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Uptake and Accumulation of Cadmium, Manganese and Zinc by Fisheries Species: Trophic Differences in Sensitivity to Environmental Metal Accumulation

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Abstract

Fishery targeted species living in estuaries face multiple anthropogenic pressures including habitat contamination. However, trace metal concentrations in aquatic organisms can be highly variable, making it difficult to interpret accumulation responses. Understanding sources for metal accumulation in these organisms and their biokinetics is important for management of local fisheries and ensuring safety and quality of consumed seafood, particularly in urbanised areas. In this study, we exposed Australian sand clams, school prawns and sand whiting to a combination of cadmium (Cd), manganese (Mn) and zinc (Zn) radioisotopes 1) dissolved in seawater, 2) adsorbed to suspended sediment particles and 3) in radiolabelled food. Sand clams were sensitive to Cd, Mn and Zn uptake and accumulation from all sources because of their filter feeding physiology. Mean Cd and Zn assimilation efficiencies (AE) were higher in clams fed benthic diatoms (51, 43, 63% for Cd, Mn and Zn, respectively) than clams fed an algal flagellate species (21, 32, 33% for Cd, Mn and Zn, respectively). Metal uptake by prawns from seawater was low, whereas assimilation from diet was high (67, 57, 52% mean AEs from Cd, Mn and Zn, respectively). Sand whiting did not accumulate metals from seawater, even after concentrations were increased. Assimilation from diet (labelled prawns) was also low for sand whiting, particularly for Cd and Zn (12, 26, 13% mean AEs from Cd, Mn and Zn, respectively). These results may help explain the persistence of sand whiting in contaminated estuaries. Suspended sediment exposures showed

that prawns and fish are less likely than clams to be negatively affected by disturbance events such as floods, which can bring metals into estuaries. The findings of this study have implications for fisheries management, both for protection and remediation of important habitats, and to ensure safe standards for seafood consumption by humans.

Keywords

Cadmium, manganese, zinc, metal, fish, prawn

1. Introduction

Estuaries and coastal bays are among the most biologically important habitats, and are some of the most degraded due to contamination from trace metals and other pollutants (Cearreta et al., 2000; Vasconcelos et al., 2007). Much of the world's seafood is produced in estuaries and food standard regulations have helped to identify key contamination issues. Understanding the pathways of trace metals from source to organism is important for not only maintaining healthy ecosystems and biodiversity, but also for preventing toxic effects on human health through consumption. Shellfish, crustaceans and fish are commonly harvested by commercial and recreational fishers in Eastern Australia (ABARES, 2018). Estuaries along this coastline vary in anthropogenic influence e.g. urban, industrial and agricultural land use, and as a result, they vary in contaminant form and concentration (Birch, 2000; Cox and Preda, 2005). Tissue metal concentrations of different organisms living in the same environment are often disparate (Dural et al., 2007; Lozano et al., 2010) and the mechanisms underlying this are poorly understood. Organism metal uptake and assimilation responses vary between trophic

levels and taxa, even those that are biologically similar, complicating our understanding of metal cycling in food webs and organism response to contamination (Pouil et al., 2017; Wang and Ke, 2002).

Aquatic organisms take up and accumulate trace metals, whether essential or not, and increased exposure has the potential to cause toxic effects (Marsden and Rainbow, 2004; Rainbow, 2007). Exposure to metals can originate from both dissolved and particulate phases. Metals dissolved in water may be accumulated by direct adsorption to body surfaces with subsequent uptake across permeable membranes, such as gills (Wang and Fisher, 1999). Particulate metals can be accumulated by animals following ingestion and digestion of contaminated food (Mathews et al., 2008). As a result of two decades of research on trophic transfer of metals, dietary exposure is now considered a major route for metal accumulation in marine animals, including both invertebrates and fish (e.g. Casado-Martinez et al., 2009; Lee et al., 2015; Zhang et al., 2011). Experimentally determining uptake and efflux rates, as well as assimilation efficiencies, allows contamination responses of wild aquatic organisms to be modelled using environmentally relevant metal concentrations.

Trace metals such as cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb) and zinc (Zn) originate from a variety of sources including industrial production, and agricultural and urban pollution, making them serial pollutants in rivers and estuaries, including those on the East Australian coastline (Brown and Peake, 2006; Moss and Constanza, 1998; O'Mara et al., 2017). To study the sources of accumulation of these metals in a simplified food chain using fisheries species, a radiotracer experiment was used. The properties of gamma-emitting radioisotopes, also referred to as radiotracers, allow metal biokinetics and bioaccumulation to

be modelled using data obtained from radioanalysis of live organisms by gamma detection under controlled laboratory conditions. Not all metal radioisotopes are suitable to use in live radiotracing experiments. A combination of ^{109}Cd , ^{54}Mn and ^{65}Zn radioisotopes was used in this experiment because of their environmental relevance and suitability in terms of spectral emissions and half-life. Mn and Zn are essential elements and are required by organisms for physiological functioning, however in high doses can have severe toxic effects (National Research Council, 2005). Cd is a non-essential element and only low concentrations are required to cause toxic effects, however the toxicity of Cd in water varies with changing environmental conditions such as water hardness and salinity (Engel and Fowler, 1979).

