Effects on survival and reproduction of *Porcellio dilatatus* exposed to different Cd species

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Abstract The woodlouse *Porcellio dilatatus* (Crustacea) is a suitable model organism as a biological indicator for the toxic effects evaluation induced by pollutants exposure in the environment. In this study, the influence of cadmium (Cd) species on survival and reproduction of isopods was investigated. Survival, growth and reproductive parameters (time to pregnancy, pregnancy duration, pregnancy and abortion occurrence, number of juveniles per female and juvenile weight) were recorded after isopods were exposed to two species of Cd deployed in food: $Cd(Cys)_2$ and $Cd(NO_3)_2$. There was a difference between survival rates of exposed males and females to both Cd species but in the case of $Cd(NO_3)_2$ these differences were more accentuated, with females having higher survival rates. In the presence of both metal species a reduction in the number of pregnancies and pregnancy duration was observed, while in the case of $Cd(Cys)_2$ all pregnancies were inconclusive. The number of juveniles delivered per female fed with $Cd(NO_3)_2$ contaminated food was lower than in the control, whereas the juvenile weights were higher. To the best of our knowledge, the present study is the first toxicity test demonstrating that metal speciation affects reproduction in isopods. $Cd(Cys)_2$ showed to be the most toxic of the contaminants tested in this long term exposure and capable of jeopardizing the reproduction efforts of isopods.

Keywords Cadmium-cysteine · Reproduction · Survival · Isopods

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Introduction

Terrestrial isopods are saprophytic detritivores that inhabit the upper layer of the soil and surface leaf litter, while playing an important role in maintaining the structure and fertility of soils (Loureiro et al. 2006). Those invertebrate-mediated processes such as drainage, aeration, incorporation and degradation of organic matter are important in improving soil quality and energy flow through ecosystems (Hornung et al. 1998b; Odendaal and Reinecke 1999).

Isopods have been frequently used as model organisms in soil ecotoxicity tests as important representatives of the invertebrate soil fauna (Calhoa et al. 2006; Caseiro et al. 2000; Drobne et al. 2008; Engenheiro et al. 2005; Jemec et al. 2010; Ribeiro et al. 2001; Sousa et al. 1998; Stanek et al. 2006). They are also easily cultured under laboratory conditions, where they can complete their entire life-cycle (Caseiro et al. 2000), and protocols for toxicity testing are available (Hornung et al. 1998a, b) although no standard methods have been developed for these organisms (i.e. ISO, ASTM, OECD). Moreover they have been widely used for the examination of metal accumulation and toxicity testing, due to their extraordinary capacity to accumulate high metal concentrations from the environment, predominantly in their hepatopancreas (Donker et al. 1990; Drobne 1997; Farkas et al. 1996; Godet et al. 2011; Hames and Hopkin 1991; Hopkin 1990; Hornung et al. 1998b; Paoletti and Hassall 1999; Udovic et al. 2009).

The most widely used toxicological endpoints in isopod testing are survival, growth, food consumption and assimilation, moult frequency, reproduction, avoidance behaviour, enzymatic biomarkers and locomotory behaviour (Drobne 1997; Drobne et al. 2008; Drobne and Strus 1996; Engenheiro et al. 2005; Hornung et al. 1997; Jansch et al. 2005; Jemec et al. 2010; Loureiro 2005; Loureiro

et al. 2006, 2009; Santos et al. 2010; Stanek et al. 2006; Vink and Kurniawati 1996). The use of reproduction patterns as response to toxicity is sometimes not convenient, as such tests are considered time-consuming and laborious. Reproduction is also difficult to assess, as females may retain the sperm for long periods of time following mating and before egg fertilization (Drobne 1997; Vink and Kurniawati 1996). On the other hand, reproduction as a response endpoint to test sublethal effects of chemicals in long-term exposures is crucial to understanding and transposing those effects to population and community levels. Similarly, evaluating changes in reproduction and survival patterns induced by stressors exposure is crucial to understanding population stability/growth and will reflect isopods' role in decomposition processes and cycling of nutrients.

