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Heavy metal exposure reduces hatching success of *Acartia pacifica* resting eggs in the sediment

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Abstract

The potential effects of three heavy metals (Cu, Pb, and Cd) on hatching success of *Acartia pacifica* resting eggs in the sediment of Xiamen Bay were experimentally investigated. The number of *A. pacifica* nauplii hatched from the sediment sharply decreased with the increase of metal concentration and exposure time from 3 to 30 d. An increase of the Cu concentration from 34.8 to 348 mg/kg, reduced the number of hatched nauplii by 46.6%–100%. An increase of the Pb concentration from 75.2 to 752 mg/kg, reduced the number of hatched nauplii by 21.4%–78.9%. An increase of the Cd concentration from 0.68 to 6.8 mg/kg, reduced the number of hatched nauplii by 31.6%–94.7%. The number of nauplii also significantly decreased with the increase of mixed-metal concentration and exposure time in the mixed-metal test. Trimmed Spearman-Karber analysis gave sediment metal 72-h LC_{50} values of 1.25 mmol Cu/kg, 1.73 mmol Pb/kg, and 0.054 mmol Cd/kg, which suggested that Cd was the most toxic to *A. pacifica* resting eggs in the three tested metals. The results indicate that heavy metals with higher concentrations can reduce recruitment of *A. pacifica* nauplii from benthic resting eggs to planktonic population.

Key words: metal; resting egg; hatching success; *Acartia pacifica*

Introduction

Many estuarine and coastal copepods actually spend a portion of their life cycle in the sediment as resting eggs to avoid the harsh environments (Grice and Marcus, 1981; Marcus, 1996). Resting eggs can be extremely abundant (10^6/m^2) in the sediment, and can persist a long period of months to years (Marcus *et al*., 1994; Marcus and Lutz, 1998; Jiang *et al*., 2004), even centuries (Hairston *et al*., 1995). These benthic resting eggs not only enable the persistence of species in the system, but also have great effects on microevolutionary dynamics (Hairston and De Stasio, 1988).

Aquatic environments are usually contaminated with high concentrations of anthropogenically introduced metals (De Groot, 1995). The most abundant metals are Cu, Cd, Ni, Pb, and Zn, and they almost occur as mixtures in sediments. It has been established that sediments often sequester high concentrations of metal mixtures (Tessier and Campbell, 1987). As a result, copepod resting eggs may be at high risk of metal toxicity. However, there is no research that has been carried out to determine the effect of metal pollution on hatching success of copepod resting eggs. Most studies of copepods focus on the survival and fecundity of adults under the metal pollution (Sunda *et al*., 1987; Hagopian-Schlekat *et al*., 2001; Hook and Fisher, 2001, 2002; Bielmyer *et al*., 2006). Gaining insight into the potential effect of pollution on the benthic resting eggs of planktonic copepods is important since the hatching of these eggs is essential for the seasonal restoration of population (Suderman and Marcus, 2002).

The calanoid copepod, *Acartia pacifica*, is the common species in coast waters of China. In Xiamen Bay, *A. pacifica* is the dominate copepod during the period of winter and spring. In summer and autumn, the species disappears from water. The mean viable resting eggs of *A. pacifica* was up to 5.2×10^4/m^2 in the sediment of Xiamen Bay (Wang *et al*., 2005a). Further, ^{210}Pb analyses suggested that the maximum age of viable eggs of *A. pacifica* in the sediment was 20.5 years and the mean egg age was 4.3 years (Jiang *et al*, 2004). These benthic resting eggs enable the persistence of species in the system, and they are important agents of local recolonization. Recent research showed that organochlorine pesticides (Jiang *et al*., 2006) and hydrocarbon contaminations (unpublished data) could reduce recruitment of *A. pacifica* nauplii from benthic resting eggs to planktonic population. The mean concentrations of Cu, Pb, Cd in sediments of Xiamen Bay are 30.7, 77.2 and 0.7 mg/kg, respectively. The concen-

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tration of Cu are high in the sediment of inner bay, while the concentrations of Pb and Cd are high in outer bay. The anthropogenic input of Cu, Pb and Cd are about 30% of the total concentration (Liu *et al*., 1995). The aim of this study is to investigate the potential effects of heavy metals on hatching success of copepod resting eggs in the sediment.

