Available online at www.sciencedirect.com
SCIENCE DIRECT®
Marine Pollution Bulletin 50 (2005) 1548–1557
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Susceptibility to oxidative stress of mussels (*Mytilus galloprovincialis*) in the Venice Lagoon (Italy)

Daniela M. Pampanin [a,*], Lionel Camus [b], Alessio Gomiero [a], Ilenia Marangon [a], Elisa Volpato [a], Cristina Nasci [a]

[a] *Institute of Marine Science, ISMAR-CNR, Castello 1363/a, Venice, Italy*
[b] *RF-Akvamiljø, Mekjarvik 12, 4070 Randaberg, Norway*

Abstract

The aim of this study was to evaluate the susceptibility to pollutant mediated oxidative stress of the Mediterranean mussel *Mytilus galloprovincialis* in the Venice lagoon (Italy).

In June 2003, mussels from a farm were transplanted to eight sites in the lagoon for five weeks. Oxidative stress responses were measured by: (i) total oxyradical scavenging capacity (TOSC) assay, for an overall evaluation of the oxidative stress response capability; (ii) catalase (CAT), as a key enzyme involved in the antioxidant defence system; (iii) malondialdehyde (MDA), as an indicator of lipid peroxidation, to evaluate an oxidative damage; (iv) metallothioneins (MTs), as they play a role in the antioxidant defence.

The TOSC analysis revealed a reduced capability to eliminate: (i) peroxyl radical in mussels transplanted at Palude della Rosa, Valle Millecampi and Chioggia; (ii) hydroxyl radical at Campalto and Valle Millecampi; (iii) peroxynitrite at Valle Millecampi.

Inhibition in CAT activity, observed in all the monitored sites, confirms the presence of an oxidative pressure in transplanted mussels.

In addition, Pearson correlation analysis was performed in order to observe possible links between the various parameters. The PCA was a powerful tool to discriminate impacted sites, suggesting that the mussels transplanted throughout the Venice lagoon were subjected to different levels of oxidative pressure. Furthermore, it provided an easy and useful tool to summarize the obtained results.

Keywords: Mussel; Oxidative stress; TOSC; Catalase; Malondialdehyde; Metallothionein; Venice Lagoon

1. Introduction

Since the discovery of the importance of radical reactions both in normal biological processes and in toxicity mechanisms of many xenobiotics, there is an increasing concern about prooxidant and antioxidant processes (see the review of Livingstone, 2001). Antioxidants represent the cellular defence mechanisms which counteract toxicity of reactive oxygen species (ROS), these mechanisms have been extensively investigated in sentinel organisms such as marine mussels (Winston et al., 1990; Viarengo et al., 1991a,b; Livingstone et al., 1992; Regoli, 1998; Camus et al., 2004). Responsiveness of antioxidants to pollutants is difficult to predict and a high degree of variability has been reported as a function of class of chemicals, kind of exposure, phase of the biological cycle (Livingstone, 2001). Nevertheless, laboratory and field studies indicated that variations in the levels or activities of antioxidants are potential biomarkers revealing a contaminant-mediated biological effect on the organisms (Porte et al., 1991; Ribera et al.,

* Corresponding author. Present address: RF-Akvamiljø, Mekjarvik 12, 4070 Randaberg, Norway. Tel.: +47 51875502; fax: +47 51875540.
E-mail address: daniela.pampanin@rf.no (D.M. Pampanin).

0025-326X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2005.06.023

1991; Livingstone, 1991; Livingstone et al., 1995; Regoli and Principato, 1995; Solè et al., 1996; Camus et al., 2004).

The analysis of the total oxyradical scavenging capacity (TOSC) has been recently demonstrated as a reliable tool for quantitatively assessing the biological resistance to toxicity of different forms of ROS, including peroxyl radicals, hydroxyl radicals and peroxynitrite decomposition products (Winston et al., 1998; Regoli and Winston, 1999). Catalase (CAT) is a commonly studied antioxidant enzyme involved in the initial anti-oxidative mechanism and widely used as a biomarker in mussel (Cajaraville et al., 2000; Khessiba et al., 2001; Nasci et al., 2002; Lau and Wong, 2003; Romèo et al., 2003). It reduces H_2O_2, produced by the superoxide dismutase enzyme (SOD), to produce water and oxygen. Moreover, high catalase activity is found in invertebrates, confirming its important role in antioxidant defence in aquatic invertebrates (Livingstone et al., 1992). Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation and prostaglandin biosynthesis that is mutagenic and carcinogenic. It reacts with DNA to form adducts (Marnett, 1999). It is particularly interesting to associate the study of antioxidant molecules (such as catalase and metallothioneins) to an oxidative damage assay, such as MDA concentration.

