Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Comparing trace metal bioaccumulation characteristics of three freshwater decapods of the genus *Macrobrachium*

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ARTICLE INFO

Article history: Received 7 February 2014 Received in revised form 9 April 2014 Accepted 9 April 2014 Available online 19 April 2014

Keywords: Uptake rates Prawn Tailings Inter-species differences

ABSTRACT

Potential sources and kinetics of metal bioaccumulation by the three Macrobrachium prawn species M. australiense, M. rosenbergii and M. latidactylus were assessed in laboratory experiments. The prawns were exposed to two scenarios: cadmium in water only; and exposure to metal-rich mine tailings in the same water. The cadmium accumulation from the dissolved exposure during 7 days, followed by depuration in cadmium-free water for 7 days, was compared with predictions from a biokinetic model that had previously been developed for M. australiense. M. australiense and M. latidactylus accumulated significant tissue cadmium during the exposure phase, albeit with different uptake rates. All three species retained >95% of the bioaccumulated cadmium during the depuration phase, indicating very slow efflux rates. Following exposure to tailings, there were significant (p < 0.05) differences in tissue arsenic, cadmium, lead and zinc concentrations among species. Cadmium and zinc concentrations were increased relative to controls for all three species but were not different between treatments (direct/indirect contact with tailings), suggesting these metals were primarily accumulated via the dissolved phase. All species bioaccumulated significantly greater arsenic and lead when in direct contact with mine tailings, demonstrating the importance of an ingestion pathway for these metals. Copper was not bioaccumulated above control concentrations for any species. The differences between the metal accumulation of the three prawns indicated that a biokinetic model of cadmium bioaccumulation for M. australiense could potentially be used to describe the metal bioaccumulation of the other two prawn species, albeit with an over-prediction of 3–9 times. Despite these being the same genus of decapod crustacean, the study highlights the issues with using surrogate species, even under controlled laboratory conditions. It is recommended that future studies using surrogate species quantify the metal bioaccumulation characteristics of each species in order to account for any differences between species.

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1. Introduction

It is widely appreciated that trace element uptake and internal distribution can be distinctive within a given taxon (Hare et al., 1991) and these differences can transcend through the genus, family and order levels (Hare, 1992). It is therefore important to undertake trace element bioaccumulation studies with different species of the same genus to identify inter-species differences in contaminant bioaccumulation patterns, especially when using a surrogate laboratory species to model aquatic field conditions. A

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http://dx.doi.org/10.1016/j.aquatox.2014.04.015

review by Pourang et al. (2004) described the patterns of metals found in the muscle tissue of several shrimp species of the Penaeus genus from a number of studies. Zinc was the trace element with the highest concentration in all species. However, the second most abundant trace element in the abdomen was either copper, iron or lead, but this differed with species. These differences may have simply been a function of the different environments the shrimps were collected from (e.g. chemistry, hydrology, food abundance) or they may have been evidence of the differences in metal bioaccumulation characteristics between species. Croteau et al. (2001) observed differences in cadmium bioaccumulation in four species of the phantom midge Chaoborus caught from the field. Based on laboratory studies, these differences between species were attributed to the differences in assimilation efficiency of cadmium from dietary items and differences in feeding habits (e.g. ecological differences). Martin et al. (2008) compared the metal concentrations in larvae







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of two *Chironomus* species from the same lake at the same water depth and time. They found that cadmium differed more than 8-fold between species, while zinc differed 2-fold and copper concentrations were comparable. The study postulated that the different diets (different sediment with differing metal availabilities) of the two species was likely to be the cause of the differences in metal concentrations.

Routine biomonitoring of the freshwater decapod crustaceans *M.* rosenbergii and *M.* latidactylus in the highly turbid receiving rivers downstream of the Porgera gold mine, Papua New Guinea, is undertaken by the Porgera Joint Venture (PJV) to determine trace metal bioaccumulation within the system. These species are used for biomonitoring in the Australasian region and are an important aquaculture species in Asia and North and South America; according to FAO (2011), approximately 220,000 tons of M. rosenbergii were produced globally in 2010, at a total value of USD\$ 1,207,000. For several years, significant increases have been detected in bioaccumulated arsenic, cadmium, copper, lead and zinc in prawns from the Strickland River downstream of the Porgera gold mine relative to those from reference tributaries (Barrick, 2013). However, trace metal concentrations in water and particulate samples collected at the same locations along the river system do not show the same differences between sites receiving mine-derived materials and reference tributaries (Barrick, 2013). Cresswell et al. (2014a) developed a biokinetic model of cadmium bioaccumulation from water, fine sediment, carrion and algae by Macrobrachium australiense with the intention that it be applied as a surrogate species to prawns present in the Strickland River. The use of M. australiense as a surrogate for the Macrobrachium species in the Strickland River was due to the inability to access sufficient numbers of the latter species. If the rates of uptake and efflux of metals from various sources were comparable between species, then the biokinetic model for M. australiense may be applicable for the Macrobrachium species in the Strickland River.