1 Materials and methods

The study site (118°02.363′E; 24°26.778′N) was located approximately in the center of Xiamen Bay, China. Five kilograms of muddy surface sediments (0–5 cm) were sampled with an Ekman Bottom Grab in June 2003. The sediments were immediately transported to the laboratory, where they were randomly mixed to ensure the homogeneous distribution of resting eggs. The sediment background metal concentrations were 34.8, 75.2, and 0.68 mg/kg, respectively, for Cu, Pb, Cd determined by an emission inductively coupled plasma instrument Perkin Elmer Plasma 40.

Sediment toxicity tests were performed for Cu, Cd, and Pb individually. Each test was composed of three treatments plus a control. The metal concentrations of three treatments were twofold, fourfold, and tenfold, individually, than the sediment background metal concentrations. In order to assess the toxicity of mixed-metal, the concentrations of the three metals were increased at the same multiple. Each treatment and control had five replicates. Before every test, a range of sediment concentrations was achieved by adding appropriate aliquots of concentrated metal chloride measured stock solution and stirring sediment enough. The treatments and the control were kept at 10°C. Resting eggs hatch synchronously if they are chilled and then warmed. Hatching success of resting eggs was investigated at three time points over a month period (3, 10, and 30 d after treatment).

Sediment sub-samples of equivalent size were obtained by filling a dish (9.1 cm^3). The mean weight of the content of the dish was 13.1 g, with a SD of 0.4 g (n=10). The contents of sediment were washed through a 200-μm mesh gauze and a 50-μm mesh gauze. The former strains out larger particles in the sediment, *A. pacifica* eggs and similarly sized particles remained on the latter while fine sediment was washed away. The material remaining was incubated in 30 ml beakers filled with sea water (25%) filtered through a 5-μm mesh gauze at room temperature (24–28°C) to allow viable eggs to hatch. The photoperiod was changed to the ambient outdoor light:dark cycle. The contents of beakers were observed every two days. The filtered sea water over the sediment was renewed. Each copepod nauplii recovered from the supernatant was individually transported to a 50-ml beaker. They were offered a mixed diet of *Isochrysis galbana* and *Phaeodactylum tricornutum* until they were identifiable. The number of *A. pacifica* was counted. The incubation lasted for 8 d because most nauplii occurred within 5 d. By thorough aeration, cleaning of the sediment and removal of finer particles, this provides a measure of the maximum hatch in the event of complete resuspension of the eggs in clean seawater (Lindley *et al*., 1998).

Mortality due to heavy metals was calculated by:

$$M = 100(1 - T/C) \tag{1}$$

Where, M is mortality, T is the mean numbers hatched from treatments and C is the mean numbers hatched from the control.

A dose response curve was generated for each metal test and used to calculate an LC_{50} plus 95% confidence interval estimation (CIE) (Hamilton *et al*., 1977).

2 Results

The number of *A. pacifica* nauplii hatched from the sediment in copper test is illustrated in Fig.1a. The one-way ANONA test indicated that the number of nauplii significantly declined with the copper concentration at each time point (Table 1). The number of nauplii emerging from the sediment samples decreased markedly with the increase of exposure time under different copper concentrations (Fig.1a). An increase of the Cu concentration from 34.8 to 348 mg/kg, reduced the number of hatched nauplii by 46.6%–100% (Table 2). Few *A. pacifica* resting eggs could survive under the high concentrations (159.2 and 348 mg/kg).

The number of *A. pacifica* nauplii hatched from the sediment in lead test is illustrated in Fig.1b. One-way ANNOA test showed that there was no significant difference among the treatments at the point of 3 d. At the point of 10 d and 30 d, the number of nauplii hatched from the sediment significantly decreased with the lead concentration (Table 2). The number of nauplii emerging from the sediment samples decreased with the increase of exposure times under different lead concentrations (Fig.1b). An increase of the Pb concentration from 75.2 to 752 mg/kg, reduced the number of hatched nauplii by 21.4%–78.9% (Table 2).

