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Effects of Heavy Metals on Clearance and Oxygen Consumption Rates of the Sea Squirt *Halocynthia roretzi* According to Various Body Sizes

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Abstract - To evaluate the biological response of sea squirt *Halocynthia roretzi* with different body size to heavy metals and its suitability for ecotoxicity assays, the effects of Cr, Cu and Zn on its clearance and oxygen consumption rates were investigated. Clearance and oxygen consumption rates of *H. roretzi* with various body sizes were calculated at different metal concentrations. Both clearance and oxygen consumption rate were negatively correlated with body sizes. Clearance rate of *H. roretzi* decreased gradually with increasing concentration of heavy metal, the decreasing rate was in an order of Cr>Cu>Zn. The oxygen consumption rate first increased at low metal concentration (below 100 μg L^{-1}) and then decreased rapidly with increasing metal concentrations. The decreasing rate was in an order of Cu>Cr=Zn. There was a trend that the clearance rate and oxygen consumption rate decreased drastically under a concentration of 400 μg L^{-1}, and then decreased smoothly when the metal ion concentration increased continually. So the oxygen consumption and clearance rate at a concentration of 400 μg L^{-1} Cu could be thought as a suitable biological tool for exotoxicology analysis.

Key words : sea squirt, *Halocynthia roretzi*, heavy metals, clearance, oxygen consumption

INTRODUCTION

A large proportion of the people in Asian depend on fishery resources for their daily livelihood. These resources are, however, declining due to over-exploitation and pollution effects brought about by an increased population pressure on the coastal areas (Lovatelli 1991). Moreover, the coastal area is also affected by the rapid industrial development that has taken place in the region. Elevated heavy metal concentrations have been recorded in both water and biota in coastal regions. Leakage from improper land-use and mining operations, together with contaminants in industrial and domestic wastes, deliver metals (e.g. copper, chromium, zinc), have caused significant influences to the marine ecosystems (Elfwing and Tedengren 2004).

The sea squirt (*Halocynthia roretzi*), an ascidian species, is a major aquacultural product in Korea (NFRDI 2008), and has been widely cultured in the eastern and southern coast. Sea squirts live in shallow water, usually attached to rocks and artificial structures. Mussels and some species of crustaceans have been extensively used in toxicological studies during last decade. However, little is known about the natural-stress tolerance of sea squirts.

In present work, we investigate the effects of copper, chromium and zinc on the sea squirt *Halocynthia roretzi*. And to determine if *H. roretzi* is a suitable biological tool for ecotoxicological assays, we test its sensitivity to sublethal concentrations of Cr, Cu and Zn, which are among the most persistent heavy metals in polluted systems in South Korea.

Clearance rate and oxygen consumption rate were chosen

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as the effect parameter since they have been described as an adequate sensitive sublethal endpoint for evaluating the biological effects of environmental stressors on many molluscs (Sobral and Widdows 2000; Rajagopal *et al.* 2005; Loayza-Muro and Elías-letts 2007). In this study, to eliminate the interference of food, we determined the clearance rate through the method of neutral red according to Gunasingh Masilamoni *et al.* (2002). We determined the clearance rate by calculating the decrease of neutral red in the water from a known solution in a given time.

The main aims of this study were to evaluate the physiological response of sea squirt when exposed to Cu, Cr and Zn, and also to investigate the sensitivity of the clearance and oxygen consumption rate of *H. roretzi*.

MATERIALS AND METHODS

1. Sea squirt acclimation and maintenance

Individuals of sea squirt were collected from Tongyong Bay, in South Korea. After collection, sea squirts were transported to the laboratory with ice in polystyrene boxes within two hours. Mortality due to transportation was negligible. Upon arrival, individuals were acclimated for seven days prior to the experiments in 200 L fibre-glass tanks in filtered (0.45 μm) sea water at 20°C, pH 7.8, with constant aeration. The natural light: dark rhythm was used. Sea squirts were fed every 2 days with *Isocrysis galbana* at an approximate concentration of 1×10^{5} cells mL^{-1}. The water from the tanks was renewed every 2 days.

Before experiment, the individuals were divided into three groups according to their body sizes, the measurements of sea squirt used in this experiment were showed in Table 1.