Two experiments including dissolved cadmium and mine tailings exposures were conducted using *M. rosenbergii, M. latidactylus,* and *M. australiense* under the same laboratory conditions. This was to enable a comparison of the trace metal bioaccumulation characteristics of the three species and to determine the applicability of the biokinetic model of cadmium bioaccumulation to the species of prawns native to the Strickland River. This appears to be the first study directly comparing the metal bioaccumulation characteristics of decapod crustaceans from the same genus.

2. Methods

High-purity acids (Tracepur, Merck, Darmstadt, Germany) were used for sample acidification (preservation) and washing of equipment. All plastic ware was acid-washed by soaking for >24 h in 10% v/v HNO₃, then rinsed with copious amounts of deionised water (18 M Ω cm, Milli-Q, Millipore, Academic Water System, Sydney, Australia) before drying in a laminar-flow cabinet (Clyde-Apac, HWS Series) prior to use. All chemicals used were analytical reagent grade or better purity.

2.1. Animal stocking and holding

M. australiense $(0.87 \pm 0.22 \text{ g})$ whole body wet weight; $12.4 \pm 3.1 \text{ mm}$ post-orbital carapace length; posterior edge of the orbital notch to the posterior of the carapace in mm) were obtained from a commercial farm (Bingera Weir Farm, Bundaberg, Australia) and were identified by Queensland Department of Primary Industries and Fisheries (Ross Lobegeiger, personal communication). *M. rosenbergii* $(3.9 \pm 2.1 \text{ g})$ whole body wet weight; $15.2 \pm 3.1 \text{ mm}$ post-orbital carapace length) and *M. latidactylus*

 $(2.0 \pm 1.3 \text{ g} \text{ whole body wet weight; } 14.6 \pm 3.3 \text{ mm post-orbital}$ carapace length) were collected from the Strickland River, Papua New Guinea, a location that receives mine-derived materials (Tiumsinawam) and also from local reference tributaries (the Baia and Tomu Rivers). These two species were identified by biologists from the PJV Environment Department. The organisms used in the experiments were not aged or sexed. Both the dissolved cadmium experiment and the tailings experiment were conducted with the same batch of M. australiense while the dissolved cadmium experiment was conducted with M. rosenbergii from the Strickland River and the tailings experiment using M. rosenbergii from the Strickland River reference tributaries. This was due to a lack of sufficient numbers of M. rosenbergii from the reference tributaries to conduct both experiments. Both experiments were carried out using M. latidactylus from reference tributaries.

All prawns were maintained in a synthetic river water (SRW-1.92 g NaHCO₃; 1.20 g CaSO₄ 2H₂O; 2.46 g MgSO₄ 7H₂O and 0.08 g KCl in 20 L deionised water) in 28 L plastic tubs with constant aeration provided via compressed air lines. SRW was chosen as a preferred holding and testing media as it had similar physicochemical properties to that of the Lagaip and Strickland Rivers (Cresswell et al., 2013, 2014b). Prawns were fed commercial food pellets (Prestige Vegi Pellet; Kirrawee, NSW, Australia: 32% crude protein; 5% crude fat; 4% crude fibre; 8% moisture) every second morning with faeces and remaining food being syphoned to waste every evening. There was daily renewal of 25% of the holding tank water. Laboratory conditions were set on a 12:12h light:dark regime. Any prawns that died during the holding period were removed, bagged and frozen immediately as were any moulted exoskeletons. Prawns that moulted during exposures were not removed from the experiment, but a note was made of the moulting event as uptake rates for at least cadmium have been shown to increase up to 3 d following moulting (Cresswell et al., 2014a).

The experiments were conducted in a temperature controlled room, in Sydney (Australia) for *M. australiense*, and at the Environment Department of Porgera mine (in the highlands of Papua New Guinea) for *M. rosenbergii* and *M. latidactylus*. The differences in the laboratory rooms resulted in a slight difference in exposure water temperatures of 21 ± 1 °C for *M. australiense* and 19 ± 2 °C for *M. rosenbergii* and *M. latidactylus*. The potential effects of these temperature differences on metal bioaccumulation rates are discussed in Section 3.2.

2.2. Dissolved cadmium exposure

A stock solution of cadmium was prepared using a plasma emission standard (AccuTrace[™] Reference Standard, AccuStandard, Newhaven, USA) in order to produce nominal test solutions of 0 (control: <0.001 µg Cd/L) and 1.0 µg Cd/L (8.9 nM Cd) in SRW $(pH=7.9\pm0.2)$. The concentration of $1 \mu g Cd/L$ was chosen as a nominal exposure concentration that was close to the concentration of cadmium in solution downstream of the Porgera Mine ($\sim 0.65 \,\mu g \, Cd/L$; Cresswell et al., 2013). The exposures were conducted in 1.125 L polypropylene (PP) exposure chambers containing internal polypropylene baskets (Décor Tellfresh), which allowed the prawns to be removed from the container without causing harm. Each chamber was filled with 400 mL of test solution and each treatment was carried out in five replicates (with each replicate consisting of an individual exposure chamber and an individual prawn). A total of fifteen test chambers were prepared for all three species which included five chambers as controls (e.g. no added cadmium) and ten as treatments ($1.0 \mu g Cd/L$; 8.9 nM Cd). Test solutions were allowed to equilibrate in exposure chambers for 24 h with continuous aeration provided by compressed air lines

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