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First data on three bivalve species exposed to an intra-harbour polymetallic contamination (La Rochelle, France)

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ABSTRACT

Evaluating diffuse sediment contamination in the environment is a major concern with the aim of reaching a good chemical and ecological state of the littoral zone. In this study the risks of chronic chemical contamination and consequences in the bivalves *Crassostrea gigas, Mytilus* sp. and *Mimachlamys varia* were evaluated in coastal environments. The objective here was to understand the anthropological phenomena that affect the functioning of the marina of La Rochelle (semi-closed environment). Harbours seeking ecomanagement accreditations (such as the international reference ISO 14001) constitute zones of interest to implement biomonitoring studies. The biological effects of chemical pollution in the Marina of La Rochelle were studied to develop a multi-biomarker biomonitoring approach on specific marine species of this site. Moreover, a genetic (DNA barcoding) approach was applied to validate the species identity of collected bivalves. Of the three species tested the scallop, *M. varia*, was the most sensitive to metal exposure.

1. Introduction

Xenobiotics produce alterations in aquatic organisms at different levels and can induce permanent damage. However, these organisms have evolved defences at the biochemical level, which protect them from contamination effects that occur during early exposure. The evaluation of the impact of pollutants in the environment is now a major concern, with the aim of reaching a good chemical and ecological state of the littoral zone. Previous studies (Milinkovitch et al., 2015; Lacroix et al., 2015; Luna-Acosta et al., 2011; Breitwieser et al., 2016) showed the risks of chronic chemical contamination and the consequences in the bivalves *Mimachlamys varia, Crassostrea gigas* and *Mytilus edulis* in an open coastal environment. In this context, the objective of this project was to assess the impacts of pollution (heavy metals) on the macrofauna of a semi-closed environment: a marina in La Rochelle (France). We also analyzed samples from two neighbouring zones: the area where muds dredged from the marina are deposited, and a less-impacted site on the coast. The department of environment of the La Rochelle Marina has started to implement focused efforts to improve the treatment of careening wastewater (filter with UV, carbon filter and sand filter). Therefore, the present study will constitute a baseline for future biomonitoring studies assessing the benefits of these efforts in terms of environmental quality. Indeed, previous studies in harbour areas from around the world (Egypt, Australia, France, Canada and Cote d'Ivoire) have identified significant enrichment of metal(loid)s in sediments and a crucial need for biomonitoring surveys (Hussein and Khaled, 2014; Walker and MacAskill, 2014; Caro et al., 2015; Bakary et al., 2015; Pippy et al., 2016). In exposed organisms, pollutant-mediated generation of reactive oxygen species (ROS) is likely to induce antioxidant defence mechanisms to prevent oxidative damages to cellular antioxidant enzymes. The measurement of the activity of antioxidant enzymes in mussels, oysters and scallops has been widely used as a biomarker of exposure to environmental pollutants (Lam, 2009; Luna-Acosta et al., 2010; Bustamante and Miramand, 2005). In the present study, we selected exposure biomarkers including glutathione S-transferase (GST), laccase immune-marker, malondialdehyde (MDA) and superoxide dismutase (SOD). Malondialdehyde and SOD are effectors of antioxidant defences and have been used to reveal the exposure to metals and a wide range of organic compounds in the environment. Glutathione S-transferases are involved in phase II detoxification of xenobiotics and toxin biotransformation, which also plays a protective

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role against oxidative stress by catalysing a selenium-dependant glutathione peroxidase (Tappel et al., 1982; Prohaska, 1980). Lipid peroxidation indicates the damage to cellular membrane lipids caused by ROS and is useful for assessing exposure to and the effects of pollutants in marine species. Moreover, there is a depletion of key enzymes such as laccase involved in the modulation of immune system in bivalve in presence of contaminants (Luna-Acosta et al., 2010, 2011; Milinkovitch et al., 2015; Breitwieser et al., 2016).

Animals were collected from several intra-harbour stations in order to: (1) quantify the content of inorganic contaminants in sessile marine organisms; and (2) identify an indicator species of intra-harbour pollution. Variations in both trace element levels and biomarker responses among sites were analyzed during winter (low energy metabolism) to assess the potential of *M. varia* as a biomonitoring species in comparison with *C. gigas* and *M. edulis*, which are used in watch programs by the National Oceanic and Atmospheric Administration (NOAA) for example. We compared these sites, impacted by anthropogenic activities, to a nearby reference site that presents much lower levels of trace metal pollution: Ré Island (Bustamante and Miramand, 2005; Milinkovitch et al., 2015).

Ecotoxicology and biomonitoring studies generally rely on comparisons (e.g. among different conditions) made within a species or subspecies, that is, within a single evolutionary unit. Unknowingly sampling different evolutionary lineages may lead to artefacts or biases when comparing bioaccumulation patterns and stress response across sites or across different years. Indeed, several studies have shown that closely related (sometimes cryptic) species can differ in sensitivity to pollutants (e.g. Sturmbauer et al., 1999; Feckler et al., 2012). Thus, we chose to include a DNA barcoding step in our study, to validate species identification made in the field and to evaluate the presence of cryptic lineages in the study area.

