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Toxicity Testing of Waterborne Mercury with Red Sea Bream (*Pagrus major*) Embryos and Larvae

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Abstract Acute toxicity tests revealed that, in red sea bream (*Pagrus major*) embryos, 24 and 48 h LC_{50} values of waterborne $HgCl_2$ were 67.3 and 39.1 μg Hg^{2+} L^{-1}. In larvae, 48, 72 and 96 h LC_{50} values were 41.9, 36.1 and 34.8 μg Hg^{2+} L^{-1}, respectively. Sub-chronic toxicity tests indicated that mercury concentrations ≥20 μg Hg^{2+} L^{-1} decreased hatching success, increased mortality and induced teratogenicity in embryos and larvae. The NOEC, LOEC and MATC values were 8.0, 16.3 and 11.4 μg Hg^{2+} L^{-1} for hatching success, mortality and teratogenicity; while those were 27.0, 36.9 and 31.6 μg Hg^{2+} L^{-1} for body length and specific growth rate, respectively.

Keywords *Pagrus major* · Early life stages (ELSs) · $HgCl_2$ · Toxicity

Due to its high toxicity to various biological processes in marine organisms, mercury pollution in the marine environment has been the subject of considerable concern for a long time (Fernández and Beiras 2001; Gopalakrishnan et al. 2008). Although many mercurials can be biologically transformed into methylmercury in the environment, the initial source of mercury compounds in ecosystems are often inorganic mercurials (Heisinger and Green 1975). Previous studies have demonstrated that aqueous mercury exposure at the ppb level can elicit toxicity, resulting in genetic, biochemical, physiological, morphological and behavioral changes in fish (Samson and Shenker 2000). Importantly, bioaccumulation of mercury induces chronic toxicity in fish, subsequently lowering both the quantity and quality of commercial fishing species.

The early life stages (ELSs) of aquatic organisms are particularly sensitive to both natural and anthropogenic stressors (Couillard et al. 2005; Gopalakrishnan et al. 2007; Witeska et al. 1995). ELS bioassays have been widely used as a cost-effective means to rapidly detect and monitor metal pollution in aquatic environments (Beiras and His 1994; Beiras et al. 2003; Weber 2006). Exposure to waterborne mercury during the ELSs of fish can result in inhibition of enzyme activities, chromosomal abnormalities, increased incidence of yolk membrane ruptures, reduced hatching success and survival, altered time to hatch, embryonic and larval teratogenicity, inhibited growth and abnormal larval behaviors (Crump and Trudeau 2009; Devlin 2006; Latif et al. 2001; Samson et al. 2001).

Although pollution is a significant problem for marine fisheries, the toxicity of ocean pollutants to the ELSs of fish in Chinese coastal waters has not been thoroughly investigated either in the laboratory or the field. In the Bohai Sea, a traditional spawning and nursery area for many commercial fishery species, degeneration of the aquatic environment caused by pollution has resulted in low fishery productivity and a decrease in the number of commercial

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fisheries in recent decades (Jin et al. 2005). Although various programs have been implemented to control water pollution in the Bohai Sea, heavy metals remain a common source of pollution in this area (Jin et al. 2005). The average mercury concentration in most areas of the Bohai Sea ranges from 0.002 to 0.15 μg L^{-1}. Much higher levels, however, have been measured at certain sites, such as sites where industrial waste water is discharged (Liu et al. 2003; Zhang 2001). In several recent fishery studies, a large number of abnormal or underdeveloped dead fish embryos were observed in spawning areas. It is believed that these findings could be due to the toxicity of heavy metals including mercury (Jin et al. 2005). Red sea bream (*Pagrus major*) were once an important commercial fishery species in the Bohai Sea, with an annual catch of 5,000 tons in the 1960s. Since the 1980s, however, the wild population has drastically decreased (Yang et al. 1990) and, currently, the red sea bream has almost disappeared from this area (Jin et al. 2005). Previous studies indicate that, during ELSs, red sea bream are sensitive to a number of heavy metals, including cadmium, copper and zinc (Cao et al. 2009, 2010; Huang et al. 2010a). At high concentrations, these metals can cause various developmental problems in embryos and larvae, including decreased hatching success, low survival, and teratogenicity. Mercury is a highly toxic metal that is found in the spawning and nursery areas of red sea bream and is believed to have deleterious effects on wild populations. The effects of mercury exposure during the ELSs of red sea bream, however, remain poorly understood.

