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Influences of Temperature, pH and Turbidity on the Behavioral Responses of *Daphnia magna* and Japanese Medaka (*Oryzias latipes*) in the Biomonitor

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Abstract

Previous studies suggested that the on-line biomonitor could record the behavioral responses of sensitive species under chemical pollution events. However, variation of general water quality parameters, such as temperature, turbidity and pH may also affect the behavioral of the animals that could result in the alarm signal. In this study, the biomonitor equipped with a multi-parameter monitor was used to evaluate the effects of temperature, turbidity and pH on the behavior changes of *Daphnia magna* and Japanese medaka (*Oryzias latipes*). The results showed that both motility strength of *Daphnia magna* and Japanese medaka were inhibited in 5h when the water temperature was lower than 5 ℃. In 50 NTU water body, the decrease of motility strength to 70% of *Daphnia magna* and Japanese medaka need 10h and 24h separately. In 100 NTU water body, the motility strength of *Daphnia magna* would decrease 3h later, and it was about 6h for Japanese medaka. If the pH value was lower than 5.5 or higher than 9.0, motility strength decreased significantly in 15h. It is concluded that in the on-line biomonitoring of accidental pollutions, the results of multi-parameter monitor should be considered.

Keywords: biomonitoring; behavioral responses; pH; temperature; turbidity.

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1. Introduction

With the development of chemical industry, there were lots of potential danger in the process of production, transportation and application of chemicals. Accidental chemical pollution, which may

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destroy the ecological balance of water environment and cause great damage to people's lives and property, has often occurred [1]. Considerable progress has been made in recent years to develop the on-line biomonitor to determine the integrated toxicity of water. The use of the biomonitor was an acceptable alternative approach based on the facts that identification of water pollutants and polluters were not possible in many circumstances [2, 3].

At present, commercially available equipments including On-line Fluorescence Monitoring System [4], Ultra-sensitive Daphnia-Toximeter [5], the Multispecies Freshwater Biomonitor (MFB) [6], and Biological Early Warning System (BEWs) [7] have been applied in in situ. Except on-line fluorescence monitoring system that depends on the fluorescence changes of luminescent bacteria, the rest of biomonitoring systems were all based on the behavioral responses of sensitive aquatic organisms, e.g. water fleas and fish. As the standard test organisms, *Daphnia magna* and Japanese Medaka (*Oryzias latipes*) were often used as the representative of water fleas and fish in the environmental assessment [8-14]. And in our previous studies, both *Daphnia magna* and Japanese Medaka were used in the on-line biomonitor (BEWs) and responses of the species to stresses of different toxic chemicals have been tested [15, 16].

Also in our previous study, it suggested that the circadian rhythms and the 24-hour behavior rhythms caused by an inner biological clock would affect the behavioral responses of *Daphnia magna* and Japanese Medaka [17]. Adjustment to the circadian rhythms and the 24-hour behavior rhythms were incorporated into the devise to avoid the false alarm. Recently, we found that a sharp change in water quality conditions may also affect the generation of the alarm signal. Therefore in this study, the influences of the water quality parameters that may affect the behavioral responses and may not affect human health were evaluated in the BEWs system, which connected to a multi-parameter water quality monitor.

2. Materials and methods

2.1. Equipment

The behavior response of the test organisms was examined by the constructed biomonitor [15, 16]. Test organisms were placed in a flow-through test chamber (2 cm long, 1.5 cm in diameter for *Daphnia magna*, and 7 cm long, 5cm in diameter for Japanese Medaka), which is closed off on both sides with nylon nets (250 μm). 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 [18]. 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 [15]. The monitor to record the behavioral responses of *Daphnia magna* and Japanese medaka were set under flow-through conditions.

The BEWs system was connected to a multi-parameter water quality monitor (made by HACH, US), which recorded the water quality data such as temperature, turbidity and pH.

2.2. Test species

The experimental *Daphnia magna* (48 h young) were cultured in laboratory for more than 3 generations. The general culture of *Daphnia magna* was maintained in the Standard Reference Water (SRW) [19], at 20±2 ℃ with 16 L: 8 D photoperiod. *Daphnia magna* were fed with a suspension of batch-cultured green algae (*Scenedesmus obliquus*).

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

2.3. Experimental setup

Water used for testing was made from SRW. A thermostat with temperature changed from 0 ℃ to 60 ℃ was applied to control the temperature of the influent. The diatomite and turbidity meter were used to adjust different turbidity of the influent. Concentrated HCl and NaOH were used to adjust pH of the influent.

Each experiment consists of three parallels or in three independent chambers. In each chamber, five healthy *Daphnia magna* neonates (48 h young) or 3 healthy medaka (2 months young) about 2.5~3.0 cm long were placed at random. All experiments were lasted for 24 h and for three repetitions. The flow rate to each test chamber was controlled to 2 L/h, which has been proved to have no effect on the motility of the test organisms [20]. During experiments, no feeding was carried out.

