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Differences in lethal response between male and female calanoid copepods and life cycle traits to cadmium toxicity

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Abstract This study determined the effect of cadmium (Cd) toxicity comparatively on two copepods, *Eurytemora affinis* (Poppe 1880) from a temperate region (Seine Estuary, France) and *Pseudodiaptomus annandalei* (Sewell 1919) from a subtropical region (Danshuei Estuary, Taiwan), according to their sex and reproductive stages. In addition, the effect of Cd to their life cycle traits was quantified. In the first experiment, both copepod sexes were exposed to 40, 80, 150, 220, and 360 μg/L of Cd and a control cultured in salinity 15, except that the temperature was 18 °C for *E. affinis* and 26 °C for *P. annandalei*. This allowed calculating median lethal concentration (LC50) of Cd after 96 h. This was 120.6 μg/L Cd for *P. annandalei* males which were almost twice as sensitive as *P. annandalei* females (LC50 = 239.5 μg/L Cd). For *E. affinis* females, the LC50 was 90.04 μg/L Cd, reflecting a 1.4 times higher sensitivity of females than of males (LC50 = 127.75 μg/L Cd). The males of both species were similarly sensitive; however, the *E. affinis* females were 2.7 times more sensitive than the *P. annandalei* females. We also compared the sensitivity of ovigerous females (OVF) and non-ovigerous females (NOF) of both species to Cd. Mortality was higher in NOF than in OVF of both copepod species in both the control and the 40 μg/L Cd treatment. Finally, the total population, fecundity and female morphology of both copepod species were estimated after exposing one generation cycle (nauplius to adult) to 40 μg/L Cd (for *E. affinis*) and 160 μg/L Cd (for *P. annandalei*). A significant decrease in cohort production, survival and clutch size but no significant difference in the prosome length of both copepod species exposed to Cd were detected. The ratio of OVF:NOF was high in both copepod species exposed to Cd. Cd toxicity did not significantly affect the M:F sex ratio and % OVF of *E. affinis*. However, the effect of Cd toxicity in *P. annandalei* was significant in the M:F sex ratio and was in favor of females and their reproductive activities due to an increase in % OVF. Moreover, there was a significant decrease in total production of *P. annandalei* due to high mortality in their nauplii and copepodid developmental stages. Toxicity to Cd appears to be affected by multiple factors including sex, reproductive life stage and species. The ecological implication of Cd toxicity on *E. affinis* and *P. annandalei* copepod ecology is more related to a skewed sex ratio, low egg production, reduced hatchability and reduced survival that affects the recruitment potential of the copepod nauplii resulting in a decreasing copepod population. Mortality, reproduction and population growth of model species may provide important bio-indicators for environmental risk assessment.

Keywords Toxicity · Cadmium · Copepods · Sex · Life cycle

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Introduction

Metals such as copper, cadmium, lead, or zinc are prevalent in marine, brackish, and freshwater environments. Some metals, such as copper and zinc, can be functionally essential at low concentrations; however, these metals can become toxic when they exceed a particular threshold. The rate of metal concentration in the environment and the level of toxicity to an organism are affected by the geochemical behavior of metals, the physiology and condition of the organism, chemical speciation, and the presence of other toxicants or environmental conditions (Ansari et al. 2004).

Heavy metals cannot be broken down in the environment, but their transfer, bioavailability, or toxicity can be affected by conditions such as low pH, low hardness, low suspended matter level, high redox potential, low salinity (Cole et al. 1999), and temperature (Boeckman and Bidwell 2006; Khan et al. 2006, 2007).

Cadmium is highly toxic to humans; it is widely distributed in the Earth's crust at an average concentration of approximately 0.1 mg/kg, and it enters the aquatic environment through geological or anthropogenic activities (FAO/BOBP 1999; Tchounwou et al. 2012). Cadmium appears to have no biological benefits to aquatic animals, and an acute or chronic exposure can result in lethal and sublethal effects (on their behavior, immune and endocrine systems, development, growth, and reproduction).

Copepods are essential trophic links among higher aquatic organisms; thus, they can be a major factor in the bioaccumulation and bio-magnification processes of toxic pollutants in the aquatic food web (Fisher et al. 2000; Watras et al. 1998). The productivity and abundance of copepods face threats from environmental toxic pollutants. However, the toxicity of metals to copepods varies because the rates of uptake, excretion, and detoxification are inter- and intra-species as well as metal specific (Fang et al. 2014; Hsiao et al. 2010; Luoma and Rainbow 2005; Ritterhoff and Zauke 1997) and can depend on their life stage and/or sex.

One of the prevalent estuarine zooplanktons is the calanoid copepod *Eurytemora affinis* (Poppe 1880) which is a dominant species in the zooplankton community of temperate regions, Northern European and North American estuaries (David et al. 2005; Quintin 2014; Winkler et al. 2011). Another calanoid copepod of interest is *Pseudodiaptomus annandalei* (Sewell 1919), a dominant euryhaline species found perennially in coastal and estuarine ecosystems in the tropical and subtropical Indo-Pacific region. *P. annandalei* is a relatively abundant and dominant brackish water species found in the Danshui estuary in northern Taiwan; this species is also abundantly present in aquaculture ponds, where they are essential live feed organisms for fish larvae (Chen et al. 2006; Doi et al. 1997; Golez et al. 2004; Hwang et al. 2010; Liao et al. 2001; Sarkar et al. 1985). *E. affinis* often exhibits different traits from distinct populations or environments. These differences are believed to be genetic, resulting from an evolutional history, to phenotypic plasticity or from acclimation to culture conditions (Souissi et al. 2016), and this is reflected in their different levels of responses or sensitivity to toxic pollutants.

