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Biomonitoring of a polluted coastal area (Bay of Muggia, Northern Adriatic Sea): A five-year study using transplanted mussels

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The subcellular effects of pollution were evaluated using two lysosomal biomarkers in mussels, *Mytilus galloprovincialis*, deployed periodically over a period of 5 years in a harbour area in the Bay of Muggia (Gulf of Trieste, North Adriatic Sea) that is strongly influenced by anthropogenic activities. Mussels were collected from a clean marine farm and analysed (sample T0). A sub-sample was transplanted to the harbour site (sample M) and analysed after about 12 weeks. An additional sub-sample was relocated within the farm as a control and was also tested at the end of the 12-week period (sample T1). The transplantation procedures were repeated twice yearly for 5 consecutive years, starting in 2009. Two well-established lysosomal biomarkers, i.e. lysosomal membrane stability and lipofuscin accumulation, were evaluated in hepatopancreas cells. The body condition index and mortality rate were also assessed. Moreover, various pollutants were determined in both mussel flesh, for a better comprehension of the biological response, and sediments, for a general characterization of the study area. As a whole, the applied biomarkers were found to be appropriate for determining the responses of mussels to environmental pollutant loads over time. Variations in lysosomal membrane stability and lipofuscin content were mostly related to total PAHs and metals respectively. Our results confirm the usefulness of active biomonitoring in evaluating pollution trends in marine coastal areas and in particular the value of lysosomal biomarkers as a rapid screening tool for highlighting pollutant effects at least at organism level.

1. Introduction

The need to integrate chemical and biological approaches in marine monitoring has received increasing attention since long, and has also become crucially important for management and regulation purposes (Moore et al., 2004). In Europe, this issue has been raised in the context of the Water Framework Directive (WFD, Directive 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/WE), both of which specifically require the achievement or maintenance of good status for marine waters, habitats and resources, to be periodically assessed with reference to a selected suite of quality elements (biological, hydrodynamic and chemical) (Borja et al., 2010). The MSFD laid additional emphasis on the importance of evaluating the effects of contaminants at various biological levels, including lower levels of organization (Lyons et al., 2010). At the organismal level, biological effects can be assessed by means of biomarkers, which signal mechanistic links between molecular, cellular and tissue alterations and environmental pollutants (Bartell, 2006). Biomarkers evaluated in various sentinel organisms have been proposed within the context of risk assessment processes related to WFD and MSFD, suggesting their use in establishing links between stress and pollution and developing a cost-effective strategy that is also accessible to Environmental Agencies (Galloway et al., 2004; Dallas and Jha, 2015; Hagger et al., 2008; Viarengo et al., 2007).

Mussels are widely used as marine biomonitoring organisms due to their sedentary and filter-feeding nature and their ability to accumulate and tolerate high concentrations of xenobiotics (Dailianis, 2010). Moreover, the extensive knowledge of their metabolic pathways makes it possible to elucidate the links between internal doses of pollutants and the elicited biological effects (Brenner et al., 2012; Brooks et al., 2015; Cajaraville et al., 2000; Cuevas et al., 2015; Sureda et al., 2011). The active biomonitoring approach, i.e. when organisms are collected from a reference population (from a natural site or marine farm) and translocated to the sites to be monitored, is widely practiced in marine monitoring programs (Cappello et al., 2015; Moschino et al., 2011; Serafim et al., 2011; Tsangaris et al.,

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2014). It is well known that difficulties in biomarker interpretation due to the genetic heterogeneity and compensatory adaptative mechanisms exhibited by native organisms living in chronically contaminated areas may be overcome by deploying a uniform batch of organisms. The active approach thus enhances the potential of biomarker responses to discriminate among sites, consequently enabling more significant comparisons between specimens from reference and potentially polluted sites (Lacroix et al., 2015; Viarengo et al., 2007). Also, the active approach is considered to be more suitable for evaluating contaminant bioaccumulation and effects in hard-to-reach areas, as well as for small-scale and site-specific studies (Hunt and Slone, 2010).

The lysosomal system of many marine species is known to be particularly sensitive to environmental perturbations and, for this reason, its alterations are widely used as indicators of cellular stress (Moore et al., 2006). Lysosomes are membrane-bound organelles, containing various hydrolytic enzymes involved in various cellular processes including digestion, defence, and reproduction (Moore, 1988; Moore et al., 2006). Specifically, the lysosomes of mollusc digestive cells are involved in detoxification, through the sequestration and accumulation of a wide range of chemicals, such as metal ions, polycyclic aromatic hydrocarbons (PAHs) and pharmaceuticals (Moore et al., 2012). Exposure to pollutants gives rise to various lysosomal alterations, namely variation in membrane permeability, changes in content (mainly accumulation of lipofuscins and neutral lipids) and increases in lysosomal size and number (Marigomez et al. 1996; Viarengo et al. 2007). Specifically, variation in membrane permeability, evaluated by the lysosomal membrane stability test, has been proposed as a core biomarker due to its high sensitivity and low cost for assessing biological effects in mussels and fish under the MSFD (ICES et al., 2015; Law et al., 2010).