We performed both acute and sub-chronic toxicity tests to investigate the effects of waterborne mercury on red sea bream embryos and larvae. The specific objectives of the present study were as follows: (1) determine the embryonic and larval LC_{50} values for mercury and (2) elucidate the effects of sub-chronic mercury exposure on hatching, morphology, survival and growth in embryos and larvae.

Materials and Methods

Fertilized eggs were obtained from a fish breeding laboratory at the Institute of Oceanology, of the Chinese Academy of Sciences, China. Immediately after fertilization, eggs were placed in 20 L incubators maintained at 18 ± 1°C at an estimated density of 800 eggs L^{-1} for 1 h. During this time, dead eggs sank to the bottom of the incubators, whereas viable eggs floated to the top. Only normally developed larvae were used for toxicity testing. The background mercury concentration (total mercury) of the rearing pond seawater was 0.076 μg L^{-1}. Other water quality parameters were as follows: hardness = 6,183 ± 360 mg $CaCO_3$ L^{-1}; pH = 8.2 ± 0.1; dissolved oxygen = 7.5 ± 0.2 mg L^{-1}; salinity = 33 ± 1‰.

The median lethal concentration (LC_{50}) for embryos was determined with a 48 h toxicity test with measured concentrations of mercury. There were seven experimental groups (10, 20, 30, 40, 50, 60 and 70 μg Hg^{2+} L^{-1}) and one control group. Four replicates were performed for each concentration tested. Analytical-grade mercuric chloride ($HgCl_2$, purity >99.5%; CAS No: 7487-94-7; Sigma–Aldrich Corporation, USA) was used for exposures. A stock solution (0.1 g Hg^{2+} L^{-1}) was prepared by dissolving $HgCl_2$ in deionized water. Aliquots of the stock solution were diluted in filtered seawater to obtain the desired final Hg^{2+} concentration. One hundred fertilized eggs were collected and transferred to experimental beakers containing 1,000 mL of the designated exposure solution. Beakers were maintained at 18 ± 1°C with a 14 h light: 10 h dark cycle. The water in the exposure beakers was static and the test solution was not renewed during the exposure period. An embryo was regarded to be dead when it sank to the bottom of the beaker and turned gray. Dead embryos were removed from each beaker at 12 h intervals and were counted to determine mortality.

The LC_{50} for larvae was determined in a 96 h exposure with measured concentrations of mercury. The experimental procedure followed guidelines set forth in the Fish Acute Toxicity Test protocol (OECD 1992a). There were seven experimental groups (20, 30, 40, 50, 60, 70 and 80 μg Hg^{2+} L^{-1}) and one control group, with four replicates for each concentration. One hundred newly hatched larvae were collected and moved to beakers containing 1,000 mL exposure solution. Throughout the exposure period, animals were not fed and the exposure solution was not changed. Larval mortality was determined in the manner described for the embryo acute toxicity test.

Sub-chronic testing was conducted to investigate the biological effects of mercury exposure on embryos and larvae. The larval rearing conditions were identical to those in the acute toxicity tests. The experimental procedure followed the guidelines set forth in the Fish, ELS Toxicity Test protocol (OECD 1992b). Based on the data derived from the acute toxicity tests, the nominal mercury concentrations were as follows: 0 (control), 10, 20, 30, 40 and 50 μg Hg^{2+} L^{-1}. One hundred fertilized eggs were randomly selected and transferred to beakers containing 1,000 mL test solution. Larvae were fed rotifers (*Brachionus plicatilis*) beginning 1 day after they started to open their mouths. Dead individuals and uneaten prey were counted and removed every 4 h during the exposure period. Once larvae began to feed, two-thirds of the test solution in each beaker was renewed daily. Two sets of sub-chronic tests were performed for embryos and larvae: (1) a hatching and survival test to determine the effects of mercury on

hatching success, survival and morphological malformation and (2) a growth test to investigate the effects of mercury exposure on growth and heart rate. Sub-chronic exposures lasted for 10 d, with three replicates for each concentration.

