Comparative effects of different metals on the Japanese medaka embryos and larvae

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Abstract

Rapid evaluation of the toxicity of metals using fish embryo acute toxicity is facilitative to ecological risk assessment of aquatic organisms. However, this approach has seldom been utilized for the comparative study on the effects of different metals to fish. In this study, acute and sub-chronic tests were used to compare the toxicity of Se(IV) and Cd in the embryos and larvae of Japanese medaka (*Oryzias latipes*). The embryos with different levels of dechorionation and/or pre-exposure were also exposed to Se(IV) and Cd at various concentrations. The results showed that the LC_{50}-144 h of Cd was 1.3–5.2 folds higher than that of Se(IV) for the embryos. In contrast, LC_{50}-96 h of Se(IV) were 200–400 folds higher than that of Cd for the larvae. Meanwhile, dechorionated embryos were more sensitive to both Se and Cd than the intact embryos. At elevated concentrations, both Se and Cd caused mortality and deformity in the embryos and larvae. In addition, pre-exposure to Cd at the embryonic stages enhanced the resistance to Cd in the larvae. However, pre-exposure to Se(IV) at the embryonic stages did not affect the toxicity of Se(IV) to the larvae. This study has distinguished the nuance differences in effects between Se(IV) and Cd after acute and sub-chronic exposures with/without chorion. The approach might have a potential in the comparative toxicology of metals (or other pollutants) and in the assessment of their risks to aquatic ecosystems.

Introduction

Understanding the effects of metals to early life stages of fish is essential for risk assessment of metals to aquatic ecosystems (Wang and Tan, 2019). Generally, the early life stages (e.g., embryo or larva) are considered one of the most sensitive stages in the life cycle of animals to various pollutants (Halbach et al. 2020; Klüver et al. 2015). Meanwhile, the relative short period of these early life stages make them ideal for the acute testing of various pollutants on a suite of endpoints related to differentiation and development, which are vital for growth and survival during this critical developmental period (Su et al. 2021). For example, the Fish Embryo Acute Toxicity (FET) (i.e., OECD test guideline (TG) 236) has been proposed as an alternative for acute fish toxicity testing such as the OECD Acute Fish Toxicity Test (TG 203) (Léonard et al. 2005; Sobanska et al. 2018), which is in accordance with the idea to reduce, refine and replace animal testing (3 R) by alternative test methods proposed by OECD (Dang et al. 2017; von Hellfeld et al. 2020). Since life cycle toxicity tests are only feasible for certain species, it is essential to use data from partial life cycle studies to estimate effects for the whole life cycle (Hutchinson et al. 1998). Data on the toxicological effects of various pollutants on non-target aquatic organisms at the sensitive early life stages can be relatively easily obtained (Belanger et al. 2013; Su et al. 2021). More importantly, data on toxicological effects on aquatic organisms at these sensitive stages are indispensable for the ecological risk assessment of pollutants due to the importance of these stages for animals (Marimuthu et al. 2013).

Prior to hatching, the fish embryo is surrounded by an acellular envelope, the chorion. Potentially, there is a risk of generating false negative results or underestimation of the developmental toxicity of some chemicals due to limited permeability of the chorion for these chemicals (Henn and

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Braunbeck, 2011). However, it is unclear whether the chorion represents an effective barrier to protect the embryo from exposure to certain chemicals or not. The simplest way to verify this is to use the embryos with the chorion removed in the toxicity testing. Additionally, it is known that the permeability of the chorion may alter during the embryonic development. For example, after hardening, the chorion becomes less permeable (Gellert and Heinrichsdorff, 2001). One earlier study suggests to integrate dechorionation with the FET test to improve the accuracy of fish embryo toxicity test (Henn and Braunbeck, 2011). However, data on metal toxicity on the early stages of fish embryos with or without chorion are still very scarce.

The aim of this study was to compare the toxicity of two inorganic substances (i.e., an essential metal (Se) and a non-essential metal (Cd)) under acute and sub-chronic exposures and with/without chorion. In this study, the embryos of Japanese medaka (*Oryzias latipes*) were dechorionated using proteinase and hatching enzymes. The dechorionated embryos and the larvae hatched were used for the determination of the acute toxicity of Se(IV) and Cd. In addition, the influence of pre-exposure at the embryonic stage on the effects of Se(IV) and Cd at the larval stage was assessed. The results of this study may help to better understand the toxicity Se and Cd to early life stages of freshwater fish such as Japanese medaka and other fish and provide insights into the different effects caused by essential and non-essential metals.