E. affinis and *P. annandalei* share a number of similarities such as their ability to be cultured in laboratory conditions; they both exhibit sexual dimorphism and optimum culture conditions including culture medium of salinity 15 for both species and a temperature range of 10–18 and 25–30 °C for *E. affinis* and *P. annandalei* respectively. Both species have similar mating behavior, where the male seizes a female by her posterior abdomen using his geniculate right antennule (Dur et al. 2011; Katona 1975). Their females are both egg carriers; *E. affinis* female have a single large egg sac, the number of egg production varies but can reach more than 30 eggs/female (in optimum conditions) (Devreker et al. 2009). Whereas, *P. annandalei* have a pair of oval-shaped egg sacs situated on each side of the female urosome. Each sac can contain about 4–14 eggs (Golez et al. 2004). Both copepod species can produce a second pair of viable clutches with high hatching success even after a single mating, but not more than a second clutch (Beyrend-Dur et al. 2011; Devreker et al. 2009). Clutch size varies widely with density, temperature and age of female (Devreker et al. 2009; Su et al. 2005). For example, maximum density of all stages of *P. annandalei* culture is around five individuals/mL (Su et al. 2005). Both species have three developmental phases, the naupliar stages (N1–N6), the copepodid stages (C1–C5) and the adult stage (C6). Development time varies in both species and usually depends on environmental conditions (Beyrend-Dur et al. 2011; Devreker et al. 2007). *P. annandalei* have higher production rate than *E. affinis* because of their shorter embryonic development and latency time (Beyrend-Dur et al. 2011; Su et al. 2005). The female morphology of both copepod species is structurally similar, the female body size in both species is larger than their males but both species have very similar body size.

Laboratory analyses of the lethal concentrations (LC) of heavy metals in aquatic organisms have revealed their quantitative responses and sensitivity levels, providing guidelines for the standardization and regulation of heavy metal influx into the aquatic environment. Several studies have highlighted the critical ecological effect of lethal and sublethal concentrations of toxic metals. However, very few studies have attempted to elucidate the responses of copepods to toxic metals based on their life stage or reproductive state. Sex can be a factor in how copepods respond to stressors (Dipinto et al. 1993; Sornom et al. 2010; Sroda et al. 2011). Michalec et al. (2013) examined the effect of sublethal concentrations of three water pollutants, including cadmium, on the swimming behavior of *E. affinis* and reported an increase in the swimming speed and activities of adult copepods, as an escape mechanism from the pollutants, and this hyperactivity was higher in males than in females. Male and female copepods may respond differently when exposed to unfavorable conditions. Therefore, investigating sex differences in copepods' responses to environmental pollutants is crucial to understand changes in their population. The sensitivity of copepods to heavy metals, particularly cadmium, varies with species (Marcus 2004) and their reproductive life stage (Hsiao and Fang 2013). The literature regarding the sex-specific sensitivity to heavy metals in both copepods, *P. annandalei* (Chen 2011; Jiang et al. 2012, 2013) and *E. affinis* (Cripe and Cripe 1990; Hall et al. 1995; Sullivan et al. 1983) is scant. Moreover, these studies have focused on the late developmental stages of copepods with little or no consideration of the sex-based sensitivity to heavy metal toxicity. Therefore, the current study investigated the sex-specific and female reproductive stage and life cycle trait responses to cadmium toxicity of *E. affinis* and *P. annandalei*.

Materials and methods

Copepod cultures

E affinis (Poppe 1880) used in this study was cultured at the LOG-Marine Station of Wimereux, France. The strain of *E. affinis* was first collected and isolated from the Seine estuary at Tancarville Bridge, France, and then acclimated in the laboratory following the protocol described by Souissi et al. (2016). *P. annandalei* (Sewell 1919) used in this study was cultured in the laboratory of the Institute of Marine Biology, National Taiwan Ocean University, Taiwan. The strain was initially collected from the coastal brackish ponds in Tungkang, Southern Taiwan, and maintained in the laboratory. The *E. affinis* and *P. annandalei* strains were maintained in 2-L beakers in a biological incubator at 18 and 26 °C, respectively, under a light regime of 12-h light and 12-h dark. We used the protocol described by Souissi et al. (2010, 2016) to maintain the copepods for generations. For the large copepod culture required for the experiment, the copepods were provided from large-scale culture systems. The seawater used for the *E. affinis* culture was pumped from the English Channel near the Wimereux marine station and filtered several times up to 1-µm. The seawater used for the *P. annandalei* culture was pumped from the ocean near the National Taiwan Ocean University (Northern Taiwan) and filtered up to 1-µm. *E. affinis* copepods were fed a microalgae mixture of *Isochrysis galbana* and *Rhodomonas baltica* cultivated in the Conway medium, and *P. annandalei* copepods were fed a mixture of *I. galbana* and *Nannochloropsis oculata* cultivated in the Conway medium, following the method described by Sadovskaya et al. (2014). The water used for both the large-stock copepod species culture and the experiment was diluted with distilled water to obtain salinity 15.

Differences in the responses of male and female copepod species to cadmium

The 50% LC (LC50) of cadmium was determined for the males and females of *E. affinis* and *P. annandalei*. Various concentrations of cadmium (40, 80, 150, 220, and 360 µg/L) were prepared in 100-mL beakers for a total of six treatments including the control (0 µg/L Cd). All treatments were prepared in triplicate. After preparing the media, 25 males and 25 females were identified under a stereomicroscope and separated into their respective prelabeled beakers. The beakers were covered with an aluminum foil and kept in the incubator at 18 °C (*E. affinis*) and 26 °C (*P. annandalei*) under a light regime of 12 h light and 12 h dark with no feeding and no aeration during the 96 h exposure. Dead copepods were identified under a stereomicroscope every 24 h; they were identified as those that were not moving for few seconds and by further touching them gently with a very fine and tiny glass tip to stimulate movement. If there was still no movement, the copepods were considered dead, recorded, and discarded.

Toxicity difference between the reproductive stages of female copepod species to cadmium

Ovigerous female (OVF) and nonovigerous female (NOF) *E. affinis* and *P. annandalei* copepods were separately tested with or without exposure to the sublethal cadmium concentration (40 µg/L). Similar environmental conditions were provided, and mortality observations were made as described in Experiment 1.

