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Temperature elevation stage-specifically increases metal toxicity through bioconcentration and impairment of antioxidant defense systems in juvenile and adult marine mysids

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ABSTRACT

Metals are of serious concern due to their toxicity, persistency, and accumulation potential in aquatic animals. However, limited information is available on the combined effects of metal with temperature elevation, which is one of the future climate changes suggested for the oceans. In this study, the effect of temperature elevation was investigated by analyzing toxicity, bioconcentration, and antioxidant response in juvenile and adult marine mysids upon exposure to 20 °C and 25 °C for 48 h and 96 h. Based on LC_{50} values, toxicity of metals was highly reliant on temperature, exposure period, and age. Elevation in temperature significantly increased the whole metal toxicity in juveniles. Bioconcentration was elevated by increasing exposure period and metal concentration. Significant elevation of malondialdehyde (MDA) and depletion of glutathione (GSH) was measured in juveniles, while significant elevation of both MDA and GSH was detected in adults. Subsequently, enzymatic activities of antioxidant enzymes in catalase (CAT) and superoxide dismutase (SOD) increased significantly in adults at 48 h and 96 h, whereas most activities were significantly lowered in juveniles at 96 h. These results suggest that the early life stage of marine mysids is more sensitive to the combined effect of metal and temperature than adult stage due to an impairment in the induction of the antioxidant defense system.

1. Introduction

Among a variety of contaminants, metals are considered as a serious threat to ecosystems due to persistency, non-degradability, pervasiveness, and inherent toxicity in living organisms (Phillips, 1977; Rainbow, 2002, 2007). High concentrations of metals affect biochemistry, physiology, and homeostasis to the tissue and cell constituents of aquatic organisms (Rainbow, 2002; Valko et al., 2005). Metal concentration can be naturally increased by weathering metal-bearing rocks and volcanic eruptions, whereas numerous anthropogenic activities in mining, agriculture, and industry contribute to metal pollution in the environment (Phillips, 1977). Among the environmental factors, temperature elevation (e.g., seasonal warming, global climate change) plays a critical role in metal accumulation and toxicity in organisms due to direct impact on all physiological processes, including an increase in metabolism and subsequent oxygen consumption and energy exhaustion (Wang and Rainbow, 2008; Tomanek, 2010). Consequently, addressing interactive effects of metal toxicity and elevated temperature in aquatic ecosystems is important to understand as it is expected to become more wide-spread in the future. Metals are proficient to interact with cellular proteins and DNA in organisms due to the rich coordination of chemistry and redox properties (Stohs and Bagchi, 1995; Valko et al., 2005). Metals have the capacity to increase reactive oxygen species (ROS) and free radicals, resulting in an alteration of cellular reduction-oxidation (redox) balance and homeostasis (Valko et al., 2005; Fanjul-Moles and Gonsebatt, 2011). When animals fail to overcome the imbalance between ROS and antioxidant/scavenging defense systems, their biological functions and metabolism can be vulnerable to oxidative stress and detrimental effects of metals.

Aquatic animals, including aquatic crustaceans have developed antioxidant defense systems for the detoxification of excessive ROS and free radicals (Livingstone, 2001; Ahearn et al., 2004; Lesser, 2006;

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Regoli and Giuliani, 2014). In normal cells, ROS and pro-oxidants are detoxified by the antioxidant defense system which comprises low molecular weight scavengers and antioxidant enzymes (Livingstone, 2001). Measurement of intracellular malondialdehyde (MDA) levels has been used as a strong biomarker for metal-induced oxidative stress in crustaceans (Stohs and Bagchi, 1995; Niki, 2008). Prominent components among these antioxidants are reduced glutathione (GSH), catalase (CAT: converts H_2O_2 to H_2O), and superoxide dismutase (SOD: converts $O_2^{\bullet-}$ to H_2O_2 and H_2O) to protect cells against concomitant detrimental effects of oxidative stress. Glutathione is involved in cell physiology, including preventing protein-SH groups from oxidization and cross-linkage, detoxification of xenobiotic and endogenous compounds, and direct scavenging of diverse oxidants (Valavanidis et al., 2006). In addition, analyzing malondialdehyde (MDA), GSH, CAT, and SOD can offer an indication of oxidative stress and anti-oxidative status of the organisms and can serve as biomarkers of several environmental pollutants (Livingstone, 2001; Valavanidis et al., 2006).

Among aquatic crustaceans, mysids have been used as model organisms in various ecotoxicological research due to their favorable characteristics of being easy to handle in the laboratory, having a wide geographic distribution, flexible physiology, phylogenetic and physiological resemblance to decapod shrimps, easy to transport, and sensitivity to environmental contaminants including metals (Verslycke et al., 2003, 2004; Haque et al., 2018). For example, in the mysid *Neomysis awatchensis*, developmental stage-specific metal effects and adaptation potential were reported by analyzing intracellular MDA concentration and enzymatic activities of the antioxidant defense system (Haque et al., 2018). Mysids also have the ability to respond to environmental variables like temperature (Verslycke et al., 2004). However, despite the high sensitivity of mysids to metals, the analysis of antioxidative responses and applications of biochemical biomarkers have yet to be performed in most mysid species. The objective of the present study was to assess the potential effects of temperature elevation (from 20 °C to 25 °C) on acute toxicity, bioconcentration, and antioxidant responses by measuring MDA, GSH, CAT, and SOD in different stages, juveniles and adults of the mysid *N. awatschensis* in response to arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn). Our results may provide a better understanding of the cumulative effects of metal and temperature on defense system and adaptive abilities of in aquatic crustaceans in general and can also provide background data for the prediction of global warming effects on metal pollution in marine ecosystems.

