

The effects of nickel on the reproductive ability of three different marine copepods

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Abstract Lethal and sublethal toxicity of Nickel (Ni) to three marine copepods *Tigriopus japonicus*, *Apocyclops borneoensis* and *Acartia pacifica* was investigated. The 48-h LC50 values were 17.70, 13.05 and 2.36 mg l⁻¹ Ni, respectively. *A. pacifica* was found to be the most sensitive to Ni in acute exposure tests. In order to assess sublethal effects of Ni on copepod reproduction, the test organisms were exposed to four nominal Ni concentrations 0, 10, 100, 1000 µg l⁻¹ Ni. The results indicated that offspring production of *T. japonicus* and *A. borneoensis* was significantly reduced after exposure to 10 µg l⁻¹ Ni. Whereas egg production and egg hatching success of *A. pacifica* were significantly reduced at 100 and 10 µg l⁻¹ Ni, respectively. Exposure of copepods to the highest Ni concentration caused a severely reduced nauplii production from *T. japonicus*, *A. borneoensis* and *A. pacifica* by 87.8, 56.9 and 65.8%, respectively, and a significantly reduced egg production of *A. pacifica* by 74.4%. These results show that Ni excess in the coastal environment can have detrimental effects on reproduction of copepods.

Keywords Metals · Nickel · Copepods · Reproductive toxicity

Introduction

Coastal and marine ecosystems worldwide are receiving a significant amount of pollutants (including heavy metals),

due to the rapid population growth, development of coastal areas, and agricultural, industrial and municipal discharge (Islam and Tanaka 2004). High metal concentrations have been reported in coastal ecosystems, which may potentially affect the water quality and biotic communities of coastal areas (Dassenakis et al. 1996; Molisani et al. 2004; Wang et al. 2008).

Nickel (Ni) is a metallic element that is ubiquitously present in the environment. Ni is released into the marine environment from the discharge of metal industries, mining, refining, power plants, waste incinerators, and direct leaching from rocks and soil (Fishbein 1981; Denkhau and Salnikow 2002). Also Ni is present in crude oils and, *in the event of an oil spill*, is released into the marine environment (Sadiq 1989). Significant amounts of Ni can be found in water where it is present as dissolved forms and suspended insoluble particles (Denkhau and Salnikow 2002). Ni concentration in surface sea water usually ranges from 15 to 20 µg l⁻¹, and deep-sea water contains 0.1–0.5 µg l⁻¹ Ni (Denkhau and Salnikow 2002).

Copepods are widespread organisms and are major secondary producers in the ocean (Zhong et al. 1989). They play an important ecological role in aquatic communities, because of their position at the base of the food chain (Zhong et al. 1989; Turner 2004). The knowledge of how copepods cope with environmental stressors is important to understand how whole aquatic ecosystems will respond. The toxicity of trace metals to marine invertebrates is related to a threshold concentration of metabolically available metal, which is different from one species to another. Once the amount of metal has passed this threshold concentration, the toxic effects will occur, initially sublethal but eventually lethal (Rainbow 2002). Several studies have been done on the lethal effects of heavy metals to marine copepods (Forget et al. 1998;

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Schleka et al. 2001; Lee et al. 2007). The sublethal effects of heavy metals to marine copepods are also well known in terms of growth, development, egg production, egg hatching rate, number of offspring, cellular and molecular responses (Barka et al. 2001; Hook and Fisher 2001; Raisuddin et al. 2007; Lee et al. 2008). The reproduction of copepods has been found to be a sensitive indicator of sublethal effects of pollutants (Reeve et al. 1977). Moreover, fecundity is important since it directly affects the population density and thus, secondary productivity (Zhong et al. 1989). Previous studies showed that metals (other than Ni) can significantly affected copepod reproduction at environmentally relevant concentrations (Toudal and Riisgard 1987; Hook and Fisher 2001, 2002; Xiaodong et al. 2007). Other than that, published information on the reproductive toxicity of Ni to the marine copepods is limited. A study by Bielmyer et al. (2006) showed that dietary Ni affected the reproduction of the marine copepod *Acartia tonsa*. For this reason, the present study primarily aimed to: (1) determine the 48-h LC50 values of Ni to the copepods *Tigriopus japonicus*, *Apocyclops borneoensis*, and *Acartia pacifica*, (2) assess the sublethal toxicity of Ni on offspring production of *T. japonicus* and *A. borneoensis* (carrying egg sac), and (3) assess the sublethal toxicity of Ni on the free spawning copepod *A. pacifica*, by applying the egg production and egg hatching rate as endpoints.

