Aquatic Toxicology 166 (2015) 42–49

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Impacts of mercury exposure on life history traits of *Tigriopus japonicus*: Multigeneration effects and recovery from pollution

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ARTICLE INFO

Article history:
Received 21 January 2015
Received in revised form 24 June 2015
Accepted 25 June 2015
Available online 15 July 2015

Keywords:
Acclimation
Life history trait
Mercury
Multigenerational toxicity
Phenotypic plasticity

ABSTRACT

Here, through a multigenerational life-cycle test, *Tigriopus japonicus* were exposed to different mercuric chloride treatments in seawater (nominal concentrations of 0, 0.5, 1, 10, and 50 μg/L) for five successive generations (F0–F4), and subsequently all the treatments were recovered in clean environments for one generation (F5). Six life history traits (survival, developmental time for nauplius phase, developmental time to maturation, fecundity, number of clutches, and number of nauplii/clutch) were examined for each generation. Mercury (Hg) accumulation was also analyzed for the adult copepods in the F1, F3, and F5. The results indicated that Hg accumulated in a dose-dependent manner for the F1, F3, and F5 generations. Moreover, higher Hg contents were observed in F3 than F1 at the same exposure levels. Among the six life history traits, only fecundity and number of nauplii/clutch showed a greater sensitivity to Hg toxicity, and the inhibitory effects worsened from F0 to F3, which was explained by a trend for higher metal accumulation with increasing generations. In the recovery generation (F5), none of the traits differed from the control, highlighting that Hg might not induce any epigenetic or parental effects in the following generations. Thus, we hypothesized that although cumulative effects might have been involved in Hg multigenerational toxicity, physiological acclimation, that is, phenotypic plasticity could explain Hg tolerance obtained by marine copepods. Impacts on important life history traits could disturb the population dynamics of some important marine copepods, hence having unexpected ecological consequences in the marine ecosystem. Yet, the Hg harmful impacts rapidly fade away as the Hg is cleared from the environment.

1. Introduction

Mercury (Hg) is one of the most hazardous and persistent environmental pollutants in the aquatic environments (Jiang et al., 2006). Hg pollution is considered as one of the primary environmental problems in China, which contributes approximately 28% of the global Hg emissions to the atmosphere. This is a result of anthropogenic activities including mining, industrialization and rapid urbanization (Pacyna et al., 2006). Atmospheric Hg is deposited through various pathways and finally aggregates into aquatic environments. In China, the average annual input of Hg into coastal environments by major rivers was about 58 tons between 2009 and 2013 (NBO, 2015). Additionally, Hg released from point sources and long-range air transport has further contaminated the marine and coastal environments in China, hence causing Hg contamination to be a serious problem in this area. Wang et al. (2009) have reported maximum levels of total Hg (T-Hg) for water and sediment in Jinzhou Bay to be 2.59 μg/L and 64 μg/g, respectively, which are three orders of magnitude higher than the background concentration. In such a highly contaminated environment, the organisms may have been exposed to the high Hg levels throughout many generations. The potent toxicity of Hg compounds is often associated with the high affinity of Hg for sulfur, causing an efficient binding to cysteine residues in proteins and enzymes, thereby perturbing their functions and subsequently displaying multiple toxicity (e.g., hepatotoxicity and neurotoxicity) in organisms (Castoldi et al., 2001; Ung et al., 2010). Therefore, data on multiple biological traits such as growth, development and reproduction are really desired in aquatic organisms when challenged with multigenerational exposure to Hg. Such information is most valuable for marine ecotoxicological studies where degenerative or adaptive responses in progeny may be the most critical impacts.

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http://dx.doi.org/10.1016/j.aquatox.2015.06.015

In recent years, more efforts have focused on the impact of multigenerational exposure to heavy metals on the growth and tolerance development of aquatic animals (Kwok et al., 2009; Lilley et al., 2012; Sun et al., 2014; Tsui and Wang, 2005; Vidal and Horne, 2003), as exposing the organisms to heavy metals through many generations might mimic the scenario that the organisms face in contaminated sites. Multigenerational studies can reveal acclimation or adaptive responses. Acclimation is a plastic physiological response driven by a stressor that can occur within a single generation at the level of an individual, and effects of acclimation can quickly be gained under a stressor and lost in the absence of the stressor (Sun et al., 2014). Physiological acclimation confers a phenomenon of phenotypic plasticity, that is, the capacity of a single genotype to exhibit a range of phenotypes in response to different environmental conditions, and it can underpin several aquatic animals' well documented capability to cope with a wide variety of stressors including heavy metals (Kwok et al., 2009; Lilley et al., 2012; Marinković et al., 2012). It should be noted that acclimation responses involve transgenerational epigenetic effects such as maternal effects (Wolf and Wade, 2009) and methylation patterns (Vandegehuchte et al., 2009), but these effects are not consistently inherited and are often limited to one generation (Youngson and Whitelaw, 2008). In contrast, adaptation of a population may include the change of genetic structure (e.g., selection of tolerant populations) (Tsui and Wang, 2005), and the inherited response could maintain for a long period even after the selective force is removed.

