 ± 2	1.9 ± 0.6
La Bouille (left bank)	*Dreissena polymorpha*	24 ± 3	2.4 ± 0.5
Caudebec (left bank)	*Dreissena polymorpha*	23 ± 2	1.7 ± 0.4
Villerville	*Mytilus edulis*	37 ± 3	5.0 ± 1.1
Le Havre	*Mytilus edulis*	45 ± 3	9.3 ± 2.9
Antifer	*Mytilus edulis*	42 ± 3	7.0 ± 1.3
Le Moulard[a]	*Mytilus edulis*	39 ± 3	6.9 ± 1.3

[a] Reference sites.
[b] Mean length ± standard deviation for 30 individuals.
[c] Mean total wet weight ± standard deviation for 30 individuals.

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