Toxicity effect of cadmium on the life cycle traits of *E. affinis* and *P. annandalei* copepods

Both species were exposed to cadmium concentrations lower than their respective female 96 h LC50 values from Table 1. The treatment culture medium included 40 µg/L (for *E. affinis*) and 160 µg/L (for *P. annandalei*) of cadmium and the control without cadmium in triplicates. Ovigerous ($n = 20$) females of both species were randomly sorted from a batch culture and transferred to a 200 µm mesh false bottom suspended in 2-liter beakers containing the aerated culture medium. *E. affinis* and *P. annandalei* were kept in their temperature-controlled environments. We used the same protocol as described in Souissi et al. (2010, 2016).

Table 1 Acute lethal concentration (LC) of cadmium (μg/L) for subtropical (*Pseudodiaptomus annandalei*) and temperate (*Eurytemora affinis*) species of copepod after 48, 72, and 96 h exposure, 95% confidence interval (CI), ($P < 0.05$)

	Male			Female		
	LC10 (95% CI)	LC20 (95% CI)	LC50 (95% CI)	LC10 (95% CI)	LC20 (95% CI)	LC50 (95% CI)
P. annandalei						
72 h	62.30 (60.29–64.31)	100.71 (99.11–102.30)	252.2 (250.82–253.59)	90.68 (89.06–92.30)	158.61 (157.18–160.03)	461.86 (460.03–463.7)
96 h	31.90 (30.09–33.70)	50.36 (48.80–51.93)	120.59 (119.30–121.89)	56.83 (55.18–58.49)	93.14 (91.72–94.56)	239.46 (238.08–240.85)
E. affinis						
48 h	–	–	–	–	–	365.54 (361.24–369.84)
72 h	61.76 (60.09–63.44)	104.04 (102.62–105.47)	281.94 (280.48–283.4)	29.15 (27.01–31.28)	52.19 (50.47–53.9)	158.88 (157.51–160.25)
96 h	41.22 (39.68–42.76)	60.79 (59.39–62.18)	127.75 (126.5–129)	21.12 (19.1–23.14)	34.75 (33.04–36.46)	90.04 (88.7–91.38)

Females were incubated until nauplii were hatching. Later the females were removed and their nauplii were allowed to develop to adults. They were fed every 2 days with 10 mL of red microalgae *Rhodomonas sp*. and *Isochysis galbana* (Tiso) (~5000 cells mL^{-1}) at its exponential growth phase. The culture water was changed once the nauplii reached copepodid stage. Algae (10 mL) were centrifuged and the supernatant was discarded and re-suspended with the respective culture medium. When individuals reached the adult stage and ovigerous females were observed, the whole population was collected and preserved in alcohol.

Life cycle traits and morphological measurements

Copepod population density and female morphology Samples were counted under a stereo microscope according to their developmental stages, as: copepodids, males, females (non-ovigerous) and ovigerous females. At least 20 ovigerous females from each treatment were sorted randomly from the fixed samples. Photos of the females were taken with an inverted microscope (OLYMPUS IX71, Tokyo, Japan), then image analysis software package Image J 1.41 (Rasband 1997–2014) was used to measure the prosome length as described in Souissi et al. (2010). Theoretical production (ThP) was calculated using the following equation:

$$\text{ThP} = \overline{CS}_{F0} \times NFemOv_{F0},$$

where CS_{F0} is the average clutch size in the stock culture (F0) and $NFemOv_{F0}$ is the number of females incubated in each beaker ($n = 20$).

Sex ratio and percentage (%) of ovigerous females

Sex ratio (males/females) and the percentages of ovigerous females (%OVF) (100 × ovigerous females/'non-ovigerous females + ovigerous females') were calculated from each treatment.

Fecundity

The fecundity of females were estimated by counting the eggs in each female's ovisac (s) (clutch size) of the same prosome size measured females from the fixed sample as in Souissi et al. (2016).

Survival rate

The survival of individuals in a generation cycle *F1* (S_1) was calculated using the following equation (as in Souissi et al. 2016):

$$S_1 = 100 \times \frac{Ntot_{F1}}{\overline{CS}_{F0} \times NFemOv_{F0}},$$

where $Ntot_{F1}$is the total number of individuals produced from generation *F1*, $\overline{CS}_{F0}$ and $NFemOv_{F0}$ are the mean clutch size and initial number of ovigerous females incubated to start generation *F1* (fixed at 20 ovigerous females).

Statistical analyses

Dead copepods were recorded as percent mortality = (no. of dead copepods/25) × 100. Probit analysis was performed using Microsoft Excel 2013, and the LC50 was calculated as described by Tlili et al. (2016). Mortality was corrected for probit analysis by using Abbot's formula. Analysis of covariance (ANCOVA) was used to compare the coefficients of male and female regression lines of both species and the sensitivity of both sexes was compared by estimating the common slope. Data are expressed as the mean ± standard deviation (SD). Significant differences were analyzed using one-way analysis of variance followed by Tukey's test. $P < 0.05$ was considered significant. SPSS, v.18.0 (SPSS Inc., Chicago, IL, USA), was used for the statistical analysis.

In the life cycle experiment, a two-sample F and T-test was used systematically to evaluate the statistical significance ($P < 0.05$) of the mean difference between all experimental treatments and species. The objective of the study was to compare the effect of Cd on the life cycle traits within both species (*E. affinis* control and Cd; *P. annandalei* control and Cd) and between both species (*E. affinis* control and *P. annandalei* control; *E. affinis* Cd and *P. annandalei* Cd). Theoretical production (ThP) was used to compare the total production (TP) (control and Cd) within and between both species.

Results

Differences in the male and female copepod responses to cadmium

Figure 1 shows the 96 h concentration–mortality regression lines for both sexes of *E. affinis* and *P. annandalei*. Mortality increased with an increase in the cadmium concentration. Mortality after 24 h Cd exposure was less than 30% for *E. affinis*, and *P. annandalei* had even lower mortality (<5% in males; none in females). Furthermore, 100% mortality was not observed at any Cd concentration tested (40–360 µg/L) after 96 h for either species.