To the best of our knowledge, multigenerational exposure of Hg has only been studied in freshwater organisms (Tsui and Wang, 2005; Vidal and Horne, 2003). For example, Vidal and Horne (2003) found that the oligochaete *Tubifex tubifex* raised in Hg-contaminated sediment developed metal resistance that could persist even when the worms were reared for three subsequent generations in clean conditions. The authors reported that adaptation to Hg pollution in the worms appeared to have both a phenotypic and genotypic basis. Therefore, it is important to investigate multigenerational effects in marine organisms following Hg exposure so as to fully understand its biological and environmental impacts in marine ecosystems.

The harpacticoid copepod *Tigriopus japonicus* has a wide geographical distribution and is regarded as a good model species commonly used in marine ecotoxicological studies (Raisuddin et al., 2007; Wang and Wang, 2010). This copepod is relatively small, the adults being approximately, 1.0 mm generally. Its life cycle includes twelve distinct postembryonic developmental stages, i.e., six nauplius stages, five copepodid stages, and one adult stage (Guo et al., 2012; Raisuddin et al., 2007). Notably, this species is easily raised in the laboratory and has a short generation time, hence making multi-generational experiments feasible (Guo et al., 2012; Kwok et al., 2009; Lee et al., 2008). In this study, via a multigenerational life-cycle test, *T. japonicus* were exposed to different mercuric chloride ($HgCl_2$) treatments (i.e., the nominal concentrations with 0, 0.5, 1, 10, and 50 μg/L in the seawater respectively) for five consecutive generations (F0–F4), and it was followed by a recovery period of one generation (F5) in seawater control conditions. Six life history traits including survival, developmental time of nauplius phase, developmental time to maturation, fecundity, number of clutches, and number of nauplii/clutch were investigated for each generation. Hg accumulation was analyzed in the adult copepods for the F1, F3, and F5. This work aimed to firstly test whether physiological acclimation and/or genetic adaptation is involved into Hg multigenerational effects, and secondly examine whether the measured effects of Hg are lost when a population exposed to this metal for five generations is reared in a clean environment; that is, to test for recovery potential after contamination removal.

2. Materials and methods

2.1. Copepod maintenance

Copepods *T. japonicus* were originally collected in rocky intertidal zone pools in Xiamen Bay (People's Republic of China) in 2007, and maintained in our laboratory before the experiments. Reference seawater used in experiments was obtained 20 km offshore in Xiamen Bay. All seawater used was filtered through a 0.22 μm polycarbonate membrane, with the background value for T-Hg concentration in the seawater being 0.0051 μg/L. The seawater characteristics were described as follows: dissolved oxygen, 6.2–6.7 mg/L; salinity, 29–30 PSU; and pH, 8.0–8.1. The copepods were maintained at a temperature of 22 °C with a 12:12 h light:dark cycle. An equal mixture of three algae, *Thalassiosira pseudonana*, *Isochrysis galbana*, and *Platymonas subcordiformis*, was used as food for copepods at a density of 8×10^5 cells/L. The algae were cultured at 22 °C, 80 μmol photons/m/s under a 12:12 h light:dark cycle in the f/2 medium using 0.22 μm filtered and sterilized seawater. All the toxicity testing conditions were the same as described above for copepod culture.

2.2. Multigenerational experiments

According to the National Standard of China for Seawater Quality GB 3097-1997 (SEPAC, 1997), four Hg treatments plus the seawater control were chosen for the life-cycle tests of copepods. $HgCl_2$ (Sigma–Aldrich, 99.5%) was added to the seawater solutions to achieve final Hg^{2+} concentrations of 0.5, 1.0, 10, and 50 μg/L. The equivalent concentrations of Hg in molar value are 0.0025, 0.005, 0.05, and 0.25 μM, respectively.

Ten nauplii (<24 h) per treatment were transferred to 6-well tissue culture plates with 8 mL working volume in four replicates (total 40 nauplii). These nauplii were raised under the above-mentioned conditions until adult females developed egg sacs. Testing solutions were daily renewed for 80% of the working volume, and *P. subcordiformis* was centrifuged to remove the culture medium (including nutrients and algal metabolites) and added at a density of approximately 6×10^5 cells/L. In total, six life history traits were investigated in this study, i.e., survival, developmental period for nauplius phase, developmental time to maturation, fecundity, number of clutches, and number of nauplii per clutch. These parameters were examined for each individual copepod in each of the five exposure concentrations.