Materials and methods

Medaka culture and embryo collection

Japanese medaka (*Oryzias latipes*) were maintained for generations under standard recirculating water conditions. Fish were maintained in moderately hard reconstituted (MHR) (114 mg/L NaHCO$_3$, 70 mg/L CaSO$_4$, 64 mg/L MgSO$_4$, and 5.1 mg/L KCl) water at 24 °C with pH 7.2–7.6 and a light: dark cycle of 14:10 h. Adult *O. latipes* were fed freshly hatched brine shrimp *Artemia* sp. nauplii twice daily during the spawning period. Embryos at the bottom of the aquarium were collected by siphoning, rinsed with dechlorinated water, and cleaned by rolling on a moistened paper towel. The cleaned embryos were examined under a dissecting microscope. The embryos at 6–7 h post fertilization (hpf) (~ at stage 10) were selected for the experiments following a previous study (Wang et al. 2020a).

Dechorionation of embryos

Healthy embryos at 6–7 hpf were dechorionated according to a previous method using a two-step protease treatment with a combination of proteinase and hatching enzyme with minor modifications (Porazinski et al. 2010). The experiment included 4 treatments depending on the degree of dechorionation. The intact embryos did not undergo any dechorionation and were used for control. For the sandpaper-treated group, the intact embryos (5–10) were placed on fine grit sandpaper (p2000 grit size, waterproof) and rolled gently for 40–60 times. The embryos after this step did not have any villi and were termed sandpaper-treated embryo. The sandpaper-treated embryos were treated with sequential digestion by protease (20 mg/mL, 27 °C, 1 h) and hatching enzyme (27 °C, 30 min). After this step, the embryos had many lunar holes on the chorion with normal shape and were termed partially dechorionated embryos. For the fully dechorionated embryos, the sandpaper-treated embryos were treated by protease (20 mg/mL, 27 °C, 1.5 h), followed by hatching enzyme at 27 °C for 50 min. After the sequential digestion, the chorion of the embryos was completely digested and/or gently removed by micro-forceps under the microscope. The embryos were termed fully dechorionated embryos.

Exposures of embryos to Se and Cd

The embryos with different levels of dechorionation were transferred to the wells in polystyrene 12-well flat-bottom plates (Corning Costar, Cambridge, MA, USA) containing 3 mL culture medium. Each level of dechorionated embryos were replicated thrice each with 5 embryos. The embryos were exposed to Se(IV) (as Na$_2$SeO$_3$ (CAS No. 10102-18-8, purity 98%), Sigma-Aldrich, St. Louis, MO, USA) ranging from 0.0 to 400.0 μM (with a spacing factor of 2) or Cd (as CdCl$_2$ (CAS No. 10043-52-4, purity 99.99%), Sigma-Aldrich, St. Louis, MO, USA) with a concentration range from 0.0 to 1600.0 μM (with a spacing factor of 2). Exposure media were prepared by mixing an appropriate volume of the stock solution of Se(IV) (100 mM) or Cd (100 mM) with culture medium. Plates were sealed with parafilm to prevent evaporation. Plates containing embryos were placed in an incubator at 27 °C with a 14 h light:10 h dark cycle. During the exposure, the exposure medium was changed every other day. Mortality was checked and recorded daily. The exposure lasted for 6 days. After the 6-d exposure, the embryos were transferred to wells with clean culture medium for hatching. The number of hatched larvae and malformation were recorded. The embryos and larvae were imaged using a Nikon Eclipse 50i light stereoscopic microscope (Nikon, Tokyo, Japan). Deformities in embryos were observed every day during the test. Different categories of abnormalities include spinal deformities, cardiovascular anomalies (incomplete or abnormal heart looping, anemia resulting in an absence of blood circulating cells, hemorrhage, and/or local accumulation of motionless blood cell), smaller body, and eye abnormality.

