INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18F–144/2019/21–2–307–313
DOI: 10.17957/IJAB/15.0895
http://www.fspublishers.org

Full Length Article

Acute Toxicity of Heavy Metals to *Onchidium struma* under Different Salinities

Mingming Zhang[1], Pei He[1,2], Pu Liu[1], Yonglong Shen[3], Guo Qiao[1*], Qiang Li[1*] and Jintian Huang[1]
[1]*Department of Marine Technology, School of Marine and Biological Engineering, Yancheng Institute of Technology, Yancheng, 224051, Jiangsu, China*
[2]*Department of Aquaculture, School of Fisheries and Life, Dalian Ocean University, Dalian 116023, Liaoning, China*
[3]*Yancheng Oceanic and Fishery Administration, Yancheng 224015, Jiangsu, China*
[*]For correspondence: qiaoguo44ok@163.com; fish790608@163.com

Abstract

This study evaluated the acute toxicity of three heavy metals Cu, Hg and Cr(VI) to *Onchidium struma* juvenile. The results showed that 24 to 96 h median lethal concentration (LC_{50}) values for Cu, Hg and Cr (VI) at 15 practical salinity units (psu) were 138.33–160.32, 6.88–9.41 and 144.47–218.97 mg/L, respectively. It demonstrated that *O. struma* was sensitive to three heavy metals: Hg > Cu > Cr (VI). Moreover, toxicity tests were conducted under different salinities (5, 15 and 25 psu) due to the seasonal salinity changes in habitat. For Cu, 96 h cumulative mortalities at 5, 15 and 25 psu salinity groups were 40, 70 and 85%, respectively. The 96 h LC_{50} values for Cu at 5 psu were much higher than those at 15 and 25 psu. For Hg, 96 h LC_{50} values were not statistically different along with salinity. For Cr (VI), 96 h LC_{50} values were significantly higher at 25 psu salinity group. These suggested that *O. struma* became more sensitive to Cu at higher salinity, and sensitive to Cr (VI) at lower salinity. The sensitivity of *O. struma* to heavy metals studied was related with the salinity. Safe concentrations for Cu, Hg and Cr (VI) were 13.78, 0.69 and 12.69 mg/L, respectively. This study provides useful information to detect environmental pollution under different salinities with this biomonitor *O. struma*.

Keywords: Salt stress; metal ion toxicity; *Onchidium struma*

Introduction

Heavy metals are the most relevant contaminants in the marine or estuarine environments (Nordberg *et al*., 2007; Dung *et al*., 2013; Pan *et al*., 2014; Brady *et al*., 2015), which is easily accumulated in sediments (Yang, 2011) and thus bioaccumulated in aquatic organisms (Bocher *et al*., 2003; De *et al*., 2010; Laxmi *et al*., 2011). Therefore, aquatic organisms could be negatively impacted by heavy metals (Bonga and Lock, 1992; Mendoza-Carranza *et al*., 2016).

Marine gastropod *Onchidium struma* belongs to Mollusca, Gastropoda, Systellommatophora, Onchidioidea, Onchidiidae (Qiu, 1991) and distributes widely in littoral-supralittoral habitats in the coastal regions of China, mainly in the south of Jiangsu province (Shen *et al*., 2011). *O. struma* feeds on sediment detritus in the semitropical coasts where various pollutants are heavily enriched (Huang and Wang, 2004). Most of *O. struma* is marine submerged in subtidal and low-tidal zones, but a few lives in brackish or freshwater. Until now, most of studies on *O. struma* have focused on ecological habitat (Huang *et al*., 2004), body size and environmental factors (Zhang *et al*., 2017), reproductive system (Wang *et al*., 2006), gonadal, embryonic and larval development (Wang *et al*., 2005). Li *et al*. (2009) reported the toxic effects of copper on antioxidative and metabolic enzymes of *O. struma*, and seasonal bioconcentration of heavy metals in Chongming Island, China. These studies demonstrated that *O. struma* can be used as a potentially valuable biomonitor to estimate the heavy metal pollutions, especially in the heavily polluted areas. *O. struma* can selectively bioconcentrate heavy metals, such as Cu. The concentrations of heavy metal are much higher in the tissues of *O. struma* than that in its habitats. The level of metal-bioconcentration is directly related with metal category (essential or non-essential), physicochemical conditions (salinity, water temperature, pH and dissolved oxygen), developmental stage, size and tissue-specificity (Li *et al*., 2009).