Cadmium has shown to be a critical pollutant in the environment as it is considered a priority substance for water and related matrices (soil, sediment, biota) in Directive 2008/105/EC. Soil contamination by Cd can have several sources like agriculture practices, by the use of contaminated water for irrigation, or mining and industry, including in urban soils aerial deposition of particulates from smelting activities, burning of fossil fuels or tyre wear (UK-EA 2002). In addition, previous dietary studies on the assimilation of Cd in the terrestrial isopod *Porcellio dilatatus* (Calhoa et al. 2006) have indicated that Cd speciation dictate the assimilation efficiency (AE) of Cd in plant-isopod food chain.

In this study, the effects of different Cd species on the survival and reproduction of *P. dilatatus* upon a long term exposure was investigated. The main goal of this study was to assess differences in toxicity of two Cd species [$Cd(Cys)_2$ and $Cd(NO_3)_2$] on the survival and reproductive effort of isopods, using as parameters the time to reach pregnancy (as an indication of fertilization ability), pregnancy duration, pregnancy and/or inconclusive pregnancy, number of juveniles per female and juvenile weight. The use of Cd-cysteinate in these studies provides an experimental tool for exploring the bioavailability of Cd (Udovic et al. 2009) that is complexed within biological tissue. Therefore Cd-cysteinate represents the most elementary form (species) of thiol-bound Cd in biological systems. In previous studies (Calhoa et al. 2011), the AE for Cd was lower in isopods fed with food containing $Cd(Cys)_2$, than for that containing $Cd(NO_3)_2$, demonstrating that the cysteine conjugate was less available.

Some vascular plants have mechanisms of tolerance that include the induction of metal-binding proteins such as phytochelatins and metallothioneins (Prasad 1995) which are small proteins with a significant concentration of cysteine (30%) (Ndayibagira et al. 2007). Cysteine contains a sulphydryl group that accounts for the Cd-metallothionein induction due to Cd high affinity for sulphur ligands (Roosens et al. 2005; Zalups and Ahmad 2003).

Materials and methods

Test organisms and culture conditions

Isopods were collected from cultures of *P. dilatatus* that have been maintained for more than 3 years under laboratory conditions and that have been derived from individuals collected in a secondary coastal dune system in Aveiro, Portugal. They were maintained on a substrate of sand in plastic containers at 20°C with a 16:8 h (light:dark) photoperiod. Oven-dried alder (*Alnus glutinosa*) leaves were provided ad libitum as a food source (Caseiro et al. 2000; Kautz et al. 2000) and distilled water was added for maintaining adequate moisture.

Lettuce and gelatine substrate

A mixture of lettuce (*Lactuca sativa*) leaves and gelatine was selected as a suitable food substrate to be used as the exposure route (Mann et al. 2005; Monteiro et al. 2008). Non-contaminated *L. sativa* leaves were reduced to powder using a mortar and pestle, and were mixed with a gelatine solution prepared from 2.5 g gelatine powder (VWR Prolabo, Fontenay Sous Bois, France) to which 12.5 ml deionised water (Milli-Q®) was added, and then mixed by vortexing (Wallace and Lopez 1996). Small portions of the mixture (gelatine discs) weighing approximately 9 mg (dry wt) were made by pipetting liquid gelatine onto Parafilm® (Pechiney Plastic Packaging, Menasha, WI, USA). These discs were stored frozen at −20 C while not in use (Wallace and Lopez 1997).

Three treatments (diets) were established based on previous studies (Calhoa et al. 2006, 2011) for this long term exposure test to evaluate the toxicity of metal speciation to the terrestrial isopod *P. dilatatus*:

- $Cd(Cys)_2$ contaminated food—gelatine contaminated with Cd-cysteinate mixed with non-contaminated leaves of *L. sativa*;
- $Cd(NO_3)_2$ contaminated food—gelatine contaminated with Cd nitrate mixed with non-contaminated leaves of *L. sativa*.
- Control food—gelatine mixed with non-contaminated leaves of *L. sativa*.

