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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

control for the accuracy of the method (cadmium concentration, 14.5 mg kg^{-1}, SD = 0.6 mg kg^{-1}). Detection limit was 0.052 μg Cd L^{-1} (n = 20). Recovery of cadmium from the certified reference material was 119% (SD = 2.3%).

2.5. Toxicokinetics modeling

The uptake and depuration kinetics of cadmium in the copepods was described using a first-order one-compartment model considering the organism as one singular compartment.

Background cadmium body concentration in the control organisms was significantly lower than the detection limit; therefore C_0 was fixed to 0 and was not included in these equations.

For the uptake phase the model used reads:

$$Q(t) = \frac{k_1}{k_2} * C_e * (1 - e^{(-k_2 * t)}) \quad (1)$$

And for the depuration phase:

$$Q(t) = \frac{k_1}{k_2} * C_e * (e^{(-k_2 * (t - t_c))} - e^{(-k_2 * t)}) \quad (2)$$

where Q(t) is the concentration in the organism in μg Cd g^{-1} dry body weight at sampling time t,

k_1 is the uptake rate constant in $mL_{medium}\ g_{organism}^{-1}\ h^{-1}$,
k_2 is the depuration rate constant in $hour^{-1}$,
C_e is the exposure concentration in the medium in μg Cd L^{-1},
t_c is the time when the organisms where transferred to fresh uncontaminated medium in hours, and
t is the sampling time in hours.

Both equations used for describing the cadmium uptake and depuration patterns were fitted simultaneously as suggested by the OECD Guideline 305 on Fish Bioaccumulation Testing (2012).

2.6. Statistical analysis

Kinetics parameters for each environmental condition were estimated using non-linear regression analysis by fitting uptake and depuration equations to the data using SPSS Statistics Package (version 20). Significance of differences in k_1 and k_2 between exposure conditions was tested applying a Generalized Likelihood Ratio Test.

The time (expressed in hours) that organisms required to eliminate half the amount of cadmium (DT_{50}), and the bioconcentration factor (BCF) in $mL_{medium}\ g_{organism}^{-1}$ were calculated as:

$$DT_{50} = \frac{\ln(2)}{k_2} \quad (5)$$

$$BCF = \frac{k_1}{k_2} \quad (6)$$

3. Results

3.1. Chemical analysis

Cadmium concentrations measured in the stock and test solutions did not differ more than 2–15% from the nominal ones, thus confirming the accuracy of the spiking technique. The cadmium free ion concentrations and cadmium free ion activities for each environmental condition estimated with the Visual MINTEQ equilibrium model, are presented in Table 1. Cadmium free ion concentrations as well as cadmium free ion activities were fairly similar across the ranges of pH and temperatures tested but decreased with increasing salinity of the test solution.

Table 1
Percentage of the total cadmium concentration present as free cadmium ions, cadmium free ion concentration and cadmium free ion activity at different pH, salinity and temperature levels of exposures used to determine the influence of environmental conditions on cadmium uptake kinetics in *Acartia tonsa*. Test solutions were spiked with a nominal cadmium concentration of 6.88 μg L^{-1}, and measured concentrations did not differ more than 15% from the nominal ones. Cadmium free ion concentrations and activities were calculated with the equilibrium model Visual Minteq ver. 3.0/3.1 (Gustafsson, 2013), using the nominal cadmium concentration, the ionic composition of the test medium and the test conditions as the starting point.

Environmental parameters			Percentage of ionic cadmium (%)	Cadmium free ion concentration (μg L^{-1})	Cadmium free ion activity (μg L^{-1})
Temperature (°C)	pH	Salinity			
20	**7.0**	**20**	8.7	0.60	0.17
20	**7.5**	**20**	8.7	0.60	0.17
20	**7.9**	**20**	8.7	0.60	0.17
20	**7.8**	**10**	15.9	1.10	0.36
20	**7.9**	**20**	8.7	0.60	0.17
20	**7.9**	**30**	5.5	0.38	0.11
15	**7.9**	**20**	8.8	0.61	0.18
20	**7.9**	**20**	8.7	0.60	0.17
25	**7.9**	**20**	8.6	0.60	0.17

3.2. Bioconcentration tests

No copepod mortality was observed during the 48-h cadmium uptake phase in any of the bioassays performed. Mortality ranged from 5% to 12% at the end of all seven bioassays, with the bioassay performed at 25 °C displaying the highest mortality after 96 h (data not shown).