The exposure concentrations of the nominal concentrations of both Se(IV) and Cd were measured according to our previous methods (Chen et al. 2019; Li et al. 2021; Wei et al. 2022; Xie et al. 2016a). The measured concentrations were listed in the supplementary file (Table S1) and were 86.5–97.3% to their nominal concentrations which remained relatively constant during the exposure. Briefly, the water samples were filtered (0.45 μm) and acidified by nitric acid before the Se or Cd determination. Total Se concentrations in the water samples were determined by atomic fluorescence spectrometer (AFS-8300, Titan Co., Ltd, Beijing, China). The instrumental detection limit was 0.1–0.2 μg/L with margin of error within ±2%. Total Cd concentrations in the water samples were measured using inductively coupled plasma mass spectrometry (ICP-MS, Agilent, 7900, USA). Other QA/QC included spiked samples and acid blanks. One blank was run after every five samples. The detection limit of the instrument was 0.1–1.0 ng/L for Cd.

Exposures of larvae to Se and Cd

The larvae hatched after the Se(IV) or Cd exospores were collected and placed in wells of 6-well flat-bottom plates in triplicates each with 5 larvae. Each well held 7 mL culture medium. The larvae were further exposed to Se(IV) at concentrations ranging from 0.0 to 400 μM (with a spacing factor of 2) or Cd at concentrations ranging from 0.0 to 400 μM (with a spacing factor of 2). The plates were sealed with parafilm to maintain constant exposure concentrations of both chemicals. The plates with larvae were placed in an incubator at 25 °C with a 12 h light:12 h dark cycle. Feed was withheld during the exposure. During the exposure, the exposure media were renewed every other day. The mortality was recorded and dead larvae were removed immediately from the wells. This acute exposure lasted for 4 days.

Additional acute toxicity testing was conducted using larvae hatched from unexposed embryos raised in clean culture medium. In this test, the experiment set up including exposure concentrations for Se(IV) and Cd, larvae per well, number of replicates, and exposure duration, was identical to the above-mentioned acute toxicity test.

Sub-chronic exposures of larvae to Se and Cd

Newly hatched larvae were placed in polystyrene petri dishes with 15 mL culture medium. The larvae were exposed to Se(IV) at 0.0, 0.01, 0.1, 1.0, 10.0 μM or Cd at 0, 0.001, 0.01, 0.1, 1.0, 10 μM for 96 h. These concentrations were chosen based on the environmental relevance in various polluted aquatic ecosystems (Xie et al. 2016a; Xie et al. 2016b). Each treatment had three replicates/wells with ten

larvae each. The exposure media were prepared by mixing stock solution of the Se(IV) or Cd with culture medium. The plates were placed in an incubator at 25 °C under a regime of 12 h light:12 h dark cycle. The exposure lasted for 21 days. During the exposure, the larvae were fed with *Artemia* sp. nauplii twice each day. The exposure media were renewed every other day. The number of dead larvae, total length, weight, and condition factor were documented after the 21 d exposure. The 14-d and 21-d LC_{50} of Se(IV) and Cd were calculated.

Statistical analyses

The LC_{50}, EC_{50}, and EC_{20} values of exposures to Se(IV) and Cd for the embryos and larvae were estimated using the logistic regression method. All data were expressed as mean ± standard error of the mean (SEM) unless otherwise stated. The differences among the treatments were analyzed using one-way analysis of variance (ANOVA). Prior to ANOVA, data were checked for normality and variance homogeneity with Kolmogorov-Smirnov test and Levene's test, respectively. Significant difference was set at $p < 0.05$.

Results

Effects of Se(IV) and Cd on embryos with 4 different levels of dechorionation

The mortality of Se(IV) and Cd on the embryos of *O. latipes* showed a concentration-dependent pattern. The LC_{50}-144h of embryos with 4 different levels of dechorionation after Se(IV) exposures was 81.1 ± 3.0, 59.0 ± 2.9, 29.7 ± 4.8, and 19.9 ± 1.1 μM, for the intact, sandpaper treated, partially dechorionated, and fully dechorionated embryos, respectively (Fig. 1A). For the Cd exposure, the mortality rate showed a similar trend to that of Se(IV) exposure. The LC_{50}-144h of Cd was 535.8 ± 8.5, 475.9 ± 29.8, 405.3 ± 6.0, and 291.1 ± 18.9 μM, for the intact, sandpaper treated, partially dechorionated, and fully dechorionated embryos, respectively (Fig. 1B). In comparison, the LC_{50}-144h of Se(IV) was much lower than that of Cd for the embryos of *O. latipes*, with approximately 7, 8, 14, and 15 folds of difference for the intact, sandpaper treated, partially dechorionated, and fully dechorionated embryos, respectively.