Lethal concentrations

Mortality was probit transformed, and lethal concentration (LC) values extrapolated from regression lines are listed in Table 1. LC10, LC20, and LC50 values and their confidence intervals for males and females of both copepod species are shown in Table 1. Because of the slow response of the copepod species to Cd, the LCs and a reasonable confidence interval (CI) could not be calculated after 24–48 h for *P. annandalei* and *E. affinis*, except for the LC50 of female *E. affinis* after 48 h (Table 1), because there was no significant mortality response to Cd exposure.

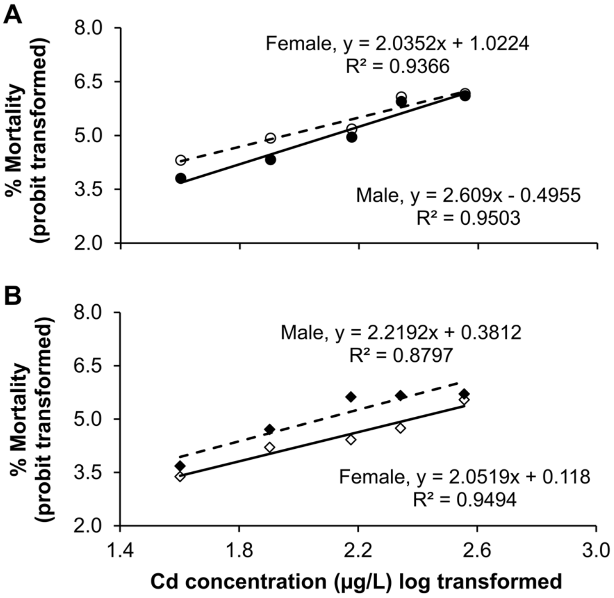

Fig. 1 Ninety-six hour concentration–mortality curves for male and female copepods exposed to cadmium. **a** *Eurytemora affinis* **b** *Pseudodiaptomus annandalei*. Symbols (closed circles (●) and diamonds (◆) for males, open circles (○) and diamonds (◇) for females) are experimental regression lines

The sensitivity of both species' sexes increased with exposure time and was significantly different ($P < 0.05$; Fig. 2). After 96 h, *P. annandalei* males (LC50 = 120.6 µg/L Cd, 95% CI = 119.3–121.9) were about twice as sensitive as *P. annandalei* females (LC50 = 239.5 µg/L Cd, CI = 238.1–240.8), and *E. affinis* females (LC50 = 90.0 µg/L Cd, 95% CI = 88.7–91.4) were approximately 1.4 times more sensitive than *E. affinis* males (LC50 = 127.75 µg/L Cd, 95% CI = 126.5–129). Although *E. affinis* males (LC50 = 127.7 µg/L Cd) and *P. annandalei* males (LC50 = 120.6 µg/L Cd) had similar sensitivity, *E. affinis* females (LC50 = 90.0 µg/L Cd) were 2.7 times more sensitive than *P. annandalei* females (LC50 = 239.5 µg/L Cd; Table 1).

Gender-specific responses within copepod species

Eurytemora affinis The females showed significantly different responses to Cd after 48 and 72 h, whereas the males only showed a significantly different response after 72 h ($P < 0.05$). The sensitivity between the males and females was also significantly different ($P < 0.05$). After 96 h, both sexes

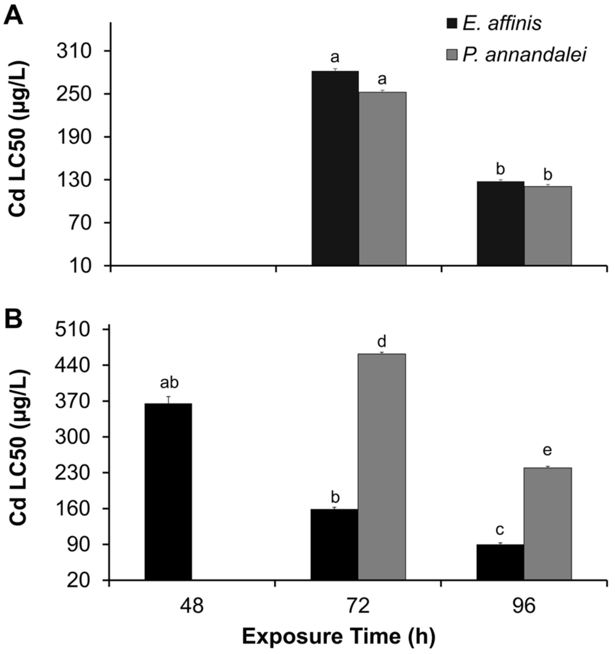

Fig. 2 Fifty-percent lethal concentration (LC50) of cadmium for temperate (*Eurytemora affinis*) and subtropical (*Pseudodiaptomus annandalei*) copepods after 96 h of exposure; **a** Male, **b** Female. Values are LC50 ± SD ($n = 3$)

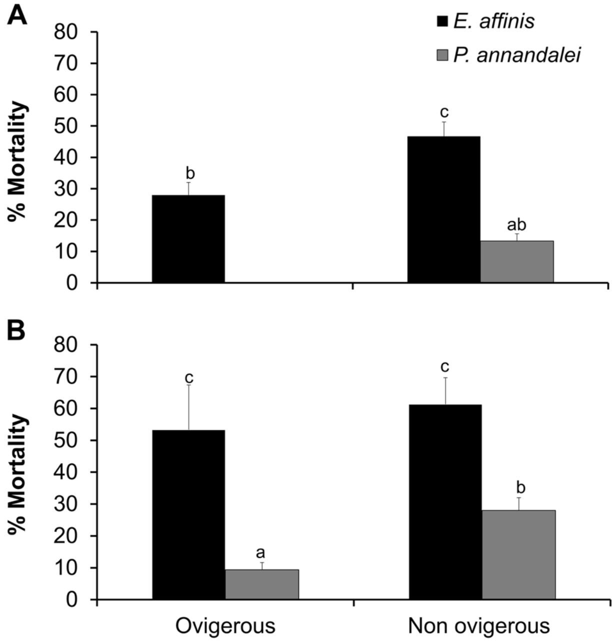

Fig. 3 Ninety-six hour percent mortality of ovigerous and non-ovigerous female copepods of two species, *Eurytemora affinis* (black) (18 °C) and *Pseudodiaptomus annandalei* (grey), (26 °C), with or without exposure to 40 µg/L cadmium; **a** Control, **b** 40 µg/L Cd. Values are mean ± SD ($n = 3$)

separately showed significant ($P < 0.05$) responses to Cd; however, the sensitivity between males and females was not significantly different ($P > 0.05$).