2. Effects of heavy metal on filtration rate

We tested the following individual metal concentrations: Cr: 0.5 (control), 50, 100, 200, 400, 800, 1600 μg L^{-1}; Cu: 3 (control), 50, 100, 200, 400, 800, 1600 μg L^{-1}; Zn: 9 (control), 50, 100, 200, 400, 800, 1600 μg L^{-1}. Stock solution of 1000 mg L^{-1} were used. Three replications were performed per treatment. The experiment was performed in translucent polycarbonate 20 L tanks containing 7 L of continuously aerated filtered sea water at 20±1°C. Test water was renewed after 24 and 48 h of exposure. Water samples were taken just before and after water renewal and analyzed to determine the actual metal exposure concentration. The filtration rate was determined using neutral red (Gunasingh Masilamoni *et al.* 2002). Five *H. roretzi* were tested for each metal concentration, and the whole experiment was performed at three different times.

Clearance rate (C) was calculated according to the equation:

$$C=\frac{V(\ln C_0-\ln C_t)}{nt}$$

where C_0 is the initial concentration of neutral red, C_t is the neutral red concentration at time t, n is the number of sea squirt and V is the culture volume. Neural red concentrations were determined at the beginning and end of experiments at 530 nm using a spectrophotometer.

3. Effect of heavy metal on oxygen consumption rate

The metal concentrations described above were tested.

Dissolved oxygen concentrations were determined at the beginning and end of experiments. The decrease in dissolved oxygen in sealed bottles was measured using a digital oxymeter. The respiration rate (O) was calculated by the following equation:

$$O=\frac{V(D_i-D_t)}{nt}$$

where D_i is the initial concentration of dissolved oxygen, D_t is the dissolved oxygen concentration at time t, n is the number of sea squirt and V is the culture volume.

After the tests, the biomass data were obtained by drying the tissue in a furnace at 60°C, for a period of 24 h. The oxygen consumption and clearance rates were corrected for a standard weight of 1 g (Resgalla 2007).

4. Statistical analysis

Two-way ANOVA was used to evaluating effects of heavy mental and body size on filtration and oxygen consumption rate. For all statistical analysis a confidence interval of 95% ($P<0.05$) was applied.

RESULTS

Fig. 1 showed the filtration rates of sea squirt *H. roretzi*

Table 1. Measurements of *Halocynthia roretzi* used in the experiments

	Body height (mm) ±SD	Body width (mm) ±SD	Total weight (g) ±SD
S	47.0±3.5 (42.4～50.9)	24.6±2.2 (21.7～27.6)	12.5±2.2 (10.0～15.9)
M	56.7±3.1 (52.2～60.4)	30.0±3.3 (27.2～34.3)	21.4±1.7 (19.9～24.5)
L	68.7±5.1 (64.3～73.4)	37.2±3.1 (35.1～39.0)	35.9±2.3 (33.4～29.8)

Values are means±SD (n=30), standard deviations; S, small group; M, middle group; L, Large group. The rages of size and weight are presented in brackets.

at different concentrations of Cr, Cu and Zn. Results indicated that both the heavy metal and body size affected the filtration rate of *H. roretzi* markedly ($P<0.05$). Fig. 1a showed the filtration rate (FR) of sea squirt under different Cr concentration. FR decreased greatly in all groups when Cr reached 400 μg L^{-1}, the small group decreased more seriously than large group. The divergence between three size groups decreased gradually when the Cr concentration increased from 400 μg L^{-1}, and there was no difference among three size groups when the Cr concentration increased to 1600 μg L^{-1}. The decreasing rate of FR in small group was 93%, while that in middle group was 91% and in large group was 88%. The decreasing trend of FR under Cu was some similar to that of Cr (Fig. 1b). The divergence between three size groups also decreased when the Cu concentration increased from 400 μg L^{-1}, and there was still slight difference among three groups when the Cu concentration increased to 1600 μg L^{-1} which was some different from Cr. And the decreasing rate was 88% in small group, 85% in middle group and 80% in large group till the end of the experiment. FR under Zn decreased less seriously compared to that in Cr and Cu (Fig. 1c). The decreasing rate was 81% in small group, 79% in middle group and 72% in large group. The divergence among three size groups decreased gradually when concentration of Zn increased from 400 μg L^{-1} to 800 μg L^{-1}. Then the FR was parallel to each other when Zn concentration increased from 800 μg L^{-1} to 1600 μg L^{-1}.