2. Materials and methods

2.1. Mysid culture

Detailed information on mysid origin and laboratory culture conditions was provided as a supplementary file and is described in our previous studies (Haque et al., 2018; Min et al., 2018; Lee et al., 2020).

The marine mysid *Neomysis awatschensis* was continuously cultured in an automated aquaculture system at Incheon National University (Incheon, South Korea). The mysids were cultured in artificial seawater (20 °C, 30 PSU, pH of 7.9–8.2, 6.8–7.3 mg DO L^{-1}) under a light:dark (L:D) photoperiod of 16:8 h and fed daily at a rate of 70–100 *Artemia* nauplii per mysid (SERA Artemia, Salt Lake, UT, USA).

2.2. Acute toxicity and metal exposure

Detailed information on acute toxicity and metal analysis is provided as a supplementary file and described in our previous study (Haque et al., 2018).

Two age groups of mysids (3rd generations in the laboratory), juveniles (≈7 days after hatching; DAH) and adults (≈ 30 DAH) were exposed to metals at 20 °C for 96 h. To determine 48 and 96 h LC_{50} values, 50 mysids were distributed into five polystyrene containers (SPL Life Science, South Korea; working volume 500 mL; n = 10 per container). Mortality rates were calculated from counts under a compound microscope (Olympus BX51, Tokyo, Japan) at 48 and 96 h.

Quantification of metal content was conducted using inductively coupled plasma mass spectroscopy (ICP-MS; NexION 300, PerkinElmer, MA, USA). The instrument was calibrated for each metal using known standards. The accumulated metal was calculated based on the dry weight of the pooled mysids (μg g^{-1} dry weight).

2.3. Antioxidant defense system

Since we have routinely performed the measurement of antioxidant responses of the same species, detailed information is provided as supplementary file and described in our previous studies (Haque et al., 2018; Min et al., 2018; Lee et al., 2020).

Intracellular TBARS concentration on each sample was determined based on a standard curve using malondialdehyde bis (dimethylacetal, Sigma-Aldrich, Inc. St. Louis, MO, USA). The MDA level was measured using the calibration curve and results were provided as nM of MDA per μg of the total sample. Intracellular GSH concentration was measured by an enzymatic method with the Glutathione Assay Kit (Catalog No. CS0260; Sigma-Aldrich, Inc.). The GSH content was determined at an absorbance of 420 nm using a Thermo Varioskan Flash spectrophotometer (Thermo Fisher Scientific, Tewksbury, MA, USA), and the standard curves were generated with GSH equivalents (0, 150, and 350 μM). The CAT and SOD activities were determined with an enzymatic method using a Catalase Assay Kit (Catalog No. CAT100; Sigma-Aldrich, Inc.) and SOD Assay Kit (Catalog No. 19160; Sigma-Aldrich Chemie, Switzerland), respectively. The total SOD and CAT activities were then measured at an absorbance of 440 nm or 520 nm at 25 °C using a Thermo Varioskan Flash spectrophotometer (Thermo Fisher Scientific), respectively.

2.4. Statistical analysis

All results were validated as mean ± standard deviation (S.D.) using the statistical software package SPSS (ver. 17.0, SPSS Inc., Chicago IL, USA). Significant difference was calculated using a one-way analysis of variance (ANOVA) followed by *post-hoc* Tukey and Dennett's multiple-comparison tests. A statistical probability of $P < 0.05$ was considered as significant.

3. Results

3.1. Acute toxicity and bio-accumulation of metals

The LC_{50} values obtained from the acute toxicity test of five metals for 48 and 96 h showed that Cu was the most toxic among the metals in juveniles at both temperatures (Table 1). The lowest 48 h- and 96 h-LC_{50} values were measured in Cu-treated juveniles as 42 μg L^{-1} and 13 μg L^{-1} at 25 °C. Based on 96 h LC_{50} values, the toxicity orders of the five metals were Cu (85 μg L^{-1}) > Zn (134 μg L^{-1}) > Cd (158 μg L^{-1}) > As (986 μg L^{-1}) > Pb (1152 μg L^{-1}) in juveniles and Cu (123 μg L^{-1}) > Cd (216 μg L^{-1}) > Zn (385 μg L^{-1}) > Pb (1152 μg L^{-1}) > As (1317 μg L^{-1}) in adults at 20 °C. In the case of 25 °C, the toxicity orders were Cu (13 μg L^{-1}) > Zn (27 μg L^{-1}) > Cd (96 μg L^{-1}) > Pb (496 μg L^{-1}) > As (688 μg L^{-1}) in juveniles and Cu (88 μg L^{-1}) > Zn (136 μg L^{-1}) > Cd (182 μg L^{-1}) > As (1107 μg L^{-1}) > Pb (2114 μg L^{-1}) in adults. Overall, juveniles were more vulnerable to metals than adults.

Bioconcentration of each metal treated with 1/10 and 1/5 of the LC_{50} values of juvenile and adult mysids was measured at 20 and 25 °C after 48 and 96 h exposure (Table 2), respectively. The bioconcentrations of metals were temperature-, exposure period-, and concentration-dependent in both juveniles and adults.

Table 1
Acute 48 and 96 h LC50 values for the mysid *Neomysis awatschensis* exposed to five metals at different temperatures.