Uptake and depuration kinetics calculated using cadmium free ion activities (Table 1) are presented in Table 2. Throughout the manuscript, results are presented and discussed according to free ions activities. For further comparison, kinetics parameters derived using total cadmium and cadmium free ion concentrations please refer to the Supplementary Data summarized in Table S1. Depuration rate constants (k_2) were independent of the type of data used in the model: total

Table 2
Uptake and elimination kinetic parameters for the bioaccumulation of cadmium in the copepod *Acartia tonsa* exposed to Cd-spiked artificial seawater, no food provided. Kinetics parameters were calculated using a one-compartment model (Eqs. 1 and 2), with estimated cadmium ion free activity in the test solutions (Table 1) as the exposure concentration; 95% confidence limits are shown in brackets. Different letters indicate significant differences between kinetics parameters estimated for a certain environmental condition (likelihood ratio test; $p < 0.05$).

Environmental Parameters	k_1 ($mL_{water}\ g_{organism}^{-1}\ h^{-1}$)	k_2 ($hour^{-1}$)	BCF (x10^3)	DT_{50} (hours)
pH				
7.0	1579[a] (1190–1968)	0.003[a] (–)	526	231
7.5	3188[b] (2833–3544)	0.0003[a] (–)	10627	2310
7.9	2904[b] (2279–3529)	0.022[b] (0.013–0.030)	132	31
Salinity				
10	2127[a] (1895–2359)	0.007[ac] (0.004–0.010)	304	99
20	2904[a] (2196–3613)	0.022[b] (0.012–0.031)	132	31
30	2637[a] (1640–3633)	0.018[bc] (0.004–0.031)	146	38
Temperature (°C)				
15	1323[a] (951–1694)	0.003[a] (–)	441	231
20	2904[b] (2262–3547)	0.022[b] (0.013–0.030)	132	31
25	4893[c] (4354–5431)	0.012[b] (0.009–0.016)	408	58

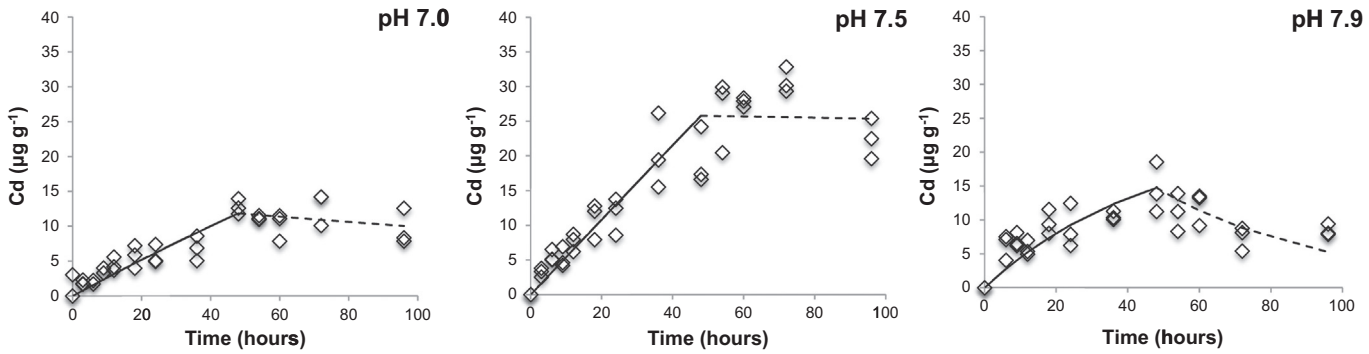

Fig. 1. Uptake and elimination kinetics of cadmium in the marine copepod *Acartia tonsa* at different pH levels. Diamonds represent measured data, continuous lines the fit of the uptake model (Eq. (1)) to the data, dotted lines the fit of the depuration model (Eq. (2)). Modeling used the estimated cadmium free ion activity in the test solutions reported in Table 1 as the measure of exposure during the uptake phase.

cadmium concentration, cadmium free ion concentration or cadmium free ion activity; therefore, k_2 values and 95% confidence intervals presented are similar for the different ways of expressing exposure concentrations in all conditions described below.

