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Metal-contaminated resuspended sediment particles are a minor metal-uptake route for the Sydney rock oyster (*Saccostrea glomerata*) — A mesocosm study, Sydney Harbour estuary, Australia

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ABSTRACT

Resuspension of surficial sediments is considered a key process influencing bioaccumulation of metals in filter-feeders in the contaminated Sydney Harbour estuary (Australia). However, previous investigations were unable to establish a significant relationship between metals in sediments or suspended particulate matter (SPM) and oyster tissue concentrations. This study used a 60-d laboratory mesocosm experiment to expose Sydney rock oysters, *Saccostrea glomerata*, to a natural range of SPM concentrations with different SPM-metal concentrations. Dissolved metal concentrations were low and the availability of algae provided as food was constant for all treatments. Tissue metal concentrations of Cu, Pb and Zn increased significantly, however, no relationship was determined between tissue metal concentrations in the oyster and either SPM or SPM-metal concentrations. The results indicated that exposure to resuspended contaminated sediment particles contributed little to the observed metal uptake. Dissolved or algae food sources appear to be more important for metal accumulation in these oysters.

1. Introduction

Sydney Harbour estuary, New South Wales, Australia, is a highly urbanised and modified waterway in which sediments are impacted by high concentrations of contaminants, particularly Cu, Pb and Zn (McCready et al., 2006). Within this environment, surface sediments are frequently resuspended into the water column (Birch and O'Hea, 2007), which occurs due to a combination of processes: meteorological and hydrological processes (tidal currents, wind-driven waves, and ocean waves); anthropogenic activities (shipping and dredging); and bioturbation by benthic organisms (Davis, 1993; Schoellhamer, 1996; Knott et al., 2009). For organisms such as filter-feeding bivalve molluscs, resuspended sediments may be an important mechanism for bioaccumulation of metals released from sediment particles or dietary ingestion of contaminated suspended particulate matter (SPM) (Griscom and Fisher, 2004; Edge et al., 2014). Bivalves generally possess high uptake and low efflux rates for metals (Wang et al., 1996; Griscom et al., 2002), thus making these animals strong metal bioaccumulators and useful as biomonitors (Rainbow, 2007; Rainbow et al., 2015).

A relationship between contaminants associated with fine sediments, SPM and tissue metal bioaccumulation has been observed for the native blue mussel (*Mytilus galloprovincialis*) in Sydney Harbour estuary (Birch and Apostolatos, 2013). However, similar relationships have proven to be elusive for the native Sydney rock oyster (*Saccostrea glomerata*) in the same estuary (Dafforn et al., 2012; Birch et al., 2014). Those observations were made in wild oysters, and a range of abiotic and biotic factors may influence the bivalve tissue metal concentrations (Luoma, 1983; Richards and Chaloupka, 2009).

A biokinetic model for zinc bioaccumulation by *S. glomerata*, developed using radio-labelled algae, sediment, and water sources, indicated that dietary exposure to suspended fine sediment particles or algae may potentially become the dominant metal exposure routes for the oyster (Lee et al., 2015). The model predicted that metal bioaccumulation may vary considerably depending on the feeding rates and food selectivity. There are many challenges in extrapolating from laboratory-based observations and model predictions to conditions experienced in the natural environment (Burton et al., 2005; Mann et al., 2010; Cresswell et al., 2014). Due to the relatively short equilibration time for the Zn-radiolabel, the zinc associated with the sediment particles used to develop the biokinetic model was expected to be more labile in the oyster's gut than may be expected for naturally contaminated sediment particles (Simpson and Batley, 2007). As a consequence, validation of the model using metal-contaminated particles collected from the field was considered necessary.

To eliminate variation in field conditions as a factor influencing metal accumulation patterns, a controlled laboratory setting was used to further investigate the role of resuspended contaminated sediments

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in metal bioaccumulation in tissues of the filter-feeding oyster *S. glomerata*. Within Sydney Harbour estuary the amounts of SPM in the water column and the SPM-metal concentrations vary both spatially and temporally, however, little information is available on concentrations of algae or metal concentrations within algae. In the present study two main variables were considered, namely SPM concentrations and SPM-metal concentrations. The range of SPM concentrations selected were chosen to represent those frequently observed in the higher energy regions of Sydney Harbour estuary (Taylor, 2000; Hatje et al., 2001; Birch and O'Hea, 2007) and the SPM-metal concentrations ranging from low to high contamination. The higher end of the ranges was expected to represent the worst case for metal exposure from this source. In all treatments, small and constant amounts of food were provided in the form of algae, and high rates of seawater renewal were used to maintain dissolved metal concentrations similar to those observed in the estuary. The laboratory-based mesocosm experiments had a duration of 60-days and at the end of the experiments the tissue metal concentrations in the oysters were related to the exposure conditions. During the experiment, analyses were made of concentrations of SPM, dissolved metals, and total and dilute-acid extractable particulate metals. The observation or absence of metal accumulation during the exposure period was expected to confirm the role of resuspended contaminated sediments in contributing to metal accumulation by the oyster species.

2. Methods

2.1. Sediment preparation and metal analysis

Sediments were collected from Iron Cove, Sydney Harbour estuary (high metal concentrations) and Bonnet Bay, Woronora River (low metal concentrations) (Birch and Taylor, 1999; Simpson et al., 2004). The sediments were wet-sieved through a 63-μm sieve (type) to isolate a high and low contamination fine sediment (<63 μm). A third sediment was prepared as a 1:1 mixture of the two sediments to provide three sediments with low medium and high levels of contamination for the laboratory experiment. Metal analyses made on the three sediments included total recoverable metal concentrations (TRM, high pressure microwave-assisted aqua regia digestion) (Dafforn et al., 2012), dilute-acid extractable metals (AEM, 1 M HCl for 60 min (Chariton et al., 2010) and dilute-acid soluble metals (ASM, 0.2% HNO_3 for 5 min (Simpson et al., 2005). All digestion methods included reagent blanks, replicates and international reference materials.

