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A comprehensive evaluation of the environmental quality of a coastal lagoon (Ravenna, Italy): Integrating chemical and physiological analyses in mussels as a biomonitoring strategy

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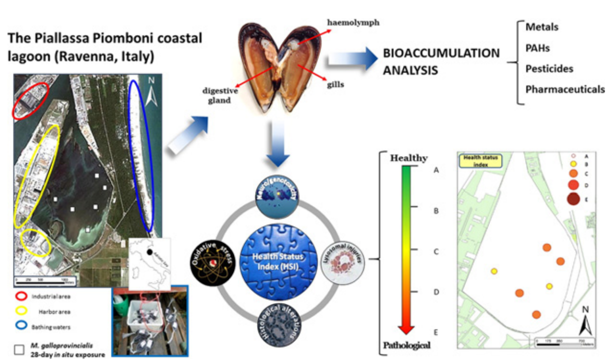
HIGHLIGHTS

- Biomarkers and bioaccumulation in mussels are proposed for coastal lagoon biomonitoring.
- Mussels accumulated high levels of PAHs and metals over a 28-day exposure in the lagoon.
- Lysosomal parameters were altered in both haemocytes and digestive gland.
- Genotoxicity and metallothionein levels were related with PAHs and metal bioaccumulation.
- Physiological and chemical endpoints proved suitable for integrated lagoon biomonitoring.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aimed at evaluating the environmental quality of a coastal lagoon (Pialassa Piomboni, NW-Adriatic, Italy) by combining analyses of biomarkers of environmental stress and bioaccumulation of contaminants in marine mussels (*Mytilus galloprovincialis*) transplanted for 28 days to six selected sites. Assessed biomarkers encompassed lysosomal endpoints, oxidative stress and detoxification parameters, specific responses to metals, neuro- and genotoxic substances; chemical analyses focused on PAHs, metals, pesticide and pharmaceuticals. Results showed up to a 67-fold bioaccumulation of 4- to 6-ring PAHs, including pyrene, fluoranthene, chrysene and benzo(*ghi*)perylene in transplanted mussels compared to reference conditions (T0). A 10-fold increase of Fe, Cr and Mn was observed, while pesticides and pharmaceuticals were not or slightly detected. The onset of a significant ($p < 0.05$) general stress syndrome occurred in exposed mussels, as outlined by a 50–57.7% decrease in haemocytes lysosomal membrane stability and an increased lysosomal volume (22.6–26.9%) and neutral lipid storage (18.9–48.8%) observed in digestive gland. Data also revealed a diffuse lipofuscin accumulation (86.5–139.3%; $p < 0.05$) in digestive gland, occasionally associated to a catalase activity inhibition in gill, indicating an increased vulnerability toward pro-oxidant factors. Higher levels of primary DNA damage (258%; $p < 0.05$) and PAH accumulation were found in mussels exposed along the eastern shoreline, hosting a petrochemical settlement. Bioaccumulated metals showed a positive correlation with increased metallothionein content (85–208%;

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$p < 0.05$) observed in mussels from most sites. Overall, the use of physiological and chemical analyses detected chronic alterations of the mussel health status induced by specific toxicological pathways, proving a suitable approach in the framework of biomonitoring programs of coastal lagoons.

1. Introduction

Coastal lagoons are among the most valuable habitats in terms of ecological relevance for the resilience of marine ecosystems. Due to their enclosed geomorphology and consistent hydrological inputs, these areas are typically characterized by highly productive waters providing a multitude of life-supporting services and natural resources to a wide array of aquatic species (Aliaume et al., 2007; Petry et al., 2016). Recently, increasing attention has been paid to the need to define standard guidelines for the protection of coastal lagoons, most of which have historically experienced a severe anthropogenic pressure owing to their relevance for industrial, harbour and other socio-economic purposes (Dolbeth et al., 2016; Newton et al., 2014).

