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Biomarkers of general stress in mussels as common indicators for marine biomonitoring programmes in Europe: The ICON experience

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ABSTRACT

This study investigated whether general stress biomarkers in mussels can be applied as common first-tier biomarkers in regional biomonitoring programmes in the North Sea (including Iceland) and western Mediterranean Sea. Stress on Stress (SoS) and lysosomal membrane stability (LMS) biomarkers were analysed in resident mussels (*Mytilus* sp.) from 8 coastal sites and in transplanted mussels (*Mytilus* galloprovincialis) from two Spanish Mediterranean coastal sites. The assessment of results, as input to pollution monitoring strategies, was performed jointly for LMS and SoS data from the two regions. Contaminant body burden of the mussels was compared with biomarker results. The results demonstrated that these two general and non-expensive stress biomarkers in mussel can be applied throughout European waters, providing a cost-effective and harmonised approach to screen contaminant-related biological effects within the framework of wide-scale pollution biomonitoring programmes, such as that proposed by the European Union, i.e. the Marine Strategy Framework Directive.

1. Introduction

At a Pan-European level, pollution monitoring methods need to be consistent across the European marine region or subregion to facilitate comparability of monitoring results. During recent decades, Regional Conventions started to recommend specific sublethal biological responses (contaminant-related biomarkers) to be measured in marine organisms (UNEP/RAMOGE, 1999; OSPAR, 2008), but to date selection of fully qualified biomarkers still lacks complete harmonisation and comparability across regional seas. Since 2008, the Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC) has put emphasis on the importance of assessing key biological responses to evaluate the health of organisms and to link any observed responses to contaminant exposure (Law et al., 2010). The key biological responses that member states can assess within the framework of MSFD are not explicitly indicated in this Directive as has been done for hazardous substances (Commission Decision 2010/477/EU). As a consequence, a range of biological effects has been addressed by member states in the national initial assessment reports and for the determination of good environmental status (Palialexis et al., 2014). It is apparent that, for a coherent of environmental assessment of contaminant effects, selection of common indicators in similar organisms within the different European regions would be the most convenient strategy to follow within the framework of the MSFD.

Filter-feeding molluscs, and particularly mussels, are good

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environmental integrators of the environmental quality of surrounding waters. Some species have a wide geographic distribution and are easily accessible, both in the field and through aquaculture, and are amenable for caging (Widdows et al., 2002; Benedicto et al., 2011; Fernández et al., 2012; Sturludottir et al., 2013). Consequently, most European countries use mussels (i.e. *Mytilus edulis, Mytilus galloprovincialis and Mytilus trossulus*) as indicator species to monitor the chemical quality of their coastal and estuarine waters (Bodin et al., 2004; Moreira and Guilhermino, 2005; Minier et al., 2006; Mubiana and Blust, 2006; Brooks et al., 2009; León et al., 2013), but only few of them have incorporated contaminant-related biomarker measurements in their mussel monitoring programs (Palialexis et al., 2014).

Mussels are considered an important component of integrated approaches for assessing marine environmental quality and, a range of different contaminants and contaminant-related biomarkers have been recommended to be monitored in the mussel component of a framework for integrated assessment of contaminants and their effects in the NE Atlantic and Mediterranean waters (UNEP/MAP, 1999; Vethaak et al., 2017). The recommended biomarkers comprise a suite of subcellular, tissue responses and whole organism responses. Lysosomal membrane stability (LMS) (subcellular response) and stress on stress (SoS) (whole organism response) are sensitive and low-cost biomarkers included in the mussel component of integrated monitoring frameworks (e.g. UNEP/MAP, 2005; Vethaak et al., 2017). Both these biomarkers (LMS and SoS) indicate general stress responses including chemical pollutants and provide simple and sensitive indicators of environmental quality (De Zwaan et al., 1995; Moore et al., 2006, 2007). The measurement of LMS using the *in vivo* neutral red retention (NRR) assay does not require expensive equipment or reagents. The reduction of LMS and lysosomal enlargement seem to be the common responses of exposure to a wide range of pollutants (Regoli et al., 1998; Moore et al., 2004), although they do not appear to be strictly interdependent (Izaguirre and Marigómez, 2009). The NRR assay reflects the potential enhanced rate of fusion of primary lysosomes with auto/hetero phagosomes, but also increased autophagy, as well as leakage of the lysosomal contents into the cytosol following damage to the membrane, and possibly, impairment of the proton pump (Seglen, 1983). SoS can be used as a physiological index of mussel health and vitality. Its application is simple and the index is calculated by the survival time when animals are exposed to air. SoS biomarker indicates whether environmental factors, including contaminants, have affected the capacity of molluscs to survive under stressful conditions, such as during exposure to air. Mussels are able to survive aerial exposure for several days, and their ability to keep their valves closed and to resist aerial exposure is thought to be related to the amount of adenosine triphosphate (ATP) available to maintain the tonus in the adductor muscle (De Zwaan and Mathiew, 1992). Exposure to pollutants can uncouple the enzymes involved in anaerobic pathways that mussels utilise during emersion in order to maintain basal metabolism (Eertman and de Zwaan, 1994).