Particle size analysis of dry sediment sub-samples was carried out by wetting approximately 0.3 g dried sub-samples with deionised water (18 MΩ·cm) and mixing with 10 mL of H_2O_2 in 50-mL centrifuge tubes to remove organic material and left to effervesce completely. Tubes were centrifuged and the supernatant liquid discarded, and the above steps were repeated three times to ensure entire removal of H_2O_2. A 40 mL aliquot of 5% $Na_6P_6O_{18}$ (sodium hexametaphosphate) was added to disperse the particles in the sub-samples and tubes were loaded onto a rotating-drum laboratory mixer to ensure thorough dispersion of samples. Samples were added to a Hydro 2000G wet dispersion bath and pumped through a Malvern Mastersizer MS-2000 to obtain particle size.

Dried test sediment sub-samples (7–10 g) were ground using a mortar and pestle for loss on ignition (LOI) (Dean, 1974) analysis. Crucibles were prepared by baking at 550 °C for 2 h, cooled and weighed. Ground samples were placed in crucibles and weighed, then placed into a muffle furnace at 550 °C for 2 h. Samples were re-weighed once cooled, and the difference between the pre- and post-LOI sample weights was calculated.

2.2. Mesocosm experiment

A mesocosm experiment was conducted in an aquarium laboratory for 60 days to mimic field conditions and to elicit responses in species that have lengthy lifespans (Forrow and Maltby, 2000). *S. glomerata* is generally observed to have an SPM limit of 15 mg L^{-1}, after which the filtration apparatus may become overloaded, inhibiting the rate of particle filtration and causing a decline in feeding (Winter, 1978; Widdows et al., 1979). However, the oysters *S. glomerata* are found in regions of Sydney Harbour estuary where SPM concentrations typically range from 10 to 30 mg L^{-1}, but are known to exceed 40 mg L^{-1} during periods of high-winds (Taylor, 2000; Hatje et al., 2001; Birch and O'Hea, 2007). As the intention of the study was to better understand the contribution of SPM ingestion to metal accumulation by *S. glomerata* within Sydney Harbour estuary, a high range of SPM concentrations was studied. By using different masses of sediment and water, low-, medium- and high-SPM treatments were prepared, characterised as 10, 20, and 40 nephelometric turbidity units (NTU), which was equivalent to approximately 15, 30, and 62 mg L^{-1} of suspended solids.

Together with the low, medium and high levels of contamination provided by the sediments, nine treatments were prepared in total to cover the natural extent of these variables in Sydney Harbour estuary. The full experiment comprised thirteen 30 L plastic aquarium tanks, comprising these nine treatments along with three control tanks with sediments not resuspended and a control tank with no sediment (Table S1 of the Supporting Information).

Three sediment entrainment or 'shaker' devices were custom built based on a modified design by Tsai and Lick (1986) to resuspend the test sediments and each consisted of a plywood frame and poly (methyl methacrylate) components (Fig. S1 of the Supporting Information). A 240 V AC motor rotated a metal-free Perspex© (PMAA, or Plexiglas©) axle, which caused a vertical oscillation of Perspex, perforated grid paddles (33 × 23.5 cm) (amplitude of 2.5 cm) and which kept sediments in suspension. The AC motors were connected to a timer set at 4 h, which replicated the duration and volume of natural resuspension in Sydney Harbour estuary. All Perspex parts and aquarium tanks were rinsed thoroughly first in Decon 90 detergent, then acid washed in 10% HNO_3 and finally rinsed in deionised water.

Three multi-parameter water quality YSI Sondes were used to monitor abiotic water conditions in the tanks. Sondes were fitted with probes to measure turbidity, temperature, pH, conductivity (salinity) and time at 5 min intervals and each sonde was calibrated prior to use. Seawater used in the experiment was pumped and filtered into aquarium facilities directly from the main Sydney Harbour estuary channel. The seawater in all tanks were drained and replaced daily and tanks were manually cleaned weekly and replenished with fresh test sediment. This was to ensure most dissolved waste products or uneaten algae, which were provided as food, did not accumulate in the tanks. Each treatment tank contained an airstone connected to a pressurised air pump system to allow sufficient aeration. Analysis for dissolved metal concentrations prior to resuspension was performed by filtering 10 mL of seawater from each tank through a 0.45 μm syringe filter into polypropylene sample vials and acidified to 0.2% *v*/v HNO_3. Blank samples of syringes, filters and sample vials were collected by washing with Milli-Q water and analysed for quality control.

One hundred and fifty *S. glomerata* oysters, aged between 18 months and 2 years, were purchased from Aquaculture Enterprises Pty Ltd. (Millingandi, NSW, Australia). Efforts were made prior to the experiment to purchase oysters of the same size, age, ploidy and brood. Upon arrival, 20 oysters were depurated and sacrificed to serve as background controls and were measured for length, width and depth using a digital calliper, shucked, then shell weight (SW) and wet soft-tissue weight (WW) were measured and soft tissues were stored frozen at −30 °C prior to the start of the experiment, oyster specimens were acclimated to aquarium conditions for 10 d (Thompson et al., 2012). Each tank contained ten oysters, which were suspended 5 cm above the tank bottom on a plastic mesh tied on to a Perspex frame. Mortality of oyster specimens was minimal with loss of three specimens throughout the experiment. Oyster diet was supplemented twice weekly, every Sunday and Wednesday morning, with a liquid microalgae food source