Gelatine discs were previously assayed for Cd radiospectrometry before being fed to isopods, and contained 335 ± 29 µg Cd/g dry wt for gelatine with $Cd(NO_3)_2$ and 744 ± 108 µg Cd/g dry wt (mean $\pm$ standard error) for gelatine with $Cd(Cys)_2$.

Experimental setup

A total of 50 non-gravid females with similar weight (35–48 mg) were selected with both antennae and no phenotypic abnormalities and separated into a test box for 1 month to guarantee that they were not pregnant at the beginning of the test. Test boxes (polyethylene terephthalate (PET); $\varnothing$ 85 mm × 43 mm) were bottom covered with a thin layer of sand in order to provide similar conditions to those in the culture boxes. After this 1 month period, a total of 30 males were also selected. At this stage, a total of 30 females and 30 males (10 females and 10 males for each treatment previously described to guarantee Cd accumulation) were exposed individually in test boxes for a period of 28 days, and fed exclusively on gelatine discs. Hereafter, this period will be named individual exposure test. Survival was checked every 2 days.

Fungi growth may alter Cd bioavailability and thus gelatine disks were replaced every week, in order to prevent consumption of those disks that had become colonized with fungi.

After this 28 day period of individual exposure, one male and one female were paired randomly per box for mating, using 10 replicates per treatment. Hereafter, this test period will be referred as reproduction test. Female reproductive cycle and survival was monitored for 54 days, 3 times a week; when pregnancy was observed females were moved into a new box alone until they gave birth. These females were still exposed to the respective food treatments. The percentage of females that successfully reached pregnancy (i.e. successful egg fertilization), the time until pregnancy, duration of pregnancy and the percentage of inconclusive pregnancies (females that successfully reached pregnancy but were unable to carry it out until the end) were recorded. The number of juveniles born per female and their individual weight were also registered. Isopods were assayed for Cd body burden by radiospectrometry to obtain Cd accumulation.

Cadmium analysis

Individual isopods and gelatine discs (dried in an oven at 60°C, for 24 h) were placed in 3.5-ml Röhren tubes (Sarstedt, Newtown, NC, USA) and were analyzed for Cd^{109} by radiospectrometry in a Genesis Gamma1 bench-top gamma counter (Laboratory Technologies, USA). The $Cd(NO_3)_2$ and $Cd(Cys)_2$ contamination solutions were analysed by inductively coupled plasma spectroscopy (ICPS) in a Jobin Ivon JY70 with a Meinard C001 nebuliser. Specific activities of the two contamination solutions were assessed by comparing gamma counts with measurements obtained by ICPS.

Statistical analysis

All data were checked for normality and homoscedasticity using SigmaStat (version 3.01, SPSS, Chicago, IL, USA). Statistical analysis was carried out at a significance level of 0.05, using t tests to compare pregnancy duration and juvenile weights or one-way analysis of variance (ANOVA) with Tukey and Dunnett's test for multiple comparisons of means to determine differences on time to reach pregnancy and number of juveniles relatively to control treatment. Whenever possible, data that was not normally distributed or whose equal variance testing failed were transformed.

The lethal time of 50% (LT_{50}) was calculated with the Probit method using the MiniTab software (2000).

Results

Survival

During the individual exposure period no mortality was observed (Fig. 1). During the 82 days of exposure, mortality did not occur among control females while 20% of the control males died during the test. This is in accordance to the 20% mortality value that is referred in Hornung et al. (1998a) as a validity criteria for controls in isopod testing. In the $Cd(Cys)_2$ treatment 70% of females and 90% of males died at the end of the test, whereas for the $Cd(NO_3)_2$ treatment mortality was of 10 and 70%. LT_{50} values for $Cd(Cys)_2$ exposures were respectively for females and males: 65.85 days (63.97–67.89 for 95%CL) and 58.35 days (56.80–59.89 for 95%CL). For the $Cd(NO_3)_2$ exposure, only data from male survival gave a LT_{50} value of 70.36 days (68.44–72.57 for 95%CL). Females exposed to $Cd(NO_3)_2$ showed a low mortality rate of 10% (Fig. 1).