Pseudodiaptomus annandalei After 48 h, the response of the individual sex to Cd and the sensitivity difference between these sexes were not statistically significant ($P > 0.05$). After 72 and 96 h, the responses of the individual sex to Cd were significantly different ($P < 0.05$); however, the difference between their sensitivity was not significant ($P > 0.05$) after 72 h but significant ($P < 0.05$) after 96 h.

Sex-specific responses between copepod species

E. affinis male and female responses were compared with *P. annandalei* male and female responses to Cd exposure by comparing their coefficient of regression lines after estimating the common slopes. The difference between the sensitivity of *E. affinis* and *P. annandalei* males to Cd was not significant ($P > 0.05$) after 48–96 h. However, *E. affinis* females were significantly ($P < 0.05$) more sensitive to Cd than *P. annandalei* females, as shown by the LC50 values in Table 1. Overall, *E. affinis* copepods appeared to be more sensitive to Cd than *P. annandalei*; however, although the males of both species did not significantly differ ($P > 0.05$) in sensitivity after 96 h, the females differed significantly ($P < 0.05$) in sensitivity after 72 and 96 h.

Toxicity differences to cadmium between female copepods at different reproductive stages

After 96 h, we observed higher mortality in the NOFs than in the OFs for both species in both the control and 40 µg/L Cd treatment group. The OF mortality of *E. affinis* was significantly higher ($P < 0.05$) than that of *P. annandalei* both in the control and 40 µg/L Cd treatment group. Similarly, the NOF mortality of *E. affinis* was significantly higher ($P < 0.05$) than that of *P. annandalei* both in the control and in 40 µg/L Cd treatment group (Fig. 3a, b). These results showed higher survival in *P. annandalei* females than in *E. affinis* females.

Toxicity effects of cadmium on the life cycle traits of *E. affinis* and *P. annandalei* copepods

Copepod population density, total production and female size

Figure 4 shows a decreasing trend in the number of individual copepods produced and the total production (TP) in both species. Copepodids and males of both species and females (non-ovigerous) of *P. annandalei* exposed to Cd were significantly lower ($P < 0.05$) than those in the control group. Ovigerous females of both species exposed to Cd and those in the control group were not significantly

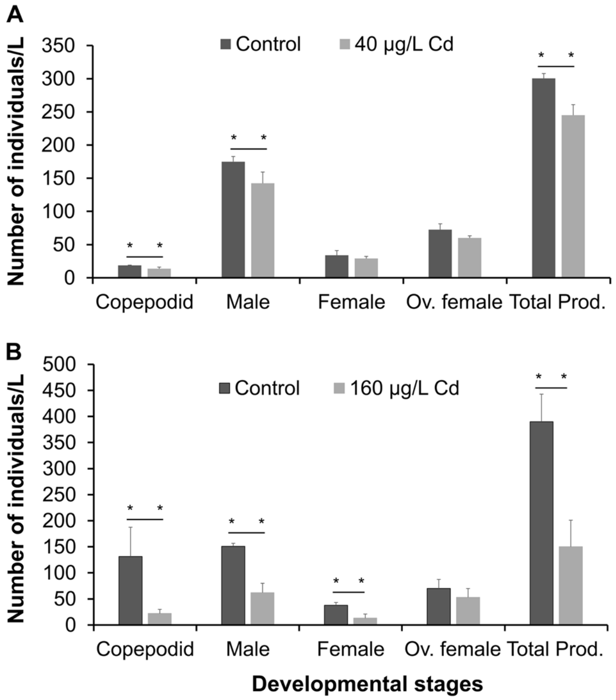

Fig. 4 Effect of cadmium on the number of individual population; copepodid, male, female (non-ovigerous), ovigerous female and total production of *Eurytemora affinis* **a** (18 °C) and *Pseudodiaptomus annandalei* **b**, (26 °C). Values are mean ± SD, asterisk (*) indicates significant difference, $P < 0.05$

different ($P > 0.05$). However, the TP of both species exposed to Cd decreased significantly ($P < 0.05$) than those in the control group. Prosome length of both species was not significantly different between those in the control and Cd exposed group (Table 2). The theoretical production of *E. affinis* was significantly higher ($P < 0.05$) than the TP of *E. affinis* exposed to Cd (Fig. 5) and those in the control group. The TP of those exposed to Cd was significantly lower ($P < 0.05$) than those in the control group. In addition, the theoretical production of *P. annandalei* was not significantly ($P > 0.05$) different from the TP of *P. annandalei* in the control group, but the TP of *P. annandalei* exposed to Cd was significantly lower ($P < 0.05$) than those in the control group.

Sex ratio and percentage of ovigerous females

Sex ratio (male: female) and percentage of ovigerous females (% OVF) of *E. affinis* showed a slight decrease when exposed to Cd compared to those in the control group, although not significantly different ($P > 0.05$). Sex ratio of *P. annandalei* (male: female) exposed to Cd similarly decreased but not significantly different ($P > 0.05$) from those in the control group. In addition, *P. annandalei* exposed to Cd showed a significant increase ($P < 0.05$) in % OVF than those in the control group. However, sex ratio (male: female) and % OVF of *E. affinis* in the control and those exposed to Cd were significantly different ($P < 0.05$) from sex ratio (male: female) and % OVF of *P. annandalei* exposed to Cd (Table 2).