We investigated three biological parameters in the hatching and survival test: (1) hatching success, defined as the percentage of hatched larvae out of the total number of exposed embryos; (2) mortality, defined as the percentage of dead individuals out of the total number of exposed embryos or larvae; (3) teratogenicity, defined as the percentage of abnormally developed larvae out of the total number of hatched larvae. All malformation types observed, including hooked or degenerated tails, spinal deformities and fin erosion, were counted using the method described by Huang et al. (2010b). Because examination of malformations in living larvae was impractical, dead larvae were removed from the experimental tanks every 4 h and checked for morphological abnormalities under a stereomicroscope (Nikon-SMZ1000; Japan).

In the growth test, three viable embryos from each test solution were randomly selected at 30 h post-fertilization (hpf) and the heart rate was measured. For all exposure conditions, hatching occurred at 38 hpf. It took a few hours for newly hatched larvae to stretch out completely and begin swimming freely. Therefore, at 44 hpf three normally developed larvae were randomly sampled from each beaker to determine the heart rate (beats 10 s^{-1}) and body length (L_0, mm) at hatching. At the end of the exposure period (244 hpf), three survivors were randomly selected from each beaker and the heart rate and final body length (L_T, mm) were measured. The specific growth rate (SGR, % d^{-1}) was used to determine larval growth: $SGR = 100 \times (e^g - 1)$. The variable g was defined as $(\ln L_T - \ln L_0)/t$ and t was the time (d) from hatching to the end of the exposure period. Selected embryos and larvae were immediately videotaped and photographed with a microscope video system (Nikon-ECLIPSE 50i and Nikon-SMZ1000; digital video camera, Nikon-DS 5 M; Software, ACT-2U) to determine the body length and heart beat. The heart rate was determined by counting the number of beats that occurred in a 10 s period. Growth and heart rate data from the three larvae selected from each experimental beaker were averaged to represent the respective values for each replicate.

In the acute toxicity tests, test solutions from each beaker were sampled at the beginning and the end of tests and the actual mercury concentration was determined. In the sub-chronic toxicity tests, test solutions were sampled from each beaker at the beginning of the test and after every solution renewal. The total mercury concentration in the exposure solutions was measured with an automated atomic fluorescence spectrometer (AFS; Titan AFS-610A, China) using the method described in the Specification for Marine Monitoring, Mercury Analysis in Seawater GB 17378.4-2007 (SAPRC 2008). The concentration error (%) of mercury in the test solutions is defined as the absolute difference between the measured and nominal concentrations divided by the nominal concentration and multiplied by 100.

Statistical analyses were performed with SPSS 15.0 for Windows (Statistical Program for Social Sciences, Chicago, IL, USA). LC_{50} values, EC_{50} values and their respective 95% confidence limits for the sub-chronic toxicity test endpoints were determined with the probit analysis described by Finney (1971). The biological parameter datasets were checked for assumptions of normality and homogeneity of variance using the Kolmogorov–Smirnov one-sample and Levene tests, respectively. Both assumptions were met for hatching success, heart rate, total length

Table 1 Nominal and measured mercury concentrations of the test solutions (μg L^{-1}; mean ± standard deviation, n = 4 × 2 for each mercury concentration in acute toxicity tests and n = 3 × 5 for each mercury concentration in sub-chronic toxicity tests)