Yancheng is located in south of Jiangsu province, China and famous for having the largest tidal area and rich tidal resources. *O. struma*, one of the dominant tidal species in this area, is being consumed by local people due to its high protein and potential medicinal values (Huang *et al*., 2004; Zhang *et al*., 2018). Success in induced breeding of *O. struma* greatly promoted its culture in a large-scale

To cite this paper: Zhang, M., P. He, P. Liu, Y. Shen, G. Qiao, Q. Li and J. Huang, 2019. Acute toxicity of heavy metals to *Onchidium struma* under different salinities. *Intl. J. Agric. Biol*., 21: 307–313

(Huang *et al*., 2004). However, tidal habitat is continuously polluted by pesticides, chemicals and antibiotics from tideland aquaculture. Thus, it is necessary to study the acute toxicity of heavy metals to this biomonitor *O. struma* in the tideland for monitoring the variation of tideland pollution and provide optimum management practices to protect the tideland from further polluted. The metal-bioconcentration changes significantly with the seasons. The heavy metal is mainly bioconcentrated in summer or autumn, since the relative humidity and salinity are frequently changed during these seasons due to the rainwater, sunlight and air temperature. There is limited information on acute toxicity of heavy metals to *O. struma* juvenile under different salinities.

The aim of this study was to compare the acute toxicities of heavy metals to *O. struma* under possible salinities (5, 15 and 25 psu) in its habitat at different seasons. Our results would serve as a reference for further eco-toxicological research on the effects of heavy metals on this species and a guide for *O. struma* culture in low-salinity inland or brackish water.

Materials and Methods

Experimental Animals and Culture

O. struma with body weight of 6.35 ± 1.56 g were obtained from Sheyang sand flats, Yancheng, Jiangsu province of China and acclimated in polyethylene tanks (70 × 50 × 40 cm) with water salinity of 15 psu (similar to natural estuary environment) for 2 days. Seawater was prepared with sea salt (Haida Ltd., Qindao, China) and aerated tap water. The experimental water with different salinities of 5, 15 and 25 psu was adjusted with the seawater and tap water. *O. struma* were then divided randomly into three groups and acclimated to the experimental salinities of 5, 15 or 25 psu by exchanging tank water twice per day. *O. struma* in each tank were acclimated for an additional week. During this period, *O. struma* were fed with *Spirulina* at 2% of body weight daily. *Spirulina* contained 2.73% crude lipid, 57.64% crude protein, 7.53% ash, 8.28% moisture, 0.2% calcium and 0.79% phosphorus. The sterilized glass slide was covered with *Spirulina* as feeding plat and cleaned at 2 h after feeding to keep tanks clean. *O. struma* were not fed within 24 h before toxicity testing. The culture temperature was 25–28°C (Shen *et al*., 2013).

Acute Toxicity Tests

The toxicity tests of 24 to 96 h median lethal concentration (LC_{50}) were carried out using the equal logarithmic basis method (Zhou and Zhang, 1989). Heavy metal [Cu, Hg and Cr (VI)] solutions were prepared with copper sulfate ($CuSO_4$), mercuric chloride ($HgCl_2$) and potassium dichromate ($K_2Cr_2O_7$) (special grade, Yixing Second Chemical Reagent Industry, Yixing, China). The stock solutions of Cu, Hg and Cr (VI) were prepared using water with 5, 15 or 25 psu salinities. Before the experiments, a primary toxicity trial was conducted to find the concentrations causing 0% and 100% mortality of *O. struma* within 96 h and the results were used as reference concentrations for making test solutions. The experimental tools such as aquaria, sea mud, fiberglass tanks and plastic film, were pre-treated with the same concentrations of heavy metals to reduce their absorption onto the surfaces of tools. The natural sediment environment was imitated in a 2 L cylindrical fiberglass tanks by coating with sea mud to 0.5 cm thick.