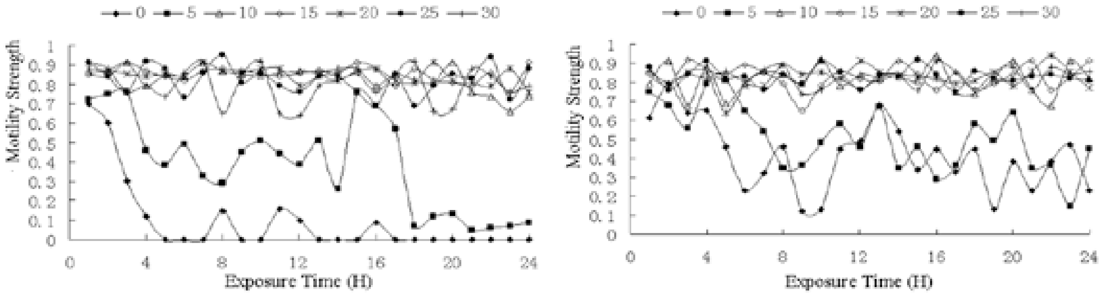
The affected signals will be received by the other pair of electrodes and Behaviour Strength (BS) values of different test chambers are got. In these studies, BS, as a scaling factor that changes from 0 (Lose the ability of movement) to 1 (Full behavior express), was introduced to illustrate the behavioral responses of *Daphnia magna* and Japanese medaka. The behavior data were sampled automatically by the monitoring system every 10 minutes and 6 data records in one hour were used to calculate BS average value. A 30% decrease of the motility strength was set up as the threshold value [16].

The temperature of the influent was adjusted to 0 ℃, 5 ℃, 10 ℃, 15 ℃, 20 ℃, 25 ℃ and 30 ℃, the turbidity of water was adjusted to 1 NTU, 2 NTU, 5 NTU, 10 NTU, 20 NTU, 50 NTU and 100 NTU and the pH of water was adjusted to 5.0, 5.5, 6.0, 7.0, 8.0, 9.0, respectively. In all of the experiments of different parameters, the other parameters were kept at the normal value.

3. Results and discussion

3.1. The effects of temperature

Fig 1. (a) showed the effects of temperature on the behavioral responses of *Daphnia magna*. Taking 20 ℃ as normal, behavioral responses of *Daphnia magna* living at other temperatures, especially at 0 ℃ and 5 ℃ changed significantly. At 0 ℃, almost all *Daphnia magna* lost their movement ability after 2 h, and the motility strength decreased sharply. After 4 h, the motility strength could only maintain at the level of less than 0.1. After 16 h, there was no signal resulting from movement of *Daphnia magna*. At 5 ℃, the motility strength would decrease to about 0.4 after 3 h and no signal was recorded after 17 h. At 10 ℃, 15 ℃, 25 ℃ and 30 ℃ and in comparison with that at 20 ℃, no significant changes of the motility strength could be observed in 24 h experiment.



a b

Fig. 1. The effects of water temperature on the behavioral responses of *Daphnia magna* (a) and Japanese medaka (b) in 24 h exposure

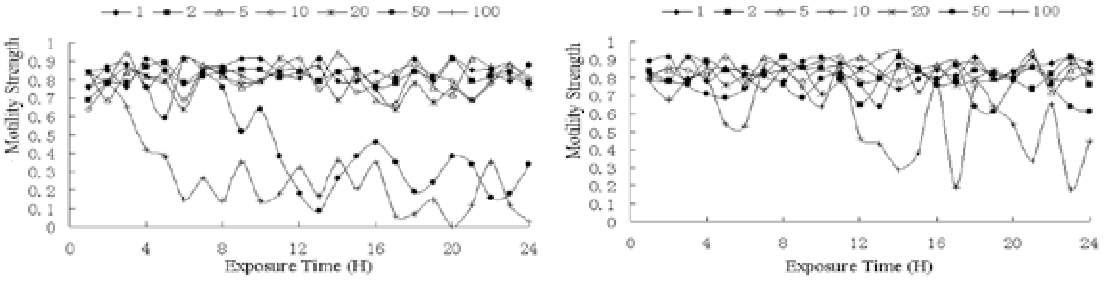
Fig 1. (b) showed the effects of temperature on the behavioral responses of Japanese medaka. The influence of temperature on motility strength of medaka was similar to that of *Daphnia magna*. In comparison with the behavior at 20 ℃, there were no significant changes occurring at 10 ℃, 15 ℃, 25 ℃ and 30 ℃. At 0 ℃ and 5 ℃, the effects were significant decrease of motility strength could be observed after 2 to 3h. Later on, the strength decreased gradually after 6 to 8 h and maintain at the level of about 0.4.

As reported by Seidl [21], *Daphnia magna* was eurythermal organism. Once the water temperature is lower than 4 ℃, diapausing eggs would help *Daphnia magna* to live at unfavorable environmental conditions [22]. Therefore, when *Daphnia magna* and Japanese medaka are to be used as the test organisms in the on-line biomonitor, temperature of influent should be maintained at above 10 ℃.