The selected copepods occupy different ecological habitats and may demonstrate differences in nickel toxicity. The intertidal harpacticoid copepod *T. japonicus* is a benthic copepod and has been commonly used in ecotoxicology and environmental genomics studies as a marine model species (Raisuddin et al. 2007). *A. borneoensis* is a cyclopoida copepod adapted to low salinity (brackish) coastal waters and often lives in the lower part of the water column. While the calanoid copepod *A. pacifica* is abundant in the pelagic marine environment of coastal waters and often lives in the upper part of the water column.

Materials and methods

Copepod collection and maintenance

All species of the genus *Tigriopus* are dominant member of shallow supratidal rock pools (Raisuddin et al. 2007). In most cases the species belong to the genus *Apocyclops* are dominant in coastal brackish waters (Støttrup 2006). Since collection the copepods *T. japonicus* and *A. borneoensis* have been maintained in cultures in our laboratory. *T. japonicus* was maintained at 18–22°C and 24–26 ppt salinity. *A. borneoensis* was maintained at 28–31°C and 18–22 ppt salinity (Suantika 2006). Species of the genus

Acartia are predominant and widespread in estuarine and coastal waters worldwide (Ohno et al. 1996; Moon et al. 2008). *A. pacifica* was collected using 64 µm mesh size plankton net and was maintained at 24–26°C and 24–26 ppt salinity in the lab for at least 48 h before the experiments began.

The copepod cultures were maintained under static-renewal conditions in 0.45 µm Millipore filtered seawater with 7–7.9 mg l⁻¹ dissolve oxygen and a pH ranging from 7.90 to 8.25, under 12D: 12L photoperiod cycle. Copepods were fed a mixed algal diet of *Isochrysis galbana* and *Platymonas subcordiformis*. The algae were cultured in filtered seawater contain f/2 enriched media at 20°C.

Test solutions

Nickel was provided as a chloride salt (NiCl₂·6H₂O) from Guang Fu Chemical Institute, China (≥98.0% pure). Stock solutions were prepared (at concentrations high enough to prevent weighing errors (=1,000 mg l⁻¹) in double distilled water. Ni concentrations were prepared by dilution of the stock solution in 0.45 µm filtered seawater. Reference seawater used in the experiments was obtained 20 km offshore in Xiamen Bay, with the background concentration of total Ni being 3.16 µg l⁻¹.

Acute toxicity test

Semi-static 48-h acute toxicity tests were performed using standard methods (ASTM 2004; Verriopoulos and Dimas 1988) with certain modifications. Experimental conditions were the same as copepod maintenance, except for temperature and salinity. The experimental temperature of *T. japonicus*, *A. borneoensis*, and *A. pacifica* was 20, 30, and 25°C, respectively. The experimental salinity of *T. japonicus*, *A. borneoensis*, and *A. pacifica* was 25, 20, and 25 ppt salinity, respectively. Only active adult copepods (females) were subjected to the acute tests. Copepods were exposed to different Ni concentrations (as nominal concentrations) in a semi-static exposure system bioassays, during which the test solutions are replaced every 24 h. Five to six toxicant Ni concentrations as nominal concentrations and control were used. With the exception of *A. pacifica*, the values of Ni concentrations are range from 0 to 64 mg l⁻¹. Ni concentrations used for the acute tests of *A. pacifica* are range from 0 to 16 mg l⁻¹. Each treatment was run in triplicate, with 10 active individuals for each replicate. Acute tests of *T. japonicus* and *A. borneoensis* were done in six well culture plates, each with 10 ml of solution and five female copepods. Culture plates used in the tests were provided from Corning Incorporated, USA.