The survival (percentage) was calculated after each exposure. Developmental stages were observed daily under a stereomicroscope and recorded to calculate the time of development from nauplii to copepodite and from nauplii to adults with egg sacs (i.e., maturation). The development of the egg sac was considered as the time of maturation. To measure fecundity, defined as the number of clutches, and number of nauplii/clutch, six females bearing an egg sac per concentration were individually transferred to a new 12-well plate containing 4 mL working volume. These females were reared under the above-mentioned conditions for 10 days. The resulting nauplii and unhatched clutches were counted and removed under the stereomicroscope.

For the second generation (F1), 10 nauplii (F1) produced by each female (F0) with first or second brood from each concentration treatment were transferred to new 6-well plates. The experimental and exposure conditions were the same as those used for the F0 generation test. The copepods of subsequent generations were treated as the same as in F0, and this multigenerational exposure was maintained until the nauplii (F4) from F3 developed to maturation.

2.3. Recovery

To test whether the Hg effect on the life history traits are lost when a population exposed to Hg for five consecutive generations is reared in a clean environment, we took the nauplii (F5) from F4 and raised them in purified environments. Ten nauplii (<24 h) per concentration treatment were cultured in 6-well plates with four replicates. The same experimental procedures were followed as in the multigenerational experiments, and the same life history traits were observed for each individual copepod.

2.4. Hg concentration analysis

To measure Hg contents in the adult copepods of the F1, F3, and F5, we simultaneously performed a multigenerational life-cycle test for this copepod exposed to the same different $HgCl_2$ concentrations. The experimental procedure was the same as described in the multigeneration exposure and recovery testing experiments. Briefly, ten nauplii (<24 h) per concentration treatment were cultured in 6-well plates with 20 replicates until these nauplii developed to maturation. Then, 10 replicates of approximately 100 adult copepods were pooled together for Hg accumulation analysis, hence producing two biological replicates for each concentration treatment. T-Hg analysis followed the protocol of the U.S. EPA method 1631 (US Environmental Protection Agency, 2002), the method of Gill and Fitzgerald (1987), and the appendix to U.S. EPA method 1631 (US Environmental Protection Agency, 2001). After freeze-drying for 2 days, the tissues were digested in 70% nitric acid in a heating block at 80 °C overnight. After digestion the solution was diluted to 50 mL, followed by the addition of BrCl solution. An aliquot of digestate was placed in a bubbler with a $SnCl_2$ solution. The reduced Hg was purged with an argon gas flow from the solution onto a gold trap. The subsequent double amalgamation process was followed by Hg detection using a cold vapor atomic fluorescence spectrometer (CVAFS) (Rayleigh AF-610B). The method detection limit was 0.78 ng/g, and recoveries for matrix spikes were within the acceptable ranges of 70–130%. The relative percentage difference of matrix spike duplicates and sample duplicates were also acceptable (<30%) (Liang et al., 2010). T-Hg content in the copepods was measured as ng/g dry weight (DW).

2.5. Statistical data processing

All experiments were replicated at least four times for the six life history traits and the data were expressed as mean values ± standard deviation (SD). Statistical analysis was carried out using SPSS 17.0 software. One-way ANOVA and the Fisher least significant difference test were used to evaluate whether the means were significantly different among the groups. Significant differences were indicated at $P < 0.05$. Prior to one-way ANOVA, data were log transformed to meet ANOVA assumptions of normality and variance homoscedasticity. It should be noted that all the data for Hg accumulation analysis were only replicated twice and the data were expressed as mean values ± semi-range, thus they were excluded in the above statistical analysis. In addition, two-way ANOVA was performed to determine the statistical significance of the copepod's offspring production (i.e., fecundity and number of nauplii/clutch) among metal treatments and generation times.

3. Results

3.1. Hg accumulation in the adult copepods

T-Hg contents were measured for the adult copepods in the multigenerational exposure and recovery testing (Table 1). The results indicated that T-Hg levels in the F1 and F3 increased with an increasing concentration. For example, the copepod's T-Hg concentrations under the 50 μg/L exposure enhanced about 100 and 150 times for the F1 and F3, respectively, in comparison to the control. Meanwhile, at the same Hg treatment level, T-Hg contents in F3 trended to be higher than F1. Interestingly, even in the recovery generation (F5), T-Hg concentrations accumulated in a dose-response manner, and it was exemplified by that the metal content under the 50 μg/L treatment was enhanced approximately 30 times when compared to the control.