Stocks of commercially fished estuarine species face multiple pressures to survive. Therefore, understanding how they accumulate contaminants is important for improving fisheries management to benefit both the fishery and human health through targeted habitat management, ultimately enhancing seafood quality. Our study aimed to determine routes of metal (Cd, Mn and Zn) uptake and accumulation in commercially important shellfish, crustaceans and fish under normal conditions and assess their vulnerability to metal uptake during disturbance events such as floods. To achieve this we had three objectives/sources for each species:

Objective A: to determine uptake and efflux rates upon exposure to metals dissolved in seawater.

Objective B: to determine metal assimilation efficiency and efflux rates from suspended sediment.

Objective C: to determine metal assimilation efficiency and efflux rates from diet.

Where possible, we also aimed to use this data to model metal accumulation from each source (seawater, suspended sediment or diet) under different environmental contamination scenarios.

2. Methods

2.1 Animal collection and housing

Juvenile sand whiting (*Sillago ciliata*) (38-64 mm standard length, fresh weight 0.79-3.2 g), juvenile school prawns (*Metapenaeus macleayi*) (9-14 mm carapace length, fresh weight 0.48-1.7 g) and sand clams (*Katelysia scalarina*) (71-84 mm shell length, fresh weight 88-141 g) were collected from Southern Moreton Bay (QLD), Wollumboola Lake (NSW) and Port Hacking (NSW), respectively. These three species were chosen because they are commercially and recreationally important species and are common and widespread in estuaries and coastal bays along the East Australian coastline from southern Queensland to Victoria and occupy different trophic levels (ABARES, 2018; Dent et al., 2016). Following collection, all organisms were held at Australia's Nuclear Science and Technology Organisation (ANSTO) Aquatic Ecosystems laboratory in several 70-90 L aquaria (with filtration and aeration). After acclimation to laboratory conditions in filtered <0.45µm seawater (temperature controlled room at 23 ± 1 °C, pH = 8.2-8.4, salinity = 35, 12h:12h light:dark lighting regime) for 1-4 w they were introduced into exposure containers. Experiments were conducted in plastic containers and organisms were fed separate diets (Supporting Information S1). Background whole-body metal concentrations (Table 1) of three replicates of each species euthanized shortly after collection (but after depurating gut contents) were determined by ICP-MS. This was done using a Varian 820MS Quadropole (all samples run with internal standard correction i.e. Indium-115 and Rhodium-103 for matrix

and drift correction) after microwave-assisted acid digestion (Concentrated HNO₃ and HCl at 3:1 ratio; Merck Suprapur) using a Milestone ETHOS1.

2.2 Dissolved cadmium, manganese and zinc exposures (Objective A)

A mixture of active (radioactive) radioisotopes ¹⁰⁹Cd, ⁵⁴Mn and ⁶⁵Zn and their respective stable metal (i.e. non-radioactive carrier metal) isotopes were added to filtered seawater to prepare exposure concentrations of 0.7, 20 and 15 µg L⁻¹ (at activities of 0.032, 0.037 and 0.034 Bq L⁻¹, respectively). These concentrations represent ANZG water quality guideline 99% species protection levels for Cd and Mn (including coral) and 95% species protection level for Zn in marine systems (ANZG, 2018) and were chosen as they represent slightly - moderately disturbed systems. Five clams, seven prawns and five fish were exposed in separate containers for 7d, followed by rinsing and 14d depuration in clean seawater. Organisms were radioanalyzed three times on the first day and then daily thereafter using a LaBr probe connected to an InSpector1000 (Canberra) spectrometer with appropriate geometry correction (Supporting Information S7 and S8). The InSpector1000 was connected to a PC and spectrum were analyzed using the Genie 2000 software (Canberra). All radioanalytical measurements were corrected for background and physical isotope decay. This radioanalysis method was used for all exposures in this study. Since fish showed no uptake of metals from solution, the metal concentration in the fish exposure containers was increased to 36, 850 and 43 µg L⁻¹ of Cd, Mn and Zn respectively after 4 d of exposure, by adding more stable metal to the solution. More details are given in Supporting Information (S2).

2.3 Sediment radiolabelling and pulse-chase analysis of clams (Objective B)

Fine sediment containing low metal concentrations collected from Bonnet Bay (South Sydney) using clean plastic falcon tubes was sieved to $<63\ \mu\text{m}$ using field-collected water and stored in a refrigerator at $<4^{\circ}\text{C}$ until use. The sediment was radiolabelled with an aliquot of ^{109}Cd , ^{54}Mn and ^{65}Zn , with stable stocks of Mn and Zn added to make up to sediment metal concentrations of 2.5, 160 and 160 mg/kg of Cd, Mn and Zn respectively. These sediment metal concentrations are commonly found in Moreton Bay (Brisbane) and Port Jackson (Sydney), two large urbanised estuaries on the East Australian coast that will be referred to throughout this study (Brady et al., 2015; Twining et al., 2008). Sediment radiolabelling was conducted as per Cresswell et al. (2014) (Supporting Information S3). Sediment was kept in suspension in 1L of seawater in each container using magnetic stirrer bars at a concentration of approx. $100\ \text{mg L}^{-1}$. Five clams were left in exposure containers for 3h before being placed in clean containers with new water and radioanalyzed at 0, 2, 4 and 6 h in the first day, then once daily for 7d to assess uptake and assimilation efficiency (AE) from suspended sediments. Since uptake from the dissolved phase was very low for prawns and fish, only two prawns and two fish were used in a sediment exposure trial, using the same method and concentrations as the clam sediment exposures (Supporting Information S3). Since there was no metal uptake in prawns or fish after 3 h, they were left in the sediment exposure containers for 24 h. From this trial, fish showed no uptake after 24 h and prawns showed very little uptake with high detector uncertainty ($>10\%$ propagated counting error for 10 min count). Therefore, no further prawns or fish were used in the pulse-chase sediment exposure.