A. pacifica acute tests were done in 100 ml glass dishes containing 50 ml of solution and five female copepods. Animals were monitored and dead animals removed at 24 and 48 h. The criteria for mortality are total lack of movement, and lack of response after repeat touches with a probe during 2 min. Because of the shorter time, copepods were not fed during the test period.

Reproductive toxicity test

Experimental conditions were the same as acute toxicity tests. Copepods were fed daily with 5×10^5 cells/ml of the algae *Isochrysis galbana*. Ni concentrations used in the sublethal toxicity test were 0, 10, 100, 1000 $\mu\text{g l}^{-1}$ Ni (as nominal concentrations). Reproductive tests were conducted using a modified ASTM protocol (ASTM 2004). For each test concentration, 20 females of *T. japonicus* and *A. borneoensis*, bearing an egg sac, were individually transferred to a new six well culture plates (Corning, USA), each with 10 ml of solution. These females were cultured for 10 days. The fecundity (offspring production) was assessed as the number of nauplii per female. Every day females were transferred to a new culture plate with fresh solutions and the resulting nauplii were counted under a stereomicroscope (Motic, series SMZ140). For each test concentration, 20 gravid females of *A. pacifica*, were individually transferred to 50 ml glass dishes with 20 ml of solution. These females were cultured for 4 days. The fecundity (egg production) was assessed as the number of egg per female. Every day females were transferred to a new glass dish with fresh solutions and the resulting eggs were counted under a stereomicroscope (Motic, series SMZ140). The test conditions for egg hatching were the same as fecundity tests. To measure egg hatching success of *A. pacifica*, there were four replicates for each treatment, with 24 eggs for each replicate (4×24 eggs). The test was done in 12 well culture plates. Egg hatching success was monitored at 24 h interval for 2 days using a stereomicroscope (Motic, series SMZ140).

Statistical analysis

LC50 values were computed using probit analysis (Finney 1971). Two statistical programs (Microsoft excel 2003 package; SPSS 11.5, Chicago, IL, USA) were used to analyze the data. The data of the reproductive toxicity tests were expressed as mean \pm standard deviation (SD). Statistical analysis was carried out using one-way ANOVA and the Fisher least significant difference test to evaluate whether the means were significantly different among the treatments. The level of significance was accepted as $p < 0.05$.

Table 1 Summary of the 48-h LC50 values and associated 95% confidence intervals of the selected copepods (adult females) exposed to Ni

Species	48-h LC50 (mg l ⁻¹)	95% Confidence intervals
<i>Tigriopus japonicus</i>	17.70	22.84–13.72
<i>Apocyclops borneoensis</i>	13.05	15.97–10.67
<i>Acartia pacifica</i>	2.36	2.68–2.07

Results

Acute toxicity test

The results of the acute toxicity test of copepods exposed to Ni are summarized in Table 1. For all selected copepods, there was no control mortality. In terms of relative sensitivity, *A. pacifica* was the most sensitive to Ni with a lower LC50 value (2.4 mg l⁻¹), whereas *T. japonicus* was least sensitive with higher LC50 value (17.7 mg l⁻¹).

Reproductive toxicity test

During the reproductive toxicity tests of *T. japonicus* and *A. borneoensis*, no mortality was observed. On the other hand, the overall mortality of *A. pacifica* in control and exposures to Ni treatments 10, 100, and 1000 $\mu\text{g l}^{-1}$ Ni was 15, 20, 25, 40%, respectively.