Based on the results from the primary trial, working solutions were prepared as follows: 1) For Cu, the logarithmic basis was set as 1.16 and tested groups included 0 (control group), 86.2, 100, 115.9, 134.4, 155.6 and 180.4 mg/L; 2) For Hg, the logarithmic basis was 1.58, and tested groups included 0, 1.6, 2.4, 3.8, 5.9, 9.2, 14.4 and 22.4 mg/L; 3) For Cr (VI), the logarithmic basis was 1.19 and the tested groups were 0, 84.4, 100.4, 119.4, 142.0, 168.9 and 200.8 mg/L. These concentrations were the real-time concentrations measured. Trials were conducted in 2 L cylindrical fiberglass tanks containing 0.5 L of the working solution at the corresponding salinities. Each group included 20 individuals and all treatments were run in triplicate. Both working solutions and sediments at different salinities were renewed daily according to the semi static renewal method for toxicity test (APHA, 1985). The mortality and animal behavior were monitored at 8 h intervals throughout the 96 h acute toxicity test. The tested *O. struma* were not fed within 96 hours post exposure (hpe) and considered normal if they were responded to the direct stimuli. *O. struma* were considered dead if they were not responsive towards the repeated touches with a glass rod according to Winner and Farrel (1976). Dead or moribund *O. struma* were removed immediately from the tanks.

Statistical Analysis

Statistical analysis was performed with SPSS 17.0 ver. software. The LC_{50} with 95% confidence interval was calculated with Trimmed Spearman-Karber method (Hamilton *et al*., 1977). Safe concentrations (SC) of heavy metals were calculated according to the formula: SC = 0.1 × 96 h LC_{50}. The effect of salinity on acute toxicity of Cu, Hg and Cr (VI) was evaluated using *t*-test at the same exposure duration. *P*-values of 0.05 or less were considered statistically significant.

Results

Clinical Signs of *O. struma* Post Exposure to Heavy Metals

The clinical signs post exposure to heavy metals included body shrunk, low activity and lack of response to stimuli

Fig. 1: Clinical signs of *O. struma* post exposure to Cu, Hg and Cr (VI) solutions. **a**, ecdysis; **b**, swollen; **c** and **d**, distortion

(Fig. 1a). In the Cu exposure groups, *O. struma* showed swollen and distended abdomen (Fig. 1b). The acute toxicity of Hg caused the tested animals ecdysis, skin darkening and swollen (Fig. 1c and d) The clinical signs of *O. struma* post exposure to Cr (VI) included ecdysis and distortion (Fig. 1).

Acute Toxicity Tests of Heavy Metals at Salinity of 15 psu

At the salinity of 15 psu (similar to the normal environment), acute toxicity results of different heavy metals are listed in Table 1, 2 and 3. The 24 to 96 h LC_{50} values for Cu, Hg and Cr (VI) in *O. struma* were 138.33–160.32, 6.88–9.41 and 144.47–218.97 mg/L, respectively. It suggests that *O. struma* were more sensitive to Hg than other two heavy metals Cu and Cr (VI). And LC_{50} values for these three heavy metals gradually decreased with the increment of exposure time.