Although the physiological role of metallothioneins (MTs) is still under debate, a fundamental involvement in essential and non essential metal pathways is certain (Roesijadi, 1992; Bebianno and Langston, 1992; Viarengo and Nott, 1993; Langston et al., 1998). MTs occur mainly in cytosol and are characterised by high cystein content, low molecular weight, heat-stability and a strong affinity for binding metals such as Ag, Cd, Cu, Hg and Zn. Moreover, MTs could protect cells from oxidative stress not only by acting as an oxyradical scavenger (Andrews, 2000; Colangelo et al., 2004), but also through metal binding/release dynamics, as suggested by Viarengo et al. (2000).

The Mediterranean mussel is widely distributed in the Venice lagoon (Italy). The Venice lagoon is a shallow coastal basin with a surface area of about 550 km^2, connected with the sea by three sea inlets (Lido, Malamocco and Chioggia). Inorganic and organic micro-pollutants enter the lagoon from industrial point sources (Porto Marghera industrial area), municipal wastewater discharges (Venice and Mestre urban areas), agricultural drainage, atmospheric deposition and illegal dumping (UNESCO, 2000).

A transplantation experiment was performed at this location, since this strategy allowed the comparison of chemical and biological properties of samples which have been collected from one population and which have been exposed to different environmental conditions (de Kock and Kramer, 1994).

The aim of this study is to gain knowledge on the susceptibility to oxidative stress of the mussel species *Mytilus galloprovincialis* in the Venice lagoon ecosystem looking at various biomarkers involved in this stress mechanism. Moreover, it is important to observe the transplantation procedure adopted that is proposed as a useful tool for environmental biomonitoring. TOSC (for an overall evaluation of the oxidative stress response capability), CAT (enzyme involved in the antioxdant defence system), MDA (representative of an oxidative damage) and MTs (as they play a role in the antioxidant defence) were then measured.

2. Materials and methods

2.1. Transplantation procedure

In June 2003 mussels (*Mytilus galloprovincialis*) of standardized shell size (4.5 ± 0.5 cm), purchased from a mussel farm, located at Alberoni, considered as a clean site (Pampanin et al., 2004) were transplanted to eight sites throughout the Venice Lagoon (from North to South): Palude della Rosa, Campalto, Tresse, Sacca Sessola, S. Pietro in Volta, Valle Millecampi, Cà Roman (reference site) and Chioggia (Fig. 1). Mussels were transported in cold boxes from the farm to the boat and, after a first sorting, they were divided into groups (of about 100 individuals each), placed in cages constituted by polyethylene netting and immersed subtidally at all sites. The transplantation procedure is the same

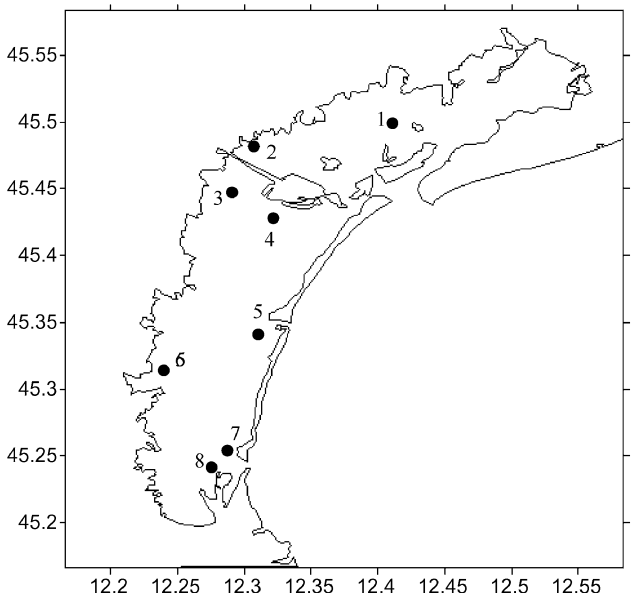

Fig. 1. Location of the sampling stations in the Venice lagoon. 1 = Palude della Rosa, 2 = Campalto, 3 = Tresse, 4 = Sacca Sessola, 5 = S. Pietro in Volta, 6 = Valle Millecampi, 7 = Cà Roman, 8 = Chioggia.