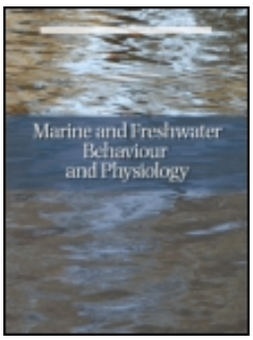

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Lead toxicity at different life stages of the giant prawn (*Macrobrachium rosenbergii,* de Man): considerations of osmoregulatory capacity and histological changes in adult gills

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ABSTRACT

This study evaluated the acute toxicity of lead in different life stages of the freshwater prawn *Macrobrachium rosenbergii*, determined the effect of sublethal Pb concentrations on osmoregulatory capacity (OC), measured the Pb level in gills, and investigated the effect of Pb on the structure of the gills of adult prawns. The 24-, 48-, and 96-h LC_{50} values for Pb to *M. rosenbergii* increased progressively with increasing life stage, from post-larvae (PL), juvenile to adult. The 24- and 48-h LC_{50} values for post-larvae ranged from 0.01- to 0.09-mg Pb/L. The 24-, 48-, and 96-h LC_{50} values for Pb were lower at 12 ppt than those at 0 ppt for either the juveniles or the adults. At 12 ppt, the 96-h LC_{50} values in PL11, juvenile and adult were 0.47-, 0.58-, and 2.03-mg Pb/L, respectively. Meanwhile, at 12 ppt, the 96-h LC_{50} values in PL11, juvenile and adult were 0.63-, 4.44-, and 7.98-mg Pb/L, respectively. In adults, the OC values of controls and prawns exposed to 2- and 4-mg Pb/L at 0 ppt were not significantly different. The OC of prawns exposed to and 2-mg Pb/L at 12 ppt increased by 72 and 109% from the OC of the control prawns. At media 12 ppt, the OC value of prawns exposed to 1-mg Pb/L was significantly different from that of prawns exposed to 2-mg Pb/L. The concentrations of Pb in gill tissues increased significantly in Pb exposed prawns both at 0 and 12 ppt. The level of Pb in gills of prawns exposed to 2-mg Pb/L at 12 ppt was not significantly different from those exposed at 0 ppt. The severe toxic actions of Pb were noted in gills of prawns exposed in media 12 ppt. Hyperplasia and necrosis were observed in gill lamellae, resulting in abnormal gill tips after Pb exposure at media 12 ppt. Since the effect of Pb is more pronounced in higher salinity (12 ppt) than in freshwater (0 ppt) it is clear that aquaculture of *M. rosenbergii* should be conducted in freshwater ponds.

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Lead; toxicity; osmoregulation; accumulation; gills; *Macrobrachium rosenbergii*

Introduction

The giant prawn, *Macrobrachium rosenbergii* (de Man), is a large prawn of family Palaemonidae, inhabits in rivers, estuaries and coastal waters, and distributed throughout

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the Indo-Pacific region (Cheng et al. 2003; Yeh et al. 2005). In nature, *M. rosenbergii* inhabits a wide range of salinities from 0 to 18 ppt during its life cycle (Nelson et al. 1977). The adult lives in the lower reaches of rivers and in the regions unaffected by tidal seawater. During the reproductive season, females migrate from freshwater habitats to estuarine regions to hatch their eggs and to develop the larvae. They return to freshwater after metamorphosing to post-larvae (Nelson et al. 1977). The biological cycle of *M. rosenbergii* consists of several developmental stages, including eleven post-larval stages, followed by juvenile and adult phases (New 2002). This species is commercially important because of its fast growth and is currently widely cultured in mainland China, the Pacific Rim, South Asian, and Southeast Asian countries including Indonesia (New 2002; Sung et al. 2003).

In Indonesia and other countries, prawn farms (both hatchery and culture pond) are often located close to the water resources (i.e. rivers and coasts). From drainage canals or pipeline systems this water is directed into the farms. However, today many water resources are contaminated by damaging compounds such as pesticides and heavy metals from human activities (Yeh et al. 2005; Amado et al. 2006). These chemicals may cause biological damage to all life stages during prawn production. The potential negative impact of damaging compounds, particularly lead (Pb) on prawn farm therefore needs to be better understood.

Lead is a highly toxic metal detectable in practically all phases of the inert environment and all biological systems and is toxic to most living things at high exposure levels (Mager et al. 2011). Lead occurs in the environment as a consequence of both natural and anthropogenic processes, with mining, smelting, coal burning, cement and battery manufacturing, and use in gasoline contributing most to the Pb contamination of aquatic environments (Amado et al. 2006; Grosell et al. 2006). Lead is one of the most used of industrial metals. The anthropogenic release of Pb to the environment is the highest of all heavy metals (Salomons & Förstner 1984; Yeh et al. 2005). The level of Pb in unpolluted waters is about 0.17 μg/L, while it reaches levels as high as 4.5 mg/L near automotive battery factories (Paek et al. 1999; Pérez-López et al. 2003; Amado et al. 2006). The Pb concentrations in coastal waters of Indonesia are varied. For example, at the coast of Palu were 0.7–0.9 mg/L (Tahril & Said 2012) and at the coast of Bangka 1–2.3 mg/L (Arifin 2011).