2.4 Microalgae radiolabelling and pulse-chase analysis of clams (Objective C)

Sand clams bury under the sediment surface and filter feed through active siphoning of surrounding benthic waters. They may ingest both benthic and planktonic microalgae. To compare dietary uptake and AE of ^{109}Cd , ^{54}Mn and ^{65}Zn from various prey, two common

microalgal species: *Tetraselmis* sp. (planktonic green alga) and *Entomoneis* cf. *punctalata* (benthic diatom), were cultured in bulk at adjacent CSIRO Land and Water laboratory under the same laboratory conditions in which the organisms in this experiment were kept. Algae were radiolabelled following a procedure adapted from Lee et al. (2015) (Supporting Information S4). Algae labelling metal concentrations were chosen with the aim of obtaining high enough radioactivity levels in the clams while ensuring that most of the algal cells remained alive and growing during the feeding exposure.

A pulse-chase experiment of five clams fed 8 mL of either *Tetraselmis* or *Entomoneis* for 3 h (10 clams in total) was used to compare uptake and AE from the two different food sources. After the 3 h pulse-feed, the clams were rinsed thoroughly with clean seawater to remove particles attached to their exterior (Supporting Information S7) and radioanalyzed at 0, 2, 4, 6 h while depurating in clean seawater to detect loss of unassimilated tracers as faeces and daily for 7 d thereafter to measure the efflux of assimilated tracers from tissues. Filtered seawater in each container was replaced daily and supplemented with non-active cultured microalgae (*Tetraselmis* sp. fed to clams in the *Tetraselmis* treatment and *Entomoneis* cf. *punctalata* fed to clams in the *Entomoneis* treatment) using 0.5 mL at a cell density of 1.21×10^7 cells mL⁻¹ every 2 d.

2.5 Artificial food pellet labelling and pulse-chase analysis of prawns (Objective C)

While this experiment aimed to study prawn uptake and assimilation of metals from sand clams, the large size of the clams made it difficult to radiolabel a portion of food (clam) with high enough activity to be detected in the prawns. Therefore, artificial food pellets (weighing 0.04 ± 0.01 g) were radiolabelled with 16.7, 9.8 and 9.5 KBq of ¹⁰⁹Cd, ⁵⁴Mn and ⁶⁵Zn, respectively (Supporting Information S5). Pellet radioactivity levels were chosen with the

aim of achieving high enough activity levels for detection in both prawns consuming pellets and fish that consumed these prawns. One pellet was given to each of five prawns that were allowed to feed for up to 3 h. They were then transferred to clean containers with filtered clean seawater and radioanalyzed three times in the first 24 h and daily thereafter for 8 d (Supporting Information S5).

2.6 Dietary exposure pulse-chase analysis of fish (Objective C)

To assess trophic transfer from both prawns and artificial pellets, 10 fish were randomly selected and fed radiolabelled pellets or radiolabelled prawns (Supporting Information S5) for 2 h before being rinsed and placed into new containers with clean seawater (Supporting Information S6). Only two of the fish fed the artificial agar-Spectrum mix pellets ingested the pellet, while the remaining three fish spat them out. The fish were radioanalyzed three times in the first 24 h and daily thereafter for 6 d. During depuration, the five fish fed active prawns were fed Ocean Nutrition Marine Mix (a frozen zooplankton/shrimp mix), while the five fish fed the artificial pellets were fed Spectrum 1 mm pellets, to keep the food sources consistent.

2.7 Metal organ distribution in clams

In order to determine which organs assimilated the metals, active clams were dissected. Clams from 1) dissolved exposure (section 2.2), 2) *Tetraselmis* pulse-chase dietary experiment (section 2.4), and 3) clams that were fed *Tetraselmis* for 2 d with no depuration or chase periods were dissected into main organ clusters: shell; muscle (including foot); gills; digestive sac; heart/rectum/kidney/other. Because the shells were large and could not be easily acid digested, a subset of the clams were radioanalyzed whole by wet organ to determine if adsorption to the shell occurred for any of the metals. After dissection and wet organ radioanalysis, organs were oven dried at 60 °C for 3 d and weighed before being

transferred to 10 mL vials and acid digested following a procedure adapted from King et al. (2004). Tissues were digested in reagent grade nitric acid (0.5 mL 0.1 g⁻¹ dw tissue) for 24 h. Vials were subsequently heated in a domestic microwave oven for 20 min (900 W; 10% power). After cooling to room temperature, hydrogen peroxide (30 %, 0.5 mL/0.1g dw tissue) was added to each vial and left to digest for 24 h. Vials were again microwaved (as above) then cooled to room temperature. Samples were transferred to 6 mL glass vials via pipette and each digestion vial was rinsed with RO water, with each rinsing transferred to the receiving vial to a final volume of 5 mL. Vials were radioanalyzed via gamma spectrometer against in-house prepared standards of the same geometry with known activity.