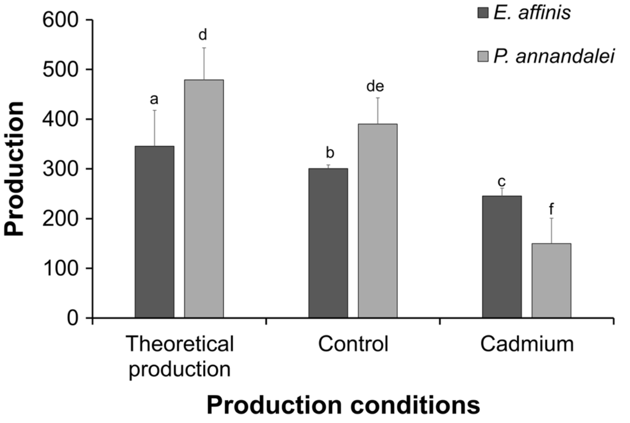

Fig. 5 Effect of cadmium on the production (F1 generation) of *Eurytemora affinis* (18 °C) and *Pseudodiaptomus annandalei* (26 °C) at different conditions. Values are mean ± SD

Fecundity and survival

Clutch size of both species exposed to Cd decreased significantly ($P < 0.05$) compared to the control groups (Table 2). However, clutch size of *P. annandalei* in the control and Cd exposed groups was significantly ($P < 0.05$) higher than that of *E. affinis*.

The percent survival of *E. affinis* and *P. annandalei* in the control groups were 87 and 81%, compared to the Cd-exposed groups with 71 and 31%, respectively.

Discussion

Differences between male and female responses to cadmium

The response of copepod population to different heavy metal concentrations has been shown to be species and gender specific (Lotufo and Fleeger 1997; Bao et al. 2008); moreover, the results of this study showed an increasing trend in mortality with time from 24 to 96 h with an increase in Cd concentration. This result suggests that the concentration and particularly the length of exposure of copepods to Cd played an important role in the increased mortality (Fig. 1a, b). Mortality was higher in *E. affinis* females exposed to 40–360 µg/L Cd than in *E. affinis* males; however, the opposite pattern was observed in *P. annandalei*. Furthermore, the 96 h LC50 of *E. affinis* females (90 µg/L Cd) was significantly lower than that of *E. affinis* males (127.8 µg/L Cd). In addition, the 96-h LC50 of *P.*

Table 2 Effect of cadmium (µg/L) on male:female sex ratio, % Ovigerous females, prosome length and clutch size of subtropical (*Pseudodiaptomus annandalei*) and temperate (*Eurytemora affinis*) species of copepod

		Sex ratio (male:female)	% Ovigerous females	Prosome length (µm)	Clutch size
E. affinis	Control	1.65 ± 0.12^{a}	68.04 ± 7.02^{a}	$775.20 \pm 2.01.53^{a}$	15 ± 1^{a}
	40 µg/L Cd	1.60 ± 0.19^{a}	67.42 ± 3.67^{a}	784.12 ± 15.41^{a}	13 ± 2^{b}
P. annandalei	Control	1.43 ± 0.23^{ab}	63.95 ± 9.35^{a}	889.75 ± 8.56^{b}	24 ± 3^{c}
	160 µg/L Cd	0.98 ± 0.10^{b}	82.36 ± 5.44^{b}	889.98 ± 21.47^{b}	20 ± 3^{d}

Different superscript from the same column are significantly different ($P < 0.05$). Values are mean ± SD

annandalei males (120.6 µg/L Cd) was significantly lower than that of *P. annandalei* females (239.5 µg/L Cd).

Multiple studies investigating Cd toxicity in copepods have reported that LC50 values vary with species and life stages (Table 3). A lower concentration results in higher female than male mortality, suggesting that *E. affinis* females are more sensitive to Cd toxicity compared with *E. affinis* males. In accordance with the findings of the current study, McCahon and Pascoe (1988) reported that the LC50 value at 48 h indicated that freshwater amphipod *Gammarus pulex* (L.) females are more sensitive to Cd compared with *G. pulex* (L.) males. Similarly, Sroda and Cossu-Leguille (2011) found that the females of two gammarid species, *G. roeseli* and *Dikerogammarus villosus*, are more sensitivity to Cu compared with the males of these two species.

Many studies carried out on the exposure of copepods to organic pollutants reported that males are more sensitive than females (Bao et al. 2008; Lotufo and Fleeger 1997; Medina et al. 2002). Similar gender response was observed for *P. annandalei* when exposed to cadmium in this study, where the males were more sensitive to Cd than females. Differences in sex-specific responses to stress are not general but are specific to both the species and the contaminant. When sexes are separated, variations in individual responses to chemical pollutants can be identified. Different chemicals and exposure times have different pathways and modes of action for different species and their sexes (Boulangé-Lecomte et al. 2014; Hinck et al. 2008; Ko et al. 2014; Stringer et al. 2012; Volz and Chandler 2004; Yu et al. 2013). Because the physiology of males and females differs, studies on individual responses to chemical pollutants can provide more insights into their tolerance level.

Size differences due to sex or species is believed to account for variations in sensitivity to contaminants; that is, the larger surface area to volume ratio of a smaller animal made them more susceptible to toxic pollutants (Chandler and Green 1996; Stringer et al. 2012; Wang and Zauke 2004). According to Hagopian-Schlekat et al. (2001), *Amphiascus tenuiremis* males are smaller than females; thus, they assumed that males could accumulate higher amounts of metals than females after observing that the survival of males following the exposure to Cu, Pb, and Ni was significantly lower than that of females. Furthermore, Stringer et al. (2012) observed that *Quinquelaophonte sp.* males were significantly more sensitive to Zn and atrazine compared with females, which was speculated to be because of different body sizes. However, in *R. propinqua*, despite the size difference between the sexes, no sex-specific differences in sensitivity to Zn, phenanthrene, or atrazine were observed, suggesting that factors other than size differences can affect sex-specific sensitivity. In addition, Medina et al. (2002) claimed that differences between sexes could not account for observed differences in tolerance because their results showed that sex-related differences in sensitivity to the pollutant pyrethroid cypermethrin changed with time.

