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Differences in the behavior characteristics between *Daphnia magna* and Japanese madaka in an on-line biomonitoring system

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Abstract

It is important to select suitable organisms to adapt the requirement of different environment monitoring purposes. Following our previous study, the behavioral responses of *Daphnia magna* and Japanese madaka (*Oryzias latipes*) were investigated and compared under flow-through conditions in an on-line biomonitoring system. The results showed that both *D. magna* and Japanese madaka had similar biological clock, but the circadian rhythms of Japanese madaka was more clearly recorded than that of *D. magna*. And the sensitivity of *D. magna* was about two orders of magnitudes higher than that of Japanese madaka in different types of toxic chemicals (dichlorovos, deltamethrin and cadmium chloride). However, when both animals were used in an on-line biomonitoring system, the life span of *D. magna* was less than 7 days and Japanese madaka could last for more than one month without feeding. Therefore, *D. magna* was proposed to be a more sensitive bioindicator and was suitable for short term monitoring the pollution events at concentration level closing to the water quality standard, while Japanese madaka was more suitable for the long-term monitoring for accidental discharges.

Key words: *Daphnia magna*; Japanese madaka; on-line biomonitoring; behavioral responses

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Introduction

Accidental chemical pollution, which may destroy the ecological balance of water environment and cause great damage to people's lives and property, has often occurred. In some circumstances, identification of water pollutants and polluters was not possible (Chai et al., 2004; Yang et al., 2008). Considerable progress has been made in recent years to develop an on-line biomonitoring capability as an acceptable alternative approach. At present, commercially available equipments including On-line Fluorescence Monitoring System (Rodriguez et al., 2002), Ultra-sensitive *Daphnia*-Toximeter (Green et al., 2003), the Multispecies Freshwater Biomonitor (MFB) (Gerhardt et al., 2002), and Biological Early Warning System (BEWs) (Li et al., 2007) have been applied in early warning of accidental pollution all over the world. Except on-line fluorescence monitoring system, which depends on the fluorescence of luminescent bacteria, the rest biomonitoring systems are all based on the behavioral responses of aquatic organisms, e.g., water fleas and fish.

Motility is a characteristic feature of many organisms that cannot be neglected as an important physiological factor in survival (Putman and Wratten, 1984; Ren et al., 2009a). Behavioral responses in organisms associated with stress and toxicant exposure provide novel information including short term and median lethal exposure effects, and the potential for mortality (Andrew et al., 2004; Ren et al., 2007), which cannot be gained from traditional toxicological methods. Therefore, in the biological early warning of accidental water environment pollution, the on-line monitoring of the behavioral responses of aquatic organisms is viable (Ren et al., 2007, 2009a, 2009b).

D. magna and Japanese madaka (*Oryzias latipes*) were often used as the representative of water fleas and fish in the on-line biomonitoring systems, e.g., *Daphnia*-Toximeter, MFB, and BEWs. The freshwater cladoceran *D. magna* Straus are normally considered as sensitive to contaminants (Sturm and Hansen, 1999; Rosa et al., 2006; Ren et al., 2007). This species has often been used in bioassays and environmental monitoring of aquatic systems due to the ease and economy of maintaining cultures (Tomasik and Warren, 1996; Martínez-Jerónimo et al., 2005; Heckmann et al., 2007). The Japanese Madaka is an extensively studied species and has been proposed as the standard test fish in the OECD guidelines (OECD, 1999). Many data related to behavior in madaka have been reported (Gray et al., 1999; Oshima et al., 2003; Gormley and Teather, 2003; Chon et al., 2005; Ren et al., 2007, 2009a, 2009b).

Since the beginning of 1980s, the behavioral responses

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of aquatic organisms had been applied in the on-line biomonitoring of aquatic systems. The results of *Daphnia*-Toximeter using D. *magna* suggested that the alarm rate of *Daphnia*-Toximeter could reach 100% in 2.5 mg/L Cd^{2+}, and 50% in 0.5 mg/L Cd^{2+} (Lechelt et al., 2000). The results of BEWs showed that the behavior strength of Japanese madaka could decrease to 0.2 in 10 min with 10 mg/L fenvalerate, and behavior strength of *D. magna* would decrease to zero within 3 hr with 50.0 μg/L malathion (Ren et al., 2008, 2009a).