2.8 Bio-kinetic modelling in clams

The relative importance of the aqueous phase (R_w , %), diet (R_f , %) and suspended sediment (R_s , %) as sources of cadmium, manganese and zinc to the clams was estimated by comparing the sum of accumulation from the aqueous phase ($C_{ss,w}$) and dietary from food ($C_{ss,f}$) or suspended sediment ($C_{ss,s}$) as described by Reinfelder et al. (1998):

$$R_w = C_{ss,w}/C_{ss} = (k_u \times C_w)/(k_{ew} \times C_{ss}) \times 100\%$$

$$R_f = C_{ss,f}/C_{ss} = (AE_f \times IR \times C_f)/(k_{ef} \times C_{ss}) \times 100\%$$

$$R_s = C_{ss,s}/C_{ss} = (AE_s \times IR \times C_s)/(k_{es} \times C_{ss}) \times 100\%$$

And

$$C_{ss} = ((k_u \times C_w)/k_{ew}) + ((AE_f \times IR \times C_f)/k_{ef}) + ((AE_s \times IR \times C_s)/k_{es})$$

Where k_u is the uptake rate constant from solution (mL g⁻¹ d⁻¹) and C_w , C_f and C_s are the metal concentrations in seawater (µg L⁻¹), algae and sediment (µg g⁻¹), respectively. AE is the metal assimilation efficiency from the food type (%), IR is the ingestion rate (g g⁻¹ d⁻¹) and k_e is the efflux rate constant (d⁻¹). Uptake rates were corrected for metal absorption to clam shells.

The biokinetic model was used to predict the relative contributions of different sources of metal accumulation in sand clams in estuaries along the east coast of Australia, within the habitat range of the clams. Since estuaries vary in degree of contamination along this coast, a range of Cd, Mn and Zn concentrations were used to see if the main source of metal accumulation varies with differing contamination levels. Low, moderate and high seawater (Cd = 0.01, 0.1, 0.5; Mn = 2, 50, 100; Zn = 2, 10, 25 $\mu\text{g g}^{-1}$) metal concentrations were used based on data from two urbanised east coast estuaries: Port Jackson, Sydney (Hatje et al., 2003) and Moreton Bay, Brisbane (Richardson and Hiles, 2015). Acid-extractable sediment concentrations (Cd = 0.1, 1, 3; Mn = 50, 100, 500; Zn = 10, 100, 500 $\mu\text{g g}^{-1}$) from these estuaries were used (Brady et al., 2015; Twining et al., 2008). The acid-extractable metal fraction was used because it better represents bioavailable metal than the total recoverable metal concentrations (Lee et al., 2015).

In terms of microalgal metal concentrations, there is a lack of relevant information in the literature on Cd, Mn or Zn concentrations in phytoplankton. Therefore a range (low, moderate and high) of values were used from global literature (Cd = 0.1, 1, 7; Mn = 20, 100, 1000; Zn = 50, 500, 5000 $\mu\text{g g}^{-1}$, Bobrov et al., 2005; Knauer and Martin, 1973) to estimate different concentrations that may be experienced through clam diet. In addition, since clam ingestion rate could not be estimated from this experiment, a range of ingestion rates (0.5, 2, 10 $\text{mg h}^{-1} \text{g}^{-1}$) were obtained from other studies that also used clams from the Veneridae family (Beiras et al., 1993; Han et al., 2008; Zhuang and Wang, 2004). These rates are lower than some studies reported for other bivalves (Hawkins et al., 1998; Navarro et al., 2000).

2.9 Data analysis

For dissolved exposures, uptake rates were calculated from the linear regression of the uptake curve over the exposure period (0 – 7d). Efflux rates from all exposures were calculated from the linear slope of the percentage of metal remaining in the organism over the slow-loss phase of depuration. The dietary slow-loss phase for clams was defined as days 3-7 for clams, days 2-8 for prawns and days 2-6 of depuration for fish. Assimilation efficiencies were calculated from pulse-chase experiments from the Y-intercept of the non-linear (exponential) regression of the ‘chase’ data from the 2-8d slow-loss phase. Statistical analyses were performed using the software R. Independent samples t -tests were used to test for statistical differences in prawn zinc concentrations between moulting and non-moulting prawns in the seawater exposure. One way ANOVAs with Tukey HSD post hocs were used to test for differences in assimilation efficiencies between sediment and diet treatments in the clam pulse-chase experiments.

3. Results and Discussion

3.1 Sand clams

Four different exposure treatments were carried out on sand clams: 1) metals dissolved in seawater (Objective A), 2) radiolabelled sediment kept in suspension (Objective B), 3) radiolabelled green phytoplankton (Objective C), and 4) radiolabelled benthic diatoms (Objective C). Using the data obtained from these experiments, a biokinetic model was produced to identify accumulation sources in estuaries of varying contamination. Organ metal distribution was also examined by dissecting, digesting and radioanalyzing different soft tissues from dissolved and green phytoplankton exposures.