3.2.1. pH

Cadmium concentrations in copepods did not reach steady state within 48 h of uptake at any of the three different pH values used in the bioconcentration tests. The internal concentration of cadmium increased at different rates during the uptake phase. Mean cadmium concentration in the copepods was 12.8 µg Cd g^{-1} at pH 7.0, 19.4 µg Cd g^{-1} at pH 7.5 and 14.6 µg Cd g^{-1} at pH 7.9 after 48 h of uptake (Fig. 1). As pH increased the uptake rate increased as well, however, it did not follow a clear pattern. Depuration rate was faster at the highest pH (Fig. 1).

The kinetics parameters for cadmium free ion activity obtained by a first-order one-compartment model are presented in Table 2. Uptake rate constant k_1 was significantly lower at pH 7 compared to higher pH levels ($\chi^2_{(1)}$ = 18.49–25.18, $p < 0.001$), but did not differ between the two highest pH levels (7.5 and 7.9) ($\chi^2_{(1)}$ = 0.40, not significant (n.s.) for ionic activity). Depuration rate constant k_2 was significantly higher at the highest pH compared to lower pH levels ($\chi^2_{(1)}$ = 16.40–18.92, $p < 0.001$), while at the two lowest pH levels no difference was observed ($\chi^2_{(1)}$ = 0.83, n.s.). As no steady state was reached in any of the treatments, bioconcentration factors were calculated from the ratio of k_1 and k_2 values. The BCF value was 526x10^3 mL_{medium} $g_{organism}^{-1}$ for pH 7, 10627x10^3 mL_{medium} $g_{organism}^{-1}$ for pH 7.5 and 132x10^3 mL_{medium} $g_{organism}^{-1}$ for pH 7.9. The time needed for the organisms to eliminate half the amount of cadmium (DT_{50}) was estimated at 231 h for pH 7, 2310 h for pH 7.5 and 31 h for pH 7.9.

3.2.2. Salinity

No steady state was reached for cadmium concentration in the copepods exposed at different salinities. Mean cadmium concentrations in *A. tonsa* decreased with increasing salinity and were 25.0, 14.6 and 8.6 µg Cd g^{-1} at salinities of 10, 20 and 30, respectively (Fig. 2). The uptake rate constant k_1 increased with increasing salinity when total cadmium concentration was used for modeling (Table S1), but did not differ among treatments when based on cadmium free ion concentrations and cadmium free ion activities in the test solutions (Table 2 and Table S1; ($\chi^2_{(1)}$ = 0.19–0.96, n.s. for cadmium free ion concentration, $\chi^2_{(1)}$ = 0.40–3.13, n.s. for cadmium free ion activity). Depuration rate constant k_2 was significantly higher at the intermediate salinity compared to the lower one ($\chi^2_{(1)}$ = 6.08, $p < 0.05$), but did not differ between the other salinities ($\chi^2_{(1)}$ = 0.44–2.37, n.s., respectively). Bioconcentration factors calculated as k_1/k_2 for cadmium free ion activity were 304x10^3, 10627x10^3 and 132x10^3 mL_{medium} $g_{organism}^{-1}$ at salinities of 10, 20 and 30, respectively. At these salinities, DT_{50} for cadmium depuration was 99, 31 and 38 h, respectively.