As the rapid development of the on-line biomonitoring of accidental pollution in recent decades, it is important to select suitable organisms to adapt the requirement of different purposes. So far, less research work has been carried out to assess the behavior characteristics of different aquatic organisms to the same stress. Therefore, in this study, the behavior rhythms, the sensitivity and the self-supportability were compared to evaluate the relative merits of *D. magna* and Japanese Madaka in the on-line biomonitoring of different purposes.

1 Materials and methods

1.1 Equipment

The movement behavior of test organisms was examined by BEWs conducted in Research Center for Eco-Environmental Science (Li et al., 2007). Test organisms were placed in a flow-through test chamber (2 cm in length, 1.5 cm in diameter), which is closed off on both sides with nylon nets (250 μm) (China Patent Number: ZL200710119338.9). One pair of electrodes at the walls of the test chambers sends a high frequency signal of altering current, which is received by a second pair of non current-carrying electrodes (Wang et al., 2007). The movement signal of test organisms is transformed by the A/D transformer and the signal changes formed by the A/D transformer were analyzed automatically by software attached to the equipment. The monitoring data were analyzed using functions of “alarm generation”, which is based on the alarm algorithm in an ARIMA-model (Ren et al., 2009b).

1.2 Test species

The experimental *D. magna* (48-hr young) were cultured in laboratory for more than 3 generations. The general culture of *D. magna* was maintained in the Standard Reference Water (ISO, 1996), at (20 ± 2)°C with 16 L: 8D photoperiod. *D. magna* were fed with a suspension of batch-cultured green algae (*Scenedesmus obliquus*).

Japanese madaka was collected from laboratory fish stock. The brood stock were kept in dechlorinated tap water at a constant temperature of (20 ± 2)°C, with a photoperiod of 16 hr:8 hr (light:dark). The brood stock was fed with newly hatched brine shrimp in the morning and flake food (Trea, Germany) in the afternoon. After 15 days, Japanese madaka was fed with flake food 2 times every day. Japanese madaka (2 months young) about 2.5–3.0 cm long was selected as the test organisms.

1.3 Test chemicals

Dichlorovos, deltamethrin and cadmium chloride were purchased from J&K Chemical Ltd., China. All compounds were technical grade (> 95% purity). Stock solutions (stored at 4°C until use) with proper concentration of each chemical were prepared in dimethyl sulfoxide (DMSO). All solvents were of analytical grade.

The concentration of DMSO in water was less than 0.5% in all experiments. A study has shown that DMSO of such concentration would neither lead to acute toxicity to *D. magna* nor affect the mobility of *D. magna* (Sandbacka et al., 2000).

1.4 Experimental setup

The on-line monitoring of the behavioral responses of *D. magna* and Japanese madaka were carried out under flow-through conditions to ensure that the dissolved oxygen in aquatic environment would not affect the normal behavior of test organisms. The differences of the behavior rhythms, the self-supportability and the sensitivity between *D. magna* and Japanese madaka were also investigated.

In these experiments, five healthy *D. magna* neonates (48-hr young) or 3 healthy madaka (2 months young) about 2.5–3.0 cm long were selected at random for each channel. Controlled flow rate of each test channel was about 2 liters per hour, which was proved to have no effect on the motility of test organisms (Guilhermino et al., 2000). The water temperature was maintained at (20 ± 2)°C with 16 hr:8 hr (light:dark) photoperiod. During these experiments, no food was added.

In these tests, the behavior strength that changed from 0 (lose the ability of movement) to 1 (full behavior express) was introduced to illustrate the differences of these behavioral responses of different organisms. The behavior strength data were sampled automatically by BEWs every 10 min and 6 data records in one hour were used to calculate the behavior strength average value. The judgment standard that behavior strength changed obviously was that: the around difference of behavior strength average value changed no less than 20% (Ren et al., 2009b).