The physiological, biochemical, and toxic effects of Pb have been determined in a number of crustacean species (Meyer et al. 1991; Bat et al. 1999; Frías-Espericueta et al. 2001; Amado et al. 2006; Offem & Ayotunde 2008; Mager et al. 2011). These studies, however, focused mainly on post-larvae or juvenile stages. Very few data are available on the effects of lead on the different life stages of decapods. The only known data concerning the effect of Pb to different life stages of decapods were reported by Usman et al. (2013) using the marine shrimp *Litopenaeus vannamei*. Asih et al. (2013) studied the effect of other metals (particularly Cu) on different life stages of *M. rosenbergii*, with particular attention to the effect of this metal on osmoregulation and to histological changes of adult gills.

Crustacean tissues are able to accumulate metals from the environment (Soegianto et al. 1999a, 1999b; Rainbow 2002). Uptake of metals occurs throughout epithelial surfaces such as the gill membranes (Scott-Fordsmand & Depledge 1997; Soegianto et al. 1999a). In crustaceans, gills are important organs of respiration, as well as of osmoregulation (Mantel & Farmer 1983; Romano & Zeng 2012). Osmoregulation in crustaceans is potentially altered by metals. The osmoregulatory role of the gills can be damaged by exposure to different levels of metals as demonstrated in several crustacean species (Lawson et al. 1995; Soegianto et al. 1999a, 1999b; Li et al. 2007; Frías-Espericueta et al. 2008; Soegianto et al. 2013). It has

been observed that the death of marine shrimp exposed to lethal Pb concentrations is due to disruption of gill function resulting in perturbation of osmoregulatory capacity (OC) (Usman et al. 2013). Many authors agree that OC can be used as a potential indicator of the physiological condition and a stress indicator among crustaceans (Bambang et al. 1995; Lignot et al. 1997; Ardiansyah et al. 2012; Usman et al. 2013).

The aim of the present study was to evaluate the acute toxicity of Pb in different life stages of the giant prawn *M. rosenbergii* and with consideration of the OC and histological changes in adult gills.

Materials and methods

Experimental organisms

Post-larvae (PL1, PL4, PL7, and PL11), juveniles, and adults of *M. rosenbergii* were obtained from a commercial prawn farm located in Trenggalek, East Java, Indonesia. The organisms were transported in separated plastic bags filled with farmed water saturated with oxygen. The values of salinity, temperature, dissolved oxygen, and pH at the prawn farm were 12 ppt, 28–29 °C, 7.0–7.6 mg/L, and 7.8–8.3, respectively. After transportation to the laboratory, the post-larvae were acclimated to laboratory conditions for about one to two hours before being used for toxicity tests. The juveniles (8.1 ± 1.3 g body weight) and adults (15.6 ± 1.4 g body weight) of *M. rosenbergii* were used for the experiments. Prior to experiments juveniles and adults were acclimated for at least four days in aerated and filtered recirculating brackish water (12 ppt salinity) and freshwater (0 ppt salinity), respectively. Only adults at intermolt or first premolt stages were considered for the experiments. Seawater was obtained from the Surabaya coast adjacent to the university, and the freshwater was obtained from municipal tap water. The tap water was dechlorinated before use in experiments, by letting it stand for at least 24 h to allow the chlorine to evaporate from the water (Putranto et al. 2014). Both seawater and freshwater were filtered through a gravel, sand and sponge filter system by airlift. Prawns were fed (*ad libitum*) twice daily with commercial fish food (30% protein, 3% fat, and 4% fiber) during the acclimation period. To maintain the quality of the ambient medium, uneaten food and feces were removed from the tank everyday. During the experiment, the water temperature was maintained at 28–29 °C, salinity at 0 and 12 ppt, pH 7.5–8.0, and dissolved oxygen at 7.4–7.8 mg/L, with a 12 h light: 12 h dark regime.

Test solution

A stock solution (1000-mg Pb/L) was prepared from 1.831 g of $Pb(CH_3COO)_2{\cdot}3H_2O$ (Merck, Germany) in 1000 mL of deionized water. Selected experimental concentrations were made by addition of adequate volumes of the stock solution to freshwater (0 ppt) or dilute seawater (12 ppt). The background dissolved Pb concentrations of control test media determined by flame atomic absorption spectrophotometer (Shimadzu, AA-6200) were 0.0025 ± 0.0005 mg/L for 12 ppt and 0.0023 ± 0.0007 mg/L for 0 ppt. Approximately 80% of medium containing Pb was renewed at 48 h intervals.