Deformities of the embryos/larvae after Se(IV) and Cd exposures were observed in the fully dechorionated embryos and in the subsequent newly hatched larvae. In the fully dechorionated group, the deformity rate of Se(IV) was about 1.7 folds higher than that of Cd, with EC_{20} of 14.3 ± 3.2 and 24.3 ± 1.5 μM for Se(IV) and Cd, respectively (Fig. 1C, D). In addition, the EC_{50} of Se(IV) for hatching rate of the embryos was decreased as the level of

Fig. 1 The toxicity of Se(IV) (0–400 μM) and Cd (0–1600 μM) to medaka embryos for 6 d with 4 different levels of dechorionation (i.e., intact, sandpaper treated, partially dechorionated, and fully dechorionated). Data are expressed as mean ± standard error of the mean (SEM, $n = 3$). Different letters indicate significant differences among groups. **A** the LC_{50} of Se(IV) for the embryos ; **B** the LC_{50} of Cd for the embryos; **C**: the EC_{20} (deformity) of Se(IV) for the embryos and hatched larvae; **D**: the EC_{20} (deformity) of Cd for the embryos and hatched larvae; **E**: the EC_{50} (hatching rate) of Se(IV); **F**: the EC_{50} (hatching rate) of Cd
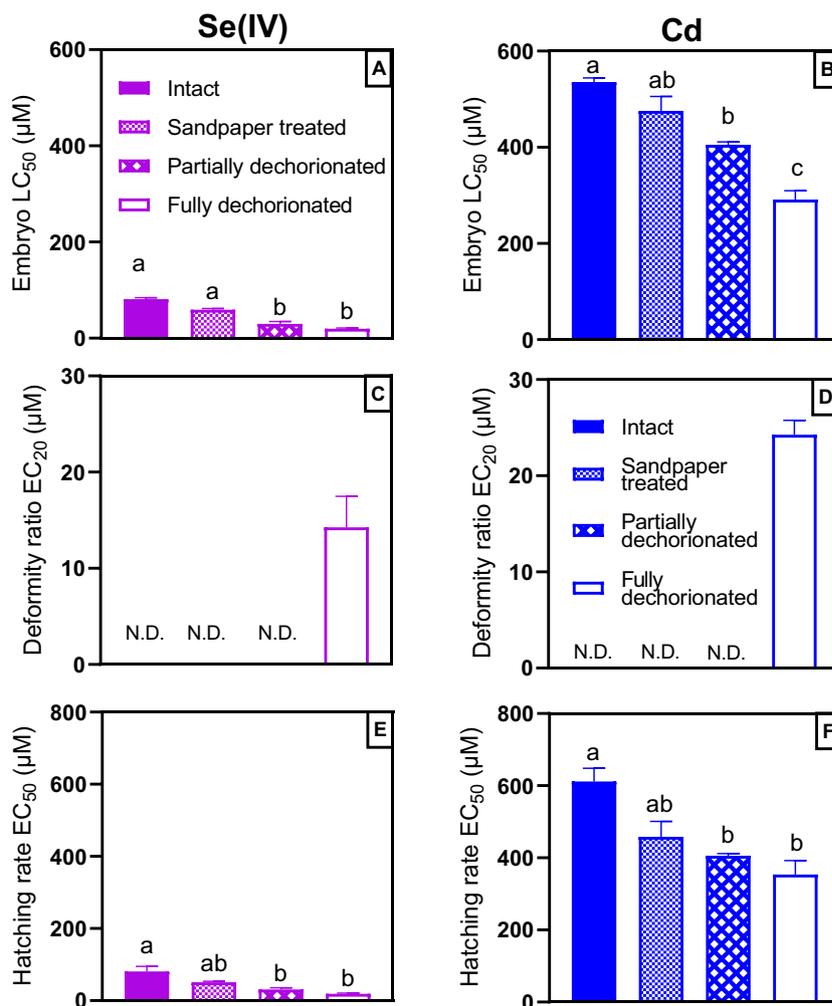
dechorionation increased (from 80.8 ± 14.4 to 18.8 ± 2.2 μM) (Fig. 1E). The EC_{50} of Cd for hatching rate showed a similar pattern, ranging from 611.8 ± 36.4 to 353.5 ± 38.9 μM (Fig. 1F).

