# Biological Monitoring to Detect Both Water Pollution and Water Pollution and Water Quality Recovery Based on Value Movements of Freshwater Bivalves (Corbicula japonica)

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# Biological Monitoring to Detect Both Water Pollution and Water Quality Recovery Based on Valve Movements of Freshwater Bivalves (*Corbicula japonica*)

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The valve movement pattern of the freshwater bivalve *Corbicula japonica* (*C. japonica*) following exposure to test chemicals was monitored. We conducted the exposure test with two heavy metal compounds, cadmium chloride ($CdCl_2$; 0, 0.1, 1, or 10 mg/L) and copper (Ⅱ) chloride ($CuCl_2$; 0, 0.1, 1, or 10 mg/L) in a continuous flow system. After a pre–exposure period in a test chamber, which was filled with dechlorinated tap water for 1 h, the bivalves were exposed to the test chemicals for 3 h. After that, only dechlorinated tap water was released again into the test chamber for 2 h. Thus, the valve movements were continuously recorded for a total of 6 h. On exposure to $CdCl_2$ (1 and 10 mg/L), the valve movement of *C. japonica* closed. However, the valve movements of *C. japonica* in all $CdCl_2$ groups recovered after termination of the exposure. *C. japonica* showed decreased distance between the valves on exposure to $CuCl_2$ (0.1, 1, and 10 mg/L). However, *C. japonica* restarted their valve movements only in the recovery period at 0.1 mg/L $CuCl_2$. Therefore, we conclude that real–time monitoring of the valve movement of *C. japonica* is a useful method to detect both the contamination with a toxicant and recovery of the water quality in an aquatic ecosystem. In addition, it can be used for the assurance of the water quality.

## INTRODUCTION

Synthetic chemicals are indispensable in the modern society, and a variety of chemicals is being used in a wide range of fields. However, contamination of the aquatic environment due to urban and industrial effluents, e.g. chemicals (including heavy metal compounds), have been reported around the world (Forstner and Muller, 1973; Singh *et al.*, 1997; Karadede and Unlu, 2000; Kaimoussi *et al.*, 2001). In addition, there is a concern that pollutants can have a harmful effect on aquatic organisms, such as depletion of their energy resources, damage of their nervous system and reproductive dysfunction.

In general, the water quality in the aquatic environments, including the water source, is validated by modern analytical methods, such as instrument analysis in laboratory. However, these methods are usually characterized as non–continuous process and the analytical results are immediately available. Unexpected toxicants or chemical complexes cannot be detected by routine chemical analysis (Borcherding and Wolf, 2001; Kramer and Foekema, 2001). Thus, there is a need to develop biological early warning systems (BEWS) using aquatic organism for water quality monitoring (Untersteiner *et al.*, 2005; Allan *et al.*, 2006).

Several BEWS have been already developed and some systems have been used for on site water quality monitoring (Benecke *et al.*, 1982; Sluyts *et al.*, 1996; Lechelt *et al.*, 2000; van der Schalie *et al.*, 2001; Kang *et al.*, 2009). Some aquatic organisms including fish, crustacean, algae and bivalves are selected as bio–monitoring organisms because of their high sensitivity and immediate response to the exposure to contaminants (Baldwin and Kramer, 1994; Sluyts *et al.*, 1996; van der Schalie *et al.*, 2001; Gerhadt *et al.*, 2006).

Bivalves have been used as bio–indicators for heavy metals and organic compounds in an aquatic environment because they stay and live in same area and accumulate contaminants in their bodies (Kramer and Botteweg, 1991, Jeng *et al.*, 2000; EL–Shenawy, 2004). The valve movements of bivalves, which are closely related to vital activities such as respiration, feeding and excretion, have been studied as an indicator of vitality and circadian rhythms (Fujii and Toda, 1991; Jorgensen, 1996). Bivalves display specific characteristics in response to pollutants and contaminants, such as closing of shells (Sluyts *et al.*, 1996; Kader *et al.*, 2001). The behavior of bivalves is reflected by their valve movement, which has been proposed as a monitoring tool that allows rapid detection of toxicants in the water system and continuous monitoring of the water quality. Thus far, several methods have been proposed for measuring the valve movements of bivalves, e.g., those using kymographs and strain–gauges (Kuwatani, 1963; Fujii, 1977; Fujii, 1979; Higgins, 1980), two small stimulation coils and electromyography (Jenner *et al.*, 1989) or impedance electrodes (Tran *et al.*, 2003).

Freshwater bivalves, *Corbicula japonica* (*C. japonica*) were selected as test organisms in this study. *C. japonica* is a small and easily available species and lives widely in rivers and brackish–water lakes in Japan. In this study, we evaluated the response of *C. japonica* to exposure to heavy metals compounds by monitoring their valve movements. In addition, the valve movements of *C. japonica* were observed under recovery conditions.

