Chemosphere 75 (2009) 1423–1428

Contents lists available at ScienceDirect

Chemosphere

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

Amphipod susceptibility to metals: Cautionary tales

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ARTICLE INFO

Article history:
Received 12 November 2008
Received in revised form 22 February 2009
Accepted 2 March 2009
Available online 29 March 2009

Keywords:
Gender
Life-stage
Crustacean
Bioaccumulation
Diet
Echinogammarus marinus

ABSTRACT

Heavy metals accumulated by aquatic crustaceans in environmental studies are normally investigated using the whole body burden, with little regard paid to uptake in different tissues, to potential gender of life stage differences, or to the influence of nutrition on the test organism. This is likely to give erroneous conclusions for a dose–response relationship within the toxicity test and potentially lead to wrong conclusions for the ecological risks of metals where species may have higher sensitivities with gender and life stage than indicated or that functionally metals may be sequestered into parts of the body so are not bioavailable. This could lead to under-estimation or over-estimation of the toxicity of metals, respectively, inaccuracy of metal budget calculations and evaluation of trophic transfers of metals. This study evaluated the influences of life stage, gender, and a priori nutritional state in the uptake of the metals zinc (an essential micro-nutrient; Zn) and cadmium (a non-essential element; Cd) in the amphipod *Echinogammarus marinus*. The study showed that life stage, and nutritional stage did significantly influence the uptake and bioaccumulation for both metals, but only Cd showed differential uptake and bioaccumulation with gender. In addition, it was concluded that there was a significant uptake and accumulation of both metals within the exoskeleton of the amphipods, which though adding to the full body burden would add little to toxicity through lack of bioavailability. These results showed that care should be taken when interpreting results from tests normally preformed on such test organisms.

1. Introduction

A great deal of effort has been put into investigating effects of metals in the environment using amphipods. However, several aspects of currently used protocols have been disregarded due to a small number of studies whose conclusions, in our opinion, are seldom reflected in recent investigations. For example the use of routine evaluation of whole body bioaccumulation rates instead of considering the differential distribution of metals taken up between target organs (Weeks et al., 1992; Rainbow, 2007) or the amount of metal embedded on and coating exoskeletons (Viarengo and Nott, 1993) may give erroneous conclusions about dose–response relationships as it does not take into account the amount of sequestered metals which are not available for metabolic processes (Rainbow, 1993, 2007). In addition, only a limited number of studies using amphipods account for differences between different life stages (males/females/juveniles and neonates) (Marsden, 2002). Data regarding variations introduced by gender on metal levels is still patchy and inconsistent, with results being frequently contradictory (e.g. Marsden, 2002; Fialkowski et al., 2003). Hence, for most studies only one life stage, adults, is used with no separation into gender (Fialkowski et al., 2003) leading Zhou et al. (2008) to state "There are overwhelming needs for the study of the gender-related differences in metal bioaccumulation". Furthermore, studies evaluating the effect of the nutritional state of the test organisms prior to metal exposure are scarce despite this potentially leading to errors in metal budgeting in organisms/populations, evaluation of trophic transfers and ecodynamic extrapolation.

From an ecological point of view not taking these factors into account could lead to biased or erroneous data. This can have significant implications for ecological risk assessment calculations of "no-effect" metal levels in environmental compartments (e.g. water, sediment or tissue), which could potentially lead to population loss.

To test some of the sensitivity aspects of the issues raised, the ubiquitous inter-tidal amphipod *Echinogammarus marinus* Leach was chosen as test organism. It has a wide distribution, reported from Norway and Iceland to Portugal (Lincoln, 1979), commonly occurs in high abundances and plays an important trophic role (Maranhão and Marques, 2003; Dick et al., 2005), feeds as both as mesograzer and predator (Dick et al., 2005), is easy to culture and manipulate and reproduces rapidly (17 d at 20 °C for production of a juvenile (Maranhão and Marques, 2003)).

The aim of this work was to investigate the influence of different life stages, of gender and of diet on uptake of metals by *E. marinus* when in presence of available essential and non-essential

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0045-6535/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2009.03.003

metals. This was achieved in three experiments: (1) exposing different life stages of *Echinogammarus* and genders to metal solutions, (2) subjecting adult *Echinogammarus* to different pre-exposure feeding regimes, and (3) evaluating the quantity of metals in the exoskeletons of *Echinogammarus*.

2. Materials and methods

2.1. Experimental design

E. marinus were collected from the Mondego estuary (40°08′N, 8°50′W), at the southernmost limits of its known distribution (Martins et al, 2002) along with *Fucus vesiculosus* L. In the laboratory, the organisms collected were placed in plastic tanks (40 × 20 cm) filled with 4 L of continuously aerated artificial seawater (SERA PREMIUM®) changed three times per week, under a 12 h dark/12 h light regime. The use of artificial seawater ensured that physic-chemical conditions affecting trace metal uptake were reproducible (Rainbow, 1997). *Echinogammarus* were maintained in these conditions for 2 weeks prior to the commencement of the experiments to allow acclimation to test conditions and allow depuration (Clason and Zauke, 2000).

All experiments consisted of 96 h static exposures to 1 mg L^{-1} cadmium ($CdCl_2 \cdot 2H_2O$, Sigma–Aldrich) or zinc ($ZnSO_4 \cdot 7H_2O$, Sigma–Aldrich) with five replicates (10 organisms per chamber) per experiment. No food was given to the organisms during the experiments. All materials (including experimental vessels) were acid washed and pre-soaked in the appropriate test medium for 24 h to saturate all adsorption sites (after Rainbow et al., 2004).