Deformities were not observed in the larvae hatched from the embryos in the control (intact), sandpaper-treated, and partially dechorionated treatments, either exposed to Se or Cd. However, obvious deformities were observed in the fully dechorionated embryos after both Se(IV) and Cd exposures (Fig. 2). Typical deformities include spinal deformities, cardiovascular anomalies, delayed abnormal development (smaller body), and eye abnormality.

## Acute toxicity of Se(IV) and Cd to larvae alone and embryo/larvae pre-exposure

The acute toxicity of Se(IV) and Cd on the larvae hatched from the Se(IV)- or Cd-exposed embryos were concentration-dependent. The LC_{50} of Se(IV) for *O. latipes* larvae was 104.2 ± 5.8 μM, while the LC_{50} of Cd was 1.3 ± 0.2 μM. The LC_{50} of Se(IV) for the pre-exposed larvae was 115.6 ± 10.9 μM, while the LC_{50} of Cd for larvae was 3.4 ± 0.6 μM. The LC_{50} of Cd was approximately 80 folds lower in magnitude compared with that of Se(IV) at the larval stage, but the difference was narrowed down to 34 folds after pre-exposure to Se(IV) and Cd at the embryonic stage. Additionally, the pre-exposure to Cd at the embryonic stages enhanced the tolerance of the larvae to Cd (with ~3 folds of difference), but not for Se(IV) (Fig. 3).

Sub-chronic toxicity of Se(IV) and Cd exposures on medaka larvae (21 d)

The total length and body weight of Cd-exposed larvae were ~10% and 21% lower than those of the Se(IV)-exposed larvae, respectively. Both Cd and Se(IV) did not affect the condition factor of the larvae. The LC_{50}-14 d and LC_{50}-21 d of Cd for the larvae were also much lower than their counterparts of Se(IV), with approximately 15 and 10 folds of differences respectively. In addition, the LC_{50}-21 d was also much lower than LC_{50}-144 h (Fig. 4).

Fig. 2 Representative images of deformity observed during embryonic development in the fully dechorionated embryos after exposure to Se(IV) (0–400 µM) and Cd (0–1600 µM) for 6 d. Black bars indicate 500 µm. Red, blue, yellow, and black arrows denote spinal deformities, cardiovascular anomalies, smaller body, and eye abnormality, respectively
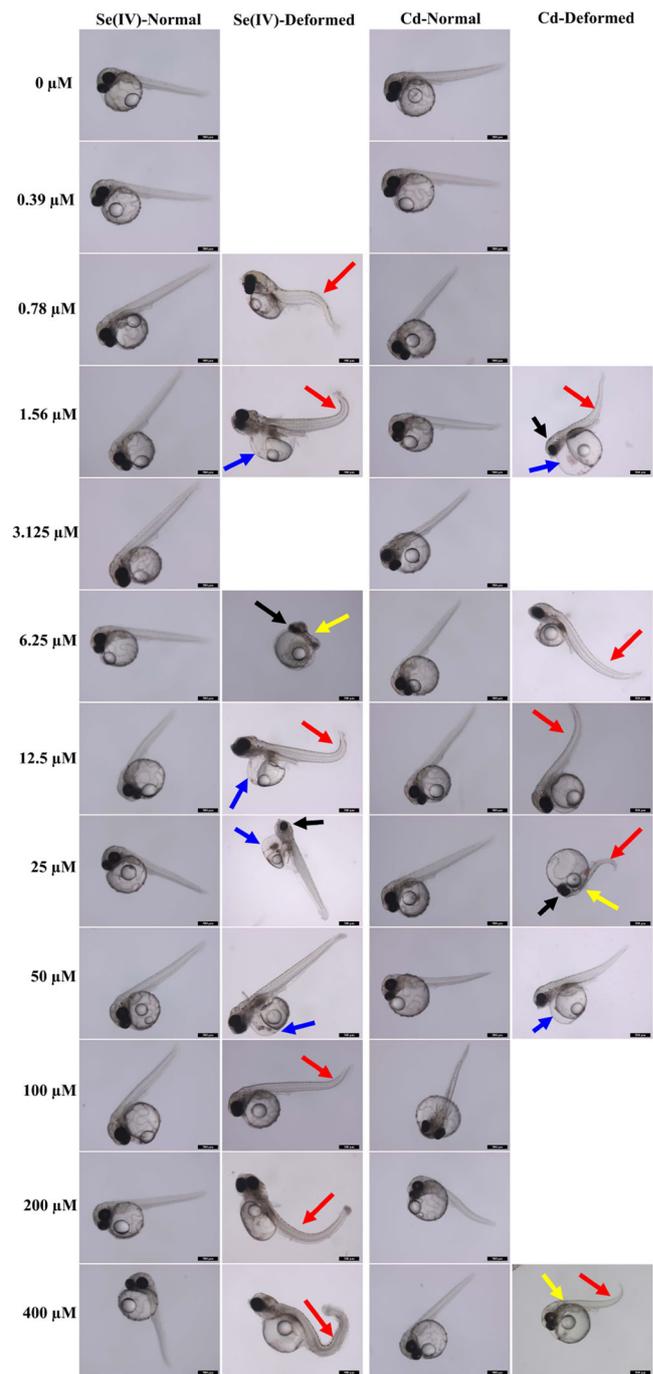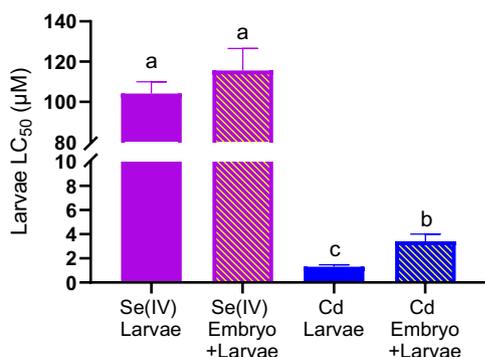