3.2. Multigenerational effects

To test the effects of five consecutive generations of exposure to waterborne Hg, six life history traits were investigated in the life-cycle test for *T. japonicus* (Figs. 1 and 2). Survival was not significantly different between treatments for five generations, except that the rate for F1 was significantly higher for the 1 μg/L Hg treatment than the control ($P < 0.05$). Similarly, Hg treatment did not significantly affect the time for nauplius phase, the development time, and number of clutches for the copepod under most circumstances during multigenerational exposure. The duration for nauplius phase was significantly delayed in the 0.5 μg/L treatment for F4, the 1 μg/L treatment for F0, and the 10 μg/L treatment for the F0 and F3 ($P < 0.05$). The total development time was just elevated by the 0.5 μg/L treatment for F3, the 10 μg/L treatment for the F2 and F3, and the 50 μg/L treatment for the F0, F1, and F2 ($P < 0.05$). Also, number of clutches was only depressed under the 10 μg/L treatment for the F2 and F4, and the 50 μg/L treatment for the F0 and F1 ($P < 0.05$).

Total fecundity and the number of nauplii per clutch were significantly inhibited by Hg toxicity in most cases during multigenerational exposure ($P < 0.05$). Furthermore, a similar response was observed for these two traits against Hg multigenerational effects, namely that the restrained effects were more severe from F0 to F3. The results of two-way ANOVA (Table 2) showed significant impacts of Hg concentration and generation time on fecundity and the number of nauplii per clutch ($P < 0.001$), and a non-significant interaction between treatment and generation.

3.3. Recovery

We tested the effect of raising copepods in clean seawater after five consecutive generations of being exposed to Hg contamination. The results indicated that all the six life traits exhibited no significant difference among all the treatments (Fig. 3 and Table 3).

4. Discussion

4.1. Hg accumulation

This study illustrated that Hg accumulated in the adult copepods with a dose-dependent manner for the F1, F3, and F5 generations following multigenerational exposure and recovery testing. Moreover, at the same level of Hg exposure, the T-Hg contents in F3 seemed to be higher than those for F1, implying that the toxic effects of Hg might worsen with the increasing number of exposed generations. In other words, accumulative effects might be correlated with Hg multigenerational toxicity in this study. Tsui and Wang (2005) examined the toxic effects of multigenerational exposure to Hg at 3.8 μg/L in the freshwater cladocera *Daphnia magna*, and found that Hg concentrations of the F1 adults were slightly higher than those in the F0 adults, although the exposure duration for the two generations was not the same (i.e., 21 days versus 17 days for F1 as compared with F0). A previous study demonstrated that chronic exposure to cadmium (Cd) of 3.0 μg/L for six

Table 1
Total-mercury contents in the adult copepod *Tigriopus japonicus* under multigenerational exposure to different mercury chloride concentrations (control, 0.5, 1, 10, and 50 μg/L). Data are expressed as mean values ± semi-range (n = 2).

Concentration (μg/L)	Mercury accumulation (ng/g)		
	F1	F3	F5 (recovery)
Control	4.27 ± 0.35	3.68 ± 1.18	4.84 ± 0.16
0.5	6.42 ± 0.84	15.50 ± 4.83	5.50 ± 0.51
1	19.24 ± 1.92	137.53 ± 16.10	50.70 ± 5.67
10	90.10 ± 7.41	283.34 ± 37.71	78.55 ± 6.50
50	451.14 ± 35.38	546.54 ± 27.33	146.78 ± 13.40