3.2.3. Temperature

In none of the three temperature exposures cadmium concentrations in the organisms reached steady state. After 48 h of exposure, mean cadmium concentration in the copepods was 11.6, 14.6, and 31.1 µg Cd g^{-1} at temperatures of 15, 20 and 25 °C, respectively (Fig. 3). There was a 3-fold and 2-fold increase in the cadmium body concentration when temperature increased from 15 to 25 °C and from 20 to 25 °C, respectively. As temperature increased, the uptake rate also increased. Cadmium depuration rate increased from 15 °C to 20 °C, while at 25 °C it was slightly lower (Fig. 3). The kinetics parameters obtained with the first-order one-compartment model are presented in Table 2. Uptake rate k_1 differed significantly between temperatures ($\chi^2_{(1)}$ = 11.51–55.57, $p < 0.001$ for cadmium free ion activity). Depuration rate k_2 did not differ between 20 °C and 25 °C ($\chi^2_{(1)}$ = 3.02, n.s.), but was significantly lower at 15 °C compared to 20 °C and 25 °C ($\chi^2_{(1)}$ = 14.26, $p < 0.001$ and $\chi^2_{(1)}$ = 4.65, $p < 0.05$, respectively). BCF values were 441x10^3, 132x10^3 and 408x10^3 mL_{medium} $g_{organism}^{-1}$ for the cadmium free ion activity at temperatures of 15, 20 and 25 °C, respectively. DT_{50} values at these temperatures were estimated to be 231, 31 and 58 h, respectively.

4. Discussion

The present study determined the effect of different environmental conditions, namely pH, salinity and temperature, on the toxicokinetics of cadmium in the marine copepod *Acartia tonsa*, mimicking conditions that commonly occur in estuarine environments. Copepods were not fed during the experiments and food deprivation did not seem to significantly influence their physiology, as confirmed by the low mortality recorded in all tests. According to Finiguerra et al. (2013), copepods can tolerate starvation for up to ~15 days until mortality peaks to 100%. The same authors have also shown that during the first days of starvation copepods rely on previous nutritional provisioning (e.g. catabolism of energy reserves) to reproduce and survive and are therefore physiologically unaffected by food deprivation. Cadmium accumulation in the marine copepod was significantly affected by each environmental parameter when compared to the culture conditions of the organism. According to Rainbow (1998) and Ray (1984), crustaceans cannot regulate their body concentration of non-essential metals, such as cadmium, which leads to a higher body concentration, high storage capacity and slow depuration of cadmium, if excreted at all. Cadmium accumulation and detoxification in the organism depends mainly on the presence of low molecular weight proteins with high affinity binding sites, known as metalloproteins or metallothioneins, which are normally induced as a response to exposure and are capable of forming stable complexes with cadmium (Rainbow, 2007). In that way, metals bound to metallothioneins are stored and prevented from possibly causing damage, e.g. in the DNA (Ray, 1984). Preston (1973), after analyzing a large set of data, observed that BCFs for cadmium

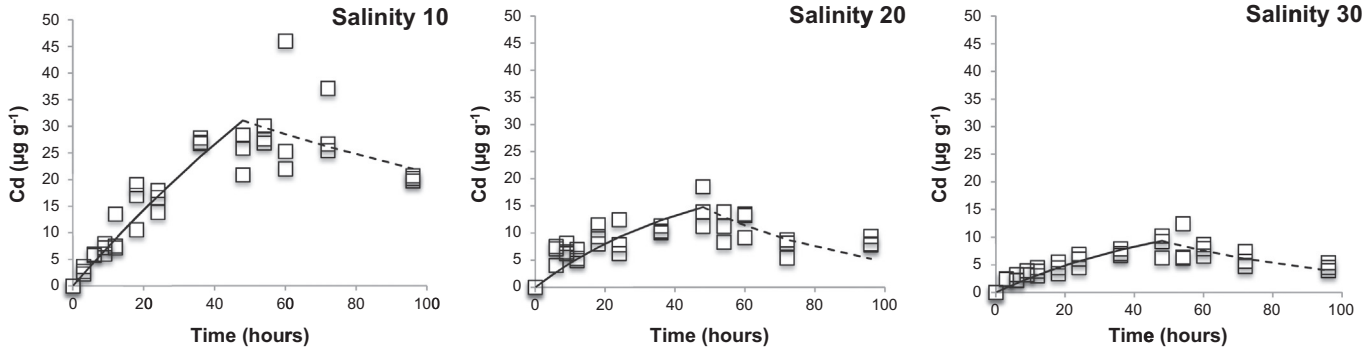

Fig. 2. Uptake and elimination kinetics of cadmium in the marine copepod *Acartia tonsa* at different salinities. Squares represent measured data, continuous lines the fit of the uptake model (Eq. (1)) to the data, dotted lines the fit of the depuration model (Eq. (2)). Modeling used the estimated cadmium free ion activity in the test solutions reported in Table 1 as the measure of exposure during the uptake phase.

