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Influence of environmental conditions on the toxicokinetics of cadmium in the marine copepod *Acartia tonsa*

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ABSTRACT

mMarine and estuarine ecosystems are highly productive areas that often act as a final sink for several pollutants, such as cadmium. Environmental conditions in these habitats can affect metal speciation, as well as its uptake and depuration by living organisms. The aim of this study was to assess cadmium uptake and depuration rates in the euryhaline calanoid copepod *Acartia tonsa* under different pH, salinity and temperature conditions. Cadmium speciation did not vary with changing pH or temperature, but varied with salinity. Free Cd^{2+} ion activity increased with decreasing salinities resulting in increased cadmium concentrations in *A. tonsa*. However, uptake rate, derived using free Cd^{2+} ion activity, showed no significant differences at different salinities indicating a simultaneous combined effect of Cd^{2+} speciation and metabolic rates for osmoregulation. Cadmium concentration in *A. tonsa* and uptake rate increased with increasing pH, showing a peak at the intermediate pH of 7.5, while depuration rate fluctuated, thus suggesting that both parameters are mediated by metabolic processes (to maintain homeostasis at pH levels lower than normal) and ion competition at membrane binding sites. Cadmium concentration in *A. tonsa*, uptake and depuration rates increased with increasing temperature, a trend that can be attributed to an increase in metabolic energy demand at higher temperatures. The present study shows that cadmium uptake and depuration rates in the marine copepod *A. tonsa* is mostly affected by biological processes, mainly driven by metabolic mechanisms, and to a lesser extent by metal speciation in the exposure medium.

1. Introduction

Zooplankton plays a key role in the trophic webs of marine and brackish ecosystems, being paramount in the bottom-up reallocation of trace elements (Fisher et al., 2000). Copepods are well represented in the zooplankton of marine and estuarine ecosystems, being widely distributed and often dominating coastal blooms (Xu et al., 2001). These organisms have been used in standardized ecotoxicological studies for several years, as they are considered to be sensitive indicators of metal pollution (Bao et al., 2013; Barka et al., 2010; Moraïtou-Apostolopoulou et al., 1979; Pedroso et al., 2007; Toudal and Riisgard, 1987; Xu et al., 2001).

Metals are common environmental pollutants and are considered to be hazardous for marine and estuarine organisms due to their persistence in water or sediment, as well as due to their high bioaccumulation potential (Mohammed et al., 2011). Previous studies have demonstrated that the accumulation of metals in marine invertebrates can differ significantly between species and under varying environmental conditions (Aksu, 2001; Mubiana and Blust, 2007; Philp, 2001; Xu et al., 2012). Different water characteristics, such as the concentration of suspended organic matter, calcium and magnesium concentration, zinc concentration, redox potential, salinity, temperature or pH, may affect the toxicity of metals (Amirthalingam et al., 2013; Di Toro et al., 2001; Engel and Fowler, 1979; United Nations Environment Programme, 2008; Frazier, 1979; Panda and Panda, 2002; Ray, 1984). These studies highlight the importance of considering not just total metal concentration but also the bioavailable fractions of metals, when presenting ecotoxicity and toxicokinetics parameters. Effects of a toxicant on the organism are related to the way uptake takes place, to what extent it is being accumulated, distributed to different body compartments, stored or metabolized and subsequently eliminated.

Cadmium is considered one of the most toxic metals to aquatic organisms (Howard and Hacker, 1990). In order to fully understand cadmium bioavailability, it is important to evaluate its uptake and

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depuration kinetics in organisms. The uptake of metals such as cadmium, cobalt or copper is known to be influenced by the availability of their free ionic form, which in turn is determined by salinity, temperature and pH (Burke et al., 2003; Mubiana and Blust, 2007; Roast et al., 2001). Mubiana and Blust (2007) demonstrated that as temperature increased the uptake and depuration rates of two non-essential metals, cadmium and lead, in the marine bivalve *Mytilus edulis* also increased. Cadmium uptake in the Asiatic clam, *Corbicula fluminea*, was significantly decreased with decreasing pH (from 7.8 to 5.0), while a positive correlation was found between cadmium uptake and increasing temperature (Graney, 1984). Mercury (Hg(II)) accumulation in the shore crab *Carcinus maenas* was found to be favored at lower salinities (Laporte et al., 1997).

Studies addressing toxicokinetics (TK) are commonly employed to describe and explain the way metal exposure can be associated to effects in the organism over time. TK studies provide information on the way the metal is being absorbed from the surrounding media and excreted from the body, as well as how toxicity develops over time (Directorate-General for Health and Food Safety and European Commission, 2013). The outcome of TK studies can provide a better understanding on how the organism is processing the chemical, enabling the calculation of uptake and depuration rate constants along with the half-life time of the chemical in the organism (i.e. residence time) (Directorate-General for Health and Food Safety and European Commission, 2013). By simulating short-term exposure conditions, TK studies can be considered a useful tool that may allow data extrapolation among species and exposure times in order to assist risk assessment (Ashauer and Escher, 2010) and regulatory procedures for protecting environmental and human health (Dorne and Renwick, 2005).

The present study aimed to determine the bioconcentration potential of cadmium in a marine calanoid copepod under different environmental conditions that are commonly recorded in estuarine environments. In this way, the uptake and depuration kinetics of cadmium were experimentally evaluated for *Acartia tonsa* stocked under different pH, salinity and temperature conditions by employing a first-order one-compartment TK model.