Effects of Salinity on the Acute Toxicity of Heavy Metals

The results of the acute toxicity of heavy metals under different salinities are shown in Table 1, 2 and 3. For Cu at salinity of 5 psu, no death was observed at 100.0 mg/L group at 96 hpe. But the death at 15 and 25 psu salinity groups was firstly observed at 48 and 24 hpe and cumulative mortalities were 10 and 35%, respectively. At the higher concentration groups (134.4 mg/L), 96 h cumulative mortalities at salinities of 5, 15 and 25 psu were 40, 70 and 85%, respectively (Table 1). At the same metal concentration, the death appeared earlier and cumulative mortality was higher at the higher salinity than those at

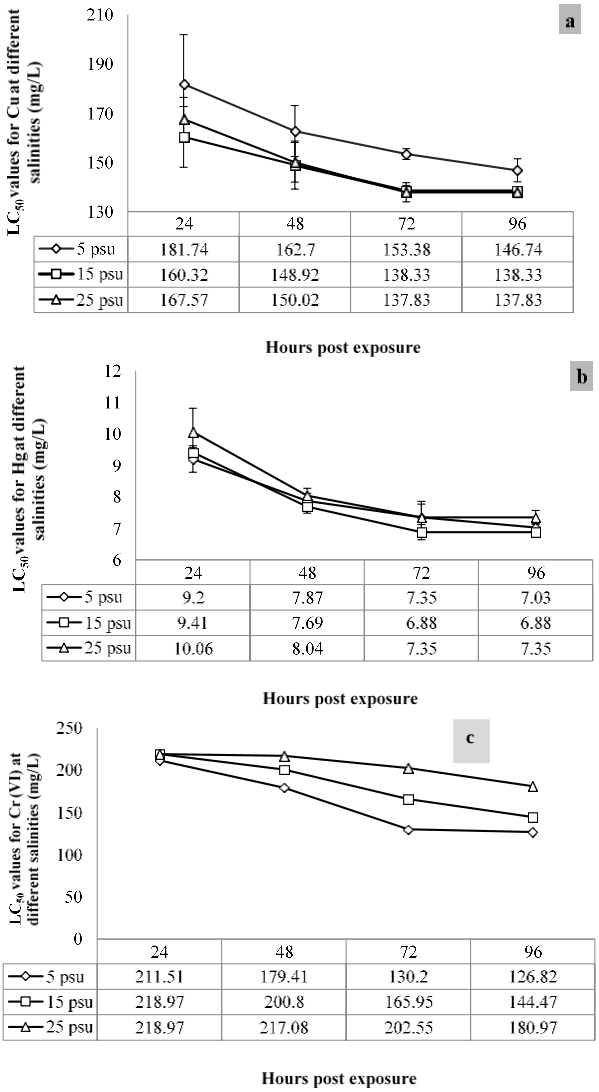

Fig. 2: LC_{50} values for Cu, Hg and Cr (VI) in *O. struma* juvenile at different exposure durations under various salinities (5, 15 and 25 psu). **a**, Cu; **b**, Hg; **c**, Cr (VI)

lower salinity. The LC_{50} values were much higher at the 5 psu salinity groups than those at the 15 and 25 psu salinity groups (Fig. 2a). *O. struma* juvenile became more sensitive to Cu while cultured at the higher salinity.

For Hg, cumulative mortalities were more than 85% at 14.4–22.4 mg/L concentration groups under different salinities within 24 hpe. The 24 h LC_{50} values at salinities 5, 15 and 25 psu groups were 9.20, 9.41 and 10.06 mg/L, respectively (Table 2). *O. struma* juveniles were more sensitive to Hg while cultured at the lower salinity. LC_{50} values were not significantly different among these three salinity groups after 48 to 96 hpe (Fig. 2b).