The experiments on the behavior rhythms and on the self-supportability of both animals were carried out in SRW, and the behavior strength of different frequency (from 0.5 to 5.0 Hz) was analyzed. In order to increase the reliability of results, the study on the behavior rhythms lasted for 4 days with 4 parallels, while on the self-supportability it was lasted for 30 days with 4 parallels.

A 48-hr exposure experiment to dichlorovos, deltamethrin and cadmium chloride, respectively, were adopted to investigate the sensitivity of different species. Toxic unit was used for comparison, i.e., the 48-hr median lethal concentration (LC_{50}) for the chemical and for the test organism was taken as one unit (1 TU). The 48-hr exposure tests under flow-through conditions were performed for 3 concentration gradients, i.e., 1, 5, and 10 TU, respectively to evaluate the difference of the sensitivity of the two test organisms with the control of SRW. The experiments were repeated three times for each

48-hr exposure.

1.5 Statistical analyses

The average values of the three repetitions of 48-hr exposure experiments were presented. If the data of the sensitivity differences of *D. magna* and Japanese madaka were distributed normally, determined by a Kolmogorov-Smirnov test, a single factor one-way analysis of variance (ANOVA) of SPSS 10.0 software was performed. If data were nonnormal, differences among the groups were tested with Kruskal-Wallis.

2 Results and discussion

2.1 Behavior rhythms

Figure 1a shows the behavior rhythms of Japanese madaka in SRW. The results suggested that the behavior changed synchronously at all frequencies. The low behavior strength could be observed for the high frequency. It was noticed that the behavior strength varied in a 24-hr photoperiod. In general, behavior strength decreased in the dark (A in Fig. 1a), and increased in light (B in Fig. 1a). Therefore, behavior responses of Japanese madaka showed evident rhythms. Such rhythms variations were constant for all frequencies and repeatable in all the 4 parallels.

Figure 1b shows the behavior rhythms of *D. magna* in SRW. The results suggested that the behavior at all frequencies kept in a relative steady level, and changed synchronously in most of time. But sometimes, the change degree at low frequency (0.5 Hz) behavior was faint (A in Fig. 1b), and behavior strength at high frequency (1.0 Hz) behavior might be higher than that at lower frequency (0.5 Hz) behavior (B in Fig. 1b). The behavior strength change, which reflected the behavior rhythms directly, showed not as strong as those of Japanese madaka.

The behavioral responses might vary with organisms and environmental conditions. Photoperiod might work on the nerve system of different organisms and cause evident 24-hr behavior rhythms, which was called "circadian rhythms". This kind of rhythms was caused by "biological clock" (Wang et al., 2006). Every living being had an inherent biological clock that controlling behavioral response. The clock worked all the time, even when there were no outside signs to mark the passing of time (Binkley et al., 1978). The behavioral responses shown in Fig. 1 suggested that both *D. magna* and Japanese madaka should have the biological clock, but the circadian rhythms was more clear in Japanese madaka than that in *D. magna*. In the light period, behavior strength at 0.5 Hz for Japanese madaka could reach about 0.9, but in the dark period, it would reach only about 0.4 (A, C in Fig. 1), or even down to zero (D in Fig. 1a). Although the behavior movement of *D. magna* showed also the circadian rhythms, the difference between light and dark periods were less evident than that of Japanese madaka (Fig. 1b). The circadian rhythms should be considered in the on-line monitoring. A calibration must be made to offset the differences of the behavior rhythms in different organisms to ensure the monitoring results and to reduce the false warning.