Acute toxicity test

The acute toxicity test in this study was evaluated by the determination of LC_{50}. Because of the short duration of some post-larval stages, the acute toxicity tests terminated 24 h for PL1

and 48 h for PL4 and PL7. For PL11, juveniles and adults, the toxicity tests were finished at 96 h. The toxicity tests for PL1, PL4, and PL7 were run in media with salinity of 12 ppt, meanwhile the assay for PL11, juveniles and adults were conducted in media with 0 and 12 ppt salinity. The different salinities used in these experiments for post-larvae, juveniles and adults were based on the salinities commonly used in the local shrimp farm practices.

Preliminary toxicity tests were conducted prior to initiating a static, acute, definitive toxicity test. Definitive toxicity tests were conducted with triplicate groups of 10 individuals (30 for each Pb concentration) kept either in plastic boxes (15 cm^3 × 10 cm^3× 5 cm^3) containing 500 mL of test solution for post-larvae (PL1, PL4, PL7, and PL11) or in plastic containers (35 cm^3 × 30 cm^3 × 25 cm^3) containing 20 L of test solution for juveniles and adults. Nominal Pb concentrations were 0 (control), 0.0025-, 0.0063-, 0.016-, 0.04-, 1-mg Pb/L for PL1, PL4, and PL7; 0 (control), 0.025-, 0.06-, 0.16-, 0.4- and, 1-mg Pb/L for PL11; 0 (control), 0.25-, 0.63-, 1.6-, 4-, and 10-mg Pb/L for juvenile and adult. Regular observations were made and dead individuals were removed 1, 3, 6, 12, 24, 48, and 96 h, after the beginning of the tests. Death was defined as prawns immobile and showing no response when touched with a glass rod.

Determination of sublethal lead concentration

Effect on OC

OC was determined in adult prawns. OC is defined as the difference between the hemolymph osmolality and the osmolality of the medium at a given salinity. Two groups of 10 adults of *M. rosenbergii* (20 for each Pb concentration) were exposed for 7 days to sublethal Pb concentrations: 0 (control), 2- and 4-mg Pb/L, at 0 ppt (fresh water); and to 0 (control), 1- and 2-mg Pb/L, at salinity of 12 ppt. These different concentrations were based on the different LC_{50} values of Pb in different salinities. Test media were aerated and renewed at 48 h intervals. The animals were fed with commercial fish food two times each day (*ad libitum*) during the test. Excess of food and feces were removed daily.

Hemolymph was sampled from the prawn on the last day of the experiment. Hemolymph was sampled by inserting a 1-ml syringe into the ventro lateral sinus of the abdomen. Approximately, 20 µl of hemolymph from each of the control and exposed animals were drawn, transferred to a Fiske® sample tube, and the osmolality was measured using a Fiske® 210 Micro-Sample Osmometer. Osmolality is expressed as mOsm/kg H_2O. The osmolality of approximately 20 prawns from each of the control and exposed animal groups was determined. The osmolalities of the medium was also measured using the same osmometer.

Lead accumulation in gill tissues

Two groups of 10 adults of *M. rosenbergii* were exposed for 7 days to 0 (control), 2- and 4-mg Pb/L, at 0 ppt (fresh water); and to 0 (control), 1- and 2-mg Pb/L, at salinity of 12 ppt. At the end of the period of Pb exposure, 12–15 adult prawns were dissected and the gills were removed from each individual of each experimental group. These tissue samples were dried at 65 °C for 48 h to a constant weight then homogenized. Approximately, 1 g of homogenized tissue sample was transferred to 3-ml concentrated nitric acid and digested at 90 °C for 4 h. After cooling, samples were filtered and diluted to 50 ml with deionized water; lead concentrations were measured on a Shimadzu type AA-6200 flame atomic absorption spectrophotometer.

Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Pb concentrations of samples were quoted as mg/kg dry weight (dw). The validity of the analytical methods was checked using dogfish muscle reference materials (DORM-2) provided by the National Research Council of Canada. The recovery for Pb in the tissue standard reference material DORM-2 was 90–95% (certified value: 0.065 ± 0.007 mg/kg dw, measured value: 0.061 ± 0.002 mg/kg dw, the values obtained for the analysis of three replicates).

Effect on gills structure

The effect of Pb on the gills was examined in gills dissected from control and Pb-exposed prawns. Prawns used in tests were for examining the sublethal effect of Pb exposure on Pb accumulation in gill tissues. After termination of the experimental period of Pb exposure, five randomly selected adult prawns were sacrificed from the treated and control groups. The gill samples of adult prawns were carefully dissected out and fixed in 4% buffered formalin, dehydrated in an ethanol series, cleared with xylene, embedded in paraffin, and 8-μm thin sections were obtained with a microtome (Microm HM 315, Germany). Each sample was mounted on slides, re-hydrated, stained with hematoxylin, and eosin, observed their histological damage with a microscope (Olympus CX41, Japan), and photographs were obtained with a digital camera.

