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A biomarker of contaminant exposure is effective in large scale assessment of ten estuaries

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HIGHLIGHTS

- Application of biomarkers in large-scale monitoring programs is assessed.
- Strong associations between lysosomal stability and metal exposure.
- Re-suspended sediments identified as a significant exposure source for bivalves.
- Lysosomal membrane stability robust to natural variations in water-quality.

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ABSTRACT

Cost-effective and sensitive measures of anthropogenic stress are necessary tools in any environmental monitoring program. When implementing new monitoring tools in a region, rigorous laboratory and field studies are essential for characterizing the sensitivity and efficacy of the approach. We exposed the oyster *Saccostrea glomerata* to various individual contaminants through multiple exposure pathways (water- and food-borne) in the laboratory and measured two biomarker responses, lysosomal membrane stability (LMS) and lipid peroxidation (LPO). LMS was sensitive to both contaminant exposure pathways. We subsequently measured this biomarker in oysters which had been experimentally deployed at multiple sites in each of ten estuaries with varying levels of contamination associated with re-suspended sediments. There was a strong association between LMS and metal exposure, despite substantial natural variation in water quality parameters. Our results illustrate the potential use of LMS as a pragmatic indicator of biotic injury in environmental monitoring programs for re-suspended contaminated sediments.

1. Introduction

It is critical that the ecological impacts of anthropogenic stressors be monitored so that trends in ecosystem health can be assessed and locations prioritised for improved management of discharges and potential remediation (Borja et al., 2008). The vast majority of monitoring strategies have focussed their efforts on describing chemical contamination in the media of concern such as air, sediments, water or biological tissue (Shulkin et al., 2003; Hedge et al., 2009). While this is a useful approach for identifying contamination hotspots and for estimating exposure risk, the resulting measurements do not necessarily translate into biological effects (Galloway et al., 2002). Increasingly researchers are seeking monitoring strategies that establish connections between external exposure levels (exposure), internal levels of tissue contamination (dose) and adverse effects (response) (van der Oost et al., 2003).

The cost and taxonomic difficulty of sampling entire biological communities (Chariton et al., 2010) has fuelled the search for monitoring tools that will provide a cost-effective and sensitive measure of anthropogenic stress with broad and consistent applicability (Ferrat et al., 2003; Sarkar et al., 2006; Sun et al., 2012). Biomarkers are a relatively novel approach to environmental monitoring, and their high value as surrogate biological measures of anthropogenic impacts is supported by numerous laboratory studies (Moore et al., 2004; Martin-Diaz et al., 2005). Typically, biomarkers are defined as quantitative measures of change in the biological system that respond to either exposure to, and/or doses of, xenobiotic substances (Lam and Gray, 2003). These can be molecular, cellular, physiological or behavioural changes. Of the numerous biomarkers proposed over the decades, those based on changes at the molecular and cellular level are

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believed to provide the earliest evidence of environmental disturbance (Martin-Diaz et al., 2004).

The efficacy of a biomarker for use in environmental monitoring requires rigorous assessment in both the laboratory and the field. Laboratory studies are useful in identifying biomarkers and investigating exposure-dose–response relationships, however biomarkers also need to be tested under field conditions where multiple stressors are present. Organisms frequently respond differently to stressors when they occur in combination, these responses can be additive, synergistic or antagonistic (Holmstrup et al., 2010). A useful biomarker for field applications requires that it be sensitive to the stressor of interest, despite substantial variations in natural stressors (e.g. temperature, salinity and pH) over large spatial scales (Dafforn et al., 2012). To date there are very few studies that evaluate biomarkers over large spatial scales in the field, where the presence of multiple stressors can create difficulties in the interpretation of biological data.

Estuaries are among the most productive of marine ecosystems and are subject to increasing pressure from anthropogenic activities (Elliott and Whitfield, 2011). Urban and industrial development is heavily concentrated in estuaries and therefore the resident biota are exposed to multiple physical and chemical stressors. Biological monitoring of estuarine environments is a critical management priority and potential biomarkers for these impacted areas are being developed. Bivalve molluscs are efficient accumulators of metal and organic contaminants, and have been integrated into biomonitoring programs such as Mussel Watch (Goldberg, 1986). Although bivalves are known to be robust organisms, they often display sub-lethal sensitivity to contaminants and are therefore potentially useful organisms with which to develop and test biomarkers (Romeo et al., 2003; Nigro et al., 2006).

Edge et al. (2012) provided initial indications that the Sydney rock oyster may be a useful biomonitoring species, with strong relationships identified between biochemical and reproductive responses. However, an investigation into the sensitivity and consistency of these biomarkers over large spatial scales is currently lacking. We use single contaminant laboratory experiments to identify potential biomarkers of water- and food-borne contaminant exposure in an oyster, and then demonstrate the feasibility of using the most promising biomarker in an extensive field study of ten estuaries separated by more than 400 km of coast. Using this biomarker we examine the exposure-dose–response relationships between contamination in suspended sediments, contamination in oyster tissues and toxicity at the cellular level. This is done within the context of large scale variability in multiple stressors related to anthropogenic modification and physico-chemical conditions. The sensitivity and environmental significance of the biomarker responses were considered in relation to sediment quality guidelines (SQGs) (ANZECC/ARMCANZ, 2000).