2.2 Sensitivity

According to previous works, LC50-48 for *D. magna* were 0.20 (0.14–0.27) μg/L for dichlorvos (Kikuchi et al., 2000), 0.45 μg/L for deltamethrin (Ren et al., 2009b), and 55 μg/L for cadmium chloride (Nebeker et al., 1986). The values of LC50-48 for Japanese madaka about 2.5–3.0 cm long were 1 mg/L for dichlorvos, and 1.8 mg/L for cadmium chloride (Canton and Slooff, 1982). As there was no explicit report about the LC50-48 of deltamethrin to Japanese madaka, based on the 48-hr exposure results of Tilapia nilotica (2.41 cm) (Golow and Godzi, 1994), 15 μg/L deltamethrin was regarded as 1 TU in the sensitivity experiments. With the control of SRW, the behavioral

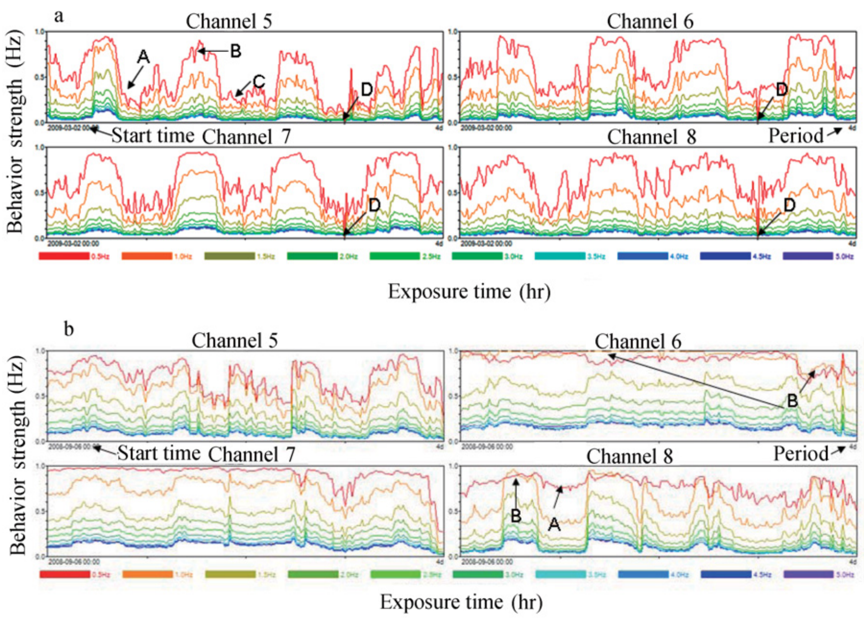

Fig. 1 Behavior rhythms of Japanese madaka (a) and *D. magna* (b) in SRW. Different curves showed in figure stand for different frequency behavior changed from 0.5 to 5.0 Hz.

responses of *D. magna* and Japanese madaka under different exposure concentrations were investigated to discuss the relative sensitivity.

Figure 2A shows the effects of dichlorvos, deltamethrin, and cadmium chloride on the behavioral responses of *D. magna* in 48-hr exposure. There was no significant change in behavioral responses in the control group. In the case of dichlorvos exposure, behavior strength of *D. magna* changed with exposure time and concentrations. Higher concentrations (5 and 10 TU) resulted in a greater decrease in the behavior strength, and a shorter response time during the first phase behavioral response, which was regarded as avoidance behavior (Sager et al., 2000; Erik et al., 2005; Ren et al., 2009a). The behavioral responses of *D. magna* to the exposures of deltamethrin, cadmium chloride and dichlorvos were similar (Fig. 2B) and they also changed with exposure time and concentration levels. If the exposure time was longer, the behavior strength of *D. magna* and Japanese madaka would decrease, especially at 5 and 10 TU. The decrease degree of behavior strength was different in different concentration levels: higher concentration (5 and 10 TU) would result in a greater decrease of behavior strength in a shorter time, and the ability of the duration of avoidance response was mainly affected by concentration levels (Ren et al., 2009b).

The results suggested that the behavioral responses of *D. magna* and Japanese madaka under different environment stresses were in accordance with the step-wise response model which was summarized in authors' previous report (Ren et al., 2009b). Namely, the movement behavior in almost all the exposures decreased step by step until intoxication, some exposure caused a gradually decreased movement behavior followed by a regulatory response or followed different adjustment/readjustment patterns. The duration of avoidance response, which was defined as time between beginning of the exposure and a significant decrease (20%) in behavior strength, varied with the concentrations of dichlorvos, deltamethrin, and cadmium chloride. Therefore, based on the step-wise response model, the behavioral responses of *D. magna* and Japanese madaka under different exposure scenarios could satisfy the pre-conditions of the early warning of an accidental pollution in aquatic environment.