3.1.1 Dissolved exposures (Objective A)

The sand clams accumulated Cd, Mn and Zn relative to dissolved concentrations (Fig. 1), with uptake rate constants ($k_u \text{ L}^{-1} \text{ g}^{-1} \text{ d}^{-1}$) of 0.0046, 0.0098 and 0.0079 for Cd, Mn and Zn respectively, and efflux rates ($k_e \text{ d}^{-1}$) of 1.14, 0.64 and 1.68 for Cd, Mn and Zn respectively. There was no uptake saturation/equilibrium reached from the concentrations of metals used, and no significant excretion of metal tracers during the exposure period. Efflux rates in our study (calculated from 1-13 d depuration) were more than 40 times greater than reported for several other bivalve species during seawater depuration of these metals (e.g. Kalman et al., 2014; Metian et al., 2009; Pan and Wang, 2008). Zn concentrations in clams were significantly lower on the last (13th) day of depuration compared to the second day ($df = 1$, $F = 38$, $p < 0.001$), however clam Cd and Mn concentrations were not (Cd: $df = 1$, $F = 2.5$, $p = 0.16$; Mn: $df = 1$, $F = 0.18$, $p = 0.69$). Greater efflux means that species can reduce accumulated metal following contamination events, such as floods and chemical spills that bring temporary elevated seawater metal concentrations. Cd, Mn and Zn concentration factors (concentration in animal vs. concentration in water) were 33, 72 and 57, respectively, after 7 d of exposure, demonstrating that sand clams effectively bioconcentrate these metals from surrounding seawater after only 7 d exposure to contaminated water. Metal uptake from seawater by bivalves, such as clams, may be an important link between dissolved and trophically available metal for predators that are more resistant to dissolved metal accumulation (Wang, 2002).

Tissue Mn concentrations (Fig. 1) are likely to be overestimated due to high Mn adsorption to clam shells. Post-depuration dissections and analysis of tissue versus shell metals showed that shells had a higher fraction of Mn tracer (24%) than Cd (7%) and Zn (4%). Similarly, Belivermiş et al. (2016) found that ^{54}Mn was mainly associated with the shells of oysters (>94%), whereas ^{109}Cd was equally distributed between the two compartments, and ^{65}Zn was mainly found in soft tissues. Shells containing more calcite than aragonite are likely to adsorb

more Mn (Soldati et al., 2016), and the strong affinity of Mn can inhibit calcite dissolution (Vinson et al., 2007). As well as adsorption, Mn may be biologically distributed to the shell: Mn^{2+} may be substituted into the shell because it has the same valence as Ca^{2+} . In the case of cadmium, the ions can cause direct disturbance of the bivalve shell calcification mechanism, mainly in the region of highest growth rate on the shell border (Faubel et al., 2008), however Zn^{2+} exposure has been shown to have no observable effect on shell structure (Lopes-Lima et al., 2012). We were unable to distinguish between metal adsorption (i.e. passive sorption from the surrounding solution) and absorption (i.e. active biodistribution) to clam shells in this study.

3.1.2 Suspended sediment (Objective B) and dietary exposures (Objective C)

Cadmium, Mn and Zn was retained by sand clams from particulate sources, including two algal species and suspended sediment (Fig. 2). Cd assimilation (i.e. AEs) differed between algal species ($df = 2$, $F = 9.62$, $p = 0.003$), however when comparing AEs of suspended sediment to algal species, there were no significant differences (Table 2). Similar to Cd, Zn AEs from *Entomoneis sp.* were significantly higher than those from *Tetraselmis sp.* ($df = 2$, $F = 7.24$, $p = 0.010$), while neither differed from suspended sediment AEs. Mn AEs between diets were not significantly different ($df = 2$, $F = 1.74$, $p = 0.216$). Rainbow and Wang (2001) suggest that AE may be correlated with the percentage of metal in the cytoplasm of the phytoplankton cell, and these distributions of metal within the cell may be partially responsible for differences seen in filter feeder AE of different phytoplankton diets.

Clam efflux rates from the three particulate sources were similar to that from the dissolved exposure (Table. 2). This indicates that retention of Cd, Mn and Zn by clams is similar between dissolved and particulate exposures, and similar physiological processes govern the

efflux of each metal. Our results from four different exposures show that sand clams accumulate metals from a variety of sources.

3.1.3 Biokinetic modelling of metal accumulation by sand clams

Biokinetic modelling showed that cadmium accumulation in the clam, *K. scalarina* is likely to be mostly from the diet, irrespective of concentration (Fig. 3). Zinc accumulation also appears to be predominately from diet, with a slight increase in this proportion as contamination levels increase or decrease relative to moderate Zn contamination concentrations. Similar results have been modelled for Zn accumulation in other bivalve species (Ke and Wang, 2001; Lee et al., 2015). Conversely, manganese accumulation at moderate contamination levels is most likely to be from seawater. In an estuary with high contamination (such as Port Jackson, Sydney), Mn accumulation is predicted to be spread between the sources (Fig. 3). The model predicts that in a clean (low contamination) estuary, suspended sediment will be the main source of Mn accumulation. However, it is likely that this would only be true in estuaries with high turbidity or if organisms were deposit feeding. In scenarios where suspended sediment appears to be a dominant source of metal contamination, it is important to note that this is dependent on suspended sediment concentrations, which are influenced by weather events, tidal resuspension and human interference such as dredging.

The chlorophyte *Tetraselmis* was used in the original model (Fig. 3), however when substituted with the diatom, *Entomoneis*, as the food source, Cd contribution from diet decreased from >90% to 40 – 60% diet contribution for the range of environmental concentrations used, suggesting that food quality may affect Cd accumulation. Modelled Zn accumulation from diet only decreased by 14 - 18% and Mn percentage from diet did not change when *Entomoneis* was used as the food source. Metian et al. (2009a) also found little

change in Mn and Zn contribution from diet with changing food sources, and Ke and Wang (2001) found larger differences in Cd source contributions with changing AE than Zn.

Determination of model components such as AE and efflux rate using laboratory experiments allows for biokinetic modelling to be applied to particular systems to identify primary sources of accumulation, and models can be validated using metal concentrations from wild organisms (Griscom et al., 2002; Pan and Wang, 2008a).