Statistical analysis

Median lethal concentration (LC_{50}) and 95% confidence intervals were calculated with trimmed Spearman–Karber method (Martins et al. 2011). LC_{50}s were calculated at 24 h for PL1, 48 h for PL4 and PL7, and 96 h for PL11, juvenile and adult. Data were expressed as means ± standard error of the mean. All data were distributed normally as shown by Kolmogorov–Smirnov tests. The comparisons of the effects of different treatments on the OC and Pb concentration in the gills were analyzed by one-way analysis of variance. When significant differences were detected ($p < 0.05$), Duncan's test was used to determine which treatments resulted in significant effects to OC and to Pb concentrations in the gills of adult prawns affected by the Pb concentrations in media with significance set at a level of 0.05. The comparisons of the effects of different treatment on lead concentration in the gills at different salinities were analyzed using a Student's *t*-test.

Results

Acute toxicity

The 24-, 48-, and 96-h LC_{50} values for Pb at 12 ppt of different life stages of *M. rosenbergii* are shown in Table 1. No deaths of control animals were observed in any experiments. The tolerance to Pb increased with increasing life stage: the least tolerant stage was PL1 and the adult was the most tolerant stage. PL11 was more tolerant than PL1, 4, and 7. The 24-h LC_{50} values were low in post-larvae (0.01–0.94-mg Pb/L), and increased substantially in juvenile (1.44-mg Pb/L) and adult (5.01-mg Pb/L). The 48-h LC_{50} values in post-larvae (PL 4, 7 and 11) ranged from 0.01- to 0.63-mg Pb/L and increased in juvenile (0.77-mg Pb/L)

Table 1. The 24-, 48-, and 96-h LC_{50} values with 95% confidence intervals of lead (mg Pb/L) to postlarvae, juvenile and adult of *M. rosenbergii* at 12 ppt salinity.

Life stage	24-h LC_{50}	48-h LC_{50}	96-h LC_{50}
Post-larvae 1	0.01 (0.01–0.01)	–	–
Post-larvae 4	0.01 (0.01–0.01)	0.01 (0.01–0.02)	–
Post-larvae 7	0.09 (0.07–0.13)	0.09 (0.06–0.14)	–
Post-larvae 11	0.94 (0.67–1.32)	0.63 (0.45–0.87)	0.47 (0.33–0.68)
Juvenile	1.44 (1.06–1.95)	0.77 (0.55–1.08)	0.58 (0.41–0.81)
Adult	5.01 (2.77–9.07)	3.13 (2.10–4.64)	2.03 (1.47–2.79)

Note: – = no data.

Table 2. The 24-, 48-, and 96-h LC_{50} values with 95% confidence intervals of lead (mg Pb/L) to juvenile and adult of *M. rosenbergii* at 0 ppt salinity (freshwater).

Life stage	24-h LC_{50}	48-h LC_{50}	96-h LC_{50}
Post-larvae 11	0.92 (0.57–1.48)	0.90 (0.50–1.60)	0.63 (0.42–0.94)
Juvenile	8.76 (6.48–11.83)	6.30 (4.78–8.29)	4.44 (3.57–5.53)
Adult	10.07 (7.34–13.83)	8.67 (6.29–11.96)	7.98 (5.68–11.23)

and adult (3.13-mg Pb/L). The 96-h LC_{50} values in PL11, juvenile and adult were 0.47-, 0.58-, and 2.03-mg Pb/L, respectively.

Table 2 shows the 24-, 48-, and 96-h LC_{50} values for Pb at 0 ppt for PL11, juvenile and adult *M. rosenbergii*. The 24-, 48-, and 96-h LC_{50} values for Pb were higher at 0 ppt than those at 12 ppt for PL11, juvenile and adult prawns, respectively. In control media (without addition of Pb) all animals survived. PL11, juvenile and adult prawns survive well in either 0 or 12 ppt. All these confirm that PL11, juvenile and adult *M. rosenbergii* are the euryhaline stages.

Effect on OC

No death of prawns was observed after exposure to 2 and 4-mg Pb/L at 0 ppt and to 1-mg Pb/L at 12 ppt, however four animals died after being exposed to 2-mg Pb/L at 12 ppt within 7 days. Figure 1 shows the values of OC of adult *M. rosenbergii* after being exposed to sublethal Pb concentrations during 7 days at 0 and 12 ppt, respectively. The *post hoc analysis* (Duncan's test) revealed that OC values of controls and prawns exposed to 2- and 4-mg Pb/L at 0 ppt were not significantly different ($p > 0.05$). After exposure to 1- and 2-mg Pb/L at 12 ppt, the OC of exposed prawns increased by 72 and 109% from the OC of the control prawns ($p < 0.05$). The OC value of prawns exposed to 1-mg Pb/L was significantly different from that of prawns exposed to 2-mg Pb/L at 12 ppt ($p < 0.05$).

Pb accumulation in gill tissues

The levels of Pb in gill tissue of control and exposed prawns are shown in Figure 2.