The number of *A. pacifica* nauplii hatched from the sediment in cadmium test is illustrated in Fig.1c. The number of nauplii significantly declined with the cadmium concentration at the points of 10 d and 30 d, however, the difference of nauplii among treatments was not significant at the point of 3 d (Table 2). The number of nauplii emerging from the sediment samples decreased with the increase of exposure time at different level of cadmium concentration (Fig.1c). An increase of the Cd

Table 1 One-way ANONA test for the effects of heavy metals on the hatching success of *Acartia pacifica* resting eggs

	Cu			Pb			Cd			Mixed-metal		
	3 d	10 d	30 d	3 d	10 d	30 d	3 d	10 d	30 d	3 d	10 d	30 d
F-value	25.26	15.16	21.39	2.47	3.60	8.23	2.53	7.16	4.856	11.25	10.01	7.04
P-value	<0.0001	<0.0001	<0.0001	0.112	0.046	0.003	0.107	0.005	0.0195	<0.0001	0.001	0.005

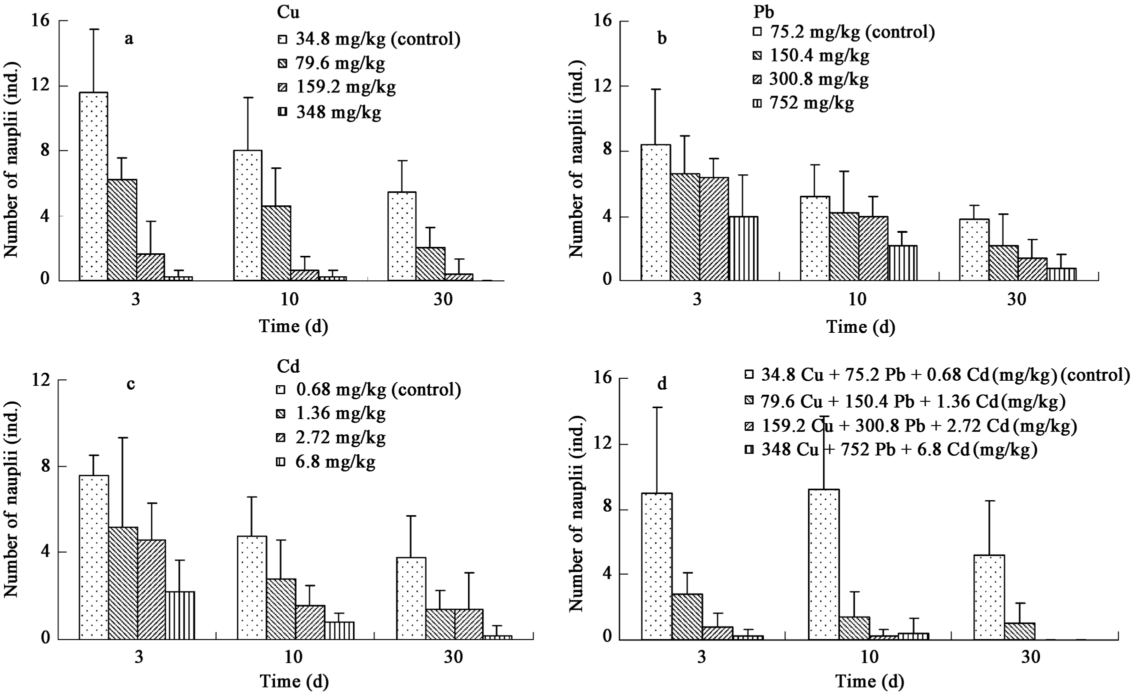

Fig. 1 Numbers of *Acartia pacifica* nauplii hatched from the sediments of copper treatment (a), lead treatment (b), cadmium treatment (c) and mixed-metal treatment (d). Error bars represent one standard deviation from the mean.

concentration from 0.68 to 6.8 mg/kg, reduced the number of hatched nauplii by 31.6%–94.7% (Table 2).

In mixed-metal test, the number of nauplii significantly decreased with the increase of mixed-metal concentration (Table 2). The number of nauplii emerging from the sediment samples decreased with the increase in exposure time at different levels of metal concentration (Fig.1d). An increase of the mixed-metal concentration, reduced the number of hatched nauplii by 68.9%–100% (Table 2).

The 72-h sediment metal LC_{50} values of three metals (Cu, Pb, Cd) for *A. pacifica* resting eggs are presented in Table 3.