Although the behavioral responses of *D. magna* and Japanese madaka under different exposures were regular and could be described with the step-wise response model, their sensitivities were different. As shown in Table 1, the sensitivities of *D. magna* and Japanese madaka were compared with the Water Quality Standards (GB3838-2002). This could be helpful in the selection of the test

Table 1 Sensitivity of *D. magna* and Japanese madaka comparing with the Water Quality Standards

Chemical	Water quality standards (GB3838-2002)	Detection limit of *D. magna*	Detection limit of Japanese madaka
Dichlorvos (μg/L)	50	0.20	1000
Deltamethrin (μg/L)	20	0.45	15
Cadmium chloride (μg/L)	17 (in $CdCl_2$)	55	1800

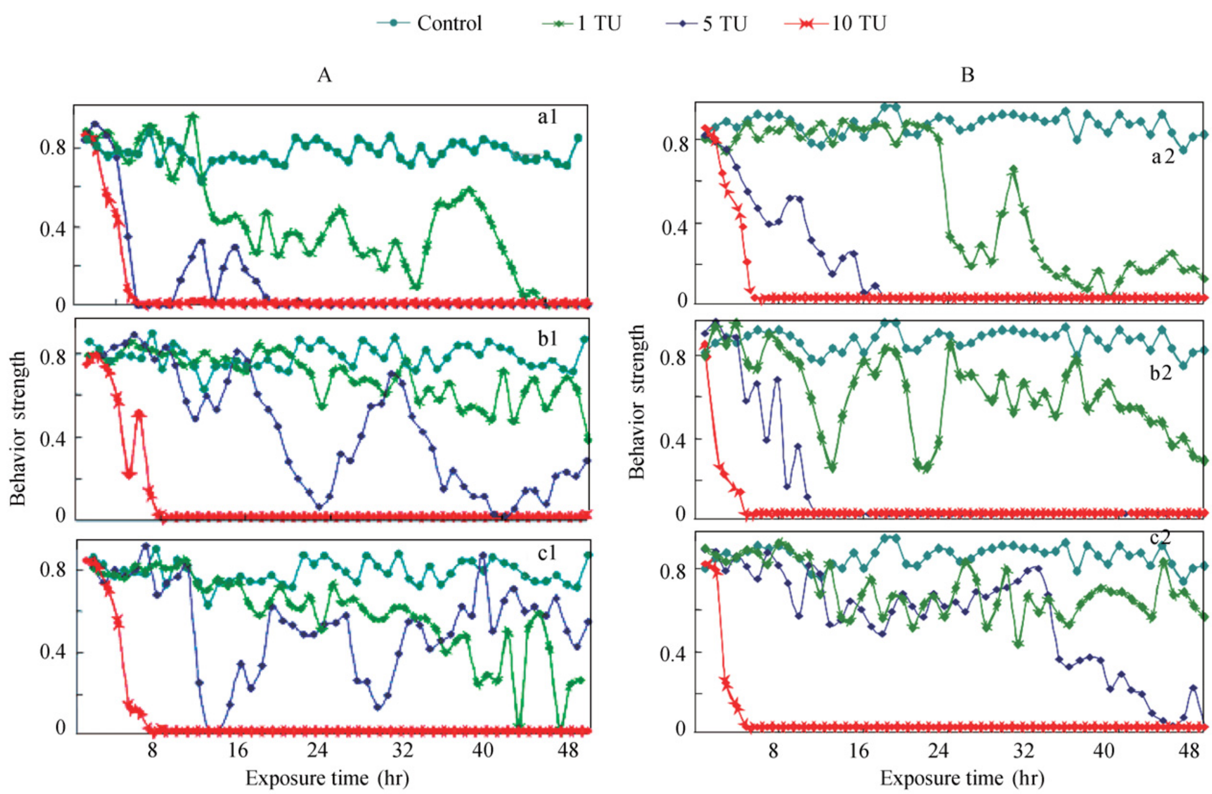

Fig. 2 Effects of dichlorvos (a1, a2), deltamethrin (b1, b2), and cadmium chloride (c1, c2) on the behavioral responses of *D. magna* (A) and Japanese madaka (B) in 48-hr exposure.