Cd accumulation in isopods

No cadmium was detected in any of the control isopods. Isopods exposed to $Cd(NO_3)_2$ and $Cd(Cys)_2$ had equivalent rates of Cd accumulation (6.4 ± 1.9 and 7.8 ± 1.7 ng Cd/mg animal, respectively) after the individual exposure period of 28 days (Fig. 2). At the end of the experiment isopods fed with $Cd(NO_3)_2$ accumulated much more Cd (17.9 ± 2.3 ng Cd/mg animal) than those from the $Cd(Cys)_2$ treatment (9.9 ± 2 ng Cd/mg animal).

Time to reach pregnancy, pregnancy duration and abortions

During the individual exposure test, plus the 54 days of the reproduction test, all animals lost weight, with the
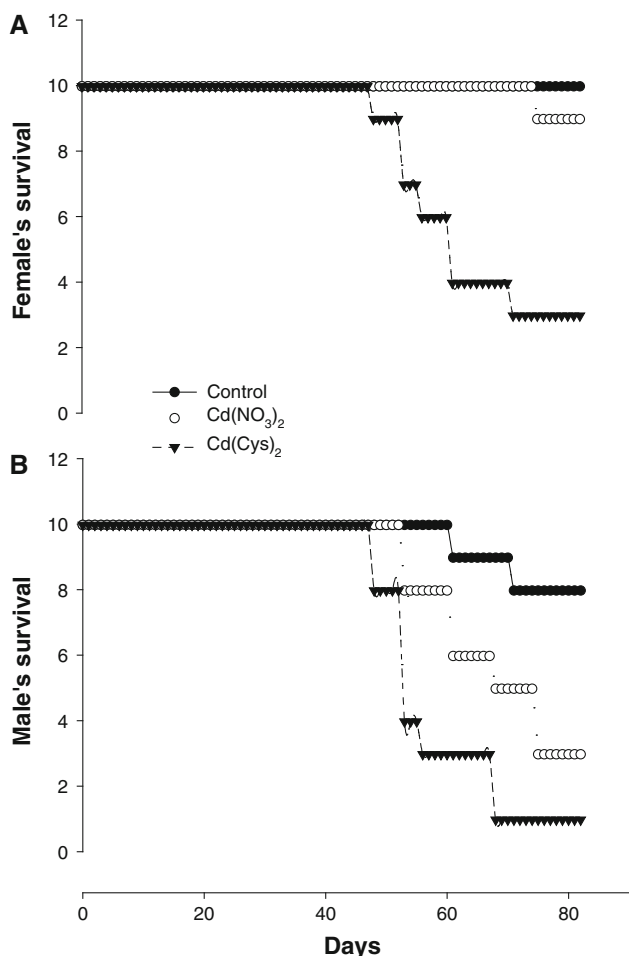
Fig. 1 Number of females (**a**) and males (**b**) of *P. dilatatus* that survived during the 82 days of the test
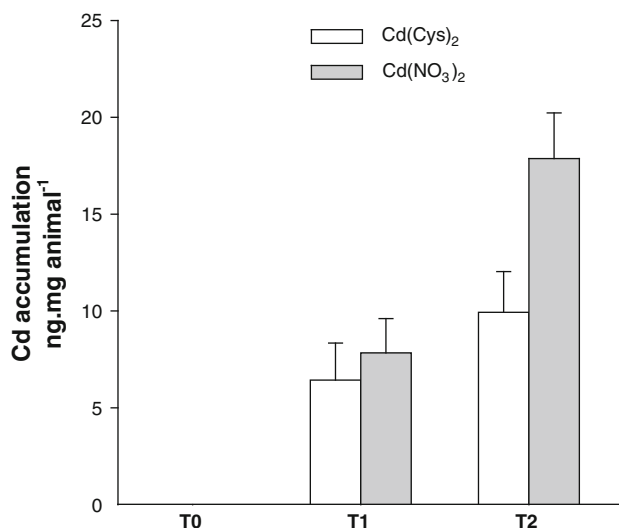