In summary, a 10 day exposure of *T. japonicus* and *A. borneoensis* resulted in a significant reduction of their reproductive output. Offspring production (i.e. number of nauplii produced) by *T. japonicus* and *A. borneoensis* decreased with increasing Ni concentrations and was significantly ($p < 0.05$) reduced at 10 $\mu\text{g l}^{-1}$ Ni (Fig. 1). Nickel (10 $\mu\text{g l}^{-1}$) reduced nauplii production from *T. japonicus* and *A. borneoensis* by 31.7 and 32.3%, respectively.

Egg production of *A. pacifica* also decreased with increasing Ni concentrations but was significantly ($p < 0.05$) reduced at 100 $\mu\text{g l}^{-1}$ Ni (Fig. 2).

The egg hatching success of *A. pacifica* showed a reduction with increasing Ni concentrations, dropping from $79.2 \pm 0.3\%$ in the control to $27.1 \pm 0.1\%$ at the highest Ni concentration (1,000 $\mu\text{g l}^{-1}$), and was significantly ($p < 0.05$) reduced at 10 $\mu\text{g l}^{-1}$ Ni (Fig. 3). Compare to control, egg hatching success of copepods exposed to Ni treatments 10, 100, and 1000 $\mu\text{g l}^{-1}$ Ni was reduced by 19.5, 40.8, and 65.8%, respectively.

Discussion

Ni is a dietary requirement to some animals, although it is toxic in higher concentrations (Zarogian and Johnson

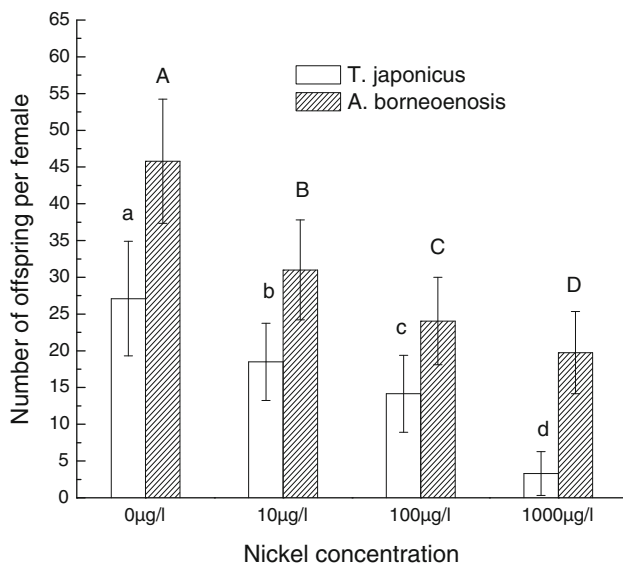


Fig. 1 Effect of Ni on offspring production of *Tigriopus japonicus* and *Apocyclops borneoensis*. Data are described as mean \pm standard deviation (n = 20). Different letters indicate a significant difference among different Ni concentrations at *p* levels < 0.05

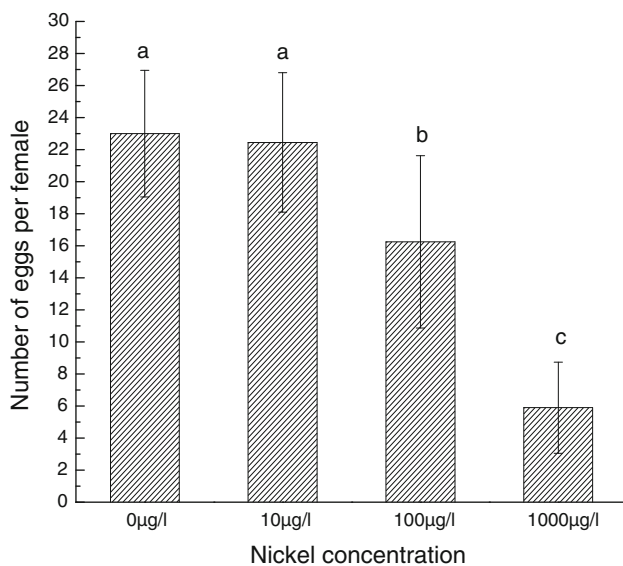


Fig. 2 Effect of Ni on egg production of *Acartia pacifica*. Data are described as mean \pm standard deviation (n = 20). Different letters indicate a significant difference among different Ni concentrations at *p* levels < 0.05

1984; Denkhaus and Salnikow 2002). Consequently, lack or excess of Ni can have adverse biological effects. For this reason, we assessed Ni toxicity to three different marine copepods.