3.1.4 Clam organ metal distribution

The organ distribution of Cd, Mn and Zn in the clam, *K. scalarina* was determined for the dissolved and dietary (fed on green algae) exposures. However, most of the stomachs of clams fed on radiolabelled algae for 2 d were used to attempt to model trophic transfer to prawns so percentages could only be calculated for one clam in this treatment, and were therefore not included in statistical tests. Organ metal distribution in clams was not uniform at the end of the depuration period in the dissolved exposure for Cd (df = 3, F = 58.2, $p < 0.0001$, Tukey's post-hoc: a. other, b. gills, bc. stomach, c. muscle), Mn (df = 3, F = 47.9, $p < 0.0001$, Tukey's post-hoc: a. other, gills, b. stomach, c. muscle) and Zn (df = 3, F = 22.7, $p < 0.0001$, Tukey's post-hoc: a. other, ab. gills, b. stomach, c. muscle). Distribution of Mn and Zn after uptake from seawater was similar, where both gills and 'other' organs (including heart, rectum and kidney) contained 30-40% of metal in each organ, whereas Cd contained in 'other' organs was more than twice the amount in the gills (Fig. 4). While gills are a primary membrane across which dissolved metal uptake occurs (Kalman et al., 2014), clam sensitivity to dissolved metals may be heightened through additional uptake from filtering water through the digestive system for food, where metal exchange through cell walls of the organs along this passage is also likely to occur. Muscle had the lowest range of metal concentrations for

all metals and exposures, which is a common observation in wild aquatic organisms (Canli et al., 2001; Roméo et al., 1999).

Unlike the dissolved treatment, Cd, Mn and Zn showed similar organ distribution patterns in clams after 8 d of pulse-chase dietary exposure (Cd: $df = 3$, $F = 4.6$, $p = 0.02$, Mn: $df = 3$, $F = 7.3$, $p = 0.005$, and Zn: $df = 3$, $F = 4.7$, $p = 0.02$). Tukey's post-hoc tests showed higher metal proportions contained in gills and 'other' organs than muscle, while metals contained in the stomach were similar to the rest of the soft tissues. These results suggest that Cd may be distributed differently when accumulated from seawater, which is supported by significantly higher Cd found in 'other' organs in dissolved compared to diet exposures ($df = 6$, $t = -2.6$, $p = 0.04$)(Fig. 4). Differential organ and subcellular distribution can cause dietary metal to exert toxicity via mechanisms different from metal in dissolved form (Frayse et al., 2006).

The low percentage distributions of radiolabelled algae signal in gills and muscle of the clam that was fed for 2 d before dissection shows that dietary Cd, Mn and Zn is moved to these organs after depuration and assimilation. Cadmium in wild *K.scalarina* from Western Australia was found to be highest in gills and lowest in muscle tissue (Hindarti et al., 2000). Our results suggest that these high concentrations in gills of wild clams could be from both dissolved and particulate sources, and that food and sediment should be considered as an important source of this accumulation. Metian et al. (2009b) found highest metal concentrations (Co, Zn, Mn) in the kidneys of scallops across dissolved, algal diet and sediment exposures. Since our 'other' organ group contained the kidney, it is likely that the kidney contained most of the metals found in this organ group.

3.2 School prawns and sand whiting

School prawns and sand whiting were exposed to radioactive Cd, Mn and Zn in seawater to study uptake from dissolved sources (Objective A). The prawns and fish were exposed to radiolabelled suspended sediment (Objective B), and were offered radiolabelled food (prawns were fed pellets and fish were fed prawns or pellets) to study uptake and accumulation from dietary sources (Objective C).

3.2.1 Dissolved exposures (Objective A)

School prawns showed very low uptake of dissolved cadmium and manganese over the 7 d exposure period, with consistently high detector uncertainty (close to or below radioactivity limits of detection). Uptake of zinc was measured but at low activities, with consistently detected radioactivity from 3 days of exposure. Although uptake of these metals from seawater was negligible (Cd and Mn) or very low (Zn), there was a difference in uptake, particularly of Mn and Zn, between prawns that moulted, and prawns that did not moult during the exposure period (Fig. 5). An increase in uptake of these metals during moulting is likely due to the substitution of calcium ions in the exoskeleton with divalent cations of similar chemistry. Notably, two of the three prawns that moulted during the exposure moulted again during the depuration phase at 15 d and 18 d, and a decrease in manganese and zinc concentrations in these prawns was measured (Fig. 5). These prawns were able to form new exoskeletons in clean water and rid some of the metals taken up during the exposure. Zinc concentrations in moulted prawns were significantly higher than Zn concentrations in prawns that did not moult at 7 d ($df = 5$, $t = 11.09$, $p < 0.0001$) and at the end of the depuration period ($df = 5$, $t = 3.12$, $p = 0.026$). Mean efflux rates ($k_{ef} \text{ d}^{-1}$) of Zn were 0.04 and 0.08 for moulted and non-moulted prawns, respectively. Statistical tests were not performed for Cd and Mn due to low uptake and activity resulting in high detector uncertainty. We

suggest that using higher water Cd and Mn concentrations would result in higher radioactivity levels, clarifying trends.