Discussion

The effects of Se(IV) on the embryos and larvae of *O. latipes*

In this study, the LC$_{50}$-144 h of Se(IV) for the embryos was about 1.3–5.2 folds lower (depending on 4 different levels of dechorionation) than that for the larvae, implying that the toxicity of Se(IV) was higher for embryos than that for larvae. A previous study shows that the toxicity of nano Se for the ilish fish (*Tenualosa ilish*) decreases as the developmental stages increases, as evidenced by the elevation of the LC$_{50}$-96 h from 0.89 mg/L for the larvae to 1.65 mg/L for fingerlings (Sadeghi and Peery, 2018). Similarly, different sensitivity to metals at different developmental stages is shown in a study revealing that the embryos are more sensitive to Cu and Zn than the larval stages in two crustaceans (*Crassostrea gigas* and *Mytilus edulis*) (Martin et al. 1981). These results suggest that fish at different developmental stages have different sensitivities to Se(IV) and other metals (Bai et al. 2021; Wang et al. 2020b).

Selenium is an essential micronutrient that acts as a building block for selenocysteine and is required for the function of more than 40 selenoproteins in teleosts, such as glutathione peroxidase and selenoprotein P, which play critical roles in various physiological functions (Pacitti et al. 2015; Roman et al. 2014). In the embryos, Se(IV) is rapidly absorbed by the yolk in the embryos. Once inside the cells, Se(IV) is metabolized via various reductions to organic Se species (i.e., seleno-methionine and seleno-cysteine) prior to incorporation into selenoproteins (Li et al. 2021; Liu et al. 2021). During the speciation of Se, reactive oxygen species (ROS) generated are considered to be responsible for the toxicity of Se (Ma et al. 2018; Sun et al. 2014; Xie

et al. 2016b). Therefore, the embryos could not counter against the toxicity of ROS produced from the speciation of Se. On the other hand, Se(IV) taken up by larvae is transported to various tissues. It is rapidly incorporated into selenoproteins. Though it undergoes the same reductions as in the embryos, the larvae have probably developed a more sophisticated system to handle the ROS generated from the metabolism (Zhu et al. 2008). For instance, liver, the major site of detoxification of metals, has been fully developed in the larvae but may not be fully functional during the embryonic stage (Mao et al. 2020). The larvae might need more Se during rapid growth period and for other biological functions (for instance, immunological need for Se). However, the exact mechanisms underlying this difference in the toxicity of Se between the embryos and the larvae deserve future investigation.