2. Materials and methods

2.1. Field collection and tissue sampling

The study sites are shown in Fig. 1. Three species (Fig. 2) were collected from four sampling sites in the harbour of La Rochelle (Rainwater, Careening, Fuels (2); 46°14′50.15″N, −1°16′67.86″W), one site right outside the harbour where muds dredged from the marina are deposited (BDM) and one site that was less contaminated (Loix-en-Ré; 46°23′09.98″N, −1°41′99.63″W). "Loix-en-Ré" situated in Ré Island was chosen as the reference site. "BDM" is the area of outlet dredging near a touristic beach and close to the harbour, "rainwaters" corresponds to the outlet of rainwaters in the harbour (without a water-filtering system), "careening" is located below the careening area, which has a water-filtering system (carbon, sand, UV and paper to remove organic, inorganic and bacterial contamination). Finally, "fuel (now)" is the recent fueling station for boaters, which has been operating the past 2 years, and "fuel (old)" was the old fueling station, which has not been functional since 2 years ago.

The digestive glands of 60 oysters (*C. gigas*), 60 mussels (*Mytilus sp.*) and 60 variegated scallops (*M. varia*) were collected (adult size, ten individuals of each species per site). A power analysis was conducted to determine the number of replicates that was sufficient to detect significant differences in biomarker activities according to thresholds from previous studies. Our power analysis revealed that eight is the minimum number of individuals per site that should be sampled to reach a power of 80% (alpha = 0.05).

Sampling and protein assays were carried out in January 2015. For each sampling site, digestive glands of bivalves were removed and stored at −80 °C for further analysis. Each digestive gland was cut into two samples and used for trace elements and biochemical assessment of biomarkers (Milinkovitch et al., 2015). Samples for trace element analysis were freeze-dried for 48 h. For each species, muscle tissue from five individuals per site (from the same batch of 60 samples) was collected and stored at −80 °C for genetic analysis.

2.2. Trace element analysis

Analyses of Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sn, V and Zn were performed with a Varian Vista-Pro ICP-OES and a Thermofisher Scientific XSeries 2 ICP-MS. To this end, aliquots weighing between 60 and 200 mg were digested using a 6:2 (v/v) 67–70% HNO_3/34–37% HCl mixture (Fisher, trace metal quality). Acidic digestion of the samples was carried out overnight at room temperature and then in a Milestone microwave oven (30 min with constantly increasing temperature up to 120 °C, then 15 min at this temperature). Each sample was made up to 50 mL with ultrapure quality water.

For samples with a weight of < 100 mg, the mixture used was 3:1 (v/v) 67–70% HNO_3/34–37% HCl, and the samples were made up to 25 mL with ultrapure water. Two certified reference materials (CRMs) and one blank, treated and analyzed in the same way as the samples, were included in each analytical batch. CRMs were DOLT-4 (dogfish liver) and TORT-2 (lobster hepatopancreas), which were both from the National Research Council Canada (NRCC). Means recovery rates were (in %) 104 (As), 103 (Cd), 98 (Co), 98 (Cr), 91 (Cu), 88 (Fe), 90 (Mn), 97 (Ni), 92 (Pb), 101 (Se), 95 (V) and 104 (Zn) for TORT2 and 96 (Ag) 92

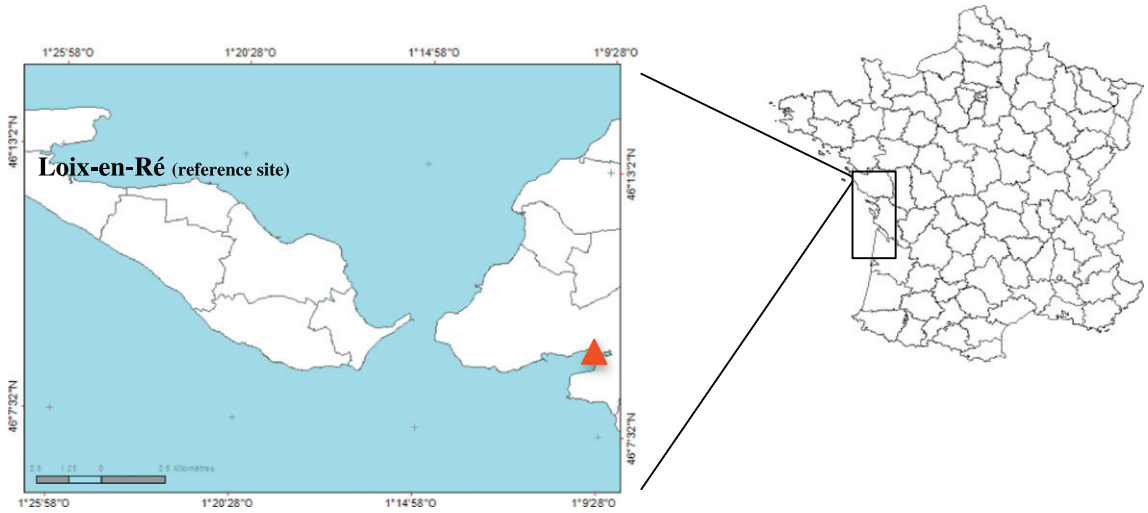

Fig. 1. Map of France with the locations of the study areas. Loix-en-Ré is the « control site (less-contaminated) and in la Rochelle Harbour ▲ are situated impacted sites. 1 : rainwaters, 2 : Careening, 3 : Fuel (now), 4 : Fuel (old), 5 : outlet dredging.