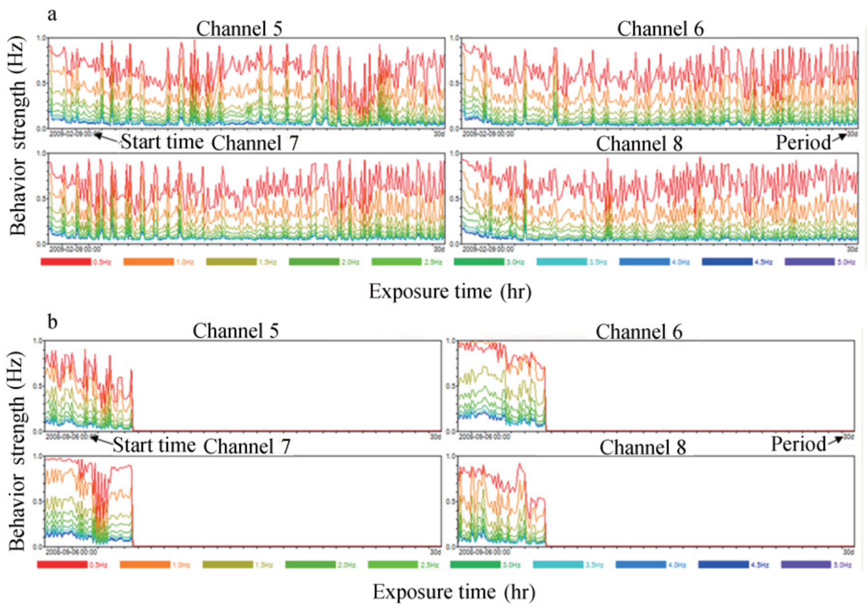

Fig. 3 Behavioral responses of Japanese madaka (a) and *D. magna* (b) in SRW with a 30-day period.

organism for certain application purpose. For the tested chemicals, *D. magna* was more sensitive, and it could be used as bioindicators to a concentration closing to the water quality standards.

2.3 Self-supportability

As no food would be added in the on-line biomonitoring of aquatic environment, the self-supportability was very important for the test organisms.

In the self-supportability test, Japanese madaka in SRW in a 30-day period (Fig. 3a) could live without feeding till the end of the experiment. In the 30-day period, the behavior movement maintained the similar behavior rhythms, and showed clear circadian rhythms. This property was useful when long term biomonitoring was required in *in-situ* conditions, where periodical feeding could increase the labor of maintenance and costs.

Figure 3b shows the behavioral responses of *D. magna* in SRW with a 30-day period. The results suggested that *D. magna* could only live about 7 days and behavior strength decreased obviously from day 5. Without feeding, the normal behavioral responses of *D. magna* could only last for about 4 days.

From the differences of the self-supportability between *D. magna* and Japanese madaka, it could be seen that Japanese madaka was more stronger than *D. magna* and was suitable for long-term biomonitoring of the accidental pollution in aquatic environment.

3 Conclusions

The movement characteristics of both aquatic organisms showed the circadian rhythms and the 24-hr behavior rhythms caused by an inner biological clock affected the behavioral responses evidently. Therefore, a calibration should be made before behavior response was used for biomonitoring.

Based on the results of the sensitivity, the minimum detect concentrations of *D. magna* were close to the Water Quality Standards, however, except deltamethrin, the minimum detect concentrations of Japanese madaka were 20 and 100 times higher than the Water Quality Standards respectively in dichlorvos and cadmium chloride. Combining the sensitivity and the self-supportability of two species, we concluded that the selection of test animal in a biomonitor depended on the purposes. Japanese madaka should be better for applications where long term, *in-situ* monitoring of accidental discharge is required; while *D. magna* should be used in circumstances where sensitive detection of toxic contamination in water with the replacement of test animals less than 4 days.

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