Metal	Age	N[a]	20 °C LC50 (μg L^{-1})[b]		25 °C LC50 (μg L^{-1})[b]	
			48 h	96 h	48 h	96 h
As	Juvenile	50	1352 (1085–1441)	968 (895–1121)	795 (615–914)	688 (607–846)
	Adult	50	2158 (1982–2335)	1317 (1034–1585)	1514 (1235–1861)	1107 (983–1316)
Cd	Juvenile	50	524 (492–558)	158 (133–172)	125 (62–415)	96 (43–382)
	Adult	50	976 (871–1142)	216 (186–237)	643 (398–925)	182 (69–245)
Cu	Juvenile	50	226 (203 − 231)	85 (71–89)	42 (0.82–84)	13 (0.77–43)
	Adult	50	891 (707–1106)	123 (108–139)	612 (507–890)	88 (39–121)
Pb	Juvenile	50	2822 (2338–3117)	1152 (1028–1491)	795 (459–1538)	496 (269–779)
	Adult	50	3868 (3396–4152)	2658 (2248–2825)	2785 (2215–3625)	2114 (1785–2894)
Zn	Juvenile	50	668 (578–691)	134 (111–153)	131 (24–256)	27 (0.41–88)
	Adult	50	889 (796–952)	385 (342–429)	613 (469–776)	136 (43–237)

[a] Number of *N. awatschensis* treated with each concentration of metals.
[b] The LC50 values are represented with the 95% confidence interval in parentheses.

3.2. MDA content

Significantly elevated MDA levels were measured in juveniles exposed to 1/5 LC_{50} of As, Cu, and Pb at 20 °C and 1/10 LC_{50} of As and Cd and 1/5 LC_{50} of As, Cd, Cu, and Zn at 25 °C for 48 h ($P < 0.05$), respectively (Fig. 1). In the case of 96 h exposure, the levels were also elevated in juveniles exposed to 1/5 LC_{50} of As, Cu, and Zn at 20 °C and 1/10 and 1/5 LC_{50} of As, Cd, Cu, Pb, and Zn at 25 °C ($P < 0.05$).

Significantly increased MDA levels were observed in adults exposed to 1/5 LC_{50} of Cu at 20 °C and 1/10 LC_{50} of Zn and 1/5 LC_{50} of As, Cd, Cu, and Zn at 25 °C for 48 h ($P < 0.05$). The levels were also elevated in response to 1/5 LC_{50} of Zn at 20 °C and 1/10 and 1/5 LC_{50} of As, Cd, Cu, Pb, and Zn at 25 °C for 96 h ($P < 0.05$).

Overall, most metals induced significant MDA production compared to the control at 25 °C. MDA elevation was age-, exposure time-, dose- and temperature-dependent, however, juveniles were more vulnerable to lipid peroxidation than adults.

3.3. GSH content

Significantly higher and lower levels of intracellular GSH were observed in adult and juvenile mysids, respectively. In juveniles, the levels of GSH were significantly depleted by 1/5 LC_{50} of As at 25 °C for 48 h, 1/10 LC_{50} of Cu and Pb and 1/5 LC_{50} of Cd and Zn at 20 °C for 96 h, and 1/10 LC_{50} of Cd and Zn and 1/5 LC_{50} of As, Cu, and Pb at 25 °C for 96 h ($P < 0.05$) (Fig. 2).

In the case of adults, significantly elevated GSH levels were measured in response to 1/5 LC_{50} of Cu and Zn at 20 °C for 48 h, 1/10 LC_{50} of As and Cd and 1/5 LC_{50} of Cu, Pb, and Zn at 25 °C for 48 h, 1/10 LC_{50} of Cu and 1/5 LC_{50} of Cd at 20 °C for 96 h, and 1/10 LC_{50} of As, Cu, Pb, and Zn and 1/5 LC_{50} of Cd at 25 °C for 96 h ($P < 0.05$).

3.4. Antioxidant enzymatic activity

Significantly increased CAT activities were observed in juveniles exposed to 1/10 LC_{50} of As, Cu, and Zn at 25 °C for 48 h, while the activities were decreased by 1/10 LC_{50} of Cu and 1/5 LC_{50} of As, Cd, Cu, Pb, and Zn at 25 °C for 96 h ($P < 0.05$) (Fig. 3). In the case of adults, significantly elevated CAT activities were measured in response to 1/5 LC_{50} of Pb at 20 °C for 48 h, 1/10 LC_{50} of As, Pb, and Cd and 1/5 LC_{50} of Cd, Cu, Pb and Zn at 25 °C for 48 h, and 1/5 LC_{50} of Cu and Zn at 20 °C for 96 h ($P < 0.05$). However, the levels were significantly decreased by 1/10 LC_{50} of Cu and 1/5 LC_{50} of Cd, Pb, and Zn at 25 °C for 96 h ($P < 0.05$).

Table 2
Exposure concentration of each metal and accumulation level measured in juvenile and adult mysids at different temperatures.