Fig. 2 Cadmium accumulation by *P. dilatatus* before the beginning of the experiment (T0), after 28 days of individual exposure (T1) and at the final of the experiment (82 days of exposure) (T2). *Grey bars* represent the accumulation in isopods fed with $Cd(NO_3)_2$ and the *white bars* represent the accumulation in isopods fed with $Cd(Cys)_2$. *Vertical error bars* represent the standard error of the mean (*n* ranges from 4 to 12)
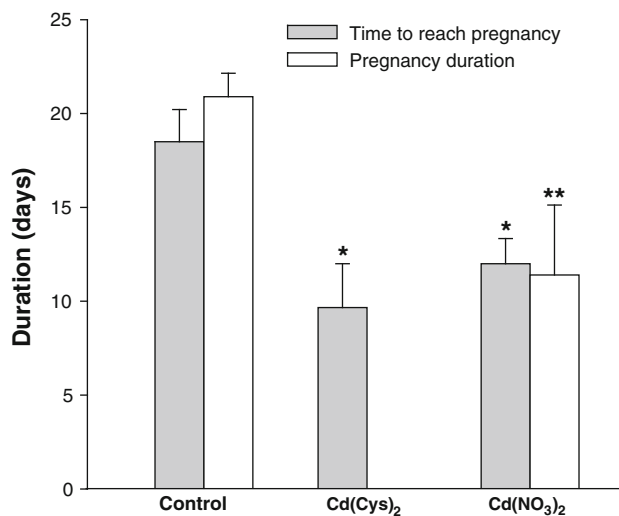

Fig. 3 Number of days until first signs of pregnancy were detected (*grey bars*), and number of days between first signs of pregnancy and release of mancae (*white bars*) of *P. dilatatus* fed with $Cd(Cys)_2$ and $Cd(NO_3)_2$ gelatine and lettuce discs. *n* ranges from 3 to 10. *Vertical error bars* represent the standard error of the mean. * Indicates a significant difference ($p < 0.05$) from the control (ANOVA, Dunnett's test); ** indicates a significant difference ($p < 0.05$) between the control and $Cd(NO_3)_2$ treatments (*t* test)

exception of the only male that survived in the $Cd(Cys)_2$ treatment, that showed a growth rate of 14.22%. There were no significant differences between treatments and within sexes (female data after exponential transformation, $p = 0.117$; males, $p = 0.376$).

As shown in Fig. 3, the average time at which the control isopods showed the first signs of pregnancy following mating was of 19 ± 2 days. This interval was narrower for isopods fed with $Cd(NO_3)_2$ (12 ± 1 days) and even more so for those in the $Cd(Cys)_2$ treatment (10 ± 2 days). Statistically significant differences were found between the control and the two Cd treatments ($p < 0.05$). Average pregnancy duration was of 21 ± 1 days for the control, and decreased in $Cd(NO_3)_2$ treatment (14 ± 3 days), while for the $Cd(Cys)_2$ treatment, and although signs of pregnancy were observed, no females were able to deliver mancae.

In the control all females successfully reached pregnancy within the test period (Fig. 4). Only 30% of the females fed with $Cd(Cys)_2$ became pregnant but none were able to take it through: two died and the one that survived,

failed to produce offspring. In the $Cd(NO_3)_2$ treatment, half of the females successfully reached pregnancy but only 80% were able to reach term.
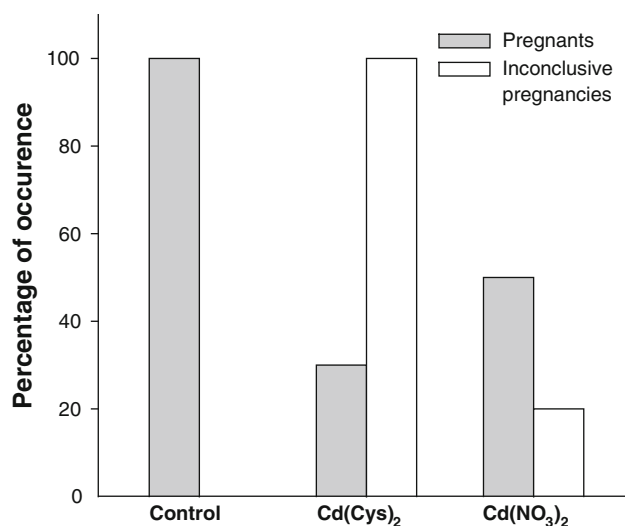
Fig. 4 Percentage of successful females achieving pregnancy (*grey bars*) and percentage of female inconclusive pregnancies (*white bars*) of *P. dilatatus* feed with $Cd(Cys)_2$ and $Cd(NO_3)_2$ gelatine and lettuce discs