Acute toxicity tests						Sub-chronic toxicity tests					
Embryos test			Larvae test			Hatching and survival test			Growth test		
Nominal values	Measured values	Error[a] (%)	Nominal values	Measured values	Error[a] (%)	Nominal values	Measured values	Error[a] (%)	Nominal values	Measured Values	Error[a] (%)
Control	0.079 ± 0.015	–	Control	0.067 ± 0.023	–	Control	0.064 ± 0.019	–	Control	0.055 ± 0.013	–
10	8.25 ± 0.29	17.5	20	17.52 ± 0.66	12.4	10	8.02 ± 0.30	19.8	10	8.18 ± 0.41	18.2
20	17.12 ± 0.45	14.4	30	27.92 ± 0.56	6.9	20	16.25 ± 1.13	18.8	20	16.71 ± 0.80	16.5
30	27.00 ± 0.74	10.0	40	39.32 ± 1.48	1.7	30	24.29 ± 2.94	19.0	30	27.01 ± 1.95	10.0
40	39.65 ± 2.02	0.9	50	48.24 ± 3.35	3.5	40	38.68 ± 1.73	3.3	40	36.88 ± 3.70	7.8
50	47.18 ± 4.86	5.6	60	56.34 ± 3.35	6.1	50	42.77 ± 3.06	14.5	50	41.74 ± 6.53	16.5
60	57.22 ± 6.34	4.6	70	67.64 ± 1.68	3.4						
70	68.20 ± 1.77	2.6	80	76.69 ± 1.13	4.1						

[a] Concentration error as defined in the text

and SGR, therefore, differences between treatment and control means for these parameters were subsequently analyzed with a one-way ANOVA followed by a Dunnett's multiple comparison test. The mortality and teratogenicity datasets, however, did not meet both of these assumptions and were subjected to a nonparametric Kruskal–Wallis test followed by a Mann–Whitney *U*-test. Statistical significance was defined as $p < 0.05$. The no observed effect concentration (NOEC), lowest observed effect concentration (LOEC) and maximum acceptable toxicant concentration (MATC, defined as the geometric mean of the NOEC and LOEC values) were determined from the results of ANOVA and nonparametric analyses of the biological parameter datasets obtained from the sub-chronic toxicity test. The calculation of all toxicity values (e.g. LC_{50}, EC_{50}, NOEC, LOEC and MATC) was based on the measured concentrations of the test solutions.

Results and Discussion

The actual and nominal mercury concentrations of test solutions are shown in Table 1. In the acute toxicity tests, the concentration errors were 0.9%–17.5% (embryos) and 1.7%–12.4% (larvae). The concentration errors were 3.3%–19.8% in the hatching and survival test and 7.8%–18.2% in the growth test. The actual mercury concentrations were within 80%–120% of the target concentrations, falling within the acceptable concentration error range defined by OECD (1992b) testing guidelines.

In the present study, the 24 and 48 h embryonic LC_{50} values were 67.3 (59.5–79.2) and 39.1 (33.8–47.5) μg L^{-1}, respectively. These values are generally lower than those reported for other species of marine and freshwater fish. The killifish (*Fundulus heteroclitus*), a marine species, has an estimated 48 h LC_{50} above 67.4 μg L^{-1}, while the 24 and 48 h LC_{50} values for the orangethroat darter (*Etheostoma spectabile*), which is a freshwater species, are 93.5 and 64.5 μg L^{-1}, respectively (Sharp and Neff 1982, USEPA 1985). The 48, 72 and 96 h larval LC_{50} values were 41.9, 36.1 and 34.8 μg L^{-1}, respectively. The 96 h LC_{50} value in red sea bream larvae was similar to most other marine fish species: 96 h LC_{50} for the tongue sole (*Cynoglossus semilaevis*) is 45 μg L^{-1} and 96 h LC_{50} value for the haddock (*Melanogrammus aeglefinus*) is 98 μg L^{-1} (Liu et al. 2006; USEPA 1985). These findings suggest that embryonic and larval red sea bream were slightly more sensitivity to mercury than other reported fish species.