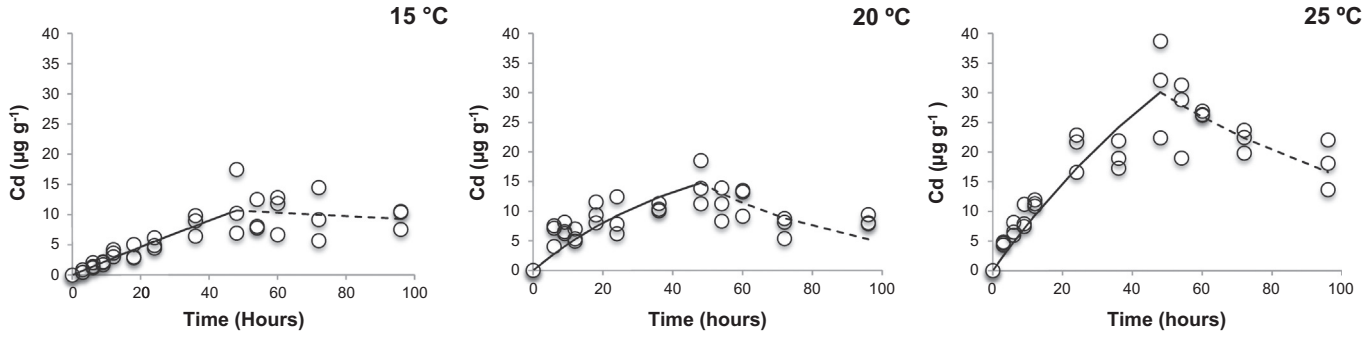

Fig. 3. Uptake and elimination kinetics of cadmium in the marine copepod *Acartia tonsa* at different temperatures. Circles represent measured data, continuous lines the fit of the uptake model (Eq. (1)) to the data, dotted lines the fit of the depuration model (Eq. (2)). Modeling used the estimated cadmium free ion activity in the test solutions reported in Table 1 as the measure of exposure during the uptake phase.

uptake in plankton and specifically crustaceans could reach levels as high as 10^4 and 10^3, respectively, which are in agreement with the values found for *A. tonsa* in the present study. Furthermore, we also showed that the highest BCF values were attained as pH, salinity and temperature decreased. This can be attributed to the slow depuration of cadmium from the copepods due to the metal being stored rather than excreted. Low depuration rates may indicate the presence of a possible storage compartment, such as the oil sac (Lee et al., 2006; van den Bosch and Gabriel, 1994), which could potentially cause metal concentrations to accumulate above the organism's threshold and eventually lead to mortality. This scenario may lead to high DT_{50} values, ranging from 31 h up to 96 days. Sick and Baptist (1979) reported an average turnover rate for cadmium in the copepod *Pseudodiaptomus pelagicus* (formerly *P. coronatus*) of 40 h at a temperature of 20 °C, pH 7.2 and salinity 25. Denton and Burdon-Jones,1981 found that cadmium half-life in the oyster *Saccostrea echinata* strongly depends on temperature and salinity fluctuations and reported values ranging from 30 to 85 days. Taking into consideration that the average lifespan of the marine copepod *A. tonsa* is approximately 80 days, higher DT_{50} values seem unrealistic, as the organism would die before being able to eliminate the excess of cadmium. In this way, it is legitimate to say that the potential of *A. tonsa* to transfer cadmium to higher trophic levels is high. Nonetheless, to the authors' knowledge and despite the trophic relevance of the copepod *A. tonsa* in marine food webs, data on bioaccumulation of cadmium is still missing for this species.