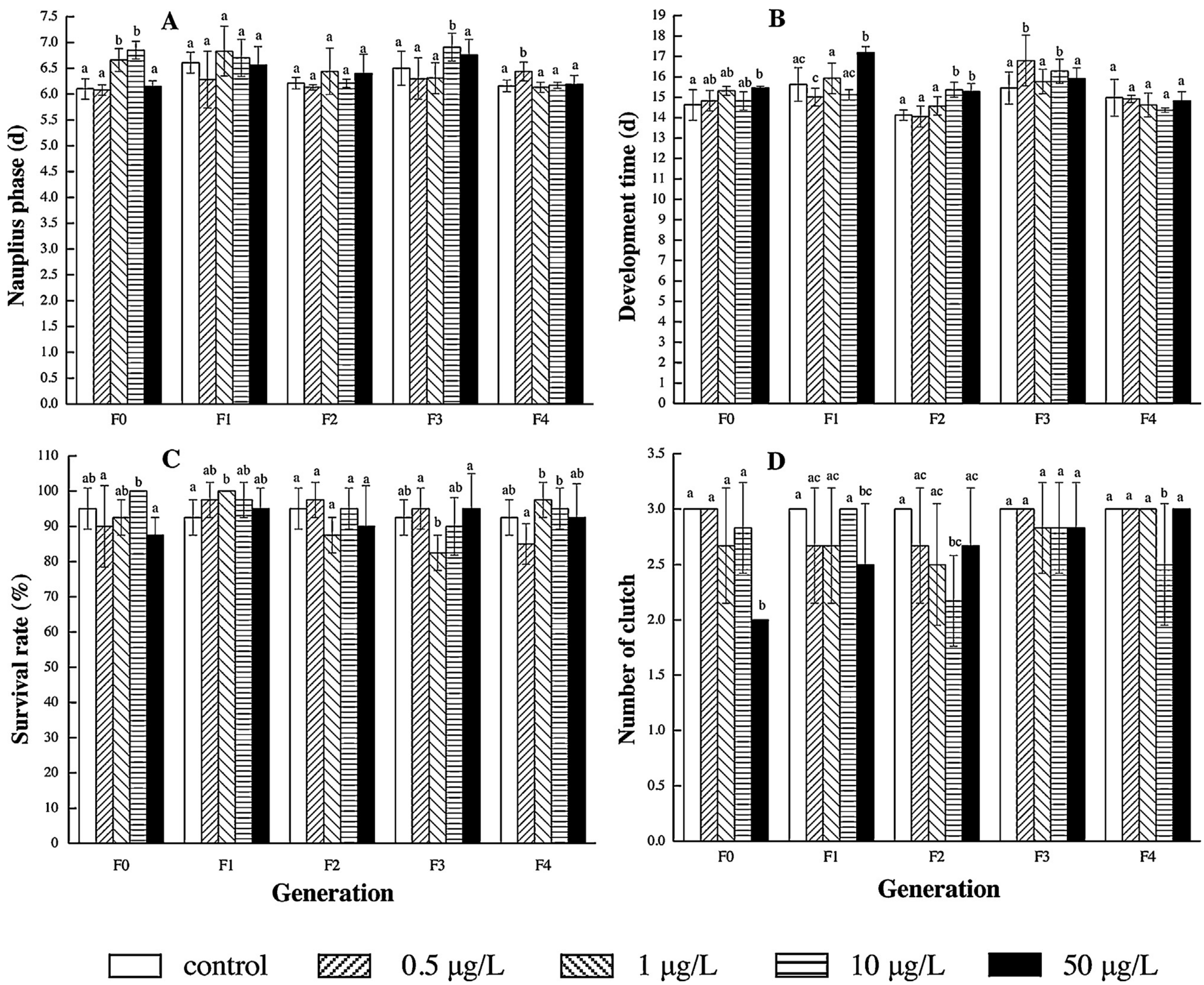

Fig. 1. Nauplius phase (A), development time (B), survival rate (C), and number of clutches (D) in five generations of *Tigriopus japonicus* exposed to different mercury chloride concentrations (control, 0.5, 1, 10, and 50 μg/L). Data are described as means ± SD (n = 6). Different letters indicate a significant difference among different mercury treatments at $P < 0.05$.

Table 2
Synthesis of the two-way factorial ANOVA displaying the effects of mercury treatment and generation time on fecundity and number of nauplii per clutch in *Tigriopus japonicus*.

Response variable	Mercury treatment			Generation time			Treatment × generation		
	F	df	P	F	df	P	F	df	P
Fecundity	23.481	4	<0.001	8.669	4	<0.001	1.286	16	0.217
Number	13.198	4	<0.001	11.178	4	<0.001	1.203	16	0.275