In the sub-chronic toxicity test, the measured mercury concentrations of the test solutions ranged from 0.055 to 42.77 μg Hg^{2+} L^{-1}, generally encompassing the ambient levels (0.002–32.00 μg Hg^{2+} L^{-1}) reported in the Bohai Sea spawning and nursery areas (Liu et al. 2003; Zhang 2001). Hatching success decreased significantly with increasing mercury concentrations (ANOVA, $p < 0.05$; Fig. 1a). Hatching success in the 10-μg Hg^{2+} L^{-1} exposure group (96%) was not significantly different from the control group (96%; Dunnett's test, $p > 0.05$). Exposure to ≥20 μg Hg^{2+} L^{-1}, however, significantly decreased hatching success (82, 75, 28 and 12% at 20-, 30-, 40- and 50-μg Hg^{2+} L^{-1} concentrations, respectively; Dunnett's test, $p < 0.05$). Hatching is a common endpoint in ELS toxicity testing (Gopalakrishnan et al. 2008). Mercuric

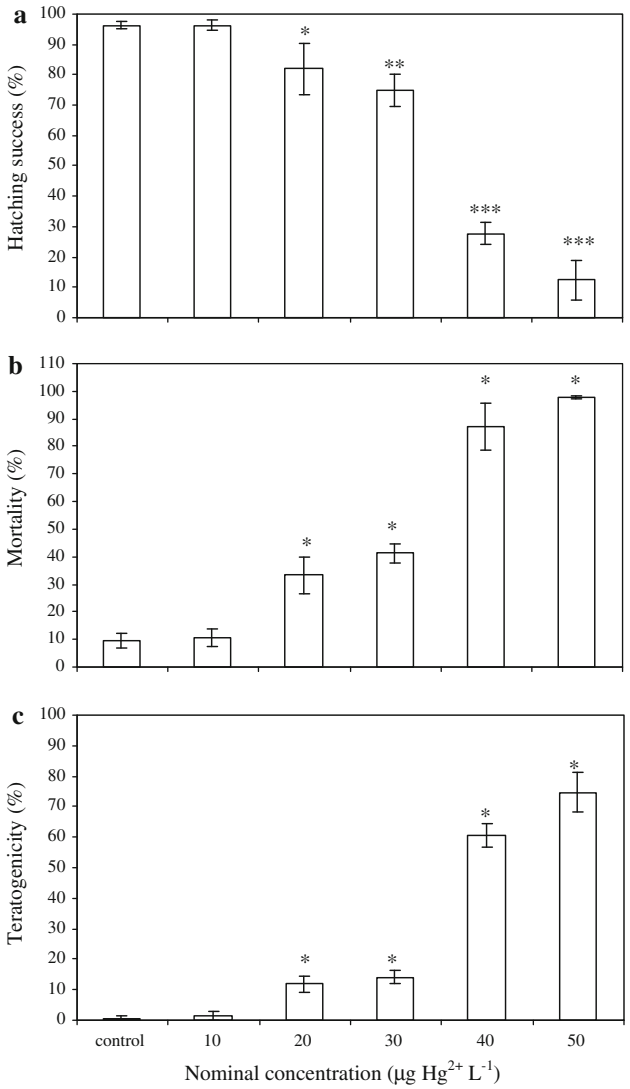

Fig. 1 Hatching success (**a**), mortality (**b**) and teratogenicity (**c**) of embryonic and larval red sea bream following a sub-chronic exposure to mercury. Data are expressed as the mean ± standard deviation (n = 3 at each concentration). The Dunnett's test was performed for the hatching success dataset, and the Mann–Whitney *U*-test was performed for the mortality and teratogenicity datasets. *, ** and *** indicating significant differences ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively) from the control group

Fig. 2 Teratogenic effects in the red sea bream larvae following a sub-chronic exposure to mercury: normally developed larvae (**a–c** 0, 2, 7 dph, respectively), V-shaped spine (**d** 20-μg Hg^{2+} L^{-1} exposure at 0 dph), curled tail (**e** 20-μg Hg^{2+} L^{-1} exposure at 2 dph), curled spine and tail (**f** 30-μg Hg^{2+} L^{-1} exposure at 7 dph), degenerated tail (**g** 30-μg Hg^{2+} L^{-1} exposure at 0 dph), erosion of fins (**h** 50-μg Hg^{2+} L^{-1} exposure at 2 dph) and curled tail (**i** 40-μg Hg^{2+} L^{-1} exposure at 7 dph)

chloride exposure is known to reduce hatching success in several fish species, including ricefish (*Oryzias latipes*; Heisinger and Green 1975) and killifish (Sharp and Neff 1980). Decreased hatching success following mercury exposure may be due to elevated levels of metal in the egg, resulting in structural and functional disturbances during embryonic development (Jezierska et al. 2009). It was also suggested that mercury may interact with the sulfhydryl (-SH) groups of proteins associated with the chromosomal spindle apparatus. This interaction could result in unequal migration of chromatids during the anaphase of mitosis, leading to elevated mortality rates in fish embryos (Latif et al. 2001; Sharp and Neff 1982).