In the control prawns, the Pb concentrations in gills are 0.0027 ± 0.0003 mg/kg (at 0 ppt) and 0.0026 ± 0.0003 mg/kg (at 12 ppt). Compared to control prawns, the concentrations of Pb in gill tissues increased significantly by 444 and 458% in prawns exposed to 2- and 4-mg Pb/L at 0 ppt ($p < 0.01$), respectively. The Pb concentration in gills of prawns exposed to 2- and 4-mg Pb/L was, however, not significantly different (Figure 2(A)). The concentration

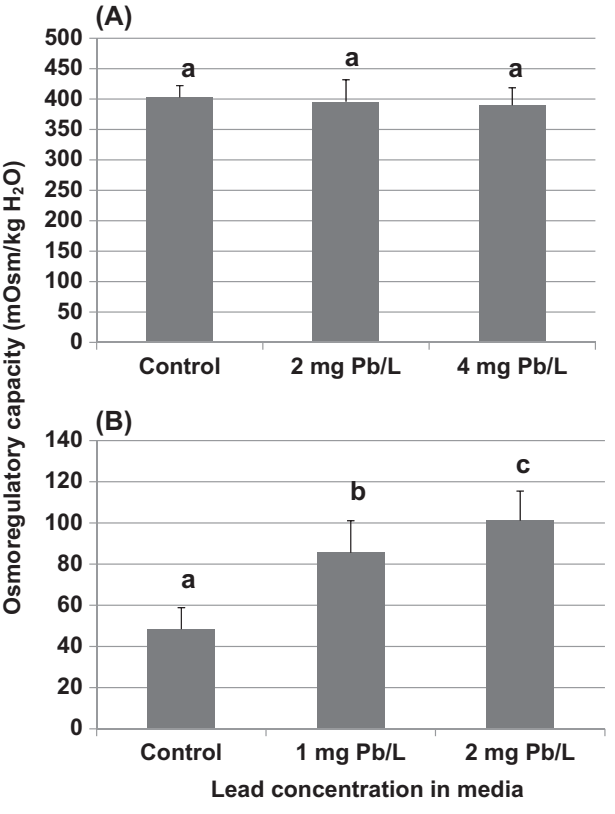

Figure 1. Osmoregulatory capacity (mean ± SE) of *M. rosenbergii*, after animals were exposed to various Pb concentrations for 7 days at 0 (A) and 12 ppt (B) salinity.
Notes: Different letters indicate significant differences ($p < 0.05$; $a < b < c$). Data are means of 20 determinations (for 0, 2, 4-mg Pb/L at 0 ppt, and 0, 1-mg Pb/L at 12 ppt) and 16 determinations (for 2-mg Pb/L at 12 ppt).

of Pb in gills of prawns exposed to 1 and 2-mg Pb/L at 12 ppt increased significantly by 431 and 573%, respectively, when compared with control prawns ($p < 0.01$). The Pb concentration in gills of prawns exposed to 1-mg Pb/L was meanwhile not significantly different from that of prawns exposed to 2-mg Pb/L ($p < 0.05$) (Figure 2(B)). The Pb concentration in gills of prawns exposed to 2-mg Pb/L at 12 ppt was not significantly different from that exposed at 0 ppt ($p < 0.05$) (Figure 3).

Effect on gills structure

Each gill lamellae of the control prawn at both 0 and 12 ppt is limited by a thin epithelium. A widened marginal canal is evident at the tip of the lamellae (Figure 4(A)). The histological structure of gills of prawn exposed to 2-mg Pb/L for 7 days at 0 ppt was similar to that of the control gills. Some marginal canals in the gills exposed to 4-mg Pb/L were, however, larger than those of the controls (Figure 4(B, C)). After exposure to 1- and 2-mg Pb/L for 7 days at 12 ppt, hyperplasia, necrosis, and swelling of lamellae were observed in the gills. This resulted in loss of marginal canal at gill tips (Figure 4(D, E)).

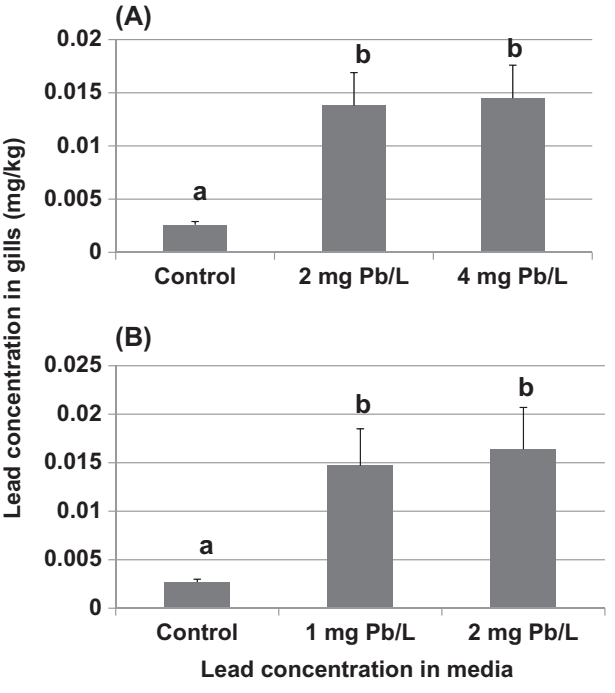

Figure 2. Lead concentrations (mean ± SE) in gill tissues of adult *M. rosenbergii* after animals were exposed to various Pb concentrations for 7 days at 0 (A) and 12 ppt (B) salinity.
Notes: Different letters indicate significant differences ($p < 0.01$; $a < b$). Data are means of 15 determinations.