Number of juveniles and individual juvenile weight

Significant differences were found between the number of juveniles in control and in Cd treatments ($p < 0.001$). Differences between the $Cd(Cys)_2$ and the $Cd(NO_3)_2$ were not statistically significant, although no mancae were delivered in females exposed to $Cd(Cys)_2$ (Fig. 5). In the control the average number of mancae delivered per female was 21 ± 2. In the $Cd(Cys)_2$ none of the females were able to carry out the pregnancy and therefore, no juveniles were
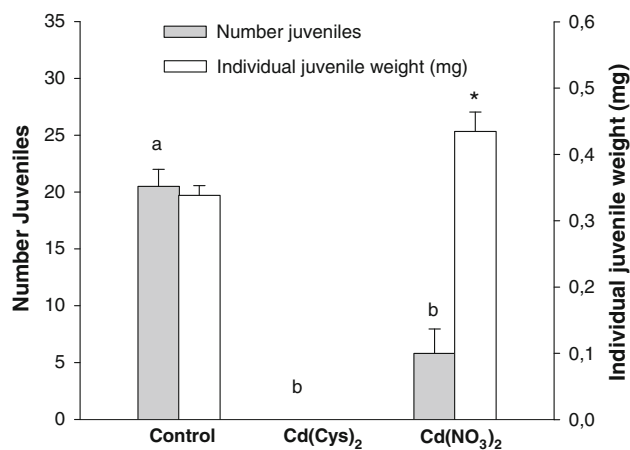

Fig. 5 Number of juveniles hatching per pregnant female (*grey bars*), and individual mancae weight (*white bars*) of *P. dilatatus* fed with $Cd(Cys)_2$ and $Cd(NO_3)_2$ gelatine and lettuce discs. *Vertical error bars* represent the standard error of the mean. (*a*) and (*b*) Indicates a significant difference between number of juveniles produced in the treatments (ANOVA, Tukey test, $p < 0.001$); *asterisk* indicates a significant difference between juvenile individual weight (t test, $p < 0.05$)

delivered. In the $Cd(NO_3)_2$, the number of mancae delivered per female was 7 ± 2.

The total juvenile weight was significantly different between the control and the $Cd(NO_3)_2$ treatment (ANOVA, Dunnett's test, $p < 0.001$). In the control, there were more juveniles delivered and therefore the total weight (6.8 ± 0.31 mg) was higher (ANOVA, Dunnett's test, $p < 0.001$) than in that exposed to $Cd(NO_3)_2$ (3.3 ± 0.96 mg). The decrease in the number of mancae per female in this later treatment lead to an individual mancae weight increase. There were significant differences between individual weight of the control juveniles and those in the $Cd(NO_3)_2$ treatment (ANOVA, Tukey test, $p = 0.007$). Although more juveniles were delivered in the control, their individual weight was lower (0.338 ± 0.015 mg) than of those exposed to $Cd(NO_3)_2$ (t test, $p < 0.05$), where fewer juveniles with higher individual weight (0.435 ± 0.029 mg) were produced.