Embryonic and larval mortality increased with increasing mercury concentrations (Kruskal–Wallis test, $p < 0.05$). Although mortality did not significantly differ between the controls (9.7%) and the 10-μg Hg^{2+} L^{-1} (10.7%) exposure group (Mann–Whitney *U*-test, $p > 0.05$), fish exposed to the higher mercury concentrations exhibited significantly increased mortality (33.3, 41.3, 87.0 and 97.7% at 20-, 30-, 40- and 50-μg Hg^{2+} L^{-1} concentrations, respectively; Mann–Whitney *U*-test, $p < 0.05$; Fig. 1b).

Teratogenicity also increased with increasing mercury concentrations (Kruskal–Wallis test, $p < 0.05$; Fig. 1c). In the 10-μg Hg^{2+} L^{-1} exposure group, 1.4% of larvae were abnormally developed. This group was not significantly different from the control group (0.7% teratogenicity; Mann–Whitney *U*-test, $p > 0.05$). However, the incidence of morphological abnormalities was significantly elevated (Mann–Whitney *U*-test, $p < 0.05$; Fig. 1c) in the higher exposure groups (11.9%, 14.2%, 60.5% and 74.7% at 20-, 30-, 40- and 50-μg Hg^{2+} L^{-1} concentrations, respectively). Mercury has been found to elicit teratogenicity in fish embryos and larvae even at low concentrations (Samson et al. 2001; Weis and Weis 1977). Embryonic exposure to mercury can cause a variety of morphological abnormalities in fish larvae that could result from abnormal development of vertebral or muscle tissues, including abnormal eye development, spine or tail flexures, cardiac malformations, jaw deformities and axial coiling (Dial 1978; Heisinger and Green 1975; Samson and Shenker 2000; Weis and Weis 1977). In the present study, a number of larval deformities were observed, including hooked or degenerated tails, spinal malformations

