Ecological Informatics 29 (2015) 107–118

Contents lists available at ScienceDirect

Ecological Informatics

journal homepage: www.elsevier.com/locate/ecolinf

Characterizing response behavior of medaka (*Oryzias latipes*) under chemical stress based on self-organizing map and filtering by integration

Lei Wang [a], Zongming Ren [a,1], Hungsoo Kim [b,c], Chunlei Xia [d], Rongshu Fu [a], Tae-Soo Chon [b,*]

[a] College of Life Science, Shandong Normal University, Ji'nan 250014, PR China
[b] Department of Biological Sciences, Pusan National University, Busan 609735, Republic of Korea
[c] Department of Materials Science and Engineering, Pusan National University, Busan 609-735, Republic of Korea
[d] Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, PR China

ARTICLE INFO

Article history:
Received 1 April 2014
Received in revised form 12 November 2014
Accepted 30 November 2014
Available online 6 December 2014

Keywords:
Acclimation
Adjustment
Behavioral responses
Intoxicating
Oryzias latipes
Stepwise response mode

ABSTRACT

Behavioral responses (BRs) of medaka (*Oryzias latipes*) were observed after exposure to low concentrations (0.1 TU (Toxic Unit, TU), 1 TU, 5 TU, and 10 TU) of trichlorfon, parathion and malathion. Overall response patterns of test organisms were reflected from surface shapes of BS (Behavior Strength) values in 3-D: parathion appeared to be most variable in presenting response behaviors whereas trichlorfon showed relatively simple response patterns. The self-organizing map (SOM) addressed the time and toxic effects efficiently. An evident circadian rhythm observed in the control diminished at a low concentration of toxic unit, and variability of toxic effects was accordingly observed according to chemicals and concentrations. Subsequently filtering by integration was conducted to time series BS values. The highly fluctuating nature of original BS values was filtered efficiently to produce linear fitting closely. Slopes of regression decreased monotonically as toxic concentrations increased. Residual curves of integral BS values from linear fitting were further used for determining different BS phases proposed by empirical observations; the positive and negative phases were in accordance with acclimation, adjustment and toxic effects in behavior response modes. According to inclination and declination periods observed in residual curves, new states of test organisms were further defined to present intoxicating and recovering tendencies Profiles based on residual curves of integral BS values were able to show landscape of response patterns across toxic concentrations in different chemicals. Computational methods for defining behavior states provide an objective ground for analyzing complex stress response and could be suitable in referencing toxic behavior modes of test organisms quantitatively.

1. Introduction

Trichlorfon, parathion and malathion are currently used throughout the world as common organophosphorous pesticides (OPs) to control pests in numerous conditions including agricultural crops, forests, and wetlands for more than four decades (Smith, 1987). The wide use of OPs is due to high toxicity and rapid environmental degradation (Eto, 1974). Unfortunately, OPs lack target specificity and can cause severe and persistent population effects on aquatic non-target species, particularly invertebrates (Schulz and Liess, 1999; Fulton and Key, 2001). Extensive use of OPs and the release into aquatic environments may alter the balance of biological communities (Ren et al., 2007).

Behavioral response (BR) has been suitable in addressing toxicological impact assessment in all test endpoints (Azizullah et al., 2011; Farr et al., 1995; Park et al., 2005). BR to contaminants is an adaptive process. Supposing the animals are placed in a closed place (i.e. no escape) and are exposed to a constant level of chemical stress, every organism has its own ability to adapt to the environmental stress by intrinsic modification, which might induce stepwise BRs including behavior stimulation, acclimation, adjustment and so on (Sun et al., 1993; Zhang et al., 2012). By far, BR of various taxa, including crustaceans (Billoir et al., 2007; Kim et al., 2006; Roast et al., 2000), snails (Coffin et al., 2008; Ibrahim et al., 1992), insects (Chon et al., 1998; Ji et al., 2007), and fish (Gray et al., 1999; Liu et al., 2011; Moore and Waring, 1996), has been reported to be sensitive to sublethal exposures to numerous chemical pollutants, which have drawn attention as a means of developing a biomonitoring tool for detecting toxic chemicals in the environment. In fact, as an efficient tool for biomonitoring in aquatic ecosystems, the automatic detection of response behaviors has received considerable attention since 1980s (Lemly and Smith, 1986). Although relationships among variables of BR may be strongly nonlinear and involve high-order interactions, behavioral monitoring was reported as a useful means of toxicity checking mechanism (Dutta et al., 1993; Inan et al., 2011), and changes in the movement behavior of organisms could be a suitable indicator to express internal state of test specimens in ecological risk assessment (Kavathekar et al., 2013; Tahedl and Häder, 2001).