Discussion

Besides being considered very toxic, Cd has no known biological function (Odendaal and Reinecke 1999). Effects on the environment have been mostly studied in the aquatic compartment, whereas in the terrestrial compartment most ecotoxicological tests have been performed using soluble Cd^{2+} salts (ECB 2007). Life-history theory predicts that habitat disturbances will increase mortality, select for early reproduction and increase reproductive effort (Donker et al. 1993). From the survival data, there was an unexpected differential response between sexes in both exposures. In several studies, differentiation between sexes is not considered (Calhoa et al. 2006; Jansch et al. 2005; Loureiro et al. 2009) due to the expected similarity on behaviour and survival in terrestrial isopods. The inexistence of differences between sexes in isopods was described in the study where the aquatic isopod *Idotea baltica* was exposed to zinc, copper and lead and no differences were observed between males and females' LT_{50} values (Bat et al. 1999). In the present work, males exposed to $Cd(Cys)_2$ died earlier than those exposed to $Cd(NO_3)_2$, showing higher acute toxicity as a response to long-term exposure.

Isopods fed with $Cd(NO_3)_2$ and $Cd(Cys)_2$ had equivalent Cd accumulation (6.4 and 7.8 ng Cd/mg animal, respectively) after the 28 days of individual exposure. It was provided a higher concentration of Cd in $Cd(Cys)_2$ gelatine discs to try to equal metal accumulation by isopods fed with both Cd species, because in previous experiments (Calhoa et al. 2011) isopods fed with $Cd(Cys)_2$ accumulated less Cd than those exposed to $Cd(NO_3)_2$ contaminated food. It was important to have similar accumulation rates for a reproduction test in order to compare toxicity

responses between both Cd species. But at the end of the experiment isopods fed with Cd(NO$_3$)$_2$ contaminated food accumulated higher Cd concentration (17.9 ng Cd/mg animal) compared to those that received the Cd(Cys)$_2$ treatment (9.9 ng Cd/mg animal). Studies made by Vijver et al. (2006) on Cd accumulation on the isopod *Porcellio scaber* showed similar accumulation rates (5.77 mg Cd/kg dry weight) after 32 days of exposure to contaminated soil and food with ^{109}Cd.

In a previous study, Calhoa et al. (2011) showed that isopods fed with Cd(Cys)$_2$ gelatine discs had a higher storage level of Cd in the metal-sensitive fractions. It is hypothesized that Cd in organelles and in heat denatured proteins can be considered potentially vulnerable fractions, and in the present study it is demonstrated that when Cd is predominantly present in these fractions it will lead to increased toxicity. The Cd(Cys)$_2$ treatment induced the highest mortality probably due to higher availability of the ingested Cd that impaired physiological processes. Isopods fed with Cd(NO$_3$)$_2$ stored Cd in metal-rich granules as a detoxification strategy so they were more efficient at detoxifying Cd, reflecting in an increased metal body burden, though being less toxic to the isopod (Calhoa et al. 2011). In this way, Cd within granules is not available for the physiological processes, thus becoming less toxic. This could be also related to metal tolerance and resistance that may be attributed to the ability of isopods to compartmentalize Cd in the hepatopancreas, acting as a detoxification mechanism and contributing to tolerance to high Cd levels.

Parental care by female isopods is a behavioural strategy that contributes to increase fitness of progeny but is energetically costly (Lardies et al. 2004). Terrestrial isopods have an extensive parental care since they carry the eggs and juveniles in a ventral marsupium, that is provisioned with fluid from the mother and allows early development to take place, independently of an external water source (Lardies et al. 2004). The tradeoff between energy losses with reproduction/maternal care and those from detoxification processes due to Cd exposure might justify a shorter isopod pregnancy, to allow shifting energy for detoxification.

Jones and Hopkin (1996) showed that terrestrial isopods (*P. scaber* and *Oniscus asellus*) in metal polluted sites had higher reproductive investment, suggesting that they were able to redirect resources from other functions, like growth, to meet the physiological costs of metal detoxification. Donker et al. (1993) also showed that isopods from a zinc smelter started to reproduce earlier and that metal contamination compromised adult survival, while the reproductive effort was higher when compared to isopods in reference sites. In our experiment, isopods were previously exposed to Cd for 28 days in order to evaluate the effects of Cd accumulation on both the isopod reproduction ability (fecundity) and on the egg fertilization phase.