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## MATERIALS AND METHODS

### Test organisms and maintenance

Adult freshwater bivalves, *C. japonica* (body weight 5.53±1.21g, length 25.3±1.94 mm, height 22.9±1.54 mm, width 14.4±0.99 mm, mean ± standard deviation) were collected between November 2007 and February 2008 from Lake Shinji (Tokushima Prefecture, Japan) and maintained in the laboratory. One hundred *C. japonica* were placed in a glass tank (360×155×155 mm), containing 18 L of dechlorinated tap water and kept under a natural photoperiod (natural daylight, filtered through a blind). There was no substrate, such as sand, at the bottom of the glass tank in order to adjust the valves to the experimental conditions. The concentration of dissolved oxygen (DO) ranged between 7.0 and 8.0 mg/L, and the water temperature was maintained between 19 and 23 °C. The water in the glass tank was renewed once a week and *C. japonica* were fed an appropriate amount of chlorella (Chlorella Industry Co., Ltd., Tokyo, Japan) twice a week. The survival of *C. japonica* during the maintaining period was over 95%.

### Measuring technique for valve movements

We measured the movements of the valves using the method described by Nagai *et al.* (2006). This method was based on the measurement of the changes in the external magnetic field, using a magnetic device called a Hall element. The Hall element is sealed in epoxy (Hall element sensor; 7×5×4 mm) and a small ferrite magnet (diameter 6 mm) is attached to each edge of the valves by a polyethylene resin and underwater paste (Fig. 1). The distance between the Hall element sensor and the magnet in the closed state was approximately 10 mm. *C. japonica* were out of water for 3 h until the glue set and hardened completely.

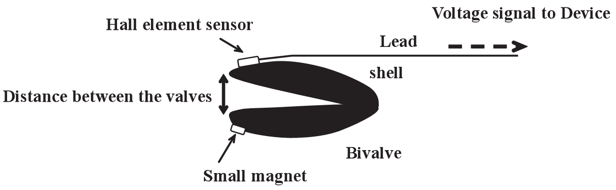

**Fig. 1.** Measuring principle of valve movements.

The valve movements of *C. japonica* were continuously recorded as output voltage from the Hall element sensor using the SL–108A (Tokyo Sokki Kenkyujo Co., Ltd., Tokyo, Japan). The distance between the sensor and the magnet is inversely correlated to the value of sensor output. Therefore, the recorded data (the sensor output value; mV) were converted into the distance between the valves (mm) with the computer software SL–7108 (Tokyo Sokki Kenkyujo Co., Ltd., Tokyo, Japan).

### Valve movements of *C. japonica* under control condition

A total of 24 *C. japonica* was monitored for their normal behavior under control conditions. Figure 2 shows the scheme of this experimental test. Eight *C. japonica* with Hall element sensors were placed into the 1 L test chamber (130×400×100 mm) that was filled with dechlorinated tap water. Only dechlorinated tap water run through the test chamber using a tube pump, RP–1000 (Iwaki Co., Ltd., Tokyo Japan) with a continuous flow (flow rate; 150 mℓ /min) in the dark. The water in test chamber was renewed 9 times per hour. After acclimatization in the test chamber for more than 12 h, the valve movements of *C. japonica* were recorded for 6 h. The water parameters (pH, DO, and temperature) were measured at 0, 3, and 6 h after the beginning of the test. The water temperature ranged between 20.4 and 22.4 °C, the concentration of DO ranged between 7.7 and 8.4 mg/L, and the pH ranged between 6.5 and 7.0. After conducting all the tests, *C. japonica* were drained on filter paper and the Hall element sensor and small magnet were removed. In addition, their body weight, length, height, and width were measured.

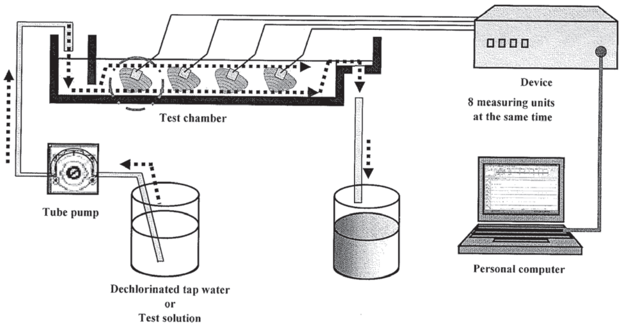

**Fig. 2.** Schematic diagram of the exposure test.

### Valve movements of *C. japonica* under exposure condition

In this study, we used copper (Ⅱ) chloride ($CuCl_2$) and cadmium chloride ($CdCl_2$) as test chemicals. $CuCl_2$ (>95% purity) and $CdCl_2$ (>95% purity) were purchased from Wako pure Chemical Industries, Ltd. (Osaka, Japan). The stock solutions of $CuCl_2$ and $CdCl_2$ (1,000 mg/L) were prepared by dissolution in dechlorinated tap water.