Previous estimations of LC50 values for Ni on marine copepods are relatively scarce. Eisler and Hennekey (1977) assessed the acute toxicity of Cd, Cr, Hg, Ni, and Zn to some estuarine macrofauna. Their 96-h LC50 values of Ni

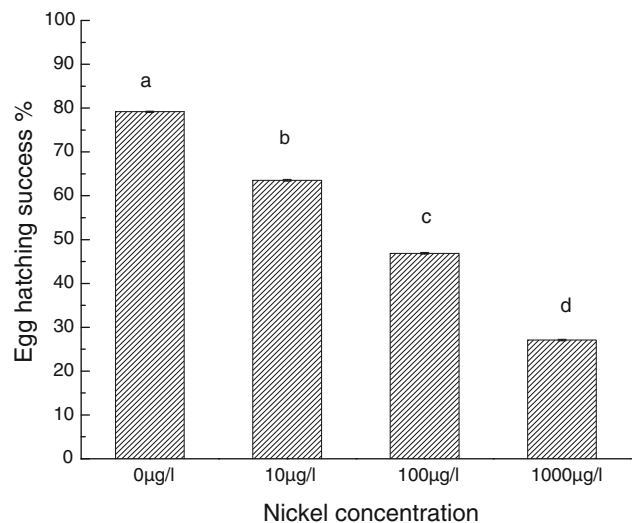


Fig. 3 Effect of Ni on egg hatching success of *Acartia pacifica*. All points are mean of four replicates, 24 eggs per replicate, \pm standard deviation. Different letters indicate a significant difference among different Ni concentrations at *p* levels < 0.05

were higher compared to 48-h LC50 of the selected copepods, suggesting that copepods are good indicators of minimal lethal concentrations of Ni. Barka et al. (2001) reported 96-h LC50 value of $206.9 \mu\text{g l}^{-1}$ Ni for the copepod *Tigriopus brevicornis*. Exact comparisons are difficult because of difference in LC50 time interval. But our result of Ni 48-h LC50 value for *T. japonicus* (17.7 mg l^{-1}) was high compared to 96-h LC50 of *T. brevicornis* ($206.9 \mu\text{g l}^{-1}$). A study by Verriopoulos and Dimas (1988) determining acute toxicity of some metals to the marine copepod *Tisbe holothuriae* in synthetic seawater, found a 48-h LC50 value for Ni of 2.6 mg l^{-1} . The later result shows that *T. holothuriae* was more sensitive to Ni than *T. japonicus* (17.7 mg l^{-1}) and *A. borneoensis* (13.1 mg l^{-1}), but not as sensitive as *A. pacifica* (2.4 mg l^{-1}).

Our results indicated that Ni significantly ($p < 0.05$) affected offspring production of *T. japonicus* and *A. borneoensis* and egg production and egg hatching success of *A. pacifica* at environmentally relevant concentrations. These results are consistent with previous findings that copepod reproductive output is significantly impacted when exposed to metals. For instance, Hook and Fisher (2001) demonstrated that dietary Hg and Cd reduced egg production and hatching rate of *Acartia tonsa* and *Acartia hudsonica*. Hook and Fisher (2002) found that egg production of *Acartia tonsa* and *Acartia hudsonica* was reduced after exposure to dietary Ag, Cd, Hg, Mn, and Zn. Sunda et al. (1987) stated that the egg laying rate of *Acartia tonsa* was sensitive to Zn and Cu. Toudal and Riisgard (1987) noticed that Cd significantly affected egg production of *Acartia tonsa*. Regarding egg hatching