To evaluate differences in metal bioaccumulation between development stage adults, juveniles and neonates were exposed in separate chambers to test solutions of the two metals. Adults were separated by gender and exposed in separated chambers. Controls containing no metals were used for each life stage/gender.

The influence of pre-exposure diet on metal uptake was evaluated by feeding two types of foods to two groups of males, females and juveniles kept in separated chambers. *F. vesiculosus* collected at a site with oceanic conditions was fed to one of the groups and *Artemia salina* (brine shrimp) hatched from commercially obtained cysts (Sanders Brine Shrimp Co.) was fed to the other group. Each of these foods was the sole diet for 1 month prior to metal exposure. Metal levels present in *F. vesiculosus* and *A. salina* used as feeds were determined.

The amount of metals present in the exoskeleton (adsorbed and embedded) was assessed using equal numbers of males and female *Echinogammarus*. At the end of the exposure individuals were frozen at −80 °C then thawed on ice and the exoskeleton stripped from the remaining tissues under a dissecting microscope. Soft and exoskeletal tissues were separated for quantification of the respective metal burdens.

2.2. Metal analysis

Extraction of metals from amphipod tissues (500 mg ww for body and 150 mg ww exoskeleton), *F. vesiculosus* (2000 mg ww) and *A. salina* (2000 mg ww) was carried out using nitric acid–hydrogen peroxide digestion. The tissues were dried at 110 °C for 24 h and ground to a fine powder. A 500 mg (*F. vesiculosus* and *A. Salina*), 50 mg (whole amphipod) or 30 mg (exoskeleton) sub-sample was added to a 20 mL Teflon screw top digestion vessel. Next 5 mL of concentrated nitric acid (69%, Aristar, BDH, 106, UK) was added and the sample was heated to 110 °C for 24 h. Once cooled, 3 mL of hydrogen peroxide (Aristar, BDH, UK) was added in 1 mL steps until the sample became totally clear and ceased effervescing. Samples were re-heated to 110 °C for a further 2 h, allowed to cool and made up to 15 mL with distilled water and centrifuged at 2000 rpm for 15 min. A 1 mL sub-aliquot was analyzed using a THERMO™ ICP-Mass Spectrophotometer (Thermo Ltd., Huntingdon, UK). Calibration of the instrument was achieved using MERK CertiPUR standards and internal quality control performed using reference material (lobster hepatopancreas tissue TORT-2, NRC-CNRC). Certified values are 26.7 ± 0.6 and 180 ± 6 mg kg^{-1} for Cd and Zn, respectively, whilst measured values were 25.3 ± 0.9 and 176 ± 7 mg kg^{-1}, respectively.

2.3. Statistical analysis

One-way Analysis of Variance (ANOVA) was performed on Log_{10} transformed data to evaluate the significance of differences between life stages and different treatments. Normality of distribution was tested using the Kolmogorov–Smirnov test. Student–Newman–Keuls (SNK) Method was applied as pairwise multiple comparison procedure to further differentiate between groups/treatments.

All statistical analysis was performed using SigmaStat (Version 3.5) statistical software.

3. Results

3.1. Life stage metal accumulation

Figs. 1 and 2 present the quantification of metal body burdens for females, males, juveniles and neonates in controls and test solutions of 1 mg L^{-1} Cd and 1 mg L^{-1} Zn, respectively.

These show that different life stages of *E. marinus* have differential bioaccumulation rates of cadmium and zinc. Post hoc results (SNK) for cadmium treatments (Fig. 1) indicate that all exposed amphipods had significantly higher bioaccumulation than the controls and, while the adults, juveniles and neonates had significantly different bioaccumulation ($P < 0.001$), there was no difference between male and female adults ($P > 0.05$). No significant differences were seen between the controls ($P > 0.05$). Post hoc results (SNK) for zinc treatments (Fig. 2) indicate that all exposed amphipods had significantly higher bioaccumulation than the controls. Adults, juveniles and neonates had significantly different bioaccumulation levels ($P < 0.001$). Significant differences were also shown between male and female adults ($P < 0.001$). No significant differences were seen between the controls ($P > 0.05$).

As a consequence, for cadmium bioaccumulation characteristics for *Echinogammarus* were:

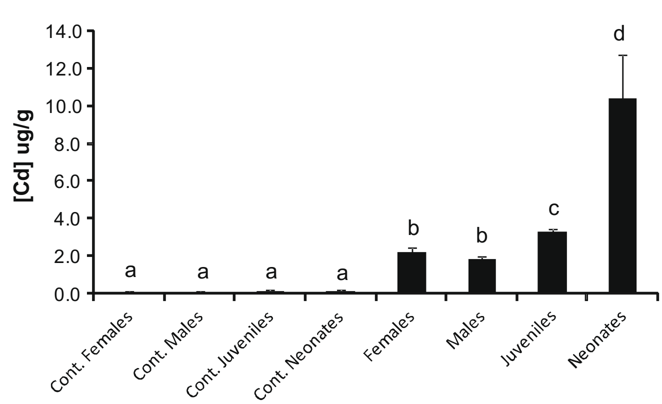

Fig. 1. Mean whole body burdens (μg g^{-1}) after a 96 h exposure to water borne cadmium (nominal 1 mg L^{-1} Cd) for *E. marinus*. Error bars are +SD. Treatments with the same letter were not significantly different; $P > 0.05$, ANOVA, post hoc SNK.