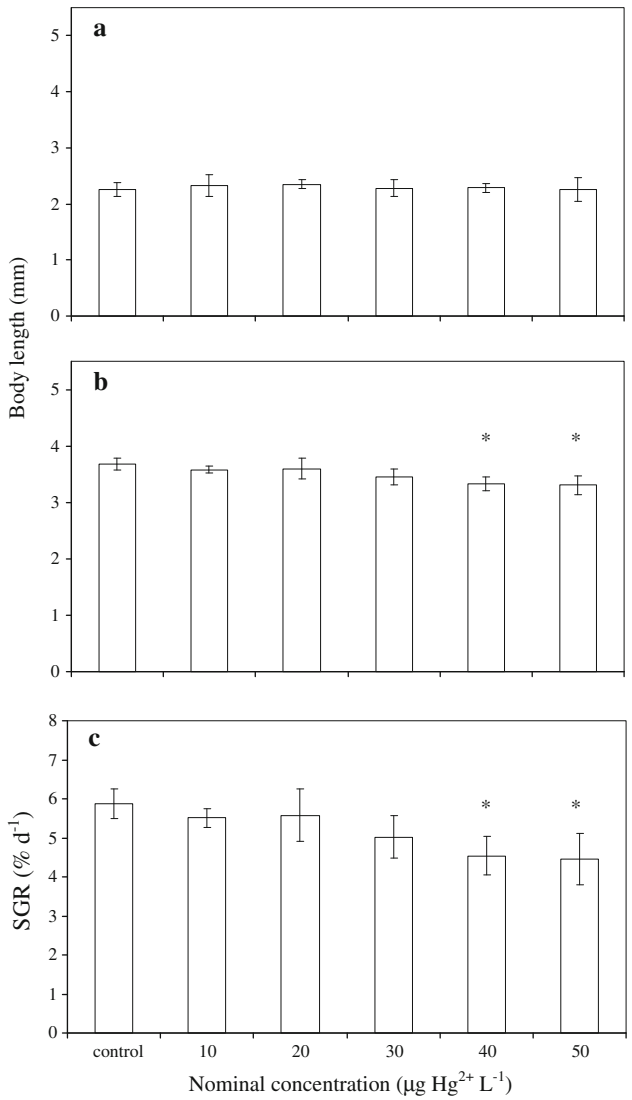

Fig. 3 Body length at 44 hpf (**a**) and 244 hpf (**b**) Specific growth rate (SGR) at 244 hpf (**c**) of the red sea bream larvae following a sub-chronic exposure to mercury. Data are expressed as the mean ± standard deviation (n = 3 at each concentration). The Dunnett's test was performed to compare differences between means. *Asterisk* indicates a significant difference ($p < 0.05$) from the control group

and fin erosion (Fig. 2). Spinal deformities and tail malformations occurred most frequently. Spinal deformities were likely caused by neurotoxic tetanic contractions of skeletal muscles in those with acute exposure, and by altered bone composition, rendering the skeleton more fragile, in those with chronic exposure (Rand and Petrocelli 1985). High levels of teratogenicity were generally accompanied by increased larval mortality, suggesting that mercury-induced teratogenicity may result in mortality. Interestingly, abnormal pigmentation, pericardial edema and visceral hemorrhage, which are effects often observed in red sea bream following cadmium or zinc exposure (Cao et al. 2009; Huang et al. 2010a), were not observed in the present study.

Mercury exposure had no effect on the heart rates of embryos (11–13 beats 10 s^{-1}; ANOVA, $p > 0.05$) or larvae (15–18 and 27–31 beats 10 s^{-1} at 44 and 244 hpf, respectively; ANOVA, $p > 0.05$). Compared to other ELS biological endpoints (Couillard et al. 2005; Gopalakrishnan et al. 2007, 2008; Witeska et al. 1995), the effects on heart rate following mercury exposure have not been thoroughly investigated. Aagaard et al. (2000) reported that, in crabs (*Gaetice depressus*), heart rates were elevated at $HgCl_2$ concentrations above the 96 h LC_{50} but depressed at concentrations below the 96 h LC_{50}. In walleye embryos, the heart rate decreased markedly as the concentration of MeHg increased (Latif et al. 2001). The mechanisms underlying mercury-induced changes in fish heart rates remain unclear. Previous studies, however, suggest that mercury exposure induces a variety of embryonic and larval heart defects that could result in heart rate disturbances, including ventricle defects, abnormal heart division, or blood tube underdevelopment (Weis et al. 1981). In red sea bream, zinc (0–2.5 mg L^{-1}), cadmium (0–3.2 mg L^{-1}) or copper (0–0.12 mg L^{-1}) exposure did not significantly affect embryonic heart rates. In contrast, larvae exhibited elevated heart rates following exposure to ≥1.0 mg Zn^{2+} L^{-1} (Huang et al. 2010a). Conversely, larval heart rate was repressed following exposure to ≥0.8 mg Cd^{2+} L^{-1} or ≥0.06 mg Cu^{2+} L^{-1} (Cao et al. 2009, 2010). In the present study, mercury exposure did not significantly affect the heart rate of either embryonic or larval red sea bream. These results suggest that, in red sea bream, the effect of ELS heavy metal exposure on heart rate varies depending on the stage of development. Heart

Table 2 Estimates of sub-chronic toxicity of mercury on red sea bream embryos and larvae

Parameters	NOEC (μg L^{-1})	LOEC (μg L^{-1})	MATC (μg L^{-1})	EC_{50} (μg L^{-1})
Hatching success	8.0	16.3	11.4	39.8
Mortality	8.0	16.3	11.4	23.7
Teratogenicity	8.0	16.3	11.4	36.2
Body length (244 hpf)	27.0	36.9	31.6	–
Specific growth rate	27.0	36.9	31.6	–

NOEC no observed effect concentration, *LOEC* lowest observed effect concentration, *MATC* maximum acceptable toxicant concentration (defined as the geometric mean of the NOEC and LOEC), EC_{50}–median effective concentration

rate, therefore, may not be an effective endpoint for assessing heavy metal toxicity in this species.