For Cr (VI), cumulative mortalities at 100.4 mg/L groups were 45%, 20% and 0 at salinities of 5, 15 and 25 psu within 96 hpe, respectively. Similar results were noted at the higher concentration of 168.9 mg/L, and cumulative

Table 1: Cumulative mortality of juvenile *O. struma* after exposed to Cu at salinities of 5, 15 and 25 psu

Salinity (psu)	Concentration (mg/L)	Cumulative mortality (%) at different exposure duration			
		24 hpe	48 hpe	72 hpe	96 hpe
5	100.0	0	0	0	0
	115.9	0	0	5	10
	134.4	0	20	30	40
	155.6	20	40	45	50
	180.4	25	60	65	90
LC_{50} (mg/L)		181.74 r = 0.912	162.70 r = 0.984	153.38 r = 0.909	146.74 r = 0.986
Confidence interval		171.4-192.0	150.2-175.2	140.8-165.9	135.2-158.3
SC (mg/L)		14.67			
15	86.2	0	0	0	0
	100	0	10	10	10
	115.9	30	40	50	50
	134.4	40	50	70	70
	155.6	60	80	100	100
LC_{50} (mg/L)		160.32 r = 0.975	148.92 r = 0.989	138.33 r = 0.990	138.33 r = 0.990
Confidence interval		147.5-173.1	136.6-161.2	128.5-148.2	128.5-148.2
SC (mg/L)		13.83			
25	86.2	0	0	0	0
	100	10	30	30	35
	115.9	10	60	65	65
	134.4	80	85	85	85
	155.6	100	100	100	100
LC_{50} (mg/L)		167.57 r = -0.188	150.02 r = -0.555	137.83 r = -0.604	137.83 r = -0.604
Confidence interval		158.2-177.0	139.0-161.0	127.0-148.7	127.0-148.7
SC (mg/L)		13.78			

Note: Cumulative mortalities were zero in control groups without addition of heavy metals and lower concentration groups of some heavy metals, and data were not listed in the Table 1. psu, practical salinity unites; hpe, hours post exposure; LC_{50}, median lethal concentration; r, coefficient correlation; SC, safe concentration

Table 2: Cumulative mortality of juvenile *O. struma* after exposed to Hg at salinities of 5, 15 and 25 psu

Salinity (psu)	Concentration (mg/L)	Cumulative mortality (%) at different exposure durations			
		24 hpe	48 hpe	72 hpe	96 hpe
5	3.8	0	0	0	0
	5.9	20	30	30	30
	9.2	45	65	80	90
	14.4	85	90	90	90
	22.4	100	100	100	100
LC_{50} (mg/L)		9.20 r = 0.995	7.87 r = 0.979	7.35 r = 0.947	7.03 r = 0.918
Confidence interval		7.2-11.1	6.2-9.5	5.9-8.8	5.8-8.3
SC (mg/L)		0.70			
15	2.4	0	0	0	0
	3.8	10	20	20	20
	5.9	20	25	25	25
	9.2	30	50	70	70
	14.4	85	95	100	100
LC_{50} (mg/L)		9.41 r = 0.973	7.69 r = 0.991	6.88 r = 0.985	6.88 r = 0.985
Confidence interval		7.3-11.5	5.9-9.5	5.4-8.4	5.4-8.4
SC (mg/L)		0.69			
25	1.6	0	0	0	0
	2.4	5	5	5	5
	3.8	5	15	15	15
	5.9	15	20	20	20
	9.2	20	45	60	60
	14.4	85	95	100	100
LC_{50} (mg/L)		10.06 r = 0.990	8.04 r = 0.990	7.35 r = 0.986	7.35 r = 0.990
Confidence interval		8.0-12.1	6.2-9.9	5.7-9.0	5.7-9.0
SC (mg/L)		0.74			

mortalities were respectively 90 and 50% at 5 and 25 psu salinity groups. Cumulative mortalities were higher at the lower salinity than those at the higher salinity (Table 3). The LC_{50} values were significantly higher at 25 psu salinity group than those at 5 and 15 psu salinity groups (Fig. 2c). The results suggests that *O. struma* juvenile became more sensitive to Cr (VI) while cultured at the lower salinity, which was different from the acute toxicity of Cu to *O. struma* (higher salinity). Safe concentrations (SC) for Cu, Hg and Cr (VI) were 13.78, 0.69 and 12.69 mg/L for all salinity groups, respectively (Table 1, 2 and 3).