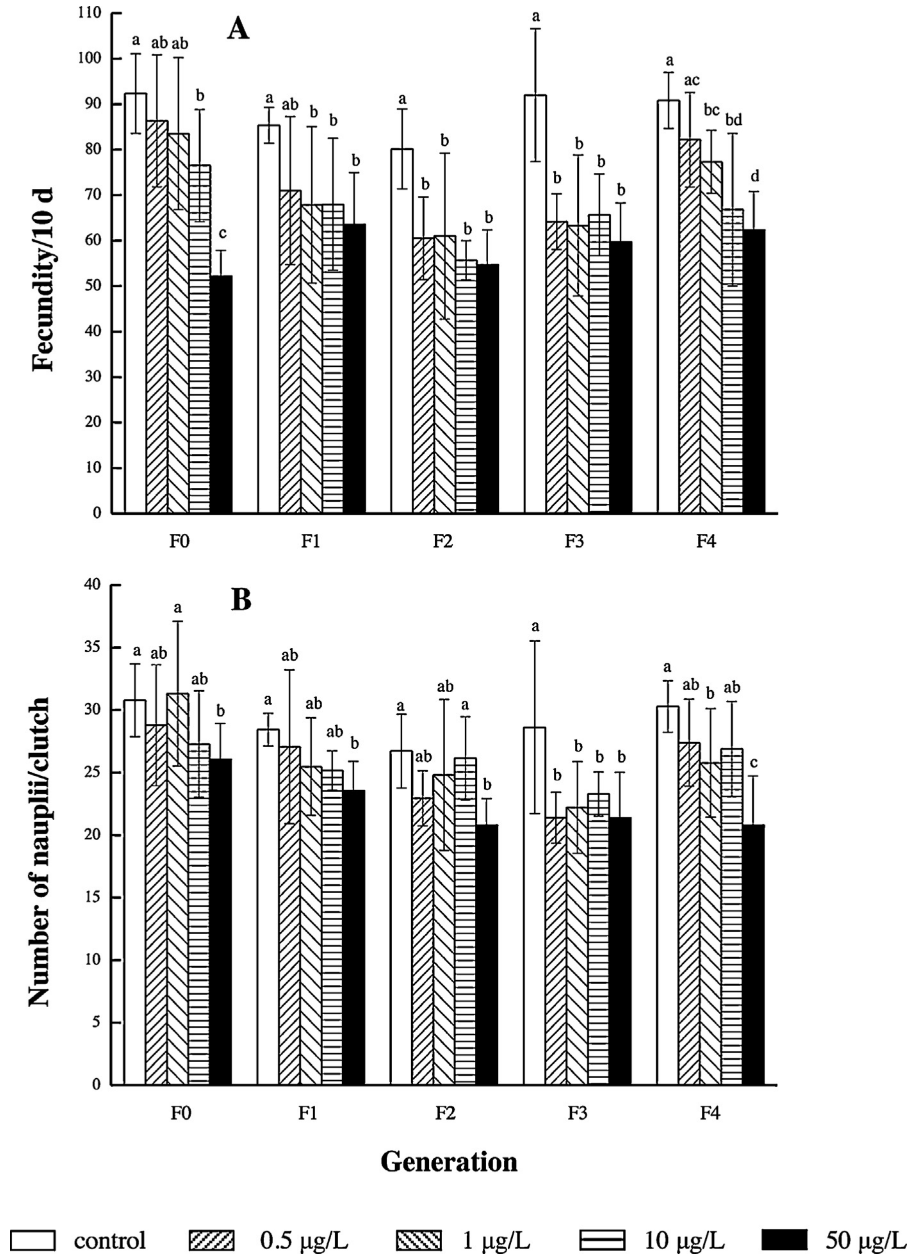

Fig. 2. Fecundity (A) and number of nauplii per clutch (B) of five generations of *Tigriopus japonicus* exposed to different mercury chloride concentrations (control, 0.5, 1, 10, and 50 μg/L). Data are described as means ± SD (n = 6). Different letters indicate a significant difference among different mercury treatments at P < 0.05.

Table 3
Nauplius phase (nauplius to copepodid), development time (nauplius to adult), survival rate and number of clutches in the copepod *Tigriopus japonicus* transferred into clean conditions after five consecutive generations of exposure to five concentrations of mercury chloride (control, 0.5, 1, 10 and 50 μg/L). Values are means ± SD.

Concentration (μg/L)	Nauplius phase (*d*)	Development time (*d*)	Survival (%)	Number of clutches
Control	6.17 ± 0.14	15.06 ± 0.86	92.50 ± 5.00	3.17 ± 0.41
0.5	6.29 ± 0.44	14.44 ± 1.08	92.50 ± 5.00	3.33 ± 0.52
1	6.08 ± 0.17	14.83 ± 0.49	97.50 ± 5.00	3.50 ± 0.55
10	6.14 ± 0.11	15.23 ± 0.36	95.00 ± 5.77	3.17 ± 0.41
50	6.06 ± 0.11	14.85 ± 0.80	95.00 ± 5.77	3.17 ± 0.41

successive generations significantly enhanced the metal accumulation of *D. magna* in all generations in contrast to the corresponding concentrations in the control groups for all generations (Guan and Wang, 2006). Also, the Cd accumulation of the exposed daphnids exhibited a biphasic pattern, e.g., an increasing trend from F1 to F4 followed by a decreasing trend for the subsequent two generations. The above previous study (Guan and Wang, 2006) taken together with our work suggests that the increasing accumulating tendency with generations can be ascribed to maternal transfer of metals (e.g., Cd and Hg) in the treated animals during multigenerational exposure. Specifically in this study, the dose-depend Hg content in the recovery generation (F5) clearly provided evidence for maternal Hg transfer in the treated copepods during the multigenerational exposure, since all the treatments were maintained