Following the same conceptual framework as applied for contaminant concentrations (OSPAR, 2008), experts have proposed categories to assess the responses of contaminant-related biomarkers using two assessment criteria (ACs): background assessment criteria (BAC) and environmental assessment criteria (EAC) (Lyons et al., 2010; see also Vethaak et al., 2017). Assessing biomarker responses against its BAC and EAC values indicate whether the responses measured are at levels not indicative of deleterious biological effects (<BAC), at levels where deleterious biological effects are possible (≥BAC but ≤EAC) or at levels that deleterious biological effects are likely in the long-term (>EAC). Baseline and threshold values of the LMS in mussels for NRR assay were initially determined from *Mytilus* sp. data based on numerous studies performed in the UK (Moore et al., 2004, 2006). Recently, LMS responses (NRR times) for reference sites in ICES waters (including Iceland, United Kingdom, Norway, Barents Sea, Denmark, Ireland, Atlantic and Spanish Mediterranean waters) were collected and compared during the ICES Working Group of Biological Effects in 2012 (ICES, 2012). In addition, it was decided not to amend the ACs of LMS in mussels on the grounds that some reference sites clearly do achieve retention times of >120 min which may be representative of the background values at these sites. Concerning SoS, background response times and response thresholds corresponding to unintended/unacceptable levels of response were stipulated from the existing UK (North Atlantic waters, *Mytilus* sp.) and Spanish national datasets (Mediterranean waters, *M. galloprovincialis*) (Davies and Vethaak, 2012). One of the challenges in assessing the health status of organisms using such criteria is how to integrate the obtained chemical and biological results. Viarengo et al. (2007) suggested an assessment method using a combination of LMS and survival rate in marine organisms as a screening tool for management decisions. An estimation of survival rate in mussels can be obtained by measuring the Stress on Stress (SoS) biomarker (Viarengo et al., 1995; Pampanin et al., 2005a, b).

Currently, the monitoring of LMS and SoS are still on a voluntary basis within the framework of the OSPAR's Coordinated Environmental Monitoring Programme (CEMP) (OSPAR Commission, Agreement 2010-1) and within the Programme for the Assessment and Control of Marine Pollution in the Mediterranean (MED POL) (UNEP(DEPI)/MED IG.21/9), and under discussion to be implemented as mandatory determinants. Within the context of the international project on Integrated Assessment of Contaminant Impacts on the North Sea (ICON) (Hylland et al., 2017), this study aimed to investigate whether LMS and SoS in mussels can be broadly applied as common biomarkers in biomonitoring programmes in western European waters (North Atlantic and western Mediterranean) to support decision making and to update national biomonitoring strategies, in particular within the context of the MSFD. This study was based on the use of mussels obtained from natural populations along western European coasts and on transplanted mussels from Spanish Mediterranean waters, according to the specificities of monitoring program of each country. Post hoc monitoring strategies combining LMS and SoS results were explored and discussed taking into consideration the availability of contaminant tissue burden data in mussels.

2. Material and methods

2.1. Sampling of native mussel

Mussels from natural populations (*Mytilus* sp.) were collected during the ICON project from seven North European coastal sites and one Mediterranean coastal site between 2008 and 2011 (Fig. 1). Sampling sites were selected on the basis that they were part of the mussel sampling networks of national monitoring/research programs of marine chemical pollution. In the present study Bjarnarhöfn (BH) and Hvassahraun (HV) (Iceland) were chosen as reference sites because of their large distance from major pollution sources. The Wadden Sea sampling site (WS) is situated in the Dutch western Wadden Sea at 1.7 nm outside the freshwater outlet sluices of Den Oever. The western Wadden Sea receives contaminants of the Rhine and Meuse rivers via the Dutch coastal zone and Lake IJssel (Laane et al., 2013). The site Honfleur (HON) is situated in the mouth of the Seine, one of the most polluted estuarine sites in France by contaminations of industrial, domestic and agricultural origins (RNO, 2006, 2013). The site Le Moulard (LM) is situated in