Discussion

To find out the appropriate concentration ranges of Cu, Hg and Cr (VI) for *O. struma* juvenile, acute toxicity tests were conducted to determine LC_{50} values under different salinities. Yancheng is famous for the longest coastline and rich tideland resources in China. However, the tideland has been contaminated mainly by tideland aquaculture and reclamation, especially the heavy metal pollution in the estuarine environments (Falahi-Ardakani, 1984; Lovley and Coates, 1997; Taylor and Kesterton, 2002; Nordberg *et al*., 2007). The heavy metal pollutions threaten the survival and growth of benthic and intertidal animals living in the estuary (Chen and Lin, 2001). *O. struma* are one of the predominant marine gastropods in the Yancheng tideland (Huang *et al*., 2004). *O. struma* are rich in protein, mineral and vitamin B1 & B2, and consumed widely by humans (Huang *et al*., 2004). *O. struma* are called 'marine Chinese medicine' and have been used to treat asthma and rheumatism, help digestion, reduce fatigue from muscle exhaustion and improve vision (Huang *et al*., 2004). Meanwhile, this species can be used as a biomonitor to detect metal pollution in the estuarine environments. Li *et al*. (2009) investigated the seasonal bioconcentration of heavy metal in *O. struma* from Chongming Island (Shanghai, China) and found that concentrations of Cu, Fe, Mn and Cr (VI) were much higher in *O. struma* than those listed in the Water Quality Standard for Fisheries (GB, 1989). This study showed that *O. struma* could bioconcentrate Cu, Hg and Cr (VI) at different salinities. The heavy metals Cu and Cr could be naturally bioconcentrated in *O. struma* at the concentrations of 375.84 and 16.12 μg/g wet weight of hepatopancreas, respectively. The 96 h LC_{50} value for Cu exposure in *O. struma* juvenile (138.33 mg/L) was higher than that in *O. struma* adult (74.80 mg/L). *O. struma* showed substantially higher LC_{50} value for Cu than that for the green mussel *Perna viridis* (0.62 mg/L) (Chan, 1988) and clams (1.25–5.74 mg/L)

Table 3: Cumulative mortality of juvenile *O. struma* after exposed to Cr (VI) at salinities of 5, 15 and 25 psu

Salinity (psu)	Concentration (mg/L)	Cumulative mortality (%) at different exposure durations			
		24 hpe	48 hpe	72 hpe	96 hpe
5	84.4	0	0	0	0
	100.4	0	0	25	45
	119.4	0	0	35	70
	142	0	10	70	80
	168.9	10	50	75	90
	200.8	10	55	95	100
LC_{50} (mg/L)		211.51 r = 0.885	179.41 r = 0.940	130.20 r = 0.996	126.82 r = 0.912
Confidence interval		201.4-221.6	163.9-194.9	116.6-142.8	116.8-137.0
SC (mg/L)		12.69			
15	84.4	0	0	0	0
	100.4	0	0	5	20
	119.4	0	5	35	40
	142	0	15	40	80
	168.9	0	30	80	90
LC_{50} (mg/L)		218.97 -	200.80 r = 0.963	165.95 r = 0.974	144.47 r = 0.992
Confidence interval		-	186.7-214.9	150.6-181.3	132.3-156.7
SC (mg/L)		14.45			
25	119.4	0	0	0	0
	142	0	0	5	5
	168.9	0	0	15	50
	200.8	0	5	25	55
LC_{50} (mg/L)		218.97 -	217.08 r = 0.963	202.55 r = 0.974	180.97 r = 0.922
Confidence interval		-	211.7-222.4	188.8-216.3	165.9-196.0
SC (mg/L)		18.10			

Table 4: Comparison of 96 h LC_{50} values between *O. struma* and other shellfish