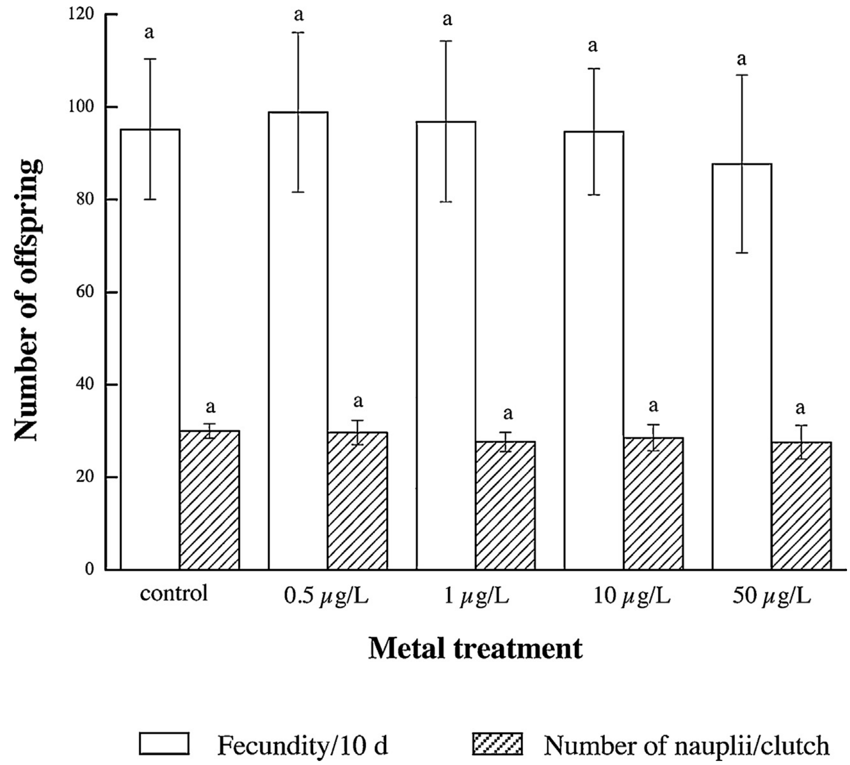

Fig. 3. Fecundity and number of nauplii per clutch in the copepod *Tigriopus japonicus* transferred into clean conditions after five generations of exposure to five concentrations of mercury chloride (control, 0.5, 1, 10, and 50 μg/L). Data are described as means ± SD (n = 6). Different letters indicate a significant difference among different mercury treatments at P < 0.05.

in clean seawater for this generation. Alternatively, an increased trend for Hg accumulation may partly be related to metallothionein (MT) induction in the copepods, since it is possible that the maternally exposed animals were more ready to synthesize more MT in order to supply more binding sites for the internal metals. Further study is, therefore, needed to determine the cause-effect of the interaction between Hg accumulation and MT induction in the copepod under multigenerational exposure. Overall, the copepods may modify physiological processes in response to ambient chronic Hg exposure, consequently accumulating more Hg. Nevertheless, the detailed mechanism really deserves a further investigation. It should be emphasized that the treated T-Hg contents in our study were within the span of Hg concentrations in several marine copepods in the environment (Cardoso et al., 2013; Hsiao and Fang, 2013; Ritterhoff and Zauke, 1997), hence enabling our study to display an environmentally relevant significance.

4.2. Multigenerational toxicity response

Our study showed that Hg exposure did not consistently impact survival, developmental time of nauplius phase, developmental time to maturation, and number of clutches under most circumstances during the multigenerational exposure, but it strongly affected fecundity and number of nauplii per clutch for the copepods. To our knowledge, there is no work to date focused on Hg multigenerational toxicity in marine animals. The few previous studies investigating Hg toxicity on marine copepods are solely concentrated on toxic effects following exposure of no more than one generation (Barka et al., 2001; Hook and Fisher, 2001). Hook and Fisher (2001) reported a decrease in egg production in *Acartia tonsa* and *Acartia hudsonica* following exposure to dissolved Hg concentrations of more than 0.05 μg/L, which is in line with this study. Consequently, it was deduced that Hg pollution can suppress fecundity of the copepods (i.e., population recruitment) and thus potentially affect their community structure and functioning in the marine ecosystem. Cardoso et al. (2013) evaluated the impact of Hg on zooplankton community structure and functioning in a temperate coastal lagoon, and reported that the most contaminated areas presented the highest Hg accumulation in zooplankton assemblages (dominated by copepods). It was further reported that these areas also had the lowest values of species richness, evenness and heterogeneity (Cardoso et al., 2013).

We noted an increased inhibitory impact of Hg on fecundity and number of nauplii/clutch from F0 to F3, and a possible explanation is the tendency for higher metal accumulation with generations. Similarly, Guo et al. (2012) found that the copepod *T. japonicus* became more sensitive to PCB 126 exposure as generations developed. Together with other studies (Guo et al., 2012; Massarin et al., 2010; Pane et al., 2004), this work highlights that the offspring may become more sensitive to pollutant exposure or that the toxic effects can accumulate with generations. Lee et al. (2008) reported that, of the seven traits (nauplius phase, development time, survival, sex ratio, number of clutch, nauplii per clutch, and fecundity), only the length of the nauplius phase and development time in *T. japonicus* displayed a greater sensitivity to several marine pollutants including copper and arsenic during a two-generation life cycle exposure, which is inconsistent with our study. The above discrepancy might be due to pollutant specificity during the exposure, and it again testifies the significance of the current study.

