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Dietary ingestion of fine sediments and microalgae represent the dominant route of exposure and metal accumulation for Sydney rock oyster (*Saccostrea glomerata*): A biokinetic model for zinc

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ABSTRACT

Past studies disagree on the extent to which dissolved or dietary uptake contribute to metal bioaccumulation in the filter-feeding Sydney rock oyster (*Saccostrea glomerata*) in urbanized estuaries. Although most data support the assumption that fine sediments are a major route of metal uptake in these bivalves, some studies based in the Sydney estuary, Australia, have indicated a poor correlation. In the present study, seawater, sediment and microalgae were radiolabelled with ^{65}Zn tracer and exposed to *S. glomerata* to assess the influence of dissolved and dietary sources to Zn bioaccumulation. Oysters in the dissolved-phase uptake experiment (5, 25 and 50 μg L^{-1} ^{65}Zn for 4 d followed by 21 days of depuration) readily accumulated ^{65}Zn for all three concentrations with an uptake rate constant of 0.160 ± 0.006 L dry weight g^{-1} d^{-1}. Oysters in the dietary assimilation experiment (1 h pulse-feed of either ^{65}Zn-radiolabelled suspended fine-fraction (<63 μm) sediment or the microalgae *Tetraselmis sp.*) accumulated ^{65}Zn, with assimilation efficiencies of 59 and 67% for fine sediment and *microalgae*, respectively. The efflux rates were low for the three experiments (0.1–0.5% d^{-1}). A bioaccumulation kinetic model predicts that uptake of Zn will occur predominantly through the dietary ingestion of contaminated fine sediment particles and microalgae within the water column, with considerably greater metal bioaccumulation predicted if oysters ingested microalgae preferentially to sediments. However, the model predicts that for dissolved Zn concentrations greater than 40 μg L^{-1}, as observed during precipitation events, the uptake of the dissolved phase may contribute ≥50% to accumulation. Overall, the results of the present study suggest that all three sources may be important exposure routes to *S. glomerata* under different environmental conditions, but contributions from dietary exposure will often dominate.

1. Introduction

The relationship between contaminated sediments and tissue metal burden in bivalves have been the focus of extensive research (Hutchins et al., 2009; King et al., 2010; Birch and Apostolatos, 2013; Taylor and Maher, 2013; Birch et al., 2014; Edge et al., 2015; Campana et al., 2015; Schmitz et al., 2015). Of significant interest in Australian waters is the Sydney rock oyster (*Saccostrea glomerata*), an inter-tidal, filter-feeding bivalve found ubiquitously along most estuaries of the east coast of Australia. Significant relationships have been observed between oyster tissue and contaminated sediments in many NSW estuaries, especially for Cu and Zn (Birch and Hogg, 2011; Dafforn et al., 2012). However, similar associations were not always observed for these organisms from Sydney Harbour estuary (Birch et al., 2014), and little understanding of the pathways of metal uptake and accumulation by this, or other, native bivalve species (Campana et al., 2013; Edge et al., 2014).

Copper, Pb and Zn are ubiquitous contaminants in the Sydney Harbour estuary (Birch and Taylor, 1999; Chariton et al., 2010), and the opportunity exists to use the gamma-emitting radioisotopes of these elements to determine metal bioaccumulation kinetics and assess the importance of dissolved and dietary routes of metal uptake. Numerous bivalve radiotracer studies from around the world can be found in the scientific literature (Reinfelder et al., 1997; Ke and Wang, 2001; Blackmore and Wang, 2004; Borretzen and Salbu, 2009; Pan and Wang, 2012), yet in Australia only one study has published the uptake and loss kinetics of trace metals (^{109}Cd and ^{64}Cu) by a native bivalve species *Tellina deltoidalis* (King

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et al., 2005). The ^{64}Cu radioisotope possesses a short half-life of 12.7 h, and while utilised previously (King et al., 2005), it is difficult to handle due to the high doses required. Radioisotopes ^{210}Pb and ^{65}Zn possess much longer half-lives of 22.3 y and 243.7 d, respectively. ^{210}Pb was not used due to insufficiently low gamma emissions, as well as high alpha emissions. The current study thus employed the radiotracer ^{65}Zn to analyse the uptake, retention and loss of Zn by the filter-feeding oyster *S. glomerata* via dissolved and dietary pathways using radioisotope tracer techniques. The acquired biokinetic parameters from exposure experiments were compared against other bivalve species reported globally and applied in bioaccumulation modelling to allow predictions of tissue metal concentrations in steady-state conditions. The dissolved and particulate metal sources were also evaluated to determine their relative contribution to overall *S. glomerata* tissue Zn bioaccumulation.

2. Methods

2.1. Oyster stocking and handling

The oysters were held in 1.125 L polypropylene containers (Décor, Tellfresh) with insertable polypropylene baskets during both the pre-test phase and radiotracer exposure experiments. Holes (5 mm diameter) were drilled into the centre of each lid to allow for aeration lines to maintain dissolved oxygen concentrations at maximum saturation levels during both holding and experimentation. Plasticware was acid-washed (10% v/v AR grade HNO_3, Merck, Germany), then rinsed in deionised water (Milli-Q, 18 MΩ cm, Millipore, Academic Water System, Sydney, Australia), and air dried. Seawater in the present study (30 PSU; pH 8.1 ± 0.1) were collected from the south coast of NSW and filtered to 1 μm.