Species	LC_{50} values (mg/L)			References
	Cu^{2+}	Hg^{2+}	Cr^{6+}	
Onchidium struma	138.24	6.88	147.07	This study
Babylonia lutosa (Lamarck)	0.4	-	-	Lin, 2012
Bullacta exarata (Philippi)	0.0011	0.63	9.44	Bao *et al*., 2007
Mactra veneriformis	-	0.207	-	Wang *et al.*, 2009
Moerella iridescens	0.0554	0.1099	19.6277	Wang *et al.*, 2007
Meretrix meretrix Linnaeus	0.0012	-	-	Zhang *et al.*, 2011
Mytilus coruscus	0.194	0.12	14.6	Zhou *et al.*, 2007
Tegillarca granosa	-	-	15.396	Liu, 2008
Haliotis discus hannai	-	0.123	-	Sui *et al.*, 1999
Bellamya purificata (Heude)	0.382	-	-	Zhang, 2008

Note: "-", data not available

(Riba *et al*., 2004). Considerable work on Cu toxicity has been done in crustaceans, yielding a broad range of 96 h LC_{50} values. The 96 h LC_{50} value for Cu in *Penaeus japonicus* juveniles was 1.20 mg/L (Bambang *et al*., 1995), whereas the corresponding value in *P. duorarum* (body length of 55 mm) was 113 mg/L (OPP, 2000). The 96 h LC_{50} values for *O. struma* juvenile were higher than them in other shellfish species, such as *Babylonia lutosa*, *Bullacta exarata*, *Haliotis discus hannai* (Sui *et al*., 1999; Bao *et al*., 2007; Wang *et al*., 2007; Liu, 2008; Zhang, 2008; Wang *et al*., 2009; Zhang *et al*., 2011; Lin, 2012). These differences might be related with the species in physiologic responses to pollutants, including differences in the rate of uptake or depuration of heavy metals (Salánki and Balogh, 1989). For the same organism species, the toxicity is related with the heavy metal category. In the present study, safe concentrations for Cu, Hg and Cr (VI) in *O. struma* juvenile were 13.78, 0.69 and 12.69 mg/L, respectively. Based on the toxicity criteria for aquatic animals (Zhang and Zhang, 1991), Hg is considered highly toxic to *O. struma*, whereas Cu and Cr (VI) show low toxicity in *O. struma*. The toxicity range in *O. struma* was: Hg > Cu > Cr (VI). The results in this study are consistent with the acute toxic values in *Mytilus coruscus* (Zhou *et al*., 2007), *Tubifex tubifex*, *Chironomus tentans* and *T. pyriformis* (Khangarot and Das, 2009). It suggests that *O. struma* can be used as an ideal biomonitor to detect the heavy metals pollution in estuaries, especially in the heavily polluted areas.

Safe concentrations for Cu, Hg and Cr (VI) in *O. struma* juvenile were 138-folds, 69-folds and 144-folds higher than those listed in the Marine Water Quality Standard for Fisheries in China (GB, 1989), indicating that *O. struma* juvenile has a strong tolerance to heavy metal pollution. Li *et al.* (2009) demonstrated that the concentrations of heavy metals in the tissues of *O. struma* were much higher than that in the sediments, water and the corresponding standards. The bioconcentration factors (BCFs) of biomass/water in *O. struma* was 103–104 for Cu and 101 for Cr (VI). The BCFs of biomass/sediment showed similar shift trend as the BCFs of biomass/water (Li *et al*., 2009). Thus, *O. struma* can be used as an ideal biomonitor to estimate the metal pollution in heavily polluted estuaries, just as some other mollusk species used to monitor the metal pollution in India (Bryan *et al*., 1980, 1985; Locarnini and Presley, 1996). However, the food safety should be considered carefully due to strong bioconcentration, popular consumption by local people and successfully artificial reproduction (Shen *et al*., 2012b). Previous studies demonstrated hepatopancreas as the dominant storage tissues for most of heavy metals, such as Cu, Cr (VI) and Zn (Li *et al*., 2009). It will be better to remove the internal organs before consuming *O. struma* since other parts of *O. struma* are also rich in protein, vitamin, mineral or essential element Cu, Fe and Zn (Li *et al*., 2009). Further studies are needed to establish stringent criteria for testing edible quality of this species in China.