* Corresponding author. Tel.: +82 51 510 2261; fax: +82 51 512 2262.
E-mail address: tschon@pusan.ac.kr (T.-S. Chon).
[1] Equal contribution to the first author.

http://dx.doi.org/10.1016/j.ecoinf.2014.11.008
1574-9541/

Due to data complexity, unbalance, and quantity in a huge amount, BR data are difficult to analyze. Sometimes, the commonly used exploratory and statistical modeling techniques may fail to find meaningful ecological patterns from data (Breiman et al., 1984; Cho et al., 2006; Richard et al., 2001; Ripley, 1996). However, once a determined behavior movement can be quantified by proper analysis methods, it has the potential to be used as a type of biomarker in the assessment of stress (Beitinger, 1990). Therefore, various computational methods have been proposed for objective and automatic analyses of the movement data, covering artificial neural networks such as multi-layer perceptron (Kwak et al., 2002) and self-organizing map (SOM) (Liu et al., 2011; Park et al., 2005), and data structure analysis including Fourier transform (Chon et al., 2004), wavelet analysis (Kim et al., 2006; Polansky et al., 2010) and fractal dimension (Ji et al., 2007; Nimkerdphol and Nakagawa, 2008). These methods all played significant roles in the analysis of movement behavior as an indicator in ecological risk assessment based on digital image processing systems (e.g., video tracking system) (Ji et al., 2007; Liu et al., 2011; Nimkerdphol and Nakagawa, 2008; Park et al., 2005). In addition to direct movement analysis signals such as electricity (Ren et al., 2007), magnetics (Gerhardt et al., 1994), and fluorescence (Rodriguez et al., 2002) based on behavioral data have been used since the method would be effective and stable in presenting the stressed state of organisms in a comprehensive manner while movement data have numerous variables and are mostly over-sensitive to noise. An online monitoring system (OMS) based on the altering current signal has been effectively applied and used for monitoring *in situ* for longer than 5 years (Ren and Wang, 2010). The continuous altering current signal could help the system to sample the behavior strength (BS) data automatically in each second. However, the stepwise responses observed during the course of toxic responses including acclimation and adjustment have not been closely examined from computational aspect to provide objective grounds for defining the modes.

Therefore, in this study, BRs were analyzed after medaka (*Oryzias latipes*) was exposed to different concentrations of trichlorfon, parathion and malathion. Behavior strength was examined to standardize the toxic effects of OPs as the time progressed after exposure. It was postulated that medaka BR would display a time-dependent sequence of different regulatory or compensatory behavioral stress responses and the stepwise responses including acclimation and adjustment would occur consequently through equilibrium between intoxicating and recovering states of the tested organisms in adapting to chemical stress. Initially three-dimensional (3-D) analysis was used to display overall toxic effects according to concentrations and kinds of chemicals (OPs). SOM was subsequently used to pattern BS values in relation to toxic effects and time after exposure. Filtering by integration was finally adopted to define response behavior states and to illustrate the computational backgrounds in determining stepwise BR and intoxicating-recovering tendencies during the course of response to chemicals.

2. Materials and methods

2.1. Test species

The individuals of medaka fish were provided by the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (Beijing, China). The brood stock was raised in a flow-through system with dechlorinated tap water (using active carbon) at a temperature of 20 ± 2 °C, and has been reared in the laboratory for more than three years. The photoperiod was maintained with 16 h of light (04:00–20:00, with a light intensity of 4000 lx) and 8 h of darkness (20:00–04:00, no light given). The brood stock was fed with newly hatched brine shrimp in the morning (7:00) and flake food (Trea®, Germany) in the afternoon (18:00). From 15 days after hatching, medaka was fed 2 times every day with flake food. Medaka individuals about 2.5–3.0 cm long (2 months after hatching) were used for test organisms. New individuals were tested for each experiment.

2.2. Chemicals

Trichlorfon, parathion and malathion were purchased from J&K Chemical Ltd. All compounds were technical grade (>95% purity). Stock solutions (stored at 4 °C until use) with proper concentrations were prepared with dimethyl sulfoxide (DMSO) as a solvent, and were diluted with appropriate aliquots. All solvents were of analytical grade. The concentration of DMSO used for test was less than 0.5% in all experiments, which would neither lead to acute toxicity to medaka nor affect the mobility (Sandbacka et al., 2000).