We exposed *C. japonica* to $CuCl_2$ (0.1, 1, or 10 mg/L) and $CdCl_2$ (0.1, 1, or 10 mg/L) in the continuous flow–through system. For each exposure test, 8 *C. japonica* were pre–conditioned for more than 12 h under the same condition as for the control test. Before each exposure, the valve movements of *C. japonica* were observed for 1 h (pre–exposure period). The test solution was released into the test chamber for 3 h and then only dechlorinated tap water was released into the test chamber for 2 h (recovery period). Thus, the valve movements were continuously recorded for a total of 6 h. During the test, the water parameters (pH, DO, and temperature) were measured at 0, 1, 4, and 6 h after the beginning of test. The water temperature ranged between 20.6 and 22.3 °C, the concentration of DO ranged between 7.6 and 8.9 mg/L, and the pH ranged between 6.0 and 7.1.

### Data analysis

The distances between the valves of *C. japonica* were statistically compared between control and exposure conditions. All data were tested for homogeneity of variance across treatments by using the Levene's test. Data were analyzed by one–way analysis of variance (ANOVA) and differences were tested using the Dunnett's test. When no homogeneity was observed, nonparametric statistical comparisons were used to detect differences among treatments (Kruskal–Wallis test). The differences between control and treatment groups were identified using the individual Mann–Whitney U–tests. A $p$ value<0.05 was considered significant; Bonferroni $p$ value was used in nonparametric tests. All statistical analyses were performed using SPSS 11.0J (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Valve movements of *C. japonica* under control condition

The distance between the valves was in the range of approximately 0.5–3 mm under control conditions for 6 h (Fig. 3A, 3B and 3C). Although the real–time response of the valves evaluated by their movements was irregular, the mean distance between the valves during each 30 min interval was in the range of approximately 1–1.5 mm (Fig. 4).

### Valve movements of *C. japonica* exposed to $CdCl_2$

Exposure to $CdCl_2$ induced the closure of the valves. When exposed to 0.1 mg/L $CdCl_2$, the bivalves began to close their valves after 2 h (Fig. 5A). The reduction in the distance between the valves was found in 3 individuals. However, no significant differences in the distance between the valves were found in all intervals compared with control conditions (Fig. 6). Out of 4 individuals, 3 who had closed their valves during the exposure period opened their valves again during the recovery period (0.1 mg/L $CdCl_2$) (Fig. 5A). In addition, their valve movements were similar to those under control conditions. The mean distance of the valves between 0 and 30 min of the recovery period was 0.7 mm. The value increased to 1.2 mm between 60 and 90 min. However, no statistical differences were observed (Fig. 6, $p<0.05$).

Out of 8 individuals, 7 began to close their valves 20 min after exposure to 1 mg/L $CdCl_2$ (Fig. 5B). The distance between the valves decreased gradually and

