Marine Environmental Research 96 (2014) 92–104

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

A multidisciplinary weight of evidence approach for environmental risk assessment at the Costa Concordia wreck: Integrative indices from Mussel Watch

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A R T I C L E I N F O

Article history:
Received 31 July 2013
Received in revised form
18 September 2013
Accepted 21 September 2013

Keywords:
Costa Concordia
Mussels
Bioavailability
Biomarkers
Weight of evidence
Environmental risk assessment

A B S T R A C T

A complex framework of chemical, biological and oceanographic activities was immediately activated after the Costa Concordia shipwreck, to assess possible contamination events and the environmental impact during both emergency and wreck removal operations. In the present paper, we describe the results obtained with caged mussels, *Mytilus galloprovincialis*, chosen as bioindicator organisms to detect variations of bioavailability and the early onset of molecular and cellular effects (biomarkers). Seven translocation experiments were carried out during the first year from the incident, with organisms deployed at 2 depths in 3 different sites. After 4–6 weeks, tissue concentrations were measured for the main classes of potentially released chemicals (trace metals, polycyclic aromatic hydrocarbons, volatile and aliphatic hydrocarbons, polychlorinated biphenyls, halogenated pesticides, organotin compounds, brominated flame retardants, anionic surfactants); a wide battery of biomarkers covered responses indicative of exposure, detoxification, oxidative stress, cell damage and genotoxic effects. Results excluded serious contamination events or a consistent increase of environmental pollution although some episodic spills with reversible effects were detected. Data were elaborated within a quantitative weight of evidence (WOE) model which provided synthetic hazard indices for each typology of data, before their overall integration in an environmental risk index, which generally ranged from slight to moderate. The proposed WOE model was confirmed a useful tool to summarize large datasets of complex data in integrative indices, and to simplify the interpretation for stakeholders and decision makers, thus supporting a more comprehensive process of "site-oriented" management decisions.

1. Introduction

On the night of 13 January 2012, the Costa Concordia ship, with 3.206 passengers and 1.023 crew members on board, collided with a submerged rock close to the Giglio Island (Tuscany, Italy), causing 32 victims. A coincidence of winds and currents prevented the ship from sinking in the deep waters, and the partially submerged wreck laid on a rock ledge at the entrance of Giglio harbour.

Immediate fears of an ecological disaster were raised from the possibility of the wreck to slip at a much greater depth, concomitantly with the risk of oil pollution and loss of other chemicals that could have devastated this highly relevant marine area. An emergency plan was rapidly activated after the incident to remove fuels and oil were from the ship, and to monitor the possible impact of such activities.

A complex framework of chemical, biological and oceanographic investigations was coordinated by the National Civil Protection and

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0141-1136/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marenvres.2013.09.016

the Italian Institute for Environmental Protection and Research (ISPRA); main activities included chemical and ecotoxicological analyses on water column and sediments, bioaccumulation and sublethal effects (biomarkers) in several invertebrates and fish species, evolution of *Posidonia oceanica* seagrass meadows, density and distribution patterns of the endangered species *Pinna nobilis*, assessment of fish assemblages by visual census, benthic cartography on ecologically relevant coastal habitats.

The present work describes the experimental design and results obtained during one year of an active Mussel Watch investigation. Mussels, *Mytilus galloprovincialis*, were caged in proximity of the Costa Concordia wreck to detect environmental changes, both through the analysis of chemicals bioaccumulation and changes of molecular and cellular responses (biomarkers). A similar approach is widely recognized as an important component of environmental risk assessment to evaluate bioavailability and early toxicological effects of anthropogenic pollutants released into the marine environment (McCarty et al., 2002): in this respect, biomarkers often represent the first warning signals of environmental disturbance, even in the absence of acutely toxic responses at organism or population levels (Galloway et al., 2004; Hagger et al., 2008; Regoli, 2000; Regoli et al., 2011; Viarengo et al., 2007).

The use of caged mussels facilitates the investigation in areas where native organisms are absent, also reducing the influence of genetic/population differences, seasonal variability or adaptive phenomena, all factors which can attenuate the capability to discriminate between different levels of anthropogenic impact (Bocchetti et al., 2008a; Brooks et al., 2012; Marigómez et al., 2013b; Nigro et al., 2006; Zorita et al., 2007). Considering the tolerance of these organisms to environmental conditions and handling, their elevated capability to accumulate both inorganic and organic pollutants and the responsiveness of molecular and cellular responses, caged mussels can typically provide a time-integrated assessment of bioavailability and early biological effects over a 4–6 weeks translocation period (Gorbi et al., 2008; Viarengo et al., 2007).

During the first year from the Costa Concordia incident, a total of 7 translocation experiments were carried out during both the emergency phase related to removal of oil and fuels, and the operations of the "Parbuckling project" aimed to refloat and tow away the wreck. Several classes of pollutants potentially released during such activities were analysed in tissues of caged mussels, i.e. trace metals, polycyclic aromatic hydrocarbons (PAHs), volatile and aliphatic hydrocarbons (C6–C10 and C10–C40), polychlorinated biphenyls (PCBs), organo-chlorinated pesticides (OCPs), organotin compounds, brominated flame retardants (BFRs) and anionic surfactants.