3 Discussion

The results of the experiment indicated that the viability of *A. pacifica* resting eggs in the sediment was sensitive to heavy metals. The mortality of resting eggs significantly increased with the increase of metal concentration and exposure time. Two types of resting eggs are commonly described: subitaneous and diapause (Grice and Marcus, 1981; Marcus, 1996). Based upon laboratory studies, quiescent subitaneous eggs are likely to remain viable in the seabed for only a few days to weeks and are a potential source of nauplii for the planktonic population only within a single reproductive season (Marcus and Lutz, 1998), while diapause eggs are important for the year-to-year perpetuation of species that regularly disappear from the water column on a seasonal basis because they can remain viable for months to years (Marcus *et al*., 1994; Hairston *et al*., 1995; Jiang *et al*., 2004). We did not distinguish resting eggs into subitaneous eggs and diapause eggs since classification of these was very difficult when they were mixed in the sediment. Diapause eggs can survive longer than subitaneous eggs when exposed to adverse environmental conditions such as anoxia, sulfide, extreme temperatures, and even digestion, possibly because of the thick chorion, outer membrane that protect the egg, or some chemical contents (Marcus, 1984; Marcus and Lutz, 1998; Wang *et al*., 2005b). Even though *A. pacifica* diapause eggs may be less sensitive than subitaneous eggs under metal pollution, neither of them can survive when continuously exposed

Table 2 Estimated percentage mortality of *Acartia pacifica* resting eggs due to treatment of single-metal and mixed-metal sediment

	Exposure time (d)	Estimated percentage mortality (%)		
		Twofold metal conc.	Fourfold metal conc.	Tenfold metal conc.
Cu	3	46.6	86.2	98.3
	10	42.5	92.5	97.5
	30	63.0	92.6	100
Pb	3	21.4	23.8	57.7
	10	19.2	23.1	57.7
	30	42.1	63.2	78.9
Cd	3	31.5	39.5	71.1
	10	41.7	66.7	83.3
	30	63.2	63.2	94.7
Mixed-metal	3	68.9	91.1	97.8
	10	84.8	97.8	94.5
	30	89.0	100	100

Table 3 The values of 72-h LC_{50} calculated by the Trimmed Spearman-Karber (SK) model

Metal	Trimmed SK LC_{50} (mg metal/kg dry sediment)	95% CIE (mg metal/ kg dry sediment)	Trim (%)
Cu	79.37	72.83–86.49	1.72
Pb	358.41	321.42–399.65	4.76
Cd	3.44	2.64–4.47	28.95

CIE: confidence interval estimation.

to the high level of metal concentration since almost no nauplii emerged under such condition in our experiment. The reproductive success of copepods is vulnerable to contamination through all stages of the life cycle (Tester and Costlow, 1981; Wright *et al*., 1996), but effects on resting eggs in the sediment are particularly important due to accumulation of contaminants including metal, oil (Suderman and Marcus, 2002), organochlorine compounds (Lindley *et al*., 1999; Jiang *et al*., 2006), and long exposure times due to dormancy during periods when the pelagic population is absent or rare. Therefore, contamination could diminish the hatching success of resting eggs in the seabed and inhibit recruitment of nauplii into pelagic population, leading to changes in the zooplankton community. Such changes could result in further ecological changes since copepods form the first vital link in the food chain that leads from the minute algal cells of the phytoplankton up to the large fishes and mammals.

The sediment metal 72-h LC_{50} values for *A. pacifica* resting eggs in Xiamen Bay were 79.37, 358.41, and 3.44 mg/kg, for Cu, Pb, Cd, respectively, which appears that *A. pacifica* resting eggs are least sensitive to Pb in three tested metals. However, when the 72-h LC_{50} values are compared on a molar basis (which is how these elements are encountered in nature), it becomes apparent that Pb and Cu have comparable toxicities to resting eggs. The sediment-metal 72-h LC_{50} values on a molar basis were 1.25 mmol Cu/kg, 1.73 mmol Pb/kg, and 0.054 mmol Cd/kg. Cu and Pb were similar in the molar concentrations that induced a toxic effect, but Cd was the most toxic of three metals to *A. pacifica* resting eggs. The hierarchical sequences for decresasing toxicity to the estuarine meiobenthic copepod adult of Amphiascus tenuiremis were Cd>Cu>Ni>Zn>Pb for sediment metal concentrations (Hagopian-Schlekat *et al*., 2001). From these data it is interesting that the decreasing toxicity of sediment metals (Cd>Cu>Pb) is not only to benthic adult copepod, but to planktonic copepod resting eggs.