Metals	Age	Temp. (°C)	Treatment to 1/10 LC50 (μg L^{-1})				Treatment to 1/5 LC50 (μg L^{-1})			
			48 h		96 h		48 h		96 h	
			Exposure (μg L^{-1})	Measured (μg g^{-1})	Exposure (μg L^{-1})	Measured (μg g^{-1})	Exposure (μg L^{-1})	Measured (μg g^{-1})	Exposure (μg L^{-1})	Measured (μg g^{-1})
As	Juvenile	20	135	20 ± 2.8	97	17 ± 1.9	270	42 ± 4.5	194	34 ± 4.5
		25	80	6 ± 1.3	69	13 ± 2.2	160	34 ± 4.1	138	38 ± 7.4
	Adult	20	216	16 ± 2.0	132	11 ± 1.2	432	32 ± 3.2	263	21 ± 3.3
		25	151	24 ± 4.8	111	9 ± 1.3	302	28 ± 2.9	222	26 ± 6.8
Cd	Juvenile	20	52	14 ± 1.7	16	9 ± 0.9	105	34 ± 4.0	32	11 ± 2.3
		25	13	3 ± 0.8	10	6 ± 0.8	26	19 ± 3.7	20	7 ± 2.8
	Adult	20	98	9 ± 1.0	22	6 ± 0.9	195	23 ± 2.9	43	5 ± 1.2
		25	64	7 ± 1.1	18	7 ± 1.1	128	16 ± 2.2	36	1 ± 2.7
Cu	Juvenile	20	23	8.9 ± 0.9	9	7 ± 0.7	45	20 ± 2.5	17	6 ± 0.7
		25	4	0.5 ± 0.6	1	0.2 ± 0.2	8	15 ± 4.7	2	3 ± 0.8
	Adult	20	89	12 ± 1.1	12	4 ± 0.5	178	11 ± 1.9	25	8 ± 0.6
		25	61	10 ± 1.3	9	3 ± 0.6	122	3 ± 0.9	18	10 ± 2.1
Pb	Juvenile	20	282	69 ± 5.3	115	27 ± 3.0	564	59 ± 6.9	230	64 ± 7.3
		25	80	11 ± 1.4	50	11 ± 2.8	160	44 ± 6.2	100	75 ± 8.9
	Adult	20	387	42 ± 4.0	266	32 ± 2.7	774	26 ± 4.4	532	40 ± 5.1
		25	279	22 ± 4.2	211	24 ± 3.9	558	29 ± 5.9	422	66 ± 8.1
Zn	Juvenile	20	67	24 ± 2.9	13	6.7 ± 0.6	134	20 ± 3.3	27	12 ± 2.4
		25	13	3.9 ± 1.0	3	5.2 ± 0.8	26	16 ± 2.7	6	8 ± 3.1
	Adult	20	89	17 ± 2.6	39	11 ± 1.2	178	15 ± 3.5	77	11 ± 1.9
		25	39	9.1 ± 1.4	14	7 ± 1.1	78	18 ± 3.8	28	12 ± 2.3