Oxygen consumption rate (OCR) of sea squirt *H. roretzi* at different concentrations of Cr, Cu and Zn were expressed in Fig. 2. Results indicated that both heavy metal and body size affected the OCR significantly ($P<0.05$). Fig. 2a showed the variation trend of OCR under different Cr concentrations. OCR increased and reached the maximum in

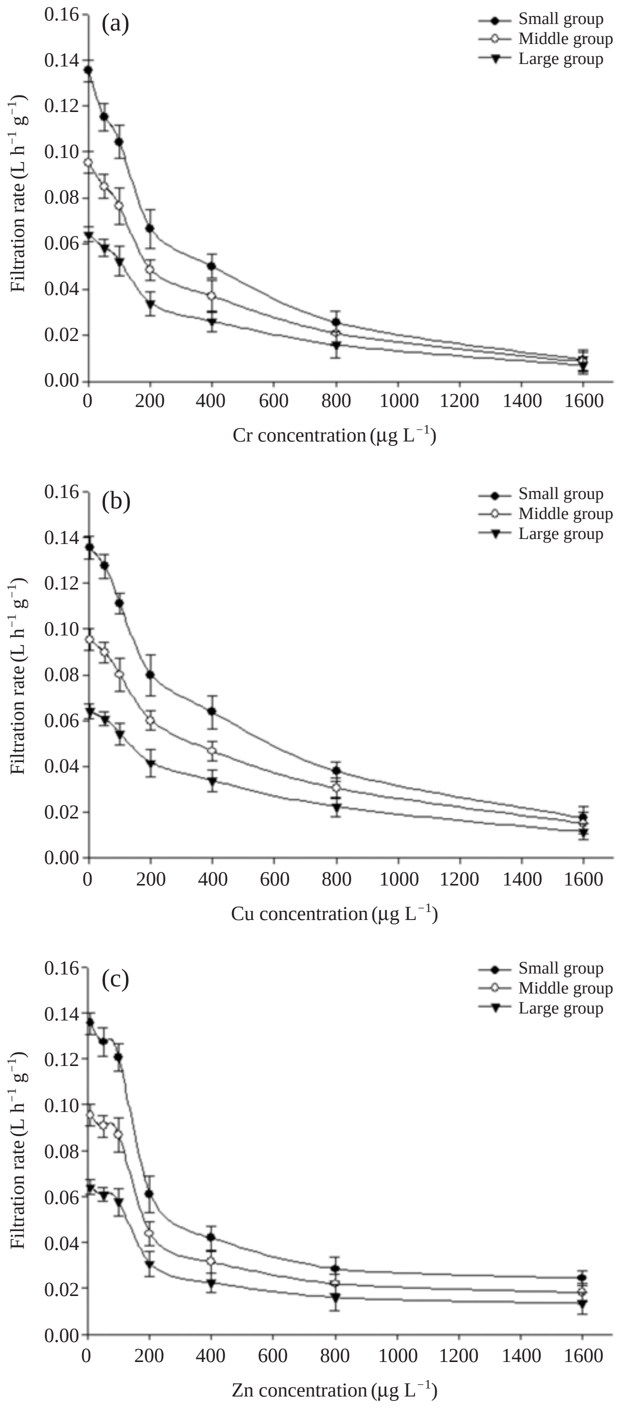

Fig. 1. Clearance rate of *Halocynthia roretzi* at different Cr (a), Cu (b) and Zn (c) concentrations after 48 h of exposure according to different body sizes. Values are means of experiments run on three occasions (±SD).

all groups when Cr concentration increased to 50 μg L^{-1}, and decreased remarkably from 50 μg L^{-1} to 400 μg L^{-1}, and then decreased smoothly from 400 μg L^{-1} to 1600 μg L^{-1}. The decreasing rate was 83% in small group, 79% in