While moulting can be a crustacean response to stress (Rao and Anjaneyulu, 2008), our results show that it may also be an effective means for these prawns to lose some metal from the exoskeleton when moving from contaminated to cleaner waters. This metal-rich exuvium can then trophically transfer if consumed by the prawn or other organisms. White and Rainbow (1984) also observed rapid Zn flux into exoskeletons of rockpool shrimp that moulted during exposure to aqueous Zn, with similar loss of the zinc tracer in shrimp that moulted again during depuration. While uptake may be affected by the moult cycle, it is unlikely that dissolved Cd, Mn and Zn is a major source of metal uptake in school prawns.

No detectable uptake of dissolved Cd, Mn or Zn by sand whiting was observed after 6 d of exposure, with consistently low counts and high detector uncertainty, even after seawater metal concentrations were increased to 36, 850 and 43 $\mu\text{g L}^{-1}$ of Cd, Mn and Zn respectively. Creighton and Twining (2010) found low Cd uptake by estuarine glassfish at low Cd concentrations (5 $\mu\text{g L}^{-1}$), however uptake was observed at 10 and 50 $\mu\text{g L}^{-1}$ Cd, and uptake of Zn at 5, 10 and 50 $\mu\text{g L}^{-1}$ Zn water concentrations. Although uptake from the dissolved phase is not considered the primary source of metal accumulation in marine fish (Jeffree et al., 2006; Mathews et al., 2008), previous studies have always observed metal uptake, usually at a rate relative to water metal concentrations (Xu and Wang, 2002; Zhang et al., 2011). Sand whiting also showed no detectable uptake of Cd, Mn or Zn from suspended sediment exposure, therefore our results suggest that sand whiting may be particularly tolerant to metal uptake across permeable membranes, such as gills.

3.2.2 *Suspended sediment (Objective B) and dietary exposures (Objective C)*

Suspended sediment exposure (using two prawns and two fish) had similar trends to exposure to dissolved metal with no clear indication of uptake (high detector uncertainty). Therefore, this exposure was not carried out with more fish and prawns. This result validates our hypothesis that suspended sediments are not directly ingested by prawns or fish. Any measured radiotracer uptake is likely to be from attachment to gills and other body surfaces, but our results suggest uptake via these pathways by these two species is negligible. These trial results reveal that pulse uptake of metals by school prawns and sand whiting from suspended sediments is unlikely, based on the particulate metal concentrations we used. This suggests that these species are resilient to floods, storm events, dredging and tidal sediment re-suspension.

Exposure to radiolabelled dietary sources of metals using school prawns and sand whiting resulted in clear differences in metal retention between the species (Fig. 6-7, Table. 2), highlighting a difference in physiological responses. Previous studies also report AEs for Cd and Mn higher than 40% for prawns (Nunez-Nogueira et al., 2006; Seebaugh and Wallace, 2009), and less than 40% for fish (Pouil et al., 2017; Wang et al., 2012). In our experiment, only two out of seven fish ate the pellets that were offered to them, providing limited data. However, it shows that the retention of Cd, Mn and Zn by sand whiting from different diets is variable (Fig. 7).

Cadmium AE of school prawns fed pellets fell within reported values for other prawn species fed a range of diets (Metian et al., 2010; Nunez-Nogueira et al., 2006; Rainbow et al., 2006). High Cd AE (67%) and relatively low efflux rate ($0.65 \text{ k}_{\text{ef}} (\text{d}^{-1})$) compared to Mn ($3.28 \text{ k}_{\text{ef}} (\text{d}^{-1})$) and Zn ($2.66 \text{ k}_{\text{ef}} (\text{d}^{-1})$) suggests prawns may be particularly susceptible to accumulation in

Cd enriched areas. However, Cd accumulation from diet is also likely to be influenced by prey availability, since AE has been shown to vary between food sources (Nunez-Nogueira et al., 2006). While Mn and Zn are essential elements for prawn growth, such high AEs may cause rapid accumulation to a threshold level, above which negative effects such as decreased growth are likely to be experienced (Cai et al., 2017; Shiao and Jiang, 2006). At moderate concentrations, the presence of Zn in prawn diet may also help to mitigate Cd toxicity through competition for binding sites on cell membrane surfaces (Wright and Welbourn, 1994; Yu et al., 2015).

3.3 Organism-level responses to metal exposure

Requiring only minimal organism numbers, radiotracer experiments are an effective way to study metal accumulation and have fast tracked the filling of knowledge gaps about biological interactions with trace metals in the environment, though they are not without limitations (Wang and Fisher, 1999). Because of the size limit of the detector used, juvenile fish and prawns were collected. However, difficulties associated with collecting desired size ranges of wild organisms meant that we were unable to collect clams that were small enough to hold enough activity per quantity of tissue edible to the prawns to model trophic transfer, limiting our multi-trophic level experiment design. Because of this, we were forced to feed prawns only an artificial diet, thereby lacking a natural diet comparison to compare assimilation efficiencies from different food sources.

Our results from multiple exposures showed that sand clams accumulated metals from seawater, food and sediment. Most of the metals were contained in the gills and 'other' organs, including the heart and kidneys. Biokinetic modelling for clams showed that in East Australian estuaries of moderate contamination, accumulation of Cd and Zn is likely to be

from diet, whereas Mn is likely to be sourced mostly from seawater. Uptake from seawater and suspended sediment was negligible for school prawns and sand whiting, but was significant from diet, with higher assimilation efficiencies for the prawns than the fish.