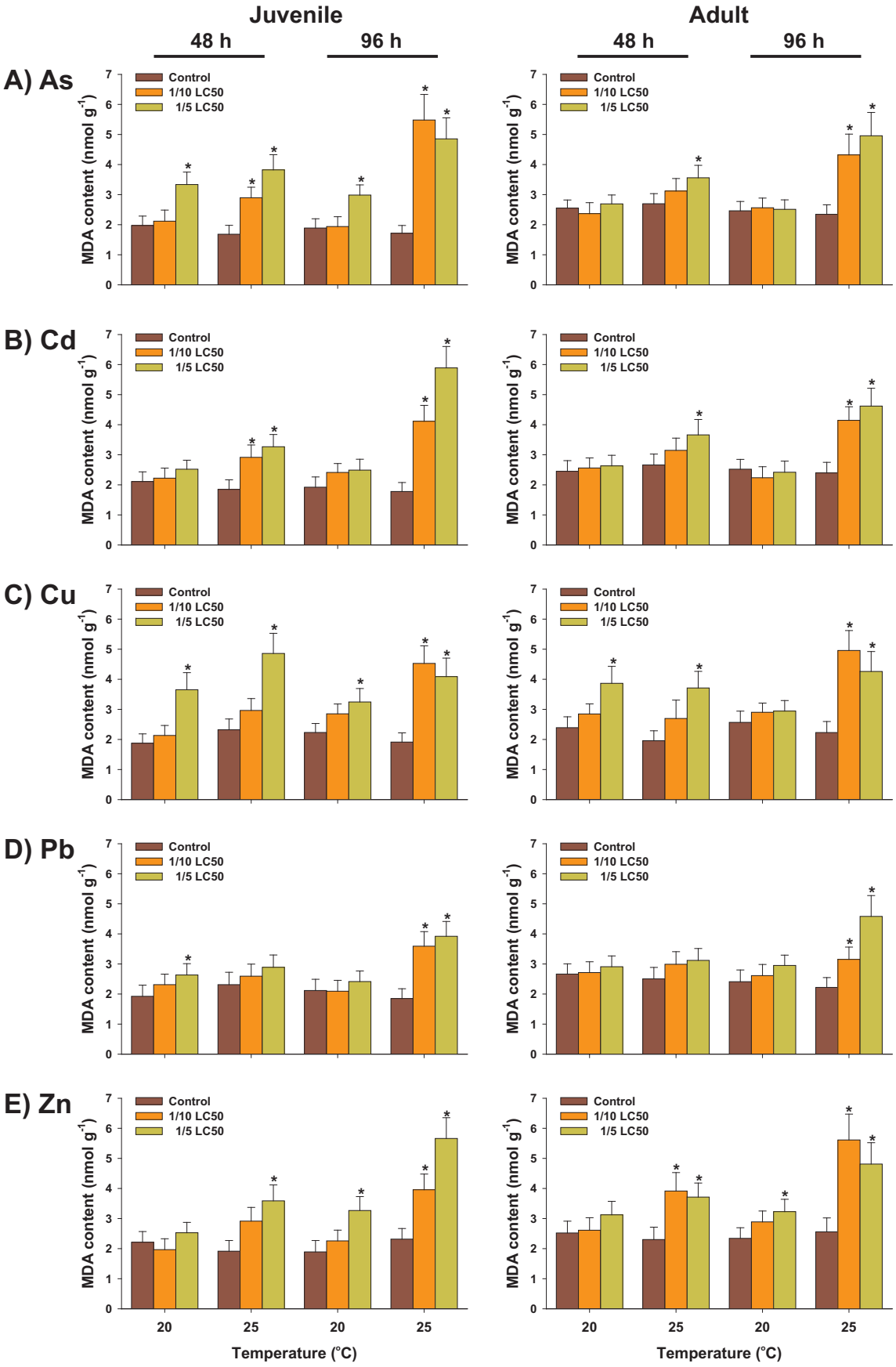

Fig. 1. Analysis of effects of temperature elevation from 20 °C to 25 °C on intracellular MDA content upon exposure to 1/5 and 1/10 of the LC_{50} values of A) As, B) Cd, C) Cu, D) Pb, and E) Pb in the juvenile and adult marine mysid *Neomysis awatschensis* for 48 h and 96 h. Data are presented as the mean ± standard deviation (S.D.). The asterisk (*) indicates statistical significance ($P < 0.05$) compared with the control value.

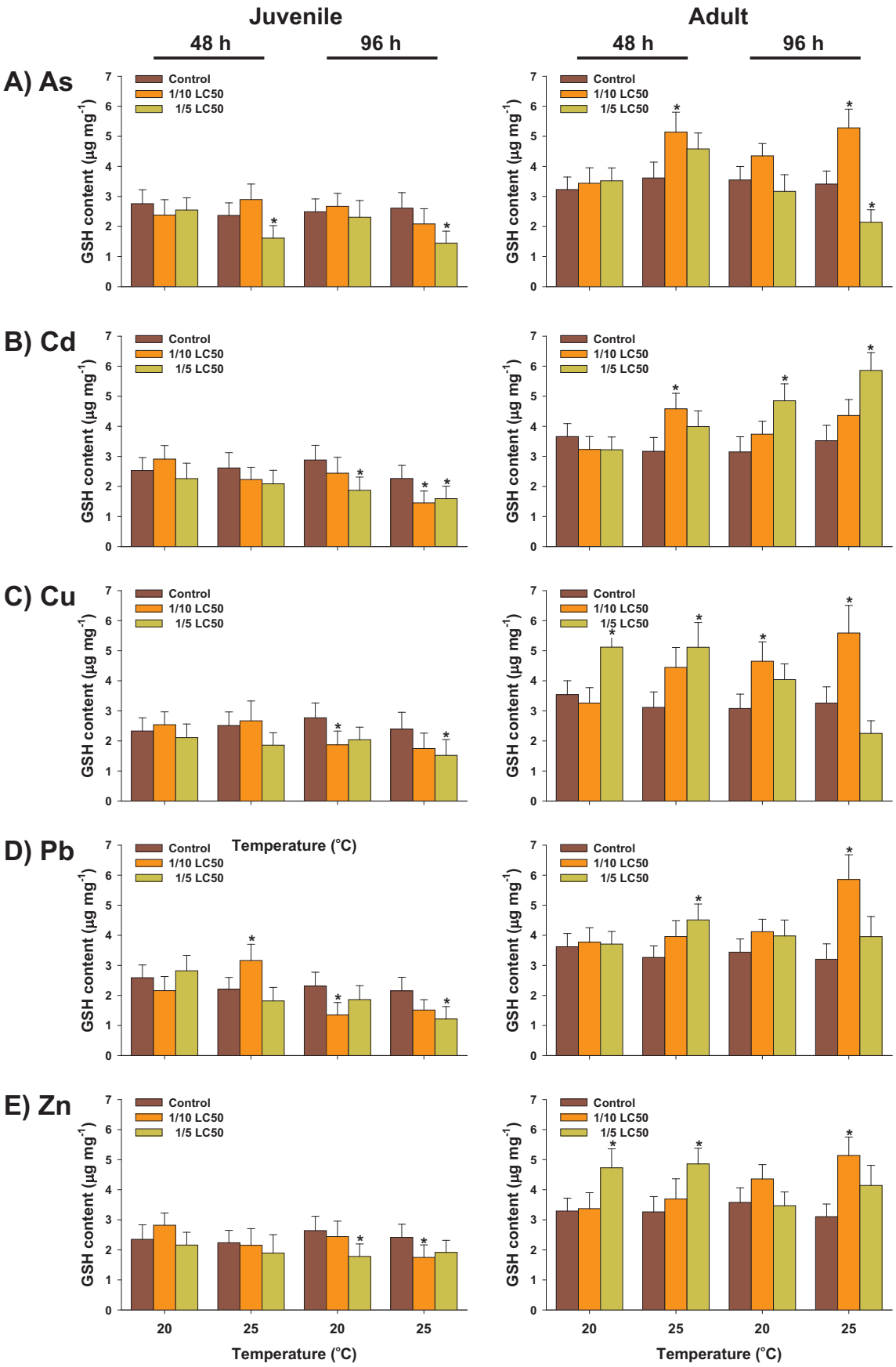

Fig. 2. Analysis of effects of temperature elevation from 20 °C to 25 °C on intracellular GSH content upon exposure to 1/5 and 1/10 of the LC_{50} values of A) As, B) Cd, C) Cu, D) Pb, and E) Pb in the juvenile and adult marine mysid *Neomysis awatschensis* for 48 h and 96 h. Data are presented as the mean ± standard deviation (S.D.). The asterisk (*) indicates statistical significance ($P < 0.05$) compared with the control value.