Zooplankton are considered as ideal targets for the aquatic toxicity test due to their ecological role, short life cycle and high fecundity. However, most attentions have been paid to the toxicity on adult zooplankton. Many other life stages, such as eggs and larva, have been ignored in toxicity tests. In natural environments, various stages of zooplankton life cycle are explored to the contaminations. Thus, it is wise to evaluate the effects of contaminations on the various zooplankton stages instead of a fraction of life cycle, such as adults. The sediment-metal 96-h LC_{50} values for *Amphiascus tenuiremis* were 3.37 μmol Cd/g, 4.44 μmol Cu/g, and 11.9 μmol Pb/g (Hagopian-Schlekat *et al*., 2001). A comparison of these LC_{50} values to our LC_{50} values shows that planktonic copepod resting eggs are much more sensitive to metals than benthic adult copepod. Thus, to establish robust sediment quality criteria, it is important to consider the frequently ignored effect of these metals on the viability of copepod resting eggs. The hatching of the rotifer *Brachionus rubens* was greatly effected by six toxic compounds (Snell and Persoone, 1989). Raikow *et al*. (2006) showed that biocide SeaKleen was toxic to resting eggs of *Brachionus plicatilis* (a marine rotifer), a freshwater copepod, *Daphnia mendotae* (a freshwater cladoceran), and *Artemia* sp. (a marine brine shrimp). The sensitivity of zooplankton resting egg to toxicity may make it a useful tool for future ecotoxicological studies.

Cadmium, a biological non-essential heavy metal, is considered as an important toxicant among the heavy metals. In natural aquatic environment, cadmium concentration is very low, but is toxic to aquatic organisms at only slightly higher concentrations, which is common especially due to various anthropogenic activities. Some studies show that copepods are extremely sensitive to cadmium (Arnott and Ahsanullah, 1979; Ghosal and Kaviraj, 2001, 2002). Unfortunately, those studies were focused on copepod adults. The present data provided evidence that Cd was the most toxic of the three metals to *A. pacifica* resting eggs in the sediment. However, little is known about the underlying toxic mechanisms of Cd to copepod resting eggs. The possible sketch is that copepod eggs exposed to cadmium-sediment may uptake ions because systems are not fully developed in these eggs. Substances, including Cd, can get into the eggs most likely via facilitated diffusion. Thus cadmium ions are easier to accumulate and toxic to the eggs. Recently, researches on the cadmium toxicity to the eggs/embryos of aquatic organisms may provide some hits for the mechanisms of copepod eggs. Molecular and cellular studies on cadmium-induced toxicity to the embryo of the model organism zebrafish showed that truck abnormalities resulting in distortions of body axis and altered axial curvatures were one of the predominant effects (Kocan, 1996; Chow and Cheng, 2003). Roccheri *et al*. (2004) showed that cadmium chloride induced the synthesis of a specific set of stress proteins in sea urchin embryos continuously exposed to sublethal concentrations of this metal.

We assumed that there was no effect of metal background concentrations on the viability of these eggs since it was very difficult to investigate it, when mortality of resting eggs was estimated. The sediment metal background concentrations were 34.8, 75.2, and 0.68 mg/kg, for Cu, Pb, Cd, respectively, at the study site. The viability of *A. pacifica* resting eggs, especially subitaneous eggs, may be reduced by the sediment metal background concentrations. Thus values of estimated percentage mortality may be underestimated, which may result in the overestimate the sediment metal 72-h LC_{50} values. Another possible error in current experiment came from the identification of *A. pacifica*. The nauplii hatched from the sediment can be identified as *Acartia* sp. The number of the nauplii of *Acartia* sp. was recorded. When the nauplii grew up, most of them were *A. pacifica*. Some of them were *A. spinicauda*, although its number was very low. The difference between the number of nauplii *Acartia* sp. and the number of adult *Acartia spinicauda* was regarded as the number of *A. pacifica*. Thus, there is a possible error that some nauplii of *A. spinicauda* may die before identification, although the number of *A. spinicauda* is very low.

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