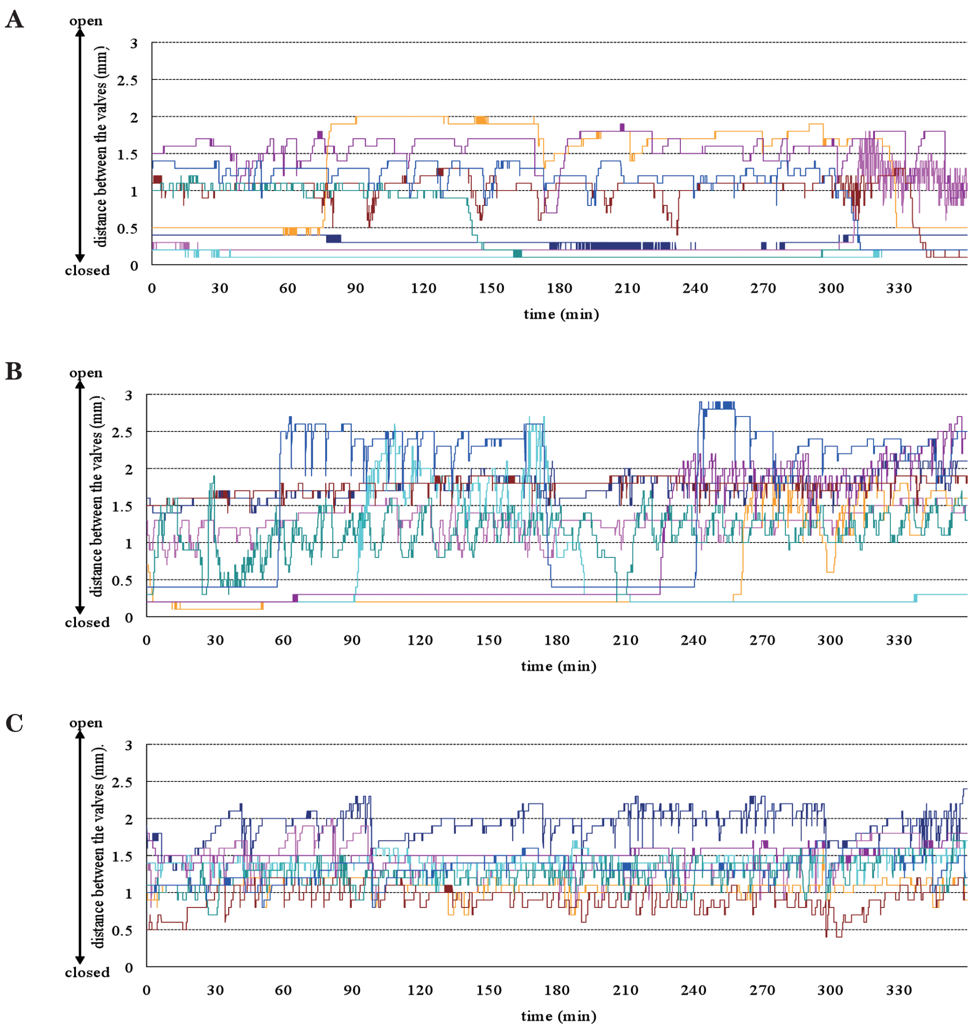

**Fig. 3** The real–time response of the valve movements of *C. japonica* under control condition

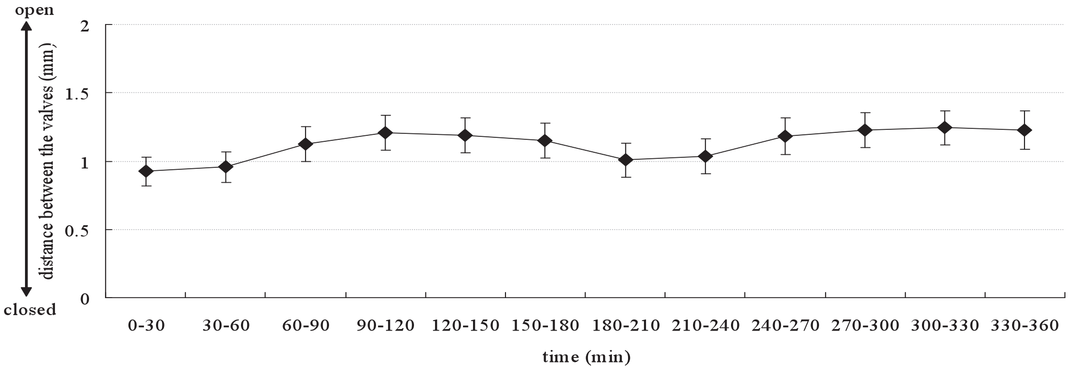

**Fig. 4.** Distance between the valves of *C. japonica* under control condition during 6 h. Data are shown as means ±SE during each 30 minutes. *: indicates significant difference compared to each section ($p<0.05$).