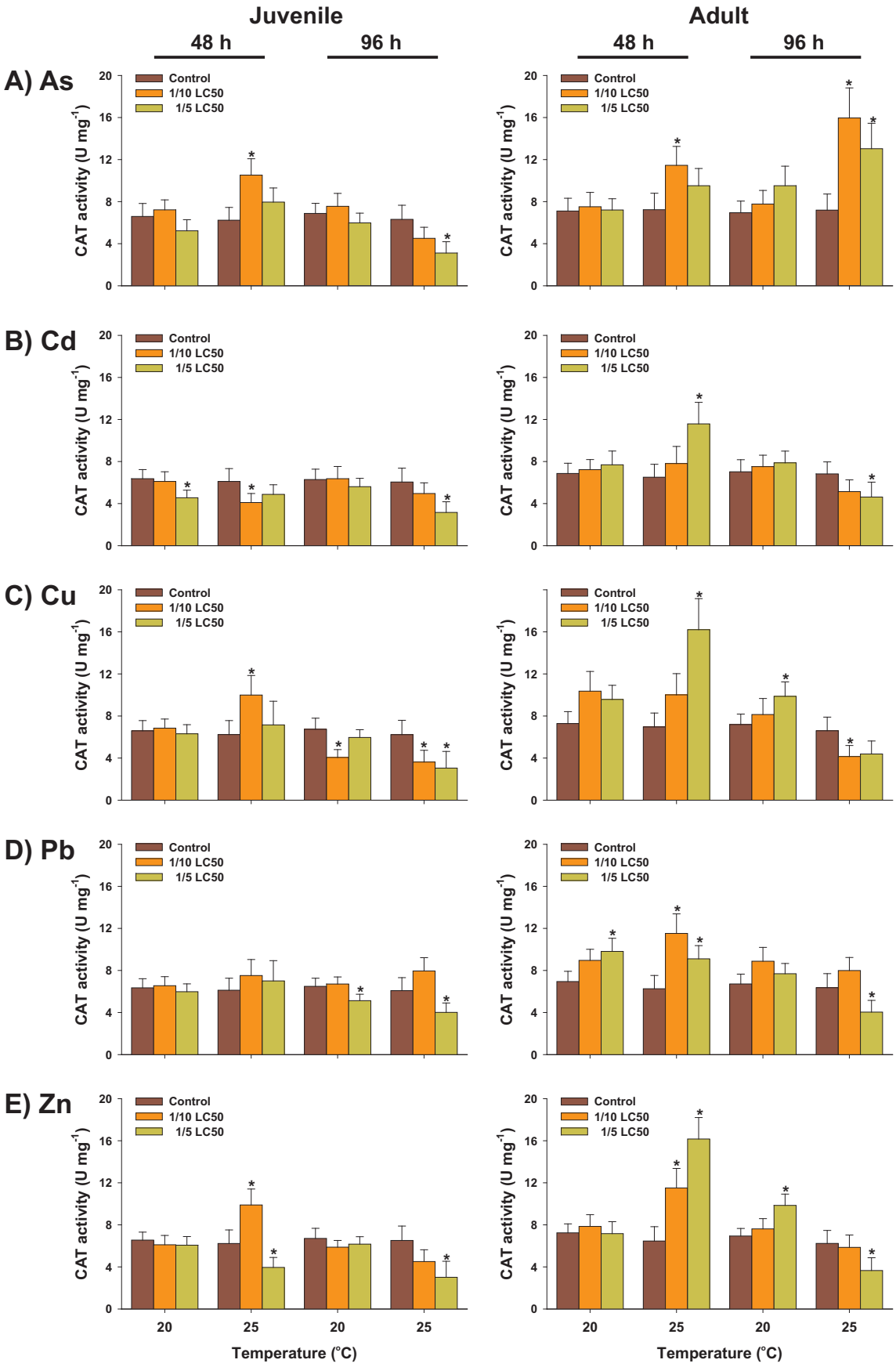

Fig. 3. Analysis of effects of temperature elevation from 20 °C to 25 °C on enzymatic activity of catalase upon exposure to 1/5 and 1/10 of the LC_{50} values of A) As, B) Cd, C) Cu, D) Pb, and E) Pb in the juvenile and adult marine mysid *Neomysis awatschensis* for 48 h and 96 h. Data are presented as the mean ± standard deviation (S.D.). The asterisk (*) indicates statistical significance ($P < 0.05$) compared with the control value.