Taxa specific accumulation of metals are common findings in the scientific literature (e.g. Wang and Ke, 2002; Weeks and Rainbow, 1994). Previous studies have shown uptake of metals by saltwater prawns and fish from seawater (e.g. Jeffree et al., 2006; Nunez-Nogueira and Rainbow, 2005; Xu and Wang, 2002). However, the fish in our study showed no measured uptake while uptake by school prawns was very low, suggesting that the general permeability of gill membranes and other external surfaces of these species to metal ions is quite low. Although it is unlikely that the dissolved Cd concentration that prawns were exposed to in our study ($0.7 \mu\text{g L}^{-1}$) was too low for significant uptake, future work may benefit from exposing the prawns to a range of dissolved concentrations. The ANZG (2018) 95% species protection guideline for Cd is $5.5 \mu\text{g L}^{-1}$, however toxicity testing largely focusses on dissolved metal toxicity. In light of our results, we suggest dietary toxicity should also be taken into consideration, particularly for saltwater prawn species since prawn AEs from diet were high.

Taxa specific accumulation responses raise concerns for the effectiveness of environmental monitoring to assess estuary health and have implications for assessing human health effects. Biokinetic models can be used to assess how much metal is actually accumulated from the environment and predict which fisheries species may pose the highest risks to human health. Biokinetic models can also be used to predict how sensitive species such as bivalves may respond to disturbance events, for example floods and dredging, to better advise where and when food safety testing should be applied. Organ distribution of metals in seafood is also

important for assessing human health risks. While all the soft tissue is usually consumed from bivalves, muscle tissue is often the only part of fish and prawns that is consumed. Muscle generally contains the lowest concentrations of metals per gram of tissue (Canli et al., 2001), however it is commonly consumed in larger quantities, therefore there is still a risk of accumulation to toxic concentrations.

Bivalves are often heavily impacted by floods, mostly by smothering and physiological stress caused by high turbidity loads, which can force them to lower filtering rates and consequently reduce feeding and breathing efficiency (Norkko et al., 2006). Accumulation of metals from contaminated water and suspended sediment carried in floodwaters presents additional stress, suggesting that bivalves living in estuaries in metal-enriched catchments may be more vulnerable to flood events. Since bivalves are known to improve water quality, a reduction in bivalve populations may further degrade these estuaries (Officer et al., 1982). Our results suggest that the prawns and fish studied here would be unlikely to display any significant uptake of Cd, Mn and Zn from turbid floodwaters, which may be an advantage when coping with rapid water quality changes that occur during flooding.

4. Conclusion

The prawn and fish species tested in our study appear to be effective at restricting certain metal uptake from seawater, therefore sand clams and other filter feeding or deposit-feeding organisms (particularly small bivalves that are a popular food source for crustaceans) are likely to be an important link between water column contaminants (especially Mn) and accumulation by higher order predators. The presence of sand clams in waterways is also likely to be an indicator of estuary health, due to their higher sensitivity to metal accumulation from a variety of sources. Biokinetic modelling identified food and suspended

sediment as the primary sources for metal accumulation in sand clams. These results further support the use of clams as a bioindicator of long-term metal contamination in estuaries, as food and suspended sediments will persist in an estuary longer than dissolved metals.

We found school prawns and sand whiting to have significant Cd, Mn and Zn uptake from diet, with high metal assimilation by prawns for all three metals, particularly Cd. School prawns require estuarine and lake habitats as an essential part of their life cycle and their stocks are considered “fully fished” in New South Wales. Food quality in estuaries is likely to have a significant effect on the health of school prawn populations, and should be considered for management of stocks. Assimilation of metals from diet by sand whiting was low, particularly for Cd and Zn, highlighting their ability to persist throughout estuaries along the east coast of Australia, including those that are considered highly contaminated. Taxa specific responses need to be considered when assessing environmental health and health risks to humans via consumption.

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Table 1. Mean $\pm$ standard error whole organism background metal concentrations ($n = 3$; fresh weight) for each of clams, prawns and fish.

	Cd (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
Clams	0.30 ± 0.068	4.7 ± 2.2	36 ± 3.5
Prawns	1.0 ± 0.46	4.6 ± 0.89	70 ± 10
Fish	0.12 ± 0.018	7.8 ± 0.80	157 ± 36

Table 2. Mean assimilation efficiencies (AE) and mean efflux rates of test species fed various diets (columns).

		<i>Entomoneis</i> <i>sp. diatom</i>		<i>Tetraselmis sp.</i> chlorophyte		Suspended fine sediment		Pellet feed		Prawn tissue	
Test		AE		AE		AE		AE		AE	
Species	Metal	%	$k_{ef} (d^{-1})$	%	$k_{ef} (d^{-1})$	%	$k_{ef} (d^{-1})$	%	$k_{ef} (d^{-1})$	%	$k_{ef} (d^{-1})$
Sand Clams	Cd	51	2.3	22	0.080	39	1.7				
	Mn	43	1.4	32	1.3	43	0.70				
	Zn	63	3.4	33	0.89	53	1.8				
School Prawns	Cd							67	0.65		
	Mn							59	3.3		
	Zn							64	2.7		
Sand Whiting	Cd							9.5	0.88	11	0.38
	Mn							6.7	0.46	26	1.2
	Zn							3.8	0.09	14	1.1

Highlights

- Our study identified heavy metal uptake pathways by fishery species
- We showed that clams were sensitive to metal accumulation from multiple sources
- Biokinetic modelling of accumulation in clams showed source differences between metals
- Prawns and fish effectively excluded metal in dissolved and suspended sediment form

ACCEPTED MANUSCRIPT

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7