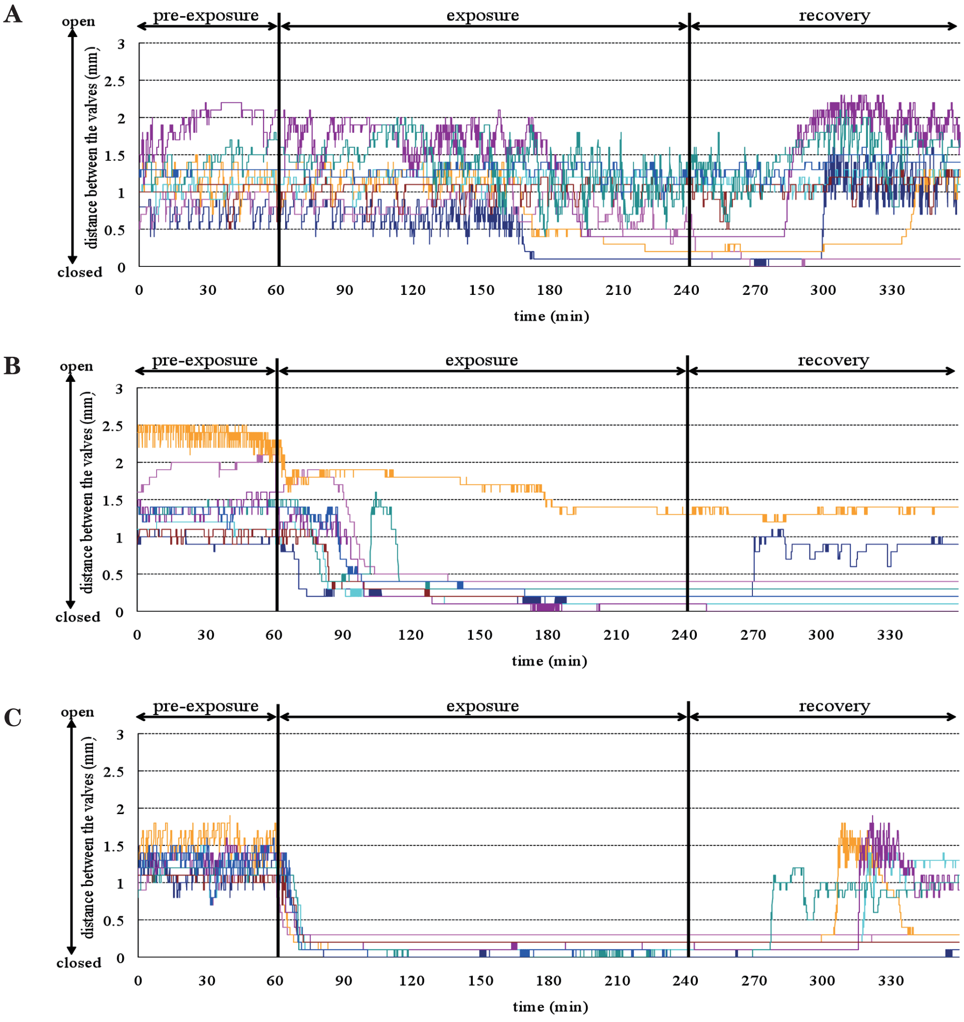

**Fig. 5.** The real–time response of the valve movements of *C. japonica* when exposed to $CdCl_2$: exposure to (A) 0.1 mg/L, (B) 1 mg/L and (C) 10 mg/L.

remained at the low values throughout the exposure period. Significant differences were observed in the distances between the valves after 60 min of the exposure compared to the distances under the control conditions (Fig. 6, $p<0.05$). After termination of the exposure, 1 out of 7 individuals that closed their valves during the exposure period opened their valves again. The valve movements were similar to those under control conditions. The mean distance between the valves during each 30 min intervals did not increase during the recovery period compared with that at the end of the exposure. The value remained at 0.4 mm (Fig. 6). The differences

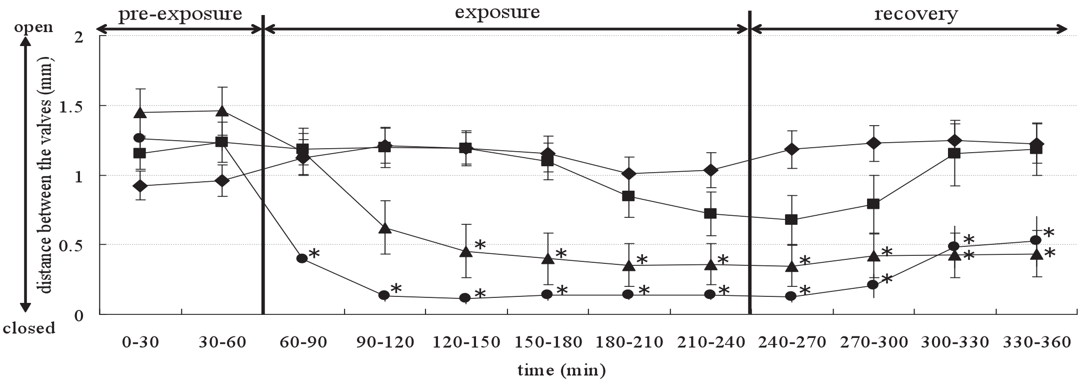

**Fig. 6.** Distance between the valves of *C. japonica* when exposed to $CdCl_2$: exposure to (◆) 0 mg/L, (■) 0.1 mg/L, (▲) 1 mg/L and (●) 10 mg/L during 6 h (unexposed period 1 h; exposure period 3 h; recovery period 2 h). Data are shown as means ±SE during each 30 minutes. *: indicates significant difference compared to control groups ($p<0.05$).