The goal of this study was to evaluate the temporal variation of pollution and the effects of pollutants on mussels in the Bay of Muggia (Gulf of Trieste), a coastal site affected by various anthropogenic pressures. To this end, two lysosomal biomarkers – lysosomal membrane stability and lipofuscin accumulation – were evaluated in the digestive glands of mussels (*M. galloprovincialis*) deployed in accordance with the active biomonitoring approach. Body condition index and mortality rate were also assessed as supporting parameters in order to characterize the mussels' general state of health (Moschino and Marin, 2006; Viarengo et al., 2007). Moreover, metals and micro-organic pollutants (i.e. PAHs, polychlorinated biphenyl (PCBs), alkyl-phenols and phthalates) were measured in mussel tissues to investigate the links between pollutants and biological responses. Lastly, the sediments were analysed in order to determine the chemical characteristics of the study area.

2. Materials and methods

2.1. Study area, mussel transplantation procedure and sampling design

The Bay of Muggia is a shallow and narrow coastal marine environment (8–20 m in depth; 7 km long to 4 km wide; Fig. 1), located in the Gulf of Trieste (northern Adriatic Sea). It has been subject to anthropogenic impacts for 2000 years, due to historic activities such as marble quarrying and oyster culture (Cibic et al., 2008). Today, the quality of water and sediments is heavily impacted by activities in the port of Trieste, discharges from a large industrial district (with an iron foundry complex of 600,000 m^2 and an oil-pipeline terminal), municipal effluents and many other economic activities (Solis-Weiss et al., 2004; Cibic et al., 2008). Discharged pollutants tend to persist in the Bay due to its morphology and hydrodynamism: its waters are indeed protected by a three-wall system, which makes them quite still (Adami et al., 2000a). Adami et al. (2000a,b) reported that total PAH levels in Trieste harbour were high and/or very high in comparison to other

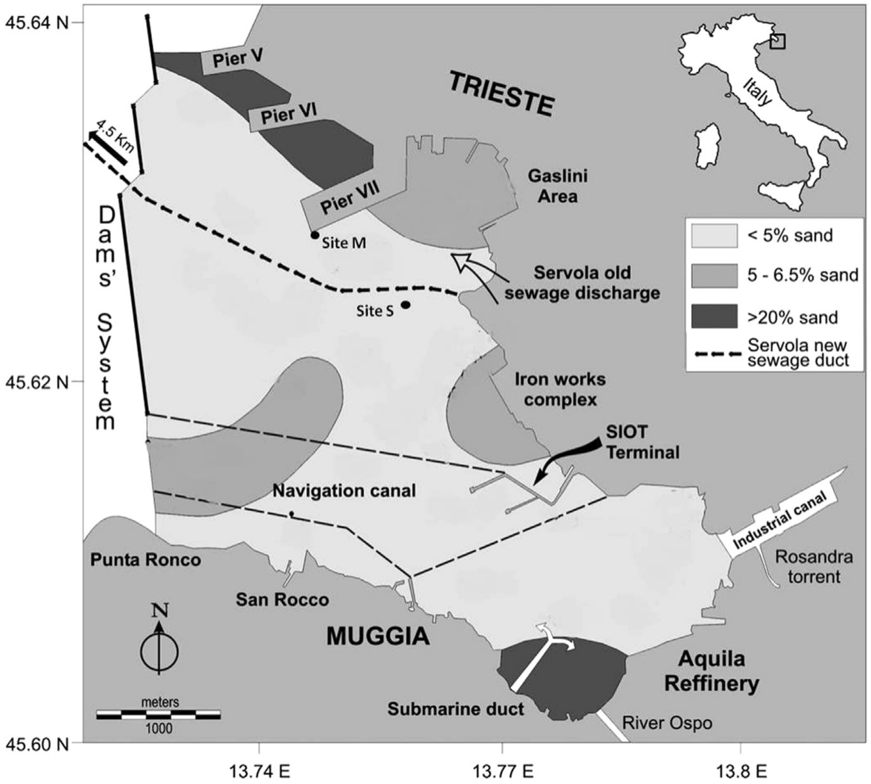

Fig. 1. Study area for sediment (S) and mussel transplantation (M) (from Solis-Weiss et al. (2004), modified).