These measurements were integrated with a multi-biomarker approach, based on a wide array of molecular and subcellular responses reflecting specific mechanisms of chemicals exposure or detoxification, cellular pathways of oxidative response to reactive oxygen species (ROS) and oxidative stress, onset of different levels of cellular unbalance and genotoxic effects. Metallothioneins-like proteins, acetylcholinesterase and Acyl CoA oxidase (AOX) were measured as typical responses to metals and several forms of organic pollutants. Metallothioneins, physiologically involved in intracellular homeostasis of essential metals, are typically induced in organisms exposed to increased environmental concentrations of these elements (Viarengo et al., 2007); the activity of acetylcholinesterase, a crucial enzyme in the neurotransmission of vertebrates and invertebrates, is inhibited by organophosphate or carbammate pesticides, and modulated by other chemicals and bioactive compounds (Gorbi et al., 2008, 2012). Peroxisomes are involved in lipid metabolism through oxidative reactions, and their proliferation in mussels exposed to organic xenobiotics is reflected by increased number and volume of organelles, and the induction of enzymes involved in fatty acid oxidation, such as AOX (Cajaraville et al., 2003; Orbea and Cajaraville, 2006).

Since a typical pathway of chemical toxicity is mediated by the enhancement of intracellular generation of reactive oxygen species (ROS), variations of antioxidant defences are rapidly activated in Mediterranean mussels to counteract enhanced prooxidant chemical challenge (Frenzilli et al., 2004; Giuliani et al., 2013; Marigómez et al., 2013b; Regoli, 1998, 2000; Regoli and Giuliani, 2014; Regoli and Principato, 1995; Viarengo et al., 2007). In the present study, oxidative effects in caged mussels were investigated through the activities of catalase, glutathione S-transferases, glutathione reductase, glutathione peroxidases, levels of glutathione; these results were integrated with the total oxyradical scavenging capacity (TOSC) toward peroxyl radicals, ROO•, and hydroxyl radicals, HO• (Regoli and Winston, 1999; Regoli et al., 1998). Compared to individual antioxidants, variations of TOSC have a greater biological relevance and prognostic value, being an impaired capability to neutralize ROS associated to the onset of various forms of oxidative damages like lysosomal dysfunctions, lipid peroxidation and genotoxic effects (Camus et al., 2003; Frenzilli et al., 2001, 2004; Gorbi and Regoli, 2003; Meyer et al., 2003; Moore et al., 2006; Regoli, 2000; Regoli et al., 2004).

Toxic effects on lysosomal systems are generally included in all the ecotoxicological studies with mussels (Moore et al., 2004, 2006). These organelles are responsible of fundamental processes in cell physiology, food digestion, intracellular turnover, immune function, sequestration and excretion of harmful compounds (Moore et al., 2006); however, they are also highly sensitive to several contaminants which can act both directly on the membranes and indirectly, through generation of ROS or signalling pathways (Regoli, 1992, 2000; Moore et al., 2006; Viarengo et al., 2007). In this study, lysosomal impairment was measured as membrane stability, while content of malondialdehyde, accumulation of lipofuscin and neutral lipids in tertiary lysosomes were investigated as further indices of lipid peroxidation processes and xenobiotic-mediated lipidosis (Regoli, 1992). The presence of a genotoxic risk around the wreck was finally evaluated in caged mussels as both DNA strand breaks, a potentially repairable effect caused by chemicals or enhanced prooxidant challenge, and as frequency of micronuclei, an irreversible genetic damage of chromatin breakage or aneuploidy during cell division (Frenzilli et al., 2009; Nigro et al., 2006).

Multidisciplinary studies which combine chemical and biological measurements, represent an added value to monitoring and management protocols, and also recent European Directives recommend the use of different quality indicators to evaluate the environmental status of aquatic ecosystems (Chapman, 2007; Chapman et al., 2013; Galloway et al., 2004; Hagger et al., 2008; Marigómez et al., 2013a). However, the combination of multiple typologies of investigations (or lines of evidence, LOEs) is often hampered by the lack of standardized procedures for the interpretation and the integration of complex datasets of heterogeneous results, which typically require various expert judgements (Benedetti et al., 2012; Dagnino et al., 2008; Linkov et al., 2009; Piva et al., 2011; Semenzin et al., 2008); additional critical issues are represented by development of qualitative and quantitative evaluations, the synthetic classification and, last but not least, the communication of risk to stakeholders (Benedetti et al., 2012; Linkov et al., 2009; Marigómez et al., 2013a). In this respect, a quantitative "Weight Of Evidence", WOE model (Sediqualsoft) has been recently developed to integrate and differently weight data from various lines of evidence, which actually include sediment chemistry, bioavailability of chemicals in key bioindicator