The seasonal bioconcentration of heavy metals in *O. struma* showed that Cu, Fe, Mn and Zn were concentrated significantly in summer or autumn. Cd, Cr (VI) and Pb increased slightly in spring and winter. The salinity is approximate 8 psu in summer or autumn, and 15 psu in spring or winter. It suggested that the heavy metal is bioconcentrated in relation with the environmental factors, such as salinity (Li *et al*., 2009). The salinity in the habitats varied at a large level, for example, from 5 to 15 psu in summer and 20 to 28 psu in winter at its key distribution zones, Sheyang coastline of Yancheng (Huang *et al*., 2004). Previous studies showed that lower salinity (5 to 25 psu) could induce the digestive enzyme activities of this species at the optimal culture temperature 25–26°C and relative

humidity above 80% (Shen *et al*., 2012a, b). The salinity can significantly impact the osmolality and ion concentration of peritoneal fluid or pericardial cavity fluid in *O. struma* (Shen *et al*., 2013). Heavy metals can be bioconcentrated in Mollusca mainly through three pathways: 1) absorbed heavy metals dissolved in the water by pallium and then transported to the various organs through blood or concentrated in the epidermal cells; 2) feeding heavy metals dissolved in water or residuals; 3) osmotic exchange interaction between water and skin surface. Heavy metals have been bioconcentrated differently through specific pathway and mechanism. Thus, acute toxicity tests were conducted under different salinities (5, 15 and 25 psu). The LC_{50} value for Cu decreased along with the increment of salinity. It might be explained that the higher salinity can inhibit the superoxide dismutase (SOD) activity in *O. struma*. The SOD activity in shrimp was noted to be influenced by factors such as pH and salinity (Su, 2007). Other studies also showed that SOD activity in *Scylla serrata* declined significantly as the salinity increased (Chen *et al*., 2007). LC_{50} value for Hg increased slightly while the salinity increased. It might be related with the bioconcentration pathway or mechanism of Hg in *O. struma*. The bioconcentration of Hg in Mollusca can destroy the ion equilibrium, change osmosis of cell membrane, inhibit Na^+/K^+-ATPase activity, and thus reduce the absorption rate of heavy metals (Čolović *et al*., 2009). The salinity less than 25 psu could impact Na^+/K^+-ATPase activity, and the lower salinity could decrease Na^+/K^+-ATPase activity slightly in epidermis, hepatopancreas and cardiac stomach (Shen *et al*., 2013) That is why LC_{50} value for Hg at the lower salinity (5 psu) was slightly lower than those at other salinities (15 and 25 psu). These results can provide useful information to detect environmental pollution under different salinities with this biomonitor *O. struma*, and also be helpful to guide its culture in brackish or freshwater. More detailed mechanism needs to be studied in the future using molecular method.

Conclusion

O. struma was sensitive to three heavy metals: Hg > Cu > Cr (VI). Toxicity of heavy metals to *O. struma* was related with salinity. *O. struma* became more sensitive to Cu at higher salinity (25 psu), and sensitive to Cr (VI) at lower salinity (5 psu). Safe concentrations for Cu, Hg and Cr (VI) were 13.78 mg/L, 0.69 mg/L and 12.69 mg/L, respectively.

Acknowledgments

This study was supported by the Major Projects of Natural Science Research in Universities and Colleges in Jiangsu Province (Grant no. 17KJB240002), and partially supported by the opening research fund of the Jiangsu Key Laboratory of Biochemistry and Biotechnology of Marine Wetland (K2016-15 and K2016-16), and Talent Introduction Program at Yancheng Institute of Technology (Grant no. xj201513).

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(Received 06 July 2018; Accepted 23 September 2018)