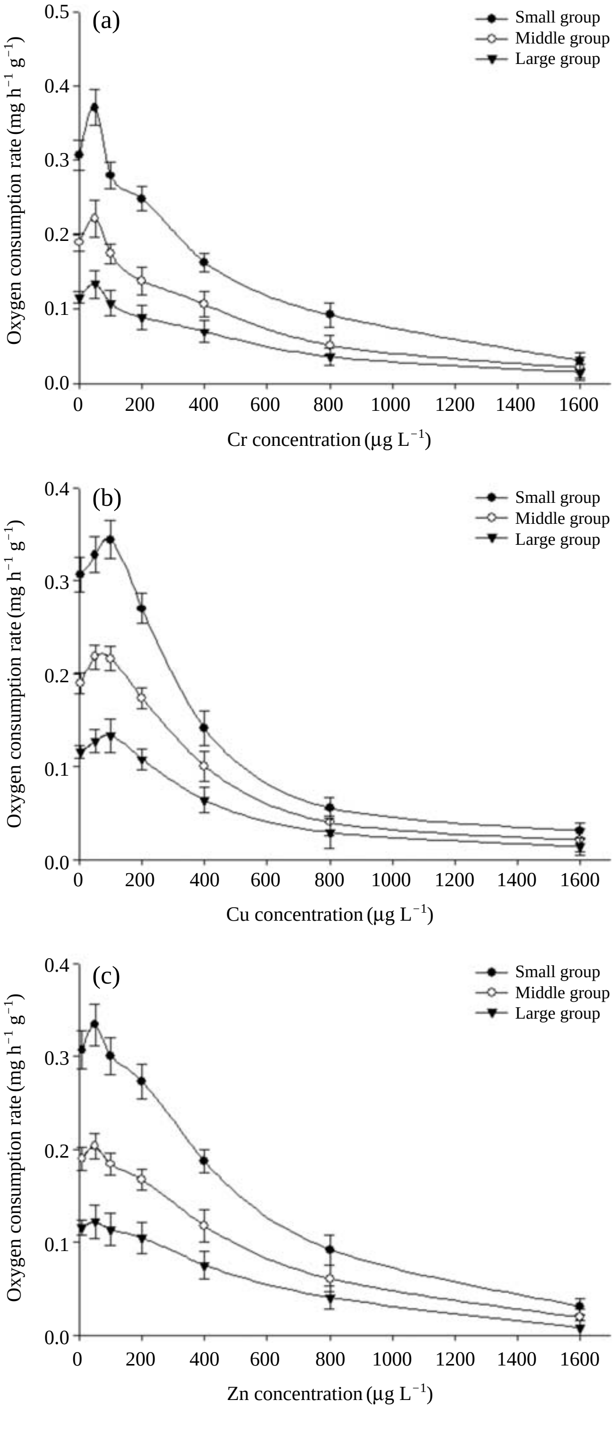

Fig. 2. Oxygen consumption rate of *Halocynthia roretzi* at different Cr (a), Cu (b) and Zn (c) concentrations after 48 h of exposure according to different body sizes. Values are means of experiments run on three occasions (±SD).

middle group and 75% in large group compared to the start point. The divergence among three size groups also decreased gradually with increasing Cr concentration. OCR increased and reached the max when Cu concentration increased to 100 μg L^{-1} (Fig. 2b), and then decreased greatly from 100 μg L^{-1} to 400 μg L^{-1}. The OCR and divergence among three size groups then decreased gradually when Cu concentration increased from 400 μg L^{-1} to 1600 μg L^{-1}. The decreasing rate was 87%, 84% and 83% respectively in small, middle and large group compared to the starting point. OCR under Zn showed similar trend to that of Cu (Fig. 2c). OCR increased until Zn concentration increased to 50 μg L^{-1}, with the smaller size had the higher increasing rate. And then decreased remarkably until 400 μg L^{-1}, the decreasing rate was 83%, 79% and 75% respectively in small, middle and large groups. And the variation trend was similar to that of Cr and Cu. In general, the filtration rate of *H. roretzi* was negatively correlated with body size ($P < 0.05$), but at the concentration of 1600 μg, there was no difference among small, middle and large groups ($P > 0.05$).