Individuals of *S. glomerata* of 18 months to 2 years in age of medium size (60–80 mm shell length) were purchased from Aquaculture Enterprises, Ltd. (Milligandi, NSW, Australia). Experiments in the present study were conducted at laboratory facilities of the Institute for Environmental Research, Australian Nuclear Science and Technology Organisation (ANSTO). Upon arrival, oysters were individually rinsed with 1 μm filtered seawater and cleaned with a toothbrush to remove any sediment or organic particles coating the exterior shell surfaces. Each oyster was then held in an individual 1.125 L polypropylene container with approximately 500 mL of filtered seawater, which was replaced daily during an acclimation period of minimum 7 d. Each oyster was fed the equivalent of 1% of its dry body weight with a commercial microalgae feed (Shellfish Diet 1800, Proaqua, Queensland, Australia). Experimentation was conducted in a temperature controlled room (21 ± 1 °C), under a 12 h: 12 h light:dark lighting regime.

2.2. Dissolved zinc exposure

The ^{65}Zn radioisotope was added to filtered seawater gravimetrically to produce three dissolved concentrations of 5, 25, and 50 μg L^{-1} (7.6×10^{-8}, 3.8×10^{-7} and 7.6×10^{-7} mol L^{-1}, respectively; 2.3, 11.7 and 23.4 kBq L^{-1}, respectively) and representing dissolved Zn concentrations naturally observed in Sydney Harbour estuary (Hatje et al., 2001, 2003; Birch and Rochford, 2010). Containers were initially conditioned overnight with 500 mL of ^{65}Zn active solution so as to saturate all possible metal binding sites on the internal surfaces of containers. This solution was then discarded to waste and replaced with fresh active solution. For each exposure concentration there were five replicates. Each one included oyster specimen within an individual container with 500 mL of active solution and aerated with silicone tubing. *S. glomerata* specimens were exposed to dissolved ^{65}Zn for a period of 4 d and radioanalysed for 5 min at 2, 4, 6, 8, 26 h and twice daily (morning and night) thereafter using LaBr probe connected to a Canberra InSpector 1000 spectrometer (Supplementary information A). The possibility of radiotracers bound to external surfaces of oyster shells, along with active solution retained within shell cavities may overestimate oyster radioactivity and these issues were addressed in detail in a pilot study (Supplementary information B). The active solution was replaced at the start of each day to maintain exposure concentration and oysters were not fed throughout the exposure. Water samples were also taken daily and radioanalysed for gamma activity before and after replacement of active solution. After 4 d, oysters were transferred into fresh (and pre-washed) containers with filtered seawater and fed 1% dw microalgae to start the 21 d depuration phase. Oysters were radioanalysed after 5 h of depuration (at night) to detect loss of unassimilated, imbibed solution and detected at 24 h intervals with the non-active seawater replaced daily.

At the end of the depuration phase oysters were radioanalysed, and then weighed and shucked to obtain length, width, height, whole weight, shell weight and wet soft tissue weights. Shells were patted dry using lint-free wipes (Kimwipes) then radioanalysed to detect ^{65}Zn adsorbed on the shell. Soft tissues were also patted dry and radioanalysed, dried in an oven at 60 °C for 6 d, weighed to obtain dry tissue weight, then radioanalysed again. Dry soft tissues samples in crucibles were placed in a muffle furnace at 500 °C for 3 h and ashed. Samples were transferred into 20 mL polyethylene scintillation vials and digested with 10 mL HNO_3 (Merck, Tracepur) and radioanalysed to convert raw gamma values from a whole oyster to a standard 10 mL geometry. Gamma counts were then converted to Becquerels by dividing by the efficiency of the gamma probe, which was then converted to μg of bioaccumulated Zn by multiplying Becquerels with the specific activity of the ^{65}Zn radiotracer.

2.3. Sediment radiolabelling and pulse-chase analysis

Sediments containing low metal concentrations from Bonnet Bay (South Sydney) were wet sieved using a plastic spoon, 63 μm nylon sieve and filtered seawater to separate the fine fraction from coarse-grained sediment and the former was radiolabelled with ^{65}Zn to assess dietary uptake and assimilation efficiency (AE) from sediments as per Cresswell et al. (2014). In a 50 mL polypropylene centrifuge tube 120 mg of wet sediment were radiolabelled with an aliquot of ^{65}Zn (112 kBq; 464 mg L^{-1}; 7 μmol L^{-1}) and made up to 50 mL using filtered seawater. A 2 mL sub-sample of the active solution was collected using a 10 mL plastic syringe and filtered through a 0.45 μm filter into a 20 mL scintillation vial and made up to 10 mL using filtered seawater to determine initial dissolved ^{65}Zn gamma activity. The 50 mL sediment slurry tube was shaken vigorously for 10 s then left to equilibrate for 7 d and 2 mL subsamples of supernatant water were collected daily for ^{65}Zn activity, as well as daily monitoring of pH, which was adjusted to 8.1 ± 0.1 using 0.1 M NaOH when necessary. An immediate binding of tracers to sediments was observed with 38% of ^{65}Zn tracer remaining in solution after the first day, 18% by the third day and 13% remaining on the seventh and final day of equilibration. The remaining active solution was decanted to waste and labelled sediments were rinsed three times by mixing with 10 mL filtered inactive seawater using a vortex mixer for 10 s and centrifuged at 4500 rpm for 5 min. Loss of ^{65}Zn after three rinses was 20% and radioactivity of dissolved ^{65}Zn tracer desorbing from the sediment in the final rinse was negligible (<1%). The final concentration of ^{65}Zn radiolabelled on to the sediment was 2260 μg g^{-1} (34.6 μmol g^{-1}) and was >90% readily-soluble in dilute acid (Supplementary information C). A high concentration of ^{65}Zn tracer was required due to a low specific gamma activity caused by decay as well as the possibility of poor adsorption of the tracer on sediment particles. However the