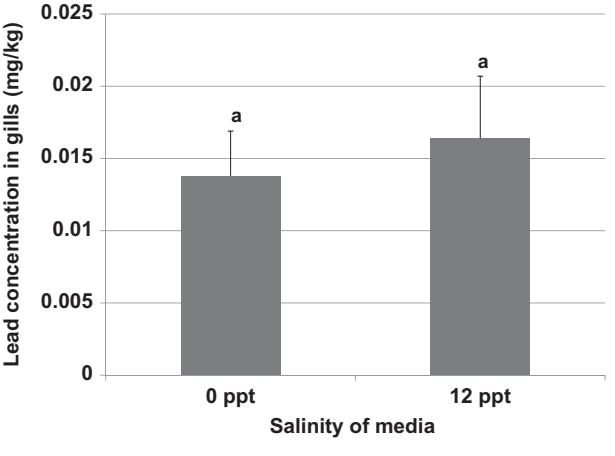

Figure 3. Lead concentrations (mean ± SE) in gill tissues of adult *M. rosenbergii* after animals were exposed to 2-mg Pb/L for 7 days at 0 and 12 ppt salinity respectively.
Notes: All treatments are not significantly different ($p > 0.05$). Data are means of 15 determinations.

Discussion

Acute sensitivity to Pb varied significantly throughout the life stages of *M. rosenbergii* in our experiments. Tolerance to Pb increased progressively from post-larval stages to juveniles

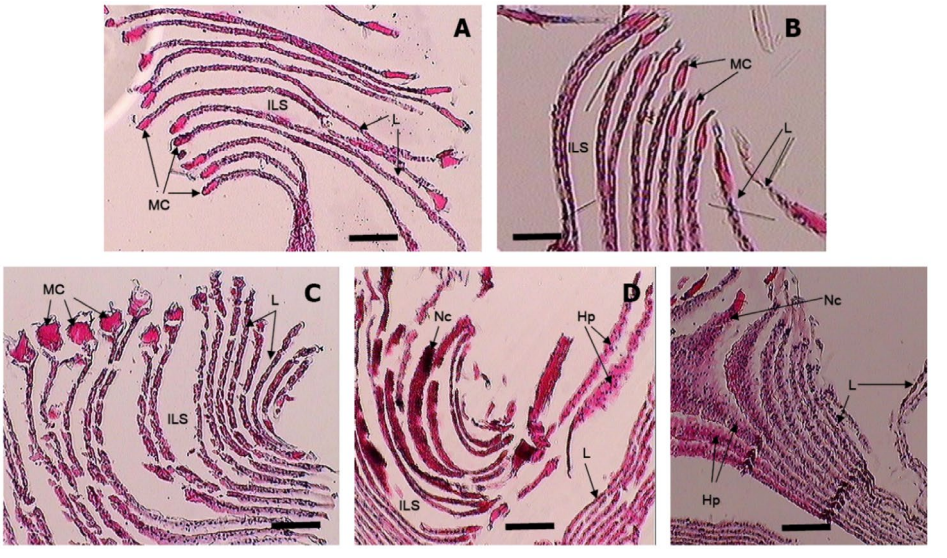

Figure 4. (Colour online) Histological structure of gills of *M. rosenbergii*.
Notes: A. Control gills at 0 ppt; B. Gills exposed to 2-mg Pb/L for 7 days at 0 ppt; C. Gills exposed to 4-mg Pb/L for 7 days at 12 ppt, some marginal canals larger than that of the control; D. Gills exposed to 1-mg Pb/L for 7 days at 12 ppt, necrosis and hyperplasia resulting swelling of gill tips (marginal canals); E. Gills exposed to 2-mg Pb/L for 7 days at 12 ppt, necrosis and hyperplasia resulting swelling of gill tips; (bar size = 10 µm; L = lamellae, MC = marginal canal, ILS = interlamellar space, Nc = necrosis, Hp = hyperplasia).

and to adult of *M. rosenbergii*. Comparison of the 24-, 48-, and 96-h LC_{50}'s data obtained between different life stages of *M. rosenbergii* showed that post-larval stages from PL1 to PL11 are the most sensitive; and adult stages are the most tolerant to Pb. Only limited studies have been conducted on the toxicity of Pb to different life stages of freshwater decapods crustacean species. Work similar to the present study has been conducted by Usman et al. (2013) on the marine decapod crustacean *L. vannamei*. Usman et al. (2013) found the same trend of tolerance to Pb across different life stages in *L. vannamei*. The most tolerant stage was the juvenile followed by post-larvae; and the less tolerant stages were the larvae. The decreasing sensitivity to metals (particularly Cd, Cu and Pb) throughout the different life stages has also been reported in the shrimps *Penaeus japonicus* (Bambang et al. 1995), *L. vannamei* (Usman et al. 2013) and the prawn *M. rosenbergii* (Asih et al. 2013).