2. Materials and methods

2.1. Copepod culture

Cultures of the marine calanoid copepod *Acartia tonsa* were kept under a continuous life cycle using artificial seawater (ASW) prepared by mixing freshwater purified by a reverse osmosis unit with the commercial marine salts Tropic Marin® Pro Reef (Tropic Marin, Wartenberg, Germany) according to the instructions provided by the manufacturer. Cultures were started from eggs kindly provided by Escola Superior de Tecnologia do Mar, IPL, Peniche, Portugal. Copepod eggs were stocked in 15-L poly(methyl methacrylate) (PMMA) cylindroconical tanks supplied with constant aeration (~3 bubbles s^{-1}) at a salinity of 20 ± 1, a temperature of 20 ± 1 °C and a photoperiod of 16 h light: 08 h dark. After hatching, different developmental stages (nauplii, copepodites and adults) of *A. tonsa* were separated in different 15-L PMMA tanks using appropriate mesh screens to retain each developmental life stage. Organisms were fed daily *ad libitum* with the cryptophyte *Rhodomonas lens* CCMP 739 (at a minimum stock density of ~2 x 10^7 cells mL^{-1}). The density of adult copepods under culture was kept at a maximum of ~130 specimens L^{-1}, with culture tanks being siphoned daily to collect eggs and remove excess of food and debris (e.g., dead organisms and fecal pellets). Water was fully renewed once a week, with collected eggs being stored at 4 °C and used to start new *A. tonsa* cultures whenever necessary (Drillet et al., 2006).

2.2. Test chemical

Cadmium chloride anhydrous (CAS No. 10108-64-2, Sigma-Aldrich, Germany) was selected to perform the trials to determine the bioconcentration potential of cadmium in *A. tonsa*. A stock solution of 100 mg Cd L^{-1} was prepared with ultrapure water using a Millipore® Academic Milli-Q system. Test concentrations were achieved through dilution in artificial seawater (ASW). Chemical analysis screening for cadmium in the ultrapure water was performed using Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). Samples from the stock solution and from the concentration in ultrapure water were acidified after spiking and sent for chemical analysis to LCA (Central Laboratory of Analysis, University of Aveiro, Portugal) to assess and confirm contamination accuracy. The chemical equilibrium model Visual MINTEQ ver. 3.0/3.1 (Gustafsson, 2013) was used to calculate the speciation of cadmium in ASW medium using the concentration of all salt constituents (information supplied by the manufacturer of the commercial marine salts employed in the present study). From the total cadmium concentration, the parameters derived and used for data analysis were the cadmium free ion concentration and the cadmium free ion activity for each environmental condition tested. Three exposure conditions were tested for each parameter: 7.0, 7.5 and 7.9 for pH, 10, 20 and 30 for salinity and 15, 20 and 25 °C for temperature. The exposure conditions used in the current study are commonly recorded in estuarine environments, where reported values vary from 10 to 35 for salinity and < 7 or up to 8.4 for pH (Riba et al., 2004; Ringwood and Keppler, 2002). For pH conditions, temperature was fixed at 20 °C, while salinity was fixed at 20. For salinity conditions, temperature was fixed at 20 °C, while pH varied according to salinity. For temperature conditions, salinity was fixed to 20 and pH to 7.9. The Visual MINTEQ ver. 3.0/3.1 chemical equilibrium model calculated the ionic strength of each test solution.

2.3. Bioconcentration tests

For the bioconcentration tests, a concentration of 6.88 µg of Cd L^{-1} was used, corresponding to the Lowest Observed Effect Concentration (LOEC) of cadmium on the hatching success of *A. tonsa*; this value was obtained from an Early Life Stage test previously performed (Pavlaki et al., 2016). Bioconcentration experiments consisted of two phases; an uptake phase where the organisms were exposed to cadmium through water contamination and a depuration/elimination phase where the organisms were allowed to depurate when stocked in non-contaminated medium. Copepods were acclimated for 24 h to each environmental condition prior to testing. The uptake and depuration phases lasted 48 h each for all exposure conditions tested. Copepods were not fed during the experiment in order to minimize possible interference from algae (uptake/adsorption). During the uptake phase 400–500 adult *A. tonsa* were exposed to cadmium in three replicate 4-L glass aquariums and then transferred to clean medium for the depuration phase. Sampling times varied for each condition tested, with 6–8 samplings being performed during the uptake phase and 4–5 during the depuration phase. Every sampling consisted of three replicates, with ~30 copepods being pooled per replicate.

2.4. Chemical analysis

Each replicate sample of *A. tonsa* was rinsed with ultrapure water to remove excess medium, freeze-dried for 24 h, weighed on a microbalance and then digested. Digestions were performed with a mixture of HNO_3 and $HClO_4$, at a ratio of 7:1 (v/v, Baker Ultrex II Ultra Pure) using four heating steps (step 1: 85 °C for 60 min, step 2: 130 °C for 60 min, step 3: 160 °C for 60 min and step 4: 180 °C until dryness) in order to destroy all organic material. Residues were taken up in 200 µL of 0.1 M HNO_3 (Baker Ultrex II Ultra Pure). Cadmium concentration was measured using Graphite Furnace Atomic Absorption Spectrophotometry (Perkin-Elmer 5100 PC). For every digestion cycle, 3–6 replicates of blanks and 3 replicates of certified reference material (CRM) (DOLT-5, Dogfish liver CRM for trace metals and other constituents) were used to