success of *A. pacifica*, the number of nauplii hatched decreased with increasing Ni concentrations at environmentally relevant levels, implying that the process of yolk accumulation (vitellogenesis) was affected (Hook and Fisher 2001). These results are in agreement with a previous study on the effects of three metals (Cu, Pb and Cd) on the hatching success of *A. pacifica* resting eggs in a sediment (Xiaodong et al. 2007). As mentioned before, Ni significantly affected the reproductive output of *T. japonicus*. Correspondingly Kwok et al. (2009) found that the reproductive output of Cu exposed *T. japonicus* decreased in a dose-dependent manner. Medina et al. (2008) found that nauplii production of *T. angulatus* was significantly different from the control at Cu exposures to 103 and 180 $\mu\text{g l}^{-1}$ Cu. Conversely, a study by Lee et al. (2008) found that Cu, As^{3+} , and As^{5+} , had no significant effects on offspring production of *T. japonicus* after 10 days exposure at the concentrations tested (0.1, 1, 10, and 100 $\mu\text{g l}^{-1}$). With the exception of salinity, they used natural seawater and the same experimental conditions. Taken together, *T. japonicus* was more sensitive to Ni than Cu, As^{3+} and As^{5+} .

Previous studies showed that Ni affected the reproduction of fresh and marine water animals (Petrich and Reish 1979; Pane et al. 2004; Evens et al. 2009). Our results showed that exposure of copepods to 10 $\mu\text{g l}^{-1}$ Ni significantly ($p < 0.05$) reduced offspring production of *T. japonicus* and *A. borneoensis*, and egg hatching success of *A. pacifica*. In contrast, egg production of *A. pacifica* was significantly reduced at 100 $\mu\text{g l}^{-1}$ Ni. Egg production of *A. pacifica* was not more sensitive to nickel exposure than the egg hatching process. Thus, egg hatching success is a determining factor for *A. pacifica* population growth. These results are in agreement with a previous study on the effects of dietary metal exposure (Ag, Zn, Cu and Ni) on reproduction of *Acartia tonsa* (Bielmyer et al. 2006). They found that no statistically significant differences in reproduction were observed expressed as total number of eggs after Ag exposure. However, exposure to Zn, Cu and Ni significantly reduced the total number of nauplii (egg hatching) at the concentrations tested.

A comparison of the effects of Ni on the fecundity of copepods occupying three different ecological habitats showed that all Ni concentrations significantly ($p < 0.05$) reduced the number of nauplii hatched of the three selected copepods. Exposure of copepods to 10 $\mu\text{g l}^{-1}$ Ni, reduced the number of hatched nauplii of *T. japonicus*, *A. borneoensis* and *A. pacifica* by 31.7, 32.3 and 19.7% respectively. The fecundity of the pelagic (often live in the upper part of the water column) and free spawning copepod *A. pacifica* was less sensitive to the lower Ni concentration than *T. japonicus* (benthic copepod carrying egg sacs) and *A. borneoensis* (often live in the lower part of the water

column and carrying egg sacs). Sensitivity differences between species can be due to different Ni uptake rates, pathway, and metabolism in copepods (Rainbow 2002).

The present study demonstrated that Ni excess in the environment can have significant and detrimental effects on reproduction of the three selected copepods. However, concentrations in estuaries and streams generally range from 1 to 75 $\mu\text{g l}^{-1}$ (Eisler 1998) and from 500 to 2,000 $\mu\text{g l}^{-1}$ in natural waters near industrial sites (Chau and Kulikovskiy-Cordeiro 1995). Thus, at the concentrations tested the population growth of the three species will be affected. To better simulate conditions in contaminated environments, future studies should examine Ni concentration in copepod tissues, egg development, and full life-cycle test following long-term exposure to Ni. Further, Ni metabolism and mechanism of toxicity to copepods should be investigated.

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