Mercury exposure did not significantly affect the body length of newly hatched larvae (44 hpf; 2.25–2.35 mm; ANOVA, $p > 0.05$; Fig. 3a). The length of 244 hpf larvae at both 40-μg (3.33 mm) and 50-μg (3.31 mm) Hg^{2+} L^{-1} concentrations, however, were significantly reduced, compared to controls (3.68 mm; ANOVA Dunnett's test, $p < 0.05$; Fig. 3b). Similarly, larval SGR at 40-μg (4.54% d^{-1}) and 50-μg (4.64% d^{-1}) Hg^{2+} L^{-1} concentrations was significantly lower than in the control group (5.86% d^{-1}; Dunnett's test, $p < 0.05$; Fig. 3c). Exposure to metals, such as copper and lead, is known to significantly decrease the size of newly hatched fish larvae (Jezierska et al. 2009). Zhou et al. (2001) found that MeHg exposure inhibits the growth of killifish. Few studies have assessed the effects of inorganic mercury exposure on larval fish growth. However, growth reduction has been reported following exposure to $HgCl_2$ in other aquatic organisms, including oysters (*Crassostrea gigas*) and hard clams (*Mercenaria mercenaria*; Beiras and His 1994; Calabrese et al. 1977). Toxicant exposure may reduce growth by impairing feeding behaviors, reducing nutrient assimilation or increasing energy expenditures. In the present study, larvae exposed to high concentrations of mercury did not feed or showed sluggish feeding behavior when initially presented with food. An inability to feed could cause starvation, adversely affecting ontogenetic development of the digestive tracts, enzymes activities and digestion of food. Excessive energy expenditures may lead to retarded larval development and growth through inhibition of ChE activity (Couillard et al. 2008) or oxidative stress responses following exposure to chemicals. In the present study, the lowest observed effect concentration (LOEC) of 36.9 μg Hg^{2+} L^{-1} is far above the concentration found in the environment (Liu et al. 2003; Zhang 2001). Importantly, mercury is readily methylated in the environment by bacteria, allowing the metal to easily cross the placental and blood brain barriers. Thus, this form of mercury is highly embryotoxic, neurotoxic and teratogenic (Heisinger and Green 1975; Zhang and Wong 2007; Zhou et al. 2001). In the present study, the test conditions may not have favored formation of the more toxic methylated form of mercury. Thus, the present study may underestimate the potential effects of mercury exposure on the growth of red sea bream larvae.

The NOEC, LOEC and MATC were 8.0, 16.3 and 11.4 μg Hg^{2+} L^{-1} for three ELS parameters of hatching success, mortality and teratogenicity, respectively. These values were lower than those observed for growth (27.0, 36.9 and 31.6 μg Hg^{2+} L^{-1}, respectively). The EC_{50} values for hatching success, mortality and teratogenicity were 39.8, 23.7 and 36.2 μg Hg^{2+} L^{-1}, respectively (Table 2).

In conclusion, the present study revealed that both embryonic and larval red sea bream are sensitive to mercury. At concentrations ≥20 μg L^{-1}, mercury induces low hatching success and increases mortality and teratogenicity in larvae. At concentrations ≥40 μg L^{-1}, 244 hpf larvae exhibited significant reductions in body length and specific growth rate. In most regions of the Bohai Sea, mercury levels range from 0.002 to 0.15 μg L^{-1}. In certain extreme sites that are near to industrial waste water outlets, however, mercury has been measured at concentrations exceeding 20 μg L^{-1} (Liu et al. 2003; Zhang 2001). With the exception of growth and heart rate, all the endpoints in the present study are useful measures to assess waterborne mercury toxicity in wild populations of red sea bream.

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