The effects of Cd on the embryos and larvae of O. latipes

In this study, the LC_{50} was ~200–400 folds higher for the embryos (depending on 4 different levels of dechorionation) than that for the larvae of O. latipes, suggesting that the toxicity of Cd was much higher for the larvae than that of embryos. This result is in opposite to the one from the Se(IV) test. An early study has shown that the larvae of 7 freshwater fish show higher sensitivity to Cd than that of their embryos (Eaton et al. 1978). Similarly, some studies find that the toxicity of Cd is the lowest to the larvae at 0 dph (days post hatching) in bocachico (Prochilodus

Fig. 3 The acute toxicity (LC_{50}-96 h) of Se(IV) and Cd to intact medaka larvae and the effects of pre-exposure (at the embryonic stages for 6 d) on the acute toxicity of Se(IV) and Cd to the larvae. Data are expressed as mean ± standard error of the mean (SEM, $n = 3$). Different letters indicate significant differences between groups

Fig. 4 The acute (0–400 μM, 4 d) and sub-chronic (0–10 μM, 21 d) effects of Se(IV) and Cd on the biometric parameters in Oryzias latipes larvae. A: total length; B: total weight (wet weight: ww); C: condition factor; D: 14 d-LC_{50}; E: 21 d-LC_{50}. Data are expressed as mean ± standard error of the mean (SEM, $n = 3$). #: significant difference between Se(IV) and Cd; *: significant difference compared with the control; ns: not significantly different. Dash line indicates the control
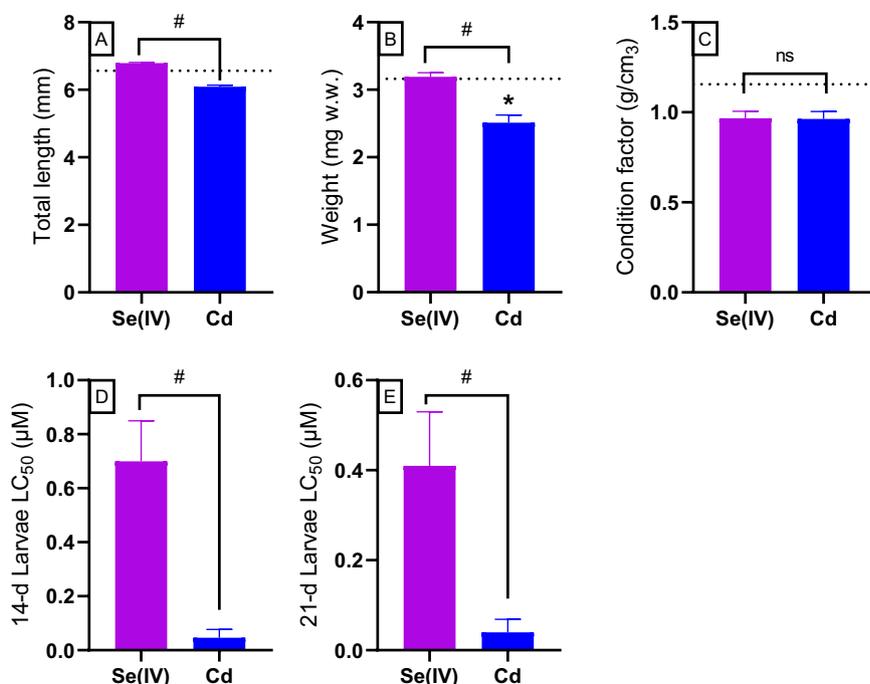
magdalenae) and the tilapia (*Oreochromis mossambicus*) but could be enhanced by approximately 10–30 folds at 7 dph than that of 0 dph (Chang et al. 1997; Hwang et al. 1995; Sierra-Marquez et al. 2019). In addition, the larvae of *C. gigas* and *M. edulis* are more sensitive to Cd than the embryos (Martin et al. 1981). All these results suggest that the developmental stages of aquatic organisms might have different sensitivity to Cd.

The higher toxicity of Cd to *O. latipes* larvae than to the embryos is interesting and unexpected. This could be due to the difference in the accumulation of Cd and the detoxification strategies between the larvae and the embryos. In the embryos, the non-essential element Cd is taken up and presumably binds to proteins (for instance, vitellogenin) in the yolk which is the major deposit site for pollutants (Halbach et al. 2020). Therefore, it is possible that Cd sequestered by the proteins in the yolk sac is not in its active ionic state to exert toxicity to the embryos. In the larvae, Cd taken up is probably redistributed to target organs of the fish to exert its toxicity (Kraal et al. 1995). In addition, Cd is taken up in the larvae by the gills, which are crucial for respiration. Cd may cause a variety of toxicity in the gills right after uptake occurs, including interference with the osmoregulation and histopathological damage prior to its accumulation in other tissues (da Silva and Martinez, 2014; Xie et al. 2016a). Apparently, future research is warranted for the test of these assumptions due to the lack of direct evidence from this study.