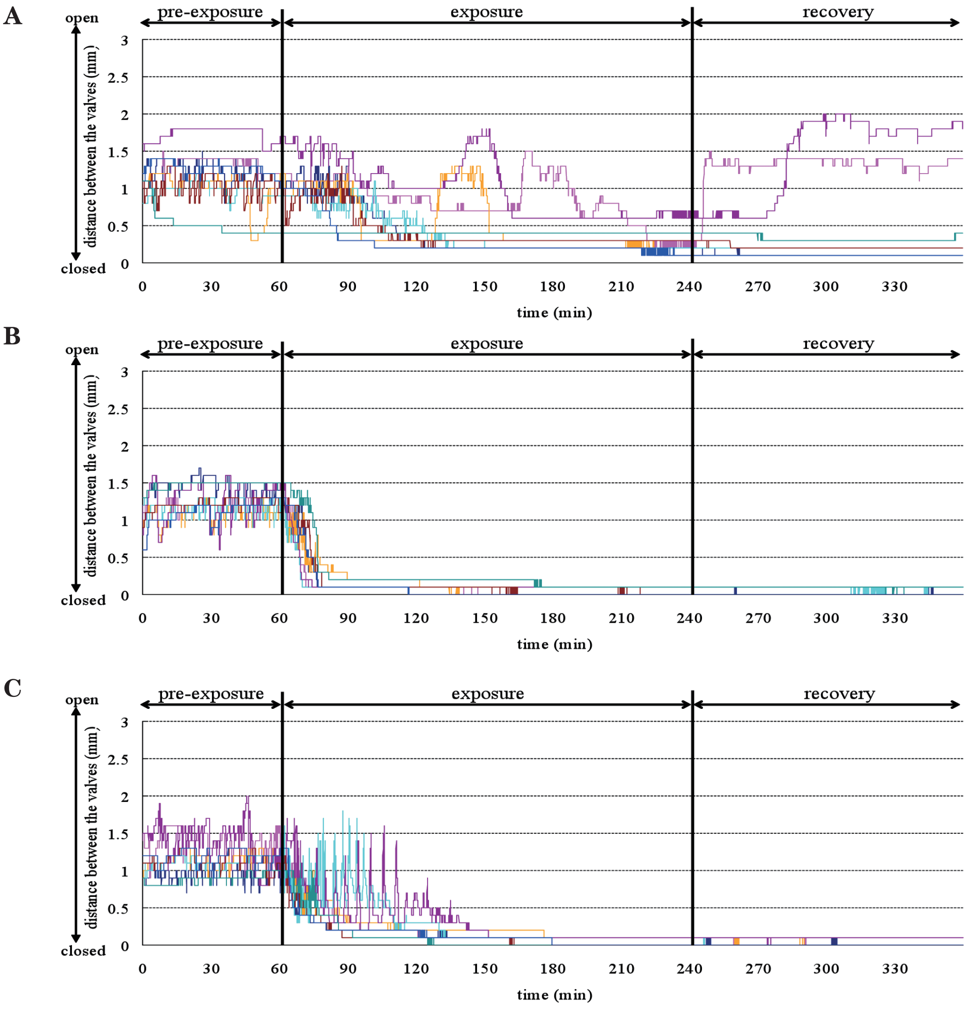

**Fig. 7.** The real–time response of the valve movements of *C. japonica* when exposed to $CuCl_2$: exposure to (A) 0.1 mg/L, (B) 1 mg/L and (C) 10 mg/L.

in the distances between the valves between recovery and control conditions were statistically significant (Fig. 6, $p<0.05$).

Moreover, all individuals closed their valves immediately after exposure to 10 mg/L $CdCl_2$ (Fig. 5C). The distance between the valves decreased drastically within 10 min of exposure. The distances between the valves were maintained throughout the exposure period (Fig. 6,

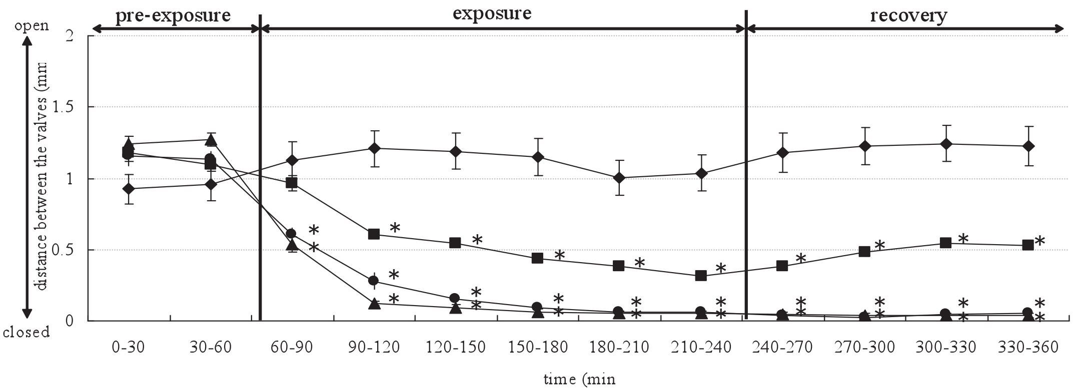

**Fig. 8.** Distance between the valves of *C. japonica* when exposed to $CuCl_2$: exposure to (◆) 0 mg/L, (■) 0.1 mg/L, (▲) 1 mg/L and (●) 10 mg/L during 6 h (unexposed period 1 h; exposure period 3 h; recovery period 2 h). Data are shown as means ±SE during each 30 minutes. *: indicates significant difference compared to control groups ($p<0.05$).