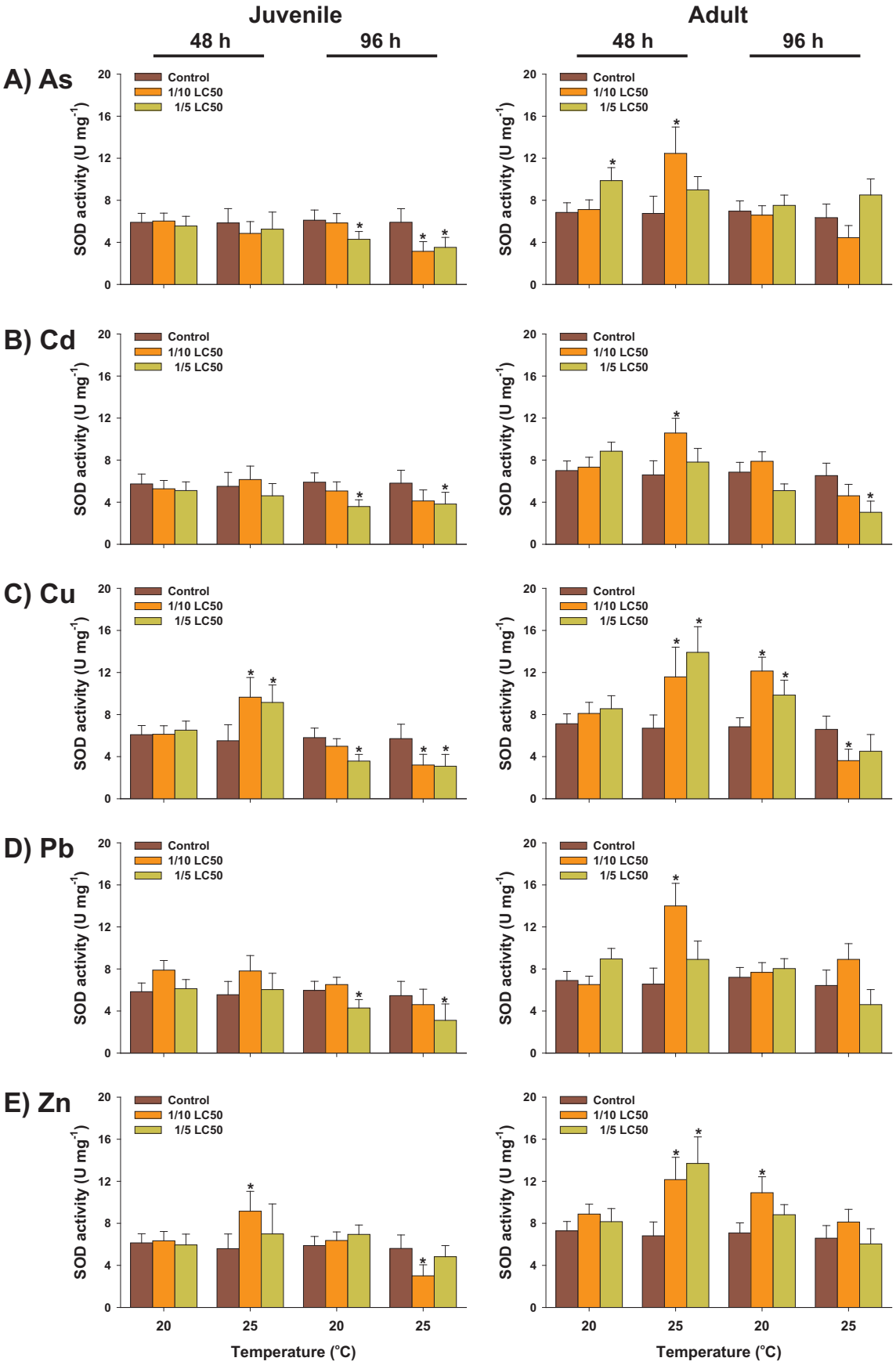

Fig. 4. Analysis of effects of temperature elevation from 20 °C to 25 °C on enzymatic activity of superoxide dismutase upon exposure to 1/5 and 1/10 of the LC_{50} values of A) As, B) Cd, C) Cu, D) Pb, and E) Pb in the juvenile and adult marine mysid *Neomysis awatschensis* for 48 h and 96 h. Data are presented as the mean ± standard deviation (S.D.). The asterisk (*) indicates statistical significance ($P < 0.05$) compared with the control value.

Significant increases in SOD activity were measured in juveniles exposed to 1/10 LC_{50} of Cu and Zn and 1/5 LC_{50} of Cu at 25 °C for 48 h, whereas the levels were decreased by 1/5 LC_{50} of As, Cd, Cu, and Pb at 20 °C and 1/10 LC_{50} of As, Cu, and Zn and 1/5 LC_{50} of As, Cd, Cu, and Pb at 25 °C for 96 h ($P < 0.05$) (Fig. 4). Significantly elevated SOD activities were observed in adults exposed to 1/5 LC_{50} of As at 20 °C for 48 h, 1/10 LC_{50} of As, Cd, Cu, Pb, and Zn and 1/5 LC_{50} of Cu and Zn at 25 °C for 48 h, and 1/10 LC_{50} of Cu and Zn at 20 °C for 96 h ($P < 0.05$). However, the levels were decreased by 1/10 LC_{50} of Cu and 1/5 LC_{50} of Cd at 25 °C for 96 h ($P < 0.05$).

4. Discussion

In this study, effects of temperature elevation on metal toxicity, bioconcentration, and the response of the antioxidant defense system were analyzed in juveniles and adults of the marine mysid *N. awatschensis*. The clear difference in LC_{50} values between juvenile and adults suggests that juveniles were more sensitive to metals than adults. Studies suggest that early developmental stages are more vulnerable to metals due to undeveloped detoxification metabolism and a high energy demand for maintaining homeostasis (Hutchinson et al., 1998; Pechenik, 1999; Muyssen and Janssen, 2007). Elevation in temperature affects toxicity and bioconcentration of metals in both mysid stages. An increase in temperature from 5.5 °C to 15 °C significantly increased Cu toxicity and survival duration in subantarctic isopod *Exosphaeroma gigas* (Lewis et al., 2016). A 7 °C increase in temperature (from 20 °C to 27 °C) increased the inhibitory effect of metals on oxygen consumption of juvenile crayfish *Orconectes immunis* (Khan et al., 2006). Increases in waterborne temperature can contribute to the acute toxicity of metals in aquatic organisms through a higher uptake and bioconcentration of metals (Sokolova and Lannig, 2008). In aquatic crustaceans such as the mud crab *Scylla serrata* and the harpacticoid copepod *Tigriopus japonicus*, it was suggested that increases in metabolic and ventilation rates may lead to a faster depletion of energy reserves at elevated temperatures and results in a higher uptake of metals (Reddy and Bhagyalakshmi, 1994; Kwok and Leung, 2005).