During the 28 days of the individual exposure test plus the 54 days of the reproduction test all animals lost weight, probably due to the long duration of this test in which a long period of stress was involved. Growth inhibition among isopods is a commonly reported consequence of Cd exposure (Odendaal and Reinecke 2004), and in this study may reflect the increased cost of detoxification of Cd (Zidar et al. 2003). Considering the high rates of mortality observed in this study and although isopods exposed to Cd(NO$_3$)$_2$ accumulated more Cd, higher female mortalities were observed in the Cd(Cys)$_2$ treatments, showing that Cd(Cys)$_2$ induced higher acute toxicity.

In the control, all females became pregnant and delivered mancae within the 54 days of the reproduction test. These values exceed those in the few reports available (e.g. Hornung and Warburg 1994; van Brummelen et al. 1996) with other isopod species but are in accordance with Lemos et al. (2010) that reported the same percentage of successfully pregnancy (100%) for *P. scaber* females.

Control females showed the first signs of pregnancy later than that in the Cd treatments which is in accordance with life-history theory that predicts a selection for early reproduction and increase reproductive effort when stressed (Donker et al. 1993).

Pregnancy duration was higher for the control treatments and decreased in Cd(NO$_3$)$_2$ treatment where juveniles were released 7 days earlier (as mean time). As Cd induced a decrease on the brood period, females exposed to Cd(NO$_3$)$_2$ had a reduced length of time to provide for their developing young. A similar observation was recorded in females of *Armadillidium vulgare* exposed to predatory ants (Castillo and Kight 2005), as well as in females of *Porcellio leavis* under physical stress (5 min of continuous locomotion daily throughout the brooding period) (Kight and Nevo 2004), thus exhibiting significantly shorter brooding periods compared with the controls and releasing juveniles almost 48 h earlier.

The isopod marsupium physically limits the area available for egg attachment, and abnormal egg growth may lead to overcrowding and consequent egg loss or reabsorption, following-on fewer offspring (Lardies et al. 2004). This phenomenon may had occurred in the present experiment in regard to the Cd(NO$_3$)$_2$ treatment. The oocyte resorption takes place when the albumin is stopped from entering the oocyte and no vitellogenin synthesis takes place. This process leaves no visible traces after the resorption has been completed. In the Cd(Cys)$_2$ none of the females were able to carry out a successful pregnancy (most animals died before breeding) and the only survivor did not deliver mancae. Hornung and Warburg (1994) reported for the first time the oosorption in an ovary of an

oniscid isopod (*P. ficulneus*) and Farkas et al. (1996) showed that *P. scaber* under stressful conditions increased disruption of oocytes within the ovarium during vitellogenesis (oosorption), resulting in fewer juveniles.

Upon $Cd(NO_3)_2$ exposure it was observed a decrease in the number of mancae delivered per female with an increase in juveniles' individual weight, which might be a response for their investment in quality rather than in quantity of juveniles. Among crustaceans, fecundity has been demonstrated to be particularly sensitive to dietary metal exposure (Hook and Fisher 2001; Mann and Hyne 2008; Mann et al. 2009). Hence we suggest that females in the $Cd(NO_3)_2$ treatment, balanced the benefits of having smaller clutch decreasing the costs of providing parental care, which implies less energy for maintenance.

Conclusion

Different metal species has been shown in previous studies to affect the AE and compartmentalization of Cd in isopods, strongly suggesting that Cd accumulates in the organism differently depending on the form it is provided in food. In this case the impairment of physiological processes by Cd in *P. dilatatus* induced a decrease in time to reach pregnancy, pregnancy duration, and also the occurrence of inconclusive pregnancies on the $Cd(Cys)_2$ treatment.

$Cd(Cys)_2$ showed to be more toxic than $Cd(NO_3)_2$ in long term exposure, demonstrating that different metal species affects differently female and male survival and that also impacts on the reproduction efforts in isopods.

The species *P. dilatatus* showed to be a good model test organism in this kind of studies where accumulation and possible trophic transfer are evaluated.

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