2. Materials and methods

2.1. Laboratory exposure experiments

Two year old Sydney rock oysters *Saccostrea glomerata* of ~7 cm length were obtained from a commercial hatchery in Port Stephens, New South Wales, Australia. Oysters were acclimated for 4 d in controlled conditions of water temperature (24 ± 1 °C), salinity (35 ± 1‰), and light:dark regime (12:12 h) prior to exposures. Oysters were fed with clean *Tetraselmis* sp. (2×10^5 algal cells oyster^{-1}) daily following a seawater change. The marine microalgae *Tetraselmis* sp. were originally obtained from the Collection of Living Microalgae, CSIRO Marine and Atmospheric Research, Hobart, Australia. Seawater (collected from Cronulla, NSW) was filtered through 0.22 μm before use in all experiments.

Following acclimation the oysters were divided up into tanks (10 L) and exposed to various treatments in the laboratory for 96 h, with three replicate tanks per treatment. Oysters were exposed to different contaminants at varying concentrations via either their food or surrounding environment (water). Each tank was held in controlled conditions (see above) for the duration of the experiment. Temperature and salinity were recorded in each tank daily using a handheld meter (340i, WTW) following calibration according to manufacturer's instructions. At the end of the 96 h exposures, 3 oysters were sampled from each of the three replicate tanks for each treatment and the digestive gland dissected out. Lysosomal membrane stability (LMS) assays were conducted immediately with pieces of digestive gland. Remaining tissues were frozen (−80 °C) and later analysed for lipid peroxidation (LPO) (details of biochemical analyses are outlined below in Section 2.3). The purpose of our laboratory experiments was to demonstrate that biomarkers respond to toxicant exposure and are therefore potentially useful candidates for field monitoring. We did not aim to establish effect levels under controlled laboratory conditions. Hence nominal concentrations of toxicants were considered acceptable and the actual concentrations in water and in food in these laboratory experiments were not measured.

2.1.1. Cu and Cd algal-borne exposure

Five treatments were applied through a food-borne contaminant exposure. Treatments were prepared for each algal-borne exposure by preparing *Tetraselmis* sp. using a 72 h dosing procedure (Levy et al., 2008). To spike microalgae, stock solutions of 50 mg Cu L^{-1} ($CuSO_4{\cdot}5H_2O$) and 50 mg Cd L^{-1} ($CdCl_4$(anhydrous) were prepared in deionised water (Milli-Q, Millipore). When required, spiking concentrations were created by diluting the appropriate amount of stock solution with a cultured test medium of filtered seawater and minimal nutrients (15 mg NO_3 L^{-1} and 1.5 mg PO_4^{3-} L^{-1}). Nominal spiking concentrations for these treatments were an unspiked cultured test medium (control), 50 and 100 μg Cu L^{-1} and 50 and 100 μg Cd L^{-1}. Algal spiking was conducted at a constant temperature of 25 ± 1 °C and allowed to grow under a Gro-Lux light set to <800 Lux. After 72 h, algal material was filtered through a 0.22 μm filter at 100 kPa, (Weksler) using an EDTA washing solution (0.01 M EDTA, 0.1 M KH_2PO_4/K_2PO_4 buffer pH 6.0, salinity adjusted to 35‰) to remove extracellular Cu and Cd. The algae retained on the filter were then resuspended in clean filtered seawater prior to the addition to tanks. Oysters were administered 2×10^5 treated algal cells per oyster daily following a fresh seawater change (0.22 μm filtered).

2.1.2. Cu water-borne exposure

Copper is one of the most abundant contaminants found in polluted estuaries, mainly due to stormwater run-off, industrial release and its use as an antifoulant on recreational and commercial vessels (Dafforn et al., 2011). Copper was used for the water-borne exposure to test if the route of exposure for this common contaminant has an effect on the biomarker response. The concentrations of Cu used in this experiment were chosen to represent an array of exposure concentrations. Dissolved Cu concentrations in estuarine waters seldom exceed 3 μg L^{-1} (Hatje et al., 2003). Yet there are extreme scenarios, such as sites of industrial discharges and highly contaminated enclosed marinas, which have resulted in much higher dissolved Cu concentrations over wide areas for prolonged periods of time, up to and above 100 μg L^{-1} (Correa et al., 1999; Stauber et al., 2000; Andrade et al., 2006; Schiff et al., 2007). Three different dissolved Cu treatments and a control (filtered seawater) were prepared from a $CuSO_4{\cdot}5H_2O$ stock solution (200 mg Cu L^{-1}). Nominal concentrations of 0, 10, 50 and 100 μg Cu L^{-1} were used. For the duration of the water-borne Cu exposure experiment, *Tetraselmis* sp. (not