DISCUSSION

In the present study, clearance and oxygen consumption rates of *H. roretzi* were negatively correlated with body sizes. This was similar to other marine molluscs (Sukhotin *et al.* 2003; Kang *et al.* 2008). However, Gunasingh Masilamoni *et al.* (2002) found that the oxygen consumption rate and filtration rate of *Brachidontesstriatulus* increased with increasing size and temperature. Tedengren *et al.* (1999) reported that smaller size Baltic mussels were most likely affected by the cadmium uptake and depuration, and possibly also the total body burden. So the size effect of sea squirt should be taken into consideration when used as ecotoxicological test organisms, since it had different response depending on the various size.

Oxygen consumption rate was observed to increase first with increasing metal concentration under a low concentration range, this may be a stress response caused by the increasing metal ion. Similar result was also observed in *Macrobrachium kistnensis* (Nagabhushanam and Kulkarni 1981), the oxygen consumption increased significantly first and then slashed severely at slightly higher concentration of $CuSO_4$ and $ZnSO_4$. However, in other reports only serious decrease was observed (Cheung and Cheung 1995; Bhamre and Desai 2012). Oxygen consumption rate decreased with increasing metal concentration and there was a negative correlation between oxygen consumption and metal concentration (Hassan 2011). The decreasing rate of oxygen consumption in our experiment was in an order of Cu > Cr = Zn. This was similar to the results of Nagabhushanam and Kulkarni (1981) that the sub Lc_{50} concentrations of $CuSO_4$ was lower than $ZnSO_4$.

Metals are known to reduce the performance of bivalve molluscs (Kramer *et al.* 1989), because in the presence of a high concentration of heavy metals, bivalve molluscs keep their shells closed for a longer period of time (Doherty *et al.* 1987), produce fewer byssus threads (Martin *et al.* 1975) and reduce heart rates (Grace and Gainey 1987), also reduce their filtration rates (Watling 1981; Grace and Gainey 1987). The exposed individuals of *S. cucullata* to cadmium and copper showed a dramatic decrease in clearance rate (Azarbad *et al.* 2010). Similar trend was observed in our experiment. Clearance rate decreased greatly when the concentration of Cr, Cu and Zn increased to 400 μg L^{-1}, and then decreased smoothly in the following period. Smaller size decreased more obviously compared to bigger size. Pynnoen and Huebner (1995) indicated that this physiological adjustment for avoiding temporal exposure to contaminants would have contributed to reduce the clearance rate of marine molluscs. Reduced clearance rate could be the result of gill damages, since one of the recorded effects of sub lethal concentrations of cadmium is structural deformations of gills (Viarengo 1989). The reduction in clearance could also be the result of avoiding the cadmium and copper through partial valve closure, although this behavior was not observed in the present study. This behavior has been reported for the blue mussel, *Mytilus edulis*, when exposed to copper (Davenport 1977; Manley 1983). Elevated metal concentration producing acute mortality are generally improper for evaluating biological effects on aquatic organisms since they are barely related to natural conditions (Loayza-Muro and Elías-letts 2007). Our results indicated that the clearance rate is an adequate sublethal endpoint for evaluating the effects of meals on *H. roretzi* since no mortality was observed.

Research on tunicates has shown that these organisms selectively accumulate certain trace elements from the marine environment. The high concentration factors found for some elements (iron, cobalt, zinc, selenium, vanadium) support the use of tunicates as models in environmental studies of trace metals (Papadopoulou and Kanias 1977). In our experiment, both clearance rate and oxygen consumption rate decreased drastically when concentration of metal ion increased to 400 μg L^{-1}, and then decreased smoothly when concentration increased continually. According to the saltwater dissolved metals criteria (EPA 1995), the maximum total recoverable metals concentration for Cr, Cu and Zn were 1079, 2.92 and 95.1 μg L^{-1} respectively. This means the toxicity was more prominent in Cu than Zn and Cr. We observed the decreasing rate of oxygen consumption rate was prominent than that of Cr and Zn, while decrease of clearance was slightly lower than Cr. And the effect was particularly obvious in small group than that in middle and large group. So the oxygen consumption and clearance rate at a concentration of 400 μg L^{-1} Cu could be thought as a suitable biological tool for exotoxicology analysis.

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