Regardless of stages, was the marine mysid *N. awatschensis* of our study highly sensitive to Cu followed by Zn, Cd, Pb, and As. Similarly, a 96 h toxicity test revealed that Cu was the most toxic metal among Cd, Zn, and Pb for the adult mysids *Americamysis bahia* and *Neomysis integer* (Verslycke et al., 2003). Among the metals, Cu can be highly toxic due to its ability to be reduced by thiol compounds and the formation of stronger ionic and complexed forms with organic ligands than other metals (Sadiq, 1992; Stohs and Bagchi, 1995). Waterborne Cu treatment significantly induced apoptosis through the formation of intracellular ROS and oxidative stress and stress responsive genes among metals (Rhee et al., 2013; Kim et al., 2014).

Metals can be bioconcentrated in aquatic crustaceans (Reichmuth et al., 2010; Adams and Engel, 2014), as shown in the marine mysid, although their bioconcentrations vary according to the magnitude of metabolic processes, experimental conditions, the type of metal, rates of uptake and excretion, physiology, and feeding mode (Bryan, 1979; Rainbow, 2002; Valko et al., 2005). Metals are accumulated in soft tissues of mysids through gut, gills, bone, liver, kidney, and exoskeletons (Mason and Jenkins, 1995). In this study, several metals were highly bioconcentrated by increased temperature, although exposed concentrations were lower at 25 °C than at 20 °C. The marine mysid might have exhausted its energy allocation for metabolic adaptation due to temperature increase, and subsequently failed detoxification and excretion of metals. The bioconcentrated metals in mysids may be of concern for the trophic biomagnification of toxicity that could be transfered to higher trophic levels, as mysids are an important food for numerous aquatic consumers (Rainbow, 2002).

Studies on synergistic effects of metal and temperature on oxidative stress and the subsequent response in antioxidant defense in aquatic crustaceans are limited. Therefore, we analyzed the whole response of the antioxidant defense system in a marine mysid. In marine mysids, higher levels in intracellular MDA were observed in juveniles than adults, suggesting metal-induced lipid peroxidation and higher susceptibility of early stages. Metal treatment induced significant lipid peroxidation in aquatic crustaceans such as the water flea *Daphnia magna*, the blue crab *Callinectes sapidus*, and the freshwater crab *Sinopotamon henanense*, resulting in the elevation of intracellular MDA levels (Brouwer and Brouwer, 1998; Barata et al., 2005; Wang et al., 2013). Damaged membranes through lipid peroxidation become inelastic and lose permeability and integrity (Lesser, 2006). Elevation in temperature clearly contributed to higher MDA levels than 20 °C in the marine mysid. Increased temperature and bioconcentrated metals may synergistically result in oxidative stress by excessive ROS production in mitochondria, inhibition of cellular antioxidant defense, as demonstrated in the meta-exposed blue crab *Callinectes amnicola* (Olakolu and Chukwuka, 2014). Cumulative effects of metals and temperature can also be associated to increase the metabolic activity of organisms, imbalance in membrane fluidity of phospholipid bilayer, and immunity inhibition by temperature-mediated reduced phagocytic activity (Parihar and Dubey, 1995; Correia et al., 2003).

To understand whether temperature- and stage-specific MDA increases are associated with toxicity and oxidative status, responses of essential components in the antioxidant defense system were measured in the marine mysid. An opposite response of GSH was observed in the marine mysid, as significant depletion of GSH was observed in juveniles, while the levels were elevated in a temperature-dependent manner in adults. Disparity of the GSH pattern suggests that the efficiency of the antioxidant defense systems and biotransformation capacity varies across life stages. Juveniles might be immature in the synthesis of GSH and unable to attain a fully developed GSH-involved antioxidant system (Dandapat et al., 2003). Prolonged GSH depletion by metals is associated with mortality in aquatic crustaceans (Brouwer and Brouwer, 1998; Ahearn et al., 2004; Regoli and Giuliani, 2014). Elevation in GSH levels in adults suggest the induction of a first line of defense against oxidative stress by a GSH-mediated detoxification process of excess ROS to maintain cellular redox homeostasis (Valko et al., 2005).

In marine mysids, enzymatic activities of CAT and SOD were significantly increased in adults in most exposures. However, their activities were decreased in juveniles by temperature elevation at 96 h. Our results demonstrated that juvenile stages are much more susceptible than adults to oxidative stress and antioxidant defense could be adversely affected during temperature elevation. Studies showed that in the case that the oxidative stress is not strong, the antioxidant defense system is properly activated. If oxidative stress is persisting or its level is high, oxidative damage on protein can be accumulated and the defense system loses its ability (Lesser, 2006). Decreased activities of CAT and SOD can lead to significant damage of principal cellular components in aquatic crustaceans (Jemec et al., 2010). The noticeable increase of CAT and SOD activation in response to metals suggests that these enzymes are actively engaged in protecting cells against metal-induced ROS and subsequent oxidative stress. We also confirmed that an increase in temperature is critical for maintaining the oxidative status and homeostasis in a marine mysid.

5. Conclusions

In summary, our results suggest that exposure of juvenile and adult mysid to metals caused acute toxicity, bioconcentration, and age-specific modifications of the antioxidant defense system. Our results also demonstrated that toxicity varied with metal type, exposure period, and life stage, juveniles showing a greater sensitivity. Temperature change obviously contributes to metal toxicity and antioxidant defense capacity. Therefore, it is reasonable to suggest that metals and temperature can synergistically induce oxidative stress and the early life stage of marine mysids are more sensitive to the combined effect of metal and

temperature than adult stages due to an impairment of the antioxidant defense system induction. These results also provide useful background data for estimating the potential effects of global warming on metal pollution in aquatic environments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpc.2020.108831.

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