$p<0.05$). After termination of the exposure, 4 out of 8 individuals opened their valves again, while the remaining 4 kept their valves closed. The mean distance between the valves between 0 and 30 min of the recovery period was 0.1 mm. The values increased gradually (Fig. 6) and reached about 0.6 mm within 90 and 120 min of recovery period (Fig. 6, $p<0.05$).

### Valve movements of *C. japonica* exposed to $CuCl_2$

The valve movements of *C. japonica* were affected when exposed to $CuCl_2$ for 6 h. When exposed to 0.1 mg/L $CuCl_2$, the bivalves began to close their valves within 30 min after exposure. The valves remained closed throughout the exposure period (Fig. 7A). During the recovery period (0.1 mg/L $CuCl_2$), 2 out of 8 individuals opened their valves again and their valve movements were similar to that under control condition. The mean distance between the valves was between 0.4 mm to 0.6 mm during the recovery period (Fig. 8, $p<0.05$).

All individuals displayed the behavior of closing their valves immediately after beginning of exposure to 1 mg/L $CuCl_2$ (Fig. 7B). Within 15 min of exposure, the distances between their valves decreased rapidly and the distance was maintained throughout the exposure period. When the valves were closed, the mean distance between the valves was < 0.1 mm. Moreover, all individuals continuously stopped their valve movements during the recovery period (Fig. 8, $p<0.05$).

When exposed to 10 mg/L $CuCl_2$, the bivalves closed their valves within 30 min after exposure and remained in that state throughout the exposure period (Fig. 7C). However, 2 out of 8 individuals showed a “switching action” in their valve movement (frequent repetition of opening and closing of the valves within a short time) before they closed their valves completely. The distance between the valves at that time was in the range between 0.4 mm and 1.8 mm. During the recovery period, the valves were not opened by any individual. In addition, we did not observe any movement. The mean distance between the valves during each 30 min interval did not increase during the recovery period compared with that at the end of the exposure period. The value remained constant at 0.1 mm and below (Fig. 8, $p<0.05$).

## DISCUSSION

This study showed that the valves of most *C. japonica* were widely open during the test period. The test organism *C. japonica* rarely closed their valves under control conditions, although irregular valve movements were observed. The distance between the valves ranged between 0.5 mm and 3 mm under control conditions. The mean distance between the valves during each 30 min interval of the test period was between 1 mm and 1.5 mm. A number of previous studies have demonstrated bivalve valve movement under normal conditions. Fujii and Toda (1991) reported that the mussel *Mytilus edulis* showed valve movements characterized by gradual fluctuations in the distance between the valves, under control conditions. Fdil *et al.* (2006) reported that the mussel *Mytilus galloprovincialis L* demonstrated continuous opening of their valves, although they occasionally showed short–term closing behavior. Ortmann and Grieshaber (2003) reported that *Corbicula fluminea* showed continuous opening of their valves during daytime. It is known that the freshwater bivalves are filter–feeder organisms. They keep opening their valves under normal conditions and extend the siphon between their valves to filtrate water-borne plankton or organic matters for food uptake. The behavior of *C. japonica* under control conditions corresponded to the normal behavior of other bivalves in the previous studies. The bivalves showed continuous valve movements and repeated open–close movements.

The avoidance behavior of *C. japonica* demonstrated by the closure of their valves was observed under exposure conditions. A significant reduction in the distance between the valves was found within 30 min from the beginning of the exposure under all exposure conditions except for 0.1 mg/L $CdCl_2$. We observed continuous closure of the valves when the exposure time increased. In addition, the mean distance between the valves was less than 0.5 mm. The distance between the valves decreased after 2 h of exposure to 0.1 mg/L $CdCl_2$. Bivalves reduce their filtrating–uptake activity by closing their valves when exposed to contaminants to avoid any toxic contamination (Wildridge *et al.*, 1998; Kadar *et al.*, 2001). Rajagopal *et al.* (1997) also reported the occurrence of long–term closure of the valves in *Mytilopsis Leucophaeta* when exposed to chlorine. In this study, the valve closing behavior of *C. japonica* when exposed to heavy metal compounds was similar to the behavior of *M. Leucophaeta* when exposed to chlorine.

Significant differences in the distance between the valves of *C. japonica* were found between control and exposure conditions. In this study, the continuous closure of the valves was observed and the distance between the valves was less than 0.5 mm under exposure conditions. Heavy mental compounds induced the rapid reduction of the distance between the valves of *C. japonica*. Based on these findings, we conclude that the distance between the valves could be a useful parameter for monitoring water quality. Therefore, we suggest that the continuous monitoring of the distance between the valves of *C. japonica* can lead to the detection of a heavy–metal contamination at an early stage.