The protective role of chorion against Se and Cd toxicity

In this study, dechorionation increases the sensitivity of embryos to both Se and Cd exposures, implying that the chorion has a protection role against toxicity of both elements. For the intact embryos, during exposure, metals are probably bound by the mucopolysaccharides in the chorion (Eddy and Talbot, 1985; Peterson and Martin-Robichaud, 1986) or present in the negatively charged colloid of the perivitelline fluid which attract cations in the exposure medium (Léonard et al. 2005). It is believed that the majority of metals (Cu, Pb, etc.) are accumulated in the chorion (70–92%) of the fish embryos, where detoxification/sequestration of metals by the proteins in the chorion occurs, thereby reducing the toxicity of metals to the embryos (Jezierska et al. 2009; Léonard et al. 2005). In addition, some studies have found that chorion can prevent the entry of other pollutants into the embryos including nanoparticles (Rotomskis et al. 2018), microplastic particles (Li et al. 2020), and organic pollutants (red sea bream *Pagrosomus major*) (Zhao et al. 2017). On the other hand, dechorionated embryos are shown to be more sensitive than their chorionated cohorts to thiobencarb in Japanese medaka

O. latipes (Villalobos et al. 2000), most probably due to loss of the protection role of chorion against thiobencarb. These results have reinforced the protection role of chorion against the toxicity of metals (including Se and Cd in this study) and other pollutants to fish embryos.

Embryo pre-exposure to Cd slightly increased the resistance of larvae

In this study, the larvae hatched from the embryos pre-exposed to Cd for 6 d had 3 folds of higher LC_{50} than the larvae hatched from the clean embryos, implying that pre-exposure to Cd at the embryonic stage may enhance the tolerance to Cd at the larval stage. It is generally believed that pre-exposure to metals may increase the tolerance to these metals in aquatic organisms. For example, the rainbow trout (*Oncorhynchus mykiss*) pre-exposed to 10.2 µg/L for 21 days showed a 20-fold difference in the incipient lethal levels compared with the larvae without pre-exposure (Stubblefield et al. 1999). In addition, the zebrafish larvae at 3 dph pre-exposed to 10 µg/L Cd for 1 day develop higher tolerance to Cd during later exposure (Gao et al. 2021). Very often, pre-exposure to certain chemicals will activate the defensive systems against the toxicity exerted by these chemicals in the exposed animals. For metals, it is assumed that metallothioneins (MTs), a class of cysteine-rich proteins play important role in the redistribution of metals in fish and the detoxification of the metal via chelating with metals. MTs are inducible upon metal exposure. Therefore, pre-exposure to Cd might induce the production of MTs in the embryos of *O. latipes*, which could handle elevated levels of Cd in the larval stage, thereby enhancing the tolerance to Cd in the larvae.

In this study, in contrast, larvae from the Se(IV) pre-exposed embryos did not show difference in tolerance to Se(IV) compared with those without pre-exposure. This reflects the differences in the biological functions between Se(IV) and Cd. Since Se is involved in many biological processes while Cd is non-essential for animals, their uptake, redistribution, tissue distribution, and metabolisms might vary greatly (Foley et al. 2021). Therefore, it is likely that no specific mechanisms against the toxicity of excess Se is necessary for most organisms. Nonetheless, the exact reasons for the observed lack of difference between the Se pre-exposed larvae and the larvae without exposure remains to be explored.

Conclusion

This study has demonstrated the feasibility of evaluating the toxicity of Se(IV) and Cd using acute and sub-chronic approaches in embryos and larvae of *O. latipes*. In addition, the effects of dechorionation on the sensitivity of fish

embryos to different metals has been evaluated. Se(IV) is more toxic than Cd to the embryos but less toxic than Cd to the larvae. The difference in the toxicity of Se(IV) and Cd in the embryos and larvae could be accounted for by their biological functions (essentiality vs non-essentiality). Meanwhile, the protective role of chorion against metal toxicity has been strengthened in this study. Furthermore, this study reveals the differences in the effects of pre-exposure on the tolerance to the same metal between Se(IV) and Cd. This study has suggested that these rapid approaches are useful for the comparisons of metal toxicity and possibly for organic pollutants, which might facilitate the assessment of ecological risk of pollutants to aquatic ecosystems.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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