In this experiment, the males of both copepod species were smaller than the females, but the females of *E. affinis* were more sensitive to Cd than the males; by contrast, the males of *P. annandalei* were more sensitive than the females. The differences in sex-specific sensitivity may not be size related but perhaps related to their physiology; that is, their individual ability to affect uptake, effectively metabolize, and eliminate contaminants can be contaminant specific (Escher and Hermens 2002). The mode of metal uptake differs and could be from ingested food or from the dissolved phase and more than 50% of cadmium accumulates from dissolved phase (Wang and Fisher 1998). In addition, mode of metal elimination differs, which could include elimination through molted exoskeleton (Dittman and Buchwalter 2010; Mirenda 1986), through deposition in eggs (Dipinto et al. 1993; Oberdörster et al. 2000) or feces (Benayoun et al. 1974). Possible elimination of cadmium by the test species in this experiment through molting or feces were not considered since the final stage of development was used and they were not fed during the experimental period.

Differences in responses to Cd toxicity between female reproductive stages of two copepods

In the first experiment, the sample of female copepods contained both OFs and NOFs. The second experiment was conducted to evaluate the reasons underlying the differences in responses to Cd toxicity between males and females. We compared the effect of Cd in OFs and NOFs with and without exposure to a sublethal concentration of Cd. The mortality of NOFs were significantly higher than that of

Table 3 Cadmium toxicity of different copepods in different environmental conditions

Copepod species	Environment	Life stage	Time (h)	Cd LC50 (μg/L)	Reference
Pseudodiaptomus annandalei	Estuarine (sub-tropical)	Adult male	96	120.6 (26 °C)	Present study
P. annandalei	Estuarine (sub-tropical)	Adult female	96	239.5 (26 °C)	Present study
P. annandalei	Estuarine (sub-tropical)		96	169	Chen 2011
Eurytemora affinis	Estuarine (temperate)	Adult male	96	127.75 (18 °C)	Present study
E. affinis	Estuarine (temperate)	Adult female	96	90.04 (18 °C)	Present study
E. affinis	Estuarine	Naupliar	96	>120	Sullivan et al. (1983)
E. affinis	Estuarine	–	96	147.7	Cripe and Cripe (1990)
E. affinis	Estuarine	–	96	60	Roberts et al. (1982)
E. affinis	Estuarine	Naupliar	96	51.6 (5ppt)	Hall et al. (1995)
E. affinis	Estuarine	Naupliar	96	213.2 (15ppt)	Hall et al. (1995)
E. affinis	Estuarine	Naupliar	96	82.9 (25ppt)	Hall et al. (1995)
Acartia tonsa	Estuarine	–	96	90	Cripe and Cripe (1990)
Oithona similis	Estuarine	–	96	20.53	Gnanamoorthy et al. (2012)
A. tonsa	Marine	–	96	380	Roberts et al. (1982)
Tigriopus brevicornis	Marine (temperate)	Adult	96	48	Barka et al. (2001)
T. brevicornis	Marine	Ovigerous female	96	47.9	Forget et al. (1998)
T. brevicornis	Marine	Naupliar	96	17.4	Forget et al. (1998)
T. brevicornis	Marine	Copepodids	96	29.7	Forget et al. (1998)
T. breuicornis	Marine	Larvae	240	78	Le Dean and Devineau (1985)
Tigriopus japonicus	–	Adult	96	25.2	Lee et al. (2007)
A. tonsa	Marine	–	96	151 (13 °C)	Toudal and RiisgArd (1987)
A. tonsa	Marine	–	96	29 (21 °C)	Toudal and RiisgArd (1987)
Tisbe holothuriae	Marine (temperate)	–	48	906	Moraitou-Apostolopoulou and Verriopoulos (1982)
T. fulvus	–	Naupliar	24	4390	Pane et al. (2008)
T. fulvus	–	Naupliar	48	2240	Pane et al. (2008)
T. fulvus	–	Naupliar	72	960	Pane et al. (2008)
T. fulvus	–	Female	48	12,360	Pane et al. (2008)
T. fulvus	–	Female	72	6540	Pane et al. (2008)
T. fulvus	–	Female	96	3320	Pane et al. (2008)
Tisbe battaglia	Temperate	Adult	96	340	Hutchinson et al. (1994)
T. battaglia	Temperate	Naupliar	96	460	Hutchinson et al. (1994)
Paralabidocera antarctica	Marine (Antarctic)	Adult	168	237 (-1 °C)	Zamora et al. (2015)
Oncaea curvata	Marine (Antarctic)	Adult	168	901(-1 °C)	Zamora et al. (2015)
Stephos longipes	Marine (Antarctic)	Adult	168	1250 (-1 °C)	Zamora et al. (2015)

LC50 Lethal concentration resulting in 50% mortality

OFs with and without exposure to Cd in both copepod species. In addition, the mortality was higher in *E. affinis* females than in *P. annandalei* females. On the cellular level, heat shock proteins (HSPs) have been shown to be expressed by aquatic organisms under stress conditions. Boulangé-Lecomte et al. (2014) found a weaker expression of HSPs in *E. affinis* males than in females on a basal level (e.g., reproduction cycle), suggesting a sex-specific stress tolerance. Therefore, it is possible that the reproductive state of female *E. affinis* can be a factor affecting the sensitivity to Cd in the present study. McCahon and Pascoe (1988) observed the LC50 of freshwater amphipod *G. pulex* exposed to Cd and found that compared with males, females with eggs were twice as sensitive and females without eggs 13 times more sensitive to Cd.