The exposure to $CuCl_2$ induced a more rapid closure of their valves than exposure to $CdCl_2$. The distance between the valves was smaller when exposed to $CuCl_2$ than when exposed to $CdCl_2$, except for the test condition using 10 mg/L $CdCl_2$. At 10 mg/L $CdCl_2$, the valves closed completely within 10 min of exposure. In addition, the time to close the valves was the shortest under this condition compared with other exposure conditions in this study. On the other hand, *C. japonica* when exposed to 10 mg/L $CuCl_2$ showed a high–frequent "switching action" in their valve movements before complete closure of their valves. Subsequently, complete closure of the valves was observed after a longer time interval. Akberali *et al.* (1982) described the contraction of the isolated siphon of the estuarine bivalve, *Scrobicularia plana* when exposed to Cu. They hypothesized that Cu facilitates the release of Ca from intracellular stores thereby inducing contraction either through effects on transmitter release in presynaptic nerve terminals or through excitation contraction in muscle cells. It is unknown why the high–frequent "switching action" occurred only in the 10 mg/L $CuCl_2$ group in this study. However, we presume that the exposure to Cu at a high concentration caused the convulsion of the adductor muscle resulting in the high–frequent "switching action". Therefore, further studies are needed to demonstrate the mechanism underlying the high–frequent "switching action" that occurred under $CuCl_2$ exposure.

Some studies reported differences in the toxicity between the two test chemicals Cu and Cd. Tsuji *et al.* (1986) demonstrated that the 48–h LC50 value for medaka (*Oryzias latipes*) was 22,000 $\mu$g/L on exposure to $CdCl_2$ and 1,100 $\mu$g/L on exposure to $CuCl_2$. In addition, Okamoto *et al.* (1999) reported the 48–h LC50 value of $CdCl_2$ for algae (Dinoflagellate) as 750 $\mu$g/L and that of $CuCl_2$ as 80 $\mu$g/L, respectively. These studies demonstrated that $CuCl_2$ has a higher toxicity than $CdCl_2$ for the same test organism and under comparable test conditions. Furthermore, Doherty *et al.* (1987) reported that the time period until an initial response could be observed (closure of the valves) was directly related to the exposure concentrations and the toxicity of the heavy metals. Exposure of *C. fluminea* to Cd resulted in the closure of their valves at a rate approximately three times faster than when exposed to Zn. Thus, we suggest that the differences in the toxicity of the test chemicals and in the exposure concentrations resulted in the different initiation responses (closure of the valves).

Moreover, we reported that various patterns of valve movements were observed during the short–term recovery period, depending on the test chemicals or their nominal concentrations. To the best of our knowledge, this is the first study to demonstrate changes in the valve movements in the recovery period after termination of the exposure. *C. japonica* did not open their valves after the exposure to 1 and 10 mg/L $CuCl_2$ was terminated. Only 1 out of 8 *C. japonica* opened their valves after exposure to 1 mg/L $CdCl_2$ was terminated. When exposed to 0.1 and 10 mg/L $CdCl_2$ and 0.1 mg/L $CuCl_2$, the number of *C. japonica* that opened their valves again increased as the water quality improved. Therefore, the mean distance between the valves increased gradually compared with that at the end of the exposure. The increase in valve distance of *C. japonica* in the recovery period indicates inception of the normal behavior which is characterized by opening their valves for filtration and food uptake. Thus, we suggest that it is possible to predict the recovery of the water quality from contaminations by observing the restart of the valve movements (opening and closing of the valves) of *C. japonica*.

In this study, we conducted a short–term exposure test using two heavy metal compounds. However, it is necessary to evaluate the valve movements of *C. japonica* when exposed to other heavy metal compounds, pesticides and organic compounds in a further study. Previous studies have reported that other factors, such as the circadian rhythm and temperature change can induced valve closure. Ortmann and Grieshaber (2003) investigated the effect of the circadian rhythm on the valve movements of *C. fluminea*. *C. fluminea* opened their valves in the afternoon and almost closed them at night at a water temperature between 19.1 °C and 22.4 °C. In addition, *C. fluminea* kept their valves closed for more than a week without any movement at a water temperature between 3.1 °C and 4.3 °C. Therefore, further investigation is needed to confirm the influence of the circadian rhythm and other environmental factors (oxygen concentration, pH and water temperature) on the valve movements of *C. japonica*. In addition, we suggest that it is necessary to evaluate the pattern of the valve movement under long–term observation.

In this study, we demonstrated that the valve movement pattern of *C. japonica* depended on the exposure concentration of dissolved copper and cadmium. Additionally, our result showed that the valve movements of *C. japonica* changed according to the recovery of the water quality after termination of the exposure period.

Many methods and systems were developed for the detection of acute toxicity and have been put to practical use for the early stage warning of water pollution. To the best of our knowledge, there is no method for detecting the recovery of the water quality after contamination. Therefore, we conclude that real–time monitoring using the valve movement of *C. japonica* is a highly useful method to detect both toxic contamination and recovery of the water quality in an aquatic ecosystem.

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