4.1. pH

The internal cadmium concentration in copepods showed a non-linear relationship with pH. This is in line with the findings reported by Goetze et al. (2014), who found that at the lowest and highest pH (7.7 and 8.2, respectively), cadmium was accumulated in oysters in a similar way, while at pH 7.9 its accumulation decreased by half. Cadmium uptake rate showed a decrease as pH decreased. This trend appears to be contradictory to the hypothesis that cadmium bioavailability increases with a lowering pH due to the dissociation of cadmium from creating complexes and consequently increasing Cd^{2+} free ion concentration. However, the decrease in pH resulted in a higher H^+ activity and increased positively charged groups, e.g. amino groups, and decreased the negatively charged groups by protonation, e.g. carboxyl and phosphate (Wang et al., 2016). This setting could eventually lead to a higher competition of protons with Cd^{2+} ions at the binding sites of biological membranes. This is in agreement with the concept of the Biotic Ligand Model (Di Toro et al., 2001; Paquin et al., 2002). A similar explanation was used by Martins et al. (2004) to explain the finding that cadmium uptake in aquatic moss increased with increasing pH from 3 to 5. Likewise, Xu et al. (2012) found that when two marine phytoplanktonic species were exposed to cadmium under decreasing pH levels the bioavailable cadmium was reduced, thus concluding that the uptake of cadmium was more likely being affected by the protonation of the binding cellular surface. The marine copepod *A. tonsa* showed low depuration rates at the three pH levels tested in the present study. Xu et al. (2001) reported that cadmium was lost mainly through feces, rather than being excreted in the calanoid copepod *Calanus sinicus*. The same authors also mention that the egestion rate of cadmium was mainly affected by food concentration. Thus, an increased food ingestion rate would likely result in an increase in cadmium egestion rate. However, since copepods were not fed during the experiments, this could have contributed to the low depuration rates recorded at the three pH levels that were tested in the present study. When comparing different pH levels, a higher depuration rate at the highest pH level implies faster cadmium depuration, which could be explained by the animal's physiology. Normal culture conditions for *A. tonsa* were at a pH of 7.9 ± 0.1, so that a decrease in pH could be considered as a stressor to its physiological state. Overall, this condition may have forced the copepods to maintain homeostasis by increasing its energy

demand (Cripps et al., 2014) at the expense of other metabolic processes, such as metal elimination. Consequently, both uptake and depuration of cadmium are mostly being mediated by biological processes, with metal speciation likely playing a minor role at tested pH levels. This assumption is in line with the small variation in cadmium free ion activity recorded at the pH levels tested in the present study (Table 1).

4.2. Salinity

Internal cadmium concentration in the copepods increased as salinity decreased due to the increased availability of cadmium (Cd^{2+}) (Table 1). Cadmium is known to have a strong affinity to chloride ions (Cl^-) to create complexes, rendering it less bioavailable, and/or affecting its competition with sodium ions (Na^+) at the binding sites of biological membranes (Di Toro et al., 2001; Newman, 2014; Paquin et al., 2002). However, cadmium uptake rate did not follow the same pattern as internal concentration, as k_1 values did not significantly differ between different salinities. Previous studies have shown that when salinity decreases from 35 to 25, and even to 15, respiration of *A. tonsa* decreased, possibly as a way to cope with changes in osmoregulation (Gaudy et al., 2000; Kinne, 1964). A similar effect was described by Hutcheson (1974), for blue crabs (*Callinectes sapidus*). The latter author reported that cadmium concentration was higher at lower salinities due to the increase in metabolic energy required to maintain an osmotic gradient and therefore allowed a lower allocation of energy to control the metal influx to the crab's tissues. Again, biological processes such as the organism's metabolic rates/respiration play a key role in cadmium uptake, since the similarity in the uptake rates could not be solely explained from the higher concentration and activity of free Cd^{2+} ions at the lower salinity of the exposure medium. Another biological process that may have allowed Cd^{2+} to enter the cells is the Ca^{2+}-transport system through Ca^{2+} channels due to its similar ionic radius and charge (Bjerregaard and Depledge, 1994). At low salinities, Cd^{2+} competes with Ca^{2+} due to the response of marine crustaceans to ionoregulate, as mentioned above, by increasing or decreasing ionic uptake when salinity diverges from normal. Therefore, a decrease/increase in salinity may result in an up- or downregulation of Ca^{2+} diffusion from the medium and the opportunistic transfer of Cd^{2+} through the same channels. Several studies have shown that cadmium accumulation in marine organisms at different salinities, such as in crabs (Burke et al., 2003), sponges (Philp, 2001), mussels (Bjerregaard and Depledge, 1994) and fish (Cinier et al., 1999), partly depends on Ca^{2+} transport channels. This suggests that the competition between the two divalent cations, Cd^{2+} and Ca^{2+}, is likely taking place at the biotic ligand sites of the organism. No difference was observed in cadmium depuration rates of *A. tonsa* exposed to cadmium at the two highest salinities, while at a salinity of 10 the depuration rate was lower. Turnover rates of cadmium in the copepod *Calanus sinicus* were in agreement with the values in this study, with copepods being exposed to a salinity of 30 and a temperature of 20 °C (Xu and Wang, 2001). According to Gaudy et al. (2000), *A. tonsa* showed no significant difference in metal excretion between salinities of 30 and 20, while when salinity dropped to 15 the excretion rate decreased significantly. This trend could explain the low depuration rate of cadmium from the copepods in our study. The abovementioned low metal excretion at lower salinities had already been identified in the copepod *A. tonsa*, as well as in the estuarine crabs *Carcinus maena* and *Uca rapax*, and is considered to be a mechanism that euryhaline organisms use to regulate and maintain isosmotic body fluids when osmotic changes occur (Gaudy et al., 2000; Wright, 1977; Zanders and Rojas, 1996).