For both juvenile and adult *M. rosenbergii*, the 24-, 48-, and 96-h LC_{50} values for Pb were lower at 12 ppt than those at 0 ppt. A similar tendency has also been reported in the case of copper (Cu) by Asih et al. (2013). That study found that juveniles and adults of *M. rosenbergii* were more sensitive to Cu at 12 ppt than at 0 ppt. An increase in salinity causes an increase in metal toxicity or uptake by juvenile and adult *M. rosenbergii*. Our findings are particularly interesting because they differ from the results of previous studies in which uptake, toxicity, and bioavailability of metals decrease with increasing salinity (e.g. Sunda et al. 1978; Nugegoda & Rainbow 1989; Bervoets et al. 1995; Ardiansyah et al. 2012; Putranto et al. 2014). The decapods *Palaemon elegans*, *Palaemonetes varians* (Nugegoda & Rainbow 1989), and *L. vannamei* (Ardiansyah et al. 2012) show increased rate of uptake or of toxicity to Zn with decreasing salinity. Cadmium toxicity or uptake is similarly raised

at reduced salinity in the crab *Uca pugilator* (O'Hara 1973), in the amphipod *Orchestia gammarellus* (Rainbow & Kwan 1995), in shrimp *L. vannamei* (Ardiansyah et al. 2012) and in the prawn *M. sintangense* (Putranto et al. 2014). In these cases, reduction in salinity reduces the chloro-complexation of trace metal ions and increases the availability of free metal ions. Because the rate of uptake is determined by the availability of external free metal ions, decrease in salinity should increase metal uptake (Rainbow 1997). On the other hand, when crustaceans are exposed to reduced salinity, their body fluids are hyperosmotic to the medium and water enters osmotically. To respond to the increased entry of water the crustacean increases the production of urine. This urine is isosmotic to the hemolymph and therefore the increased expulsion of water is associated with a concomitant loss of major ions like Na^+ and Ca^{2+} in the urine (Mantel & Farmer 1983). This loss is balanced by active uptake of major ions across the gill. A decrease in salinity would thus cause increased uptake of calcium and therefore potentially of any metal ions entering via this calcium uptake route (Rainbow 1997).

With regard to our findings, *M. rosenbergii* may respond physiologically to reduced salinity that more than counteracts the expected physicochemical effects of increased free metal ion availability at lower salinities. It is possible that the integumental permeability of the prawn plays a prominent role in metal uptake. The integumental permeabilities of crustaceans decrease along the habitat salinity gradient from marine via estuarine to freshwater (Mantel & Farmer 1983), and metal uptake rates by crustaceans similarly decrease along this habitat gradient (Rainbow 1997). Crustaceans including juvenile and adult *M. rosenbergii* exposed to higher salinities will therefore have a higher permeability for the major cations and so that the uptake of other metals which use the same pathways could be expected to increase (Rainbow 1997). Thus, although Pb bioavailability decreased along with the increasing salinity, the prawns became increasingly permeable to Pb, so that the uptake and toxicity of Pb increased. This study showed that the concentration of Pb in gills of prawns exposed to 2-mg Pb/L at 12 ppt was significantly higher than that exposed at 0 ppt. A similar finding was reported in *M. rosenbergii* (Asih et al. 2013), in the crabs *Carcinus maenas* (Chan et al. 1992), and in the shrimps *P. varians* and *P. elegans* (Nugegoda & Rainbow 1989) exposed to metals in different salinities. Asih et al. (2013) reported that the concentration of Cu in gills of *M. rosenbergii* exposed to 0.5 and 0.75 mg Cu/L at 12 ppt was significantly higher than those exposed at 0 ppt. The brackish-water decapod *P. varians* had a lower zinc uptake rate under identical physicochemical conditions than its more marine relative *P. elegans* (Nugegoda & Rainbow 1989). In the crab, *C. maenas* exposed to Cd at salinities of 33, 25, and 15 ppt, the Cd uptake rate was highest at a salinity of 33 ppt (Chan et al. 1992). It appeared that any apparent reduction in water permeability (AWP) at 15 ppt was sufficient to limit Zn and Cd uptake despite greater bioavailability (Chan et al. 1992). Other crustaceans, for example, the amphipod *Gammarus duebeni* (Bolt 1983) and the shrimp *P. longirostris* (Campbell & Jones 1990) also showed a decrease in AWP in low salinity.