It should be emphasized that, at the lower exposures to Hg the first generation effects did not reflect the changes in subsequent generations, while at the highest concentration the first generation response predicted the response in future generations. This is in agreement with a previous study where toxicity of quantum dots and cadmium salt was investigated in the nematode *Caenorhabditis elegans* under multigenerational exposure (Contreras et al., 2013). These results suggest that over generations, aquatic animals can show more potential to acclimate to lower metal exposure rather than higher concentrations. Consequently, environmental risk assessment of contaminants must consider exposure history to provide a realistic measurement of the influences of pollutants on aquatic life.

4.3. Recovery

After five generations of exposure to four concentrations of Hg, the nauplii from the fifth generation (F4) were transferred into clean seawater. In this recovery generation, none of the measured traits differed from the seawater control regardless of Hg accumulation increasing with the increased Hg concentration used in the pre-multigenerational exposure, hinting that Hg toxicity might not produce any epigenetic or parental effects on the later generations. A full recovery within one generation for the copepods excluded the effect of fast adaptation to Hg exposure as a possible cause. Additionally, a sign of recovery was observed in F4 under multigenerational exposure, since at most cases the restrained effects of Hg on the copepod's fecundity trended to decrease in this generation when compared with F3. This provides some evidence that phenotypic plasticity played an important role in Hg acclimation obtained by marine copepods after multigenerational exposure, which is illustrated by several previous studies on copper (Kwok et al., 2009; Sun et al., 2014) and Hg acclimation (Tsui and Wang, 2005). Kwok et al. (2009) found that a single generation exposed to copper strikingly enhanced metal tolerance in *T. japonicus*, which was then lost when the tolerant lines' offspring were reared in absence of copper. The acquired copper resistance in *T. japonicus* through acclimation seems more likely to be a result of physiological acclimation rather than a genetic adaptation, and is considered to be phenotypically plastic. A previous study examined the toxic effects of multigenerational exposure to Hg in a population of freshwater cladocera *D. magna*, indicating that Hg tolerance in the animals could partially be explained by enhanced MT synthesis, which is only a phenotypic phenomenon (Tsui and Wang, 2005). Similarly, this phenomenon has been found in other multigenerational toxicity studies of chemical pollutants including endocrine disrupters and pharmaceutical waste (Clubbs and Brooks, 2007; Tominaga et al., 2003). Further studies are required to determine how marine animals are acclimating to Hg pollution, particularly focusing on the hypothesis that this acclimation is linked to MT induction. The present study suggests that the harmful effects of Hg on population viability of *T. japonicus* may end as soon as Hg pollution is removed from the environment, since this species exhibits potential for phenotypic plasticity.

5. Conclusions

Our study indicated that Hg accumulated in the adult copepods in a dose-dependent manner during the multigenerational exposure and recovery testing experiments. For the exposure concentrations used, higher T-Hg contents were reported in F3 as compared to F1, highlighting that Hg attack might exert cumulative toxic effects in the treated animals during multigenerational exposure. Among the six life history traits, only fecundity and number of nauplii per clutch were sensitive to Hg toxicity, and the observed effect worsened in consecutive generations. This may be explained by the increased metal accumulation with generations. Interestingly, all the traits were not correlated with Hg toxicity after one generation of recovery in clean seawater, though the copepod's Hg levels increased with increased metal concentrations used in the pre-multigenerational exposure. Even though cumulative damage was observed in Hg multigenerational effects, metal acclimation could also be acquired by marine copepods and it seemed to be underpinned by phenotypic plasticity. However, an omics-based study should be utilized to elucidate the detailed mechanism of action for phenotypic plasticity exhibited by marine copepods under long-term Hg exposure. Overall, alterations in the important life cycle traits could interfere with the population dynamics of some important copepods, and subsequently cause unexpected ecological impacts in the marine ecosystem. Yet, as a result of displaying phenotypic plasticity, the toxic effects of Hg pollution rapidly disappear in the copepod as the Hg is cleared from the environment.

Acknowledgments

We thank the two anonymous reviewers for their comments on this work. This study was supported by the National Natural Science Foundation of China (No. 41476094), the Fundamental Research Funds for the Central Universities (No. 2013121043), and the National Programme on Global Change and Air-Sea Interaction (No. GASI-03-01-03-01). The authors greatly appreciate the kind help from Pro. Dr. Dongxing Yuan (Xiamen University) in Hg analysis.

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