4.3. Temperature

As temperature increased, the uptake rate of cadmium as well as the internal cadmium concentration in the copepod *A. tonsa* increased. Cadmium assimilation in copepods showed a 3-fold increase when temperature shifted from 15 to 25 °C. These findings are consistent with several studies that confirm an increased cadmium uptake and accumulation rate with increasing temperatures in a number of marine taxa (e.g., marine bivalves (Ali and Taylor, 2010; Mubiana and Blust, 2007), marine crustaceans (O'Hara, 1973; White and Rainbow, 1986)). At higher temperatures, the metabolic rate of the organism increases and with that protein synthesis, which can result in enhanced consumption, assimilation and increased formation of metal-binding proteins (e.g. metallothioneins) in the presence of metals. Howard and Hacker (1990) reported that an increase in temperature also resulted in an increase in the level of metallothionein-like cadmium binding proteins in the estuarine ditch shrimp (*Palaemonetes pugio*) when exposed to cadmium. These authors highlight the role played by metal-binding proteins, which are considered a defense/detoxification mechanism against metal toxicity. In the present study, cadmium depuration rate increased with temperature from 15 to 20 °C, while no significant difference was recorded on the depuration rate when temperature was raised from 20 to 25 °C. This trend can be attributed to the faster metabolism of *A. tonsa* at higher temperatures. A study on the respiratory and excretion rates of *A. tonsa* under different temperatures and salinities showed that both parameters decreased with decreasing temperature (Gaudy et al., 2000) as a result of decreasing the metabolic energetic demand by the organism.

5. Conclusions, implications and future perspectives

The present study shows that biological processes, likely metabolism/respiration, play a key-role in cadmium uptake and depuration rates when the marine copepod *Acartia tonsa* is exposed to different environmental conditions. In general, *A. tonsa* appears to have a high capacity to bioaccumulate cadmium under the environmental conditions tested in the present work. The low depuration rates recorded may indicate the existence of a potential storage compartment, which could subsequently cause metal concentrations to reach above the organisms' threshold and eventually lead to mortality. In this way, the metal uptake and storage capacity displayed by marine copepods can pose a threat to higher trophic levels in food webs through the occurrence of food-chain transfer.

The experimental design employed in the present study was able to assess individually the influence of three important environmental factors (pH, salinity and temperature) on the patterns of cadmium accumulation in this copepod. We suggest that this experimental approach can be used as a foundation for a more in-depth understanding on how cadmium accumulation may vary under fluctuating environmental conditions. Future studies should advance the state of the art by assessing more complex and realistic scenarios, such as the combined and/or interacting effects of different abiotic factors on the uptake and depuration of cadmium. Such approach could then be used for the development of predictive models that may improve the accuracy of risk assessment within the frame of multiple environmental scenarios.

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Conflict of interest

The authors declare no conflict of interests.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Appendix A. Supporting information

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