The low sensitivity of females with eggs to Cd toxicity indicates that they have a more effective mode of toxic elimination. The difference found between OFs and NOFs points to the possibility of OFs eliminating Cd through the eggs they carried. The process of detoxification by depositing toxic waste in female eggs was referred to as ovodeposition by Dipinto et al. (1993), which could account for their higher tolerance to environmental contaminants (Oberdörster et al. 2000). Roberts and Leggett (1980) reported an example of ovodeposition in which eggs produced by the blue crab *Callinectes sapidus* contained more toxic contaminants (Kepone) compared with muscles. Egg production was concluded to be a major route for eliminating Kepone from female blue crabs (Roberts and Leggett 1980). The theoretical explanation for these results is that lipophilic compounds such as Kepone have an affinity for lipid-rich eggs. Therefore, whether sensitivity to Cd toxicity is higher or lower in females than in males can have an ecological impact. For example, if female copepods are more sensitive than males, and if the concentration and bioavailability of metal increase in aquatic environments influenced by changes in physiochemical parameters, the rate of female mortality could increase consequently impeding the production of new recruits. Moreover, even when the females of a copepod species seem to be less sensitive than the males (as with *P. annandalei* in the second experiment), continuous exposure to metal pollution can result in bioaccumulation.

Toxicity effect of cadmium on the life cycle traits of *E. affinis* and *P. annandalei*

When assessing the impact of contaminant in the environment, mortality is usually the first endpoint to be considered. Other bioindicators such as reproduction and development of model test species have recently become an important endpoint in the risk assessment of aquatic pollutants (Kwok et al. 2015). Cadmium in the aquatic environment can cause a reduction in recruitment potential of copepod nauplii either through decreasing egg production, reduced hatching success or high mortality at the nauplii or copepodid stages and the degree of effect varies with the level of concentration, exposure duration and species (Jiang et al. 2007; Mohammed et al. 2011; present study). In our complete life-cycle experiment, a decrease in the number of individual copepod developmental stages and an overall decrease in the total population of both copepod species exposed to Cd were observed. Results from this experiment showed that chronic exposure of Cd negatively affected the population and total production of both copepod species. These were significantly lower than their theoretical production. Survival of both species in the control groups were less than 20% of the theoretical production. However, survival of *P. annandalei* exposed to Cd was 50% lower than those of the control groups, whereas *E. affinis* was 20% lower. LC50 values of Cd in the first experiment showed *E. affinis* to be more sensitive than *P. annandalei*. However, we observed a more significant decrease in the population and survival of *P. annandalei* when exposed to Cd. This is due to mortality occurring through the life cycle, which means that nauplii and/or copepodids of *P. annandalei* are more sensitive than the adult stage. Lira et al. (2011) observed a similar sensitivity change with a decrease in the population density of marine nematode *Rhabditis (Pellioditis) marina* when exposed to Cd.

Constantly changing environmental conditions are commonly unfavorable to inhabiting species. The sex ratio is skewed towards the gender with which shows a better tolerance or adaptive capabilities (Krupa 2005). The Seine (France) and Danshuei (Taiwan) estuaries have particularly high pollution levels of heavy metals (Dauvin 2008; Fang and Lin 2002; Fang et al. 2006, 2014; Hwang et al. 2010; Jeng and Han 1994). In both estuaries, the sex ratio of *E affinis* and *P. annandalei* is skewed in favor of males and varies during the year (Beyrend-Dur et al. 2013; Devreker et al. 2010). In this study, there were more males than females in the control and Cd exposed groups of both copepod species except those of *P. annandalei* exposed to Cd. In addition, the male proportion was higher in *E. affinis* than in *P.annandalei*. *P. annandalei* male:female sex ratio was skewed in favor of females, which could be a response to the toxicity of Cd they were exposed to.

An increase in clutch size usually correlates positively with an increase in prosome length (Souissi et al. 2016). This experiment shows a significant decrease in clutch size. However, a slight increase in prosome length was observed, although not significant. This could be due to the fact that only one generation was observed in our study compared to the multigenerational study by Souissi et al. (2016).

The higher ratio of OVF: NOF and the higher % OVF observed in *P. annandalei* show that even though the population density was lower in the Cd treatment, the surviving copepods were in favor of the female population and their reproductive activities. A high percentage of Cd was reported to be associated with the capsule membrane of the eggs of cuttlefish *Sepia officinalis* (Bustamante et al. 2002) and accumulates in the chorion of *Oncorhynchus mykiss* (Beattie and Pascoe 1978) and *Oryzias latipes* (Michibata 1981). If toxic waste is indeed eliminated by the deposition in eggs (De Loof 2015), this may affect the eggs' hatching success or naupliar viability. However, this hypothesis has to be tested in the future. Jiang et al. (2007) observed a significant reduction in the number of hatched nauplii of *Acartia pacifica* copepod resting eggs exposed to increasing Cd concentrations. Therefore, the significant reduction observed in *P. annandalei* TP when exposed to Cd (Fig. 5) could be a result of low hatching success or increased mortality from chronic exposure. The experiment was conducted for one generation and the total population was collected after ovigerous females were observed in high number. Ovigerous females were majorly carrying their first egg sacs. Moreover, female copepods can produce a second clutch even after a single mating. This means that more eggs could be produced as a means of reducing the contamination load. Gismondi et al. (2013) suggested that one of the possible reasons for higher survival observed in *Gammarus roeseli* females than in males could be a result of ovodeposition. The trade-off between reducing the fitness of one clutch and increasing female survival could become an added ecological advantage for female copepods. A study on Cd effects on several generations could, therefore, shed more light on the ecological significance and adaptive potentials to Cd contamination.

In conclusion, the ecological implication of Cd toxicity on copepod ecology is more related to a skewed sex ratio, low egg production, reduced hatchability, and reduced survival that affects the recruitment potential of copepod nauplii resulting in a decreasing copepod population. Korsman et al. (2014) modeling on environmental stress factors in *E. affinis* suggest that exposure to zinc and copper was largely responsible for reduced population densities in a contaminated estuary. As a major link in the aquatic food web, copepod decline could result in major disruptions of ecosystem structure and functioning. To conclude, mortality, reproduction and population growth of model species may provide important bio-indicators for environmental risk assessment.

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Compliance with ethical standards

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