3.2. The effects of turbidity

Water turbidity was correlated with not only the content of suspended matter, but also the impurity elements, particle size, and so on [23]. Turbidity of natural water may vary from 0 NTU to more than 1000NTU in rain events.

Fig 2. (a) showed the effects of turbidity on the behavioral responses of *Daphnia magna*. The behavioral strength decreased significantly when turbidity increased to 50 NTU or 100 NTU. Compared with 1 NTU in 24 h exposure, the motility strength would decrease 3h later in 100NTU water body, and it would decrease 8 h later in 50 NTU water body. After the adjustment of behavior movement, the motility strength would keep about 0.3, which was obviously lower than that in 1, 2, 5, 10, and 20 NTU. These results illustrated that the water turbidity might cause abnormal behavioral responses of *Daphnia magna*.



a b

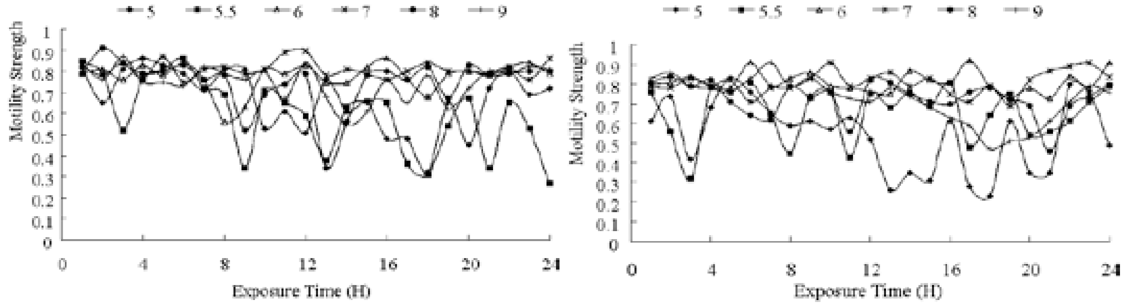
Fig. 2. The effects of water turbidity on the behavioral responses of *Daphnia magna* (a) and Japanese medaka (b) in 24 h exposure

Fig 2. (b) showed the effects of turbidity on the behavioral responses of Japanese medaka. Compared with 1 NTU in 24 h exposure, the behavior change trends in 1, 2, 5, 10, and 20 NTU were similar to *Daphnia magna*, and it showed little effects on behavioral responses in 24 h. But in 50 NTU, great difference was presented between Japanese medaka and *Daphnia magna*: the effects of 50 NTU turbidity water body on Japanese medaka were only a little in 24 h, and at the end of the exposure, it did not show significant motility strength decrease. In 100 NTU water body, Japanese medaka showed intensive behavioral response.

These results suggested that the water turbidity could affect the normal behavioral responses of aquatic organisms. Therefore, turbidity effects must be discussed, and to decrease the effects of turbidity on the behavioral responses, some water filtration technologies, e.g. sand filtration and coagulation technologies, should be developed in the use of BEWs.

3.3. The effects of pH

Fig 3. (a) showed the effects of pH on the behavioral responses of *Daphnia magna*. These results suggested that acid stress on the motility strength was significant. With the control of pH 7 in 24 h exposure, it showed evident effects in pH 5.0 and 5.5, especially after about 8h exposure, but there was no *Daphnia magna* dead in all of the experiments, which was consistent with the reports of Zhuang [24]. In pH 8.0 and 9.0, some effects could be detected by BEWs, but were not observed by eyes directly.



a b

Fig. 3. The effects of water pH on the behavioral responses of *Daphnia magna* (a) and Japanese medaka (b) in 24 h exposure

Fig 3. (b) showed the effects of pH on the behavioral responses of Japanese medaka. In all of the experiments, the behavior change trends were similar to those of *Daphnia magna*. In pH 6.0 and 7.0, the motility strength kept about 0.8, which was same as *Daphnia magna*. In pH 5.0, 5.5, 8.0 and 9.0, some abnormal behavioral responses were detected by BEWs, but similar to the studies on *Daphnia magna*, no evident behavior changes were observed by eyes.

Previous studies on the acid stress had showed that water bodies of different pH value might affect the survival, growth and reproduction of *Daphnia magna* [24, 25]. These results advised that there were some effects of pH stress on the behavioral responses of aquatic organisms. But in general, pH value of water bodies in normal environment were changed from 6.0 to 8.0, and theses pH of water bodies could not cause obvious abnormal behavior changes. Therefore, once the alarm of BEWs was started because of the abnormal of pH value, the results suggest that some pollution events might happen.

4. Conclusions

The movement behavior of *Daphnia magna* and Japanese medaka could be affected by water temperature, turbidity and pH. Therefore, we suggested that physical parameter modification such as maintaining of temperature and pre-filtration should be required for the on-line biomonitor. An alternative approach is to connect the biomonitor to a multi-parameters water quality monitoring system, which could record variations of all parameters simultaneously. In later case, integrated data analysis will help to avoid the false alarm and monitor the water quality more effectively.

Acknowledgments

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