A wide spectrum of urban and industrial chemicals is in fact reported in lagoons, including metals (Khalil and El-Gharabawy, 2016; Mendoza-Carranza et al., 2016; Romano et al., 2015), PCBs (Castro-Jiménez et al., 2008; Sfriso et al., 2014), PAHs (León et al., 2013; Liu and Kiwanuka, 2016; Sogbanmu et al., 2016), pesticides (Campillo et al., 2013; Pinto et al., 2016; Salem et al., 2016), and emerging contaminants, such as pharmaceuticals (Moreno-González et al., 2016; Niemi et al., 2013) and endocrine disruptors (Jonkers et al., 2010; Rocha et al., 2016a). In the context of the legislative interventions undertaken in compliance with the EU Water Framework Directive (WFD, 2000/60/EC), specific environmental quality standards (EQS) have been defined for a set of priority substances (Directive 2013/39/EC). EQS-based assessments have been adopted in monitoring plans and interventions aimed at achieving the good environmental status within EU surface waters, including estuaries and lagoons (Vorkamp and Sanderson, 2016). However, increasing evidence have suggested that chemical assessments do not allow *per se* an accurate and reliable appraisal of related risks for the quality of lagoon ecosystems. (Carvalho et al., 2014; Dahms, 2014). Physico-chemical perturbations and diversified geological conditions typical of lagoons may shape the effectual xenobiotic bioavailability and toxicity for exposed organisms, inducing potential interactive effects (Piggott et al., 2015). Chemical assessments also fail in detecting biotransformation products, that may result more toxic than the parental compounds (Buryskova et al., 2006), and in estimating synergistic/antagonistic effects induced on the fitness of the exposed biota (Lari et al., 2017). Under these assumptions, the implementation of an innovative chemical-biological approach to reinforce remediation and conservation strategies of coastal lagoons has been recommended by environmental protection agency within EU (Acom, 2011; Law et al., 2010). Scientific efforts expended over the past two decades contributed to identify a suite of molecular, cellular and/or physiological parameters modulated in response to various chemical stressors. These parameters, defined biomarkers, have successfully been used in association to bioaccumulation analyses to characterize sub-organism toxicological dynamics occurring in highly contaminated environments and provide early-warning information (Ben-Khedher et al., 2014; Chalkiadaki et al., 2014). The loss of the homeostatic capacity (Fernandes et al., 2007) and the suppression of cellular defence mechanisms (Hamza-Chaffai, 2014; Pereira et al., 2010) were related to an excessive metal uptake in fish; also, in bivalves and crustaceans, the bioaccumulation of various contaminants, including metals, PAHs and pharmaceuticals, was described in relation to impairments of the animal endo-lysosomal system (Moore et al., 2006; Franzellitti et al., 2014), pro-oxidant effects (Maranho et al., 2015; Regoli and Giuliani, 2014), and modulations of neuro- and/or genotoxic pathways (Viarengo et al., 2007). The simultaneous analysis of a wide set of biomarkers coupled to selected integrative tools, provides synthetic but accurate information on the sub-lethal alterations induced by multiple stressors on the exposed biota, and allows to correlate impacts with specific classes of contaminants (Shaw et al., 2011). On these basis, the contribution given by biomarker-based assessments in the framework of monitoring surveys of marine and transitional environments is currently emphasized by the scientific community (Ielmini et al., 2014; Piló et al., 2017).

In the present study, a combined analysis of biological and chemical endpoints was performed on marine mussels (*Mytilus galloprovincialis*) transplanted for 28 days in different sites of the Piallassa Piomboni (Ravenna Italy), a shallow coastal lagoon hosting an industrial/harbour complex. Being highly adaptable to changing environmental conditions and efficient bioaccumulators, marine mussels have extensively been employed as bioindicators of subtle/chronic alterations triggered by toxicants in transitional ecosystems (Vidal-Liñán et al., 2014). The *in situ* deployment approach was preferred to the direct sampling of specimens from natural populations to exclude potential bias owing to differences in the animal nutritional/reproductive status, and to allow data interpretation with respect to preliminary assessed baseline conditions. Employed biological endpoints consisted of a set of biomarkers widely validated in mussels (Viarengo et al., 2007). More specifically, the applied battery encompassed (i) lysosomal parameters of general stress, such as the lysosomal membrane stability (LMS), the neutral lipids (NL) accumulation and the lysosome to cytoplasm volume ratio (LYS/CYT); (ii) oxidative stress and biotransformation biomarkers, including the activities of the enzymes glutathione S-transferase (GST) and catalase (CAT), as well as the lipofuscin (LF) content; (iii) specific responses activated by the exposure to metals, neuro- and genotoxic substances, such as the metallothionein content (MT), the acetylcholinesterase activity (AChE) and levels of primary DNA damages. In completion to selected biomarkers, the bioaccumulation of metals, PAHs, pharmaceuticals, and agricultural products (*i.e.* pesticides, herbicides, and fungicides), was analysed following the *in situ* exposure. Finally, the environmental quality was ranked by means of the Mussel Expert System (MES) data integration (Dagnino et al., 2007), which assigns a unique A-E scaled mussel health status index (HSI) based upon the observed biomarker modulation(s).

The present study had three main objectives: (i) to assess the suitability of the integration of biomarkers and bioaccumulation data from *in situ* deployed mussels as a valid biomonitoring strategy for coastal lagoons, (ii) to identify and characterize the toxicological pathways activated by chemical mixtures in a dynamic and highly diversified ecosystem, and (iii) to outline the main anthropogenic stress sources within the Piallassa Piomboni coastal lagoon.

2. Materials and methods

2.1. Study area

The Piallassa Piomboni (Fig. 1) is a brackish coastal lagoon located along the North-western Adriatic seacoast at about 6–8 km east from the town of Ravenna (Italy). It has been included in the list of the sites of community importance (SCIs = SIC) and special protection areas (SPAs), by virtue of its relevant natural heritage (European Council, 92/43/EEC; 79/409/EEC). Together with the Piallassa Baiona, other transitional basin positioned slightly further north, it is part of an extended lagoon system originated about 250 years ago and progressively subject to a series of anthropogenic interventions which dramatically altered its initial ecological, physicochemical and geomorphological conditions. In