The present study showed that increasing Pb concentration in media at 0 ppt did not affect the OC values of adult *M. rosenbergii*. However, at 12 ppt, increasing Pb in the medium significantly increased the OC values of adult prawn. Adult *M. rosenbergii* may have little ability to maintain hemolymph osmoregulation when affected by sublethal concentrations of Pb at 12 ppt. This indicated that imbalance in plasma and ions occurred in prawns exposed to sublethal levels of Pb at 12 ppt. In salinity 0 ppt, it is possible that the integumental

permeability of this prawn is not sufficient to increase the rate of trace metal uptake. Similar findings have been found in the crabs *Dilocarcinus pagei* exposed to 2.7-mg Pb/L at salinities 0 and 15 ppt (Amado et al. 2006). There was a significant increase in osmolality of crabs after exposure to Pb at 15 ppt. In contrast, no change was detected when *D. pagei* was exposed to the same Pb concentration at 0 ppt (Amado et al. 2006). In the case of copper, Asih et al. (2013) reported that the OC values of controls and prawns exposed to 0.5- and 0.75-mg Cu/L at 0 ppt were not significantly different. Meanwhile, the OC of prawns exposed to 0.5- and 0.75-mg Cu/L at 12 ppt was significantly different from the OC of the control animals.

Imbalance in the plasma ion concentrations has also been observed in crustaceans after exposure to various metal concentrations. After exposure to sublethal Pb concentrations (3.25-, 6.5-, and 9.75-mg Pb/L) for 15 days at a salinity of 22 ppt, the OC of *L. vannamei* significantly reduced compared with control animals (Usman et al. 2013). Wu and Chen (2004) reported that after exposure to 3-mg Cd/L or 3-mg Zn/L for 12 h, the osmolality of *L. vannamei* was lower than those of control animals. Exposure for 24 h caused greater effects on the osmolality of exposed shrimps. Exposure to sublethal concentration of Cd and Zn reduced the hyper-OC of *L. vannamei* at 5 and 15 ppt salinity and increased the hypo-OC of exposed shrimps at 27 ppt salinity (Ardiansyah et al. 2012). The hyper-OC of *P. japonicus* in dilute seawater, and its hypo-OC in seawater, decreased significantly with increasing Cu concentrations in the media (Bambang et al. 1995).

Many factors such as the tissue volume regulation, the integumental permeability, the ionic permeability of exchange surface epithelia, and the ratio of surface to volume of the organism affect the hemolymph osmolality value of crustaceans (Chan et al. 1992; Lignot et al. 1997; Amado et al. 2006). The perturbation in OC observed in this study could be due to direct inhibition of the osmoregulation mechanisms, for example, inhibition of water and ion transport mechanisms (Rainbow & Black 2005; Amado et al. 2006). Lead is known to affect negatively the hemolymph concentration of Na^+, K^+, Cl^-, osmolality of crabs *D. pagei* (Amado et al. 2006), and inhibit the branchial Na^+–K^+-ATPase activity of crayfish *Cherax destructor* (Ahern & Morris 1998). The alteration of OC of *M. rosenbergii* after exposure to Pb could be caused by direct disruption by Pb of hemolymph ionic concentrations and the alteration on gill Na^+–K^+-ATPase activity.

This study showed that Pb significantly accumulated in the gills of adult *M. rosenbergii* after sublethal exposures for 7 days at either 0 or 12 ppt. The level of Pb in gills from media 12 ppt was, however, slightly higher than that from media 0 ppt. With respect to the effects of Pb on gill structure, we note that the severe toxic actions of Pb observed in gills of prawns exposed in media 12 ppt. Gill hyperplasia, necrosis, swelling of lamellae, and abnormality of gill tips were observed in the Pb exposed prawns at 12 ppt. Similar histological damages had been observed in other crustaceans exposed to various heavy metals (Li et al. 2007; Frías-Espericueta et al. 2008; Wu et al. 2009; Asih et al. 2013; Putranto et al. 2014). Gill hyperplasia, swelling of gill filaments, necrosis of gill cells resulting in narrowed or obstructed hemolymphatic lacuna at gill tips, and loss of regular structure of the epithelium had been observed in crustaceans exposed to different levels of metals. The structural changes in the epithelial cells of gills disrupt their physiological function (Pequeux 1995). The structural changes due to the sublethal level of heavy metals affect the reduction in ion uptake by branchial epithelium (Pequeux 1995; Ahern & Morris 1998). In the experiment using media 12 ppt, significant osmoregulatory change was noted in Pb exposed prawns whereas no evidence of osmoregulatory change was recorded in the prawns exposed in media 0 ppt. It is important

to note that the adverse effects of Pb on gill structures of exposed *M. rosenbergii* might be one of the main factors responsible for the alteration of OC reported in this study.

In conclusion, our study demonstrates that based on the LC_{50} values the early life stages (post larvae) of *M. rosenbergii* are sensitive to lead. Some coastal waters of Indonesia have levels from 0.7- to 2.3-mg Pb/L and in the more polluted waters up to 4.5-mg Pb/L, levels that could negatively impact their survival. The later stages (juveniles and adults) are, however, substantially better adapted to withstand lead pollution, particularly in fresh or low-salinity water. The results suggest that use of brackish water in prawn hatcheries should be reconsidered because of its potential negative impacts on larval growth and survival, particularly in areas with elevated lead levels. Treating of source waters entering hatcheries to remove lead may also be considered.

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