2.3. Experimental setup

Behavior responses of medaka were monitored by an OMS built in Research Center for Eco-Environmental Science, Chinese Academy of Sciences (Ren and Wang, 2010). Three healthy medaka individuals were selected at random and each individual was placed separately within a flow-through test chamber (7 cm long, 5 cm in diameter) for each treatment (3 chemicals × 4 concentrations) according to previous experimental design and corresponding research (Ren and Wang, 2010; Ren et al., 2007, 2015). The test chamber was closed off with nylon nets (250 μm) on both sides (Ren and Wang, 2010). The flow rate of each test chamber was controlled at about 2 l per hour, which has no effect on the motility of test organisms (Guilhermino et al., 2000). One pair of electrodes were located at the inside walls of the test chambers and sent a high frequency signal of altering current, which was received by a second pair of noncurrent-carrying electrodes located at the other side of the test chamber (Wang et al., 2007). Overall response activity of test organisms inside the chamber is reflected on the signal and is transformed by the A/D converter. Detailed mechanism in OMS can be referred to Zhang et al. (2012). We define the converted data observed in OMS as behavior strength (BS) in the chamber responding to environmental stimuli (Ren and Wang, 2010). BS was automatically sampled by OMS in each second, and average values every 6 min were produced as output. Values of BS were normalized, ranging from 0 (loss of motility) to 1 (full behavior expressed).

The observation started at 16:00, and was completed at 16:00 of the third day for each treatment (48 h). During the observation period, no food was provided in order to minimize noise. Light and temperature conditions were the same as those given in rearing stock populations. Chemical Toxic Unit (TU) to test organisms was determined for comparing toxic effects. The 48-hour median Lethal Concentration ($LC_{50\text{-}48}$) was taken as one unit (1 TU) based on the previous reports (Kong et al, 2000; Nishiuchi and Hashimoto, 1967; Tsuda et al., 1997). The calculation of TU value is shown as follows:

$$TU = \sum \frac{C_i}{LC^i_{50\text{-}48}} \tag{1}$$

where C_i and $LC^i_{50\text{-}48}$ were the total concentration and 48-hour median lethal concentration for chemical i, respectively. The $LC_{50\text{-}48}$ values, regarded as 1 TU in this study, were 10 mg/L, 2.9 mg/L, and 0.75 mg/L for trichlorfon, parathion and malathion, respectively. Four concentrations of 0.1 TU, 1 TU, 5 TU, and 10 TU were treated for each chemical. In order to observe various toxic phases including death across different levels of toxic concentrations, test individuals were dead at higher concentrations, 5 TU and 10 TU, at the final phase during the observation period. We additionally defined “death mode” in this study. Due to the experimental conditions of the OMS, however, low saturation line was consistently produced from the observation system. Once the observed BS value was lower than 0.1 and no follow-up recovery occurred (i.e., BS value increased higher than 20%), all individuals in the test chamber were considered not moving and were regarded as dead organisms, according to authors' observation experience.

2.4. Data presentation, self-organizing map (SOM), and filtering by integration

Initially, the behavior data was analyzed by a three dimensional surface plot of MATLAB 2009 (© 1984–2009 The MathWorks, Inc.). "Surf (*X*, *Y*, *Z*)" creates a shaded surface using Z for the behavior strength of medaka as well as surface height. X and Y are vectors defining experimental conditions for different treatments ("chemical" and "concentration") and time progress after exposure to chemicals, respectively. In this case, the vertices of the surface are triples of (time; $X(n)$, concentration; $Y(m)$, and BS; $Z(m, n)$).

The SOM was subsequently used to classify movement patterns by training the continuous movement data of BS. The learning process of the SOM was conducted using the SOM Toolbox developed by the Laboratory of Information and Computer Science, Helsinki University of Technology in MATLAB environments (Vesanto et al., 2000). The SOM has been used effectively for extracting information from ecological and behavioral data (Chon et al., 2004; Liu et al., 2011). Through preliminary training, the size of 14 × 10 nodes was used in this study. A detailed description regarding the application of the SOM to behavioral data can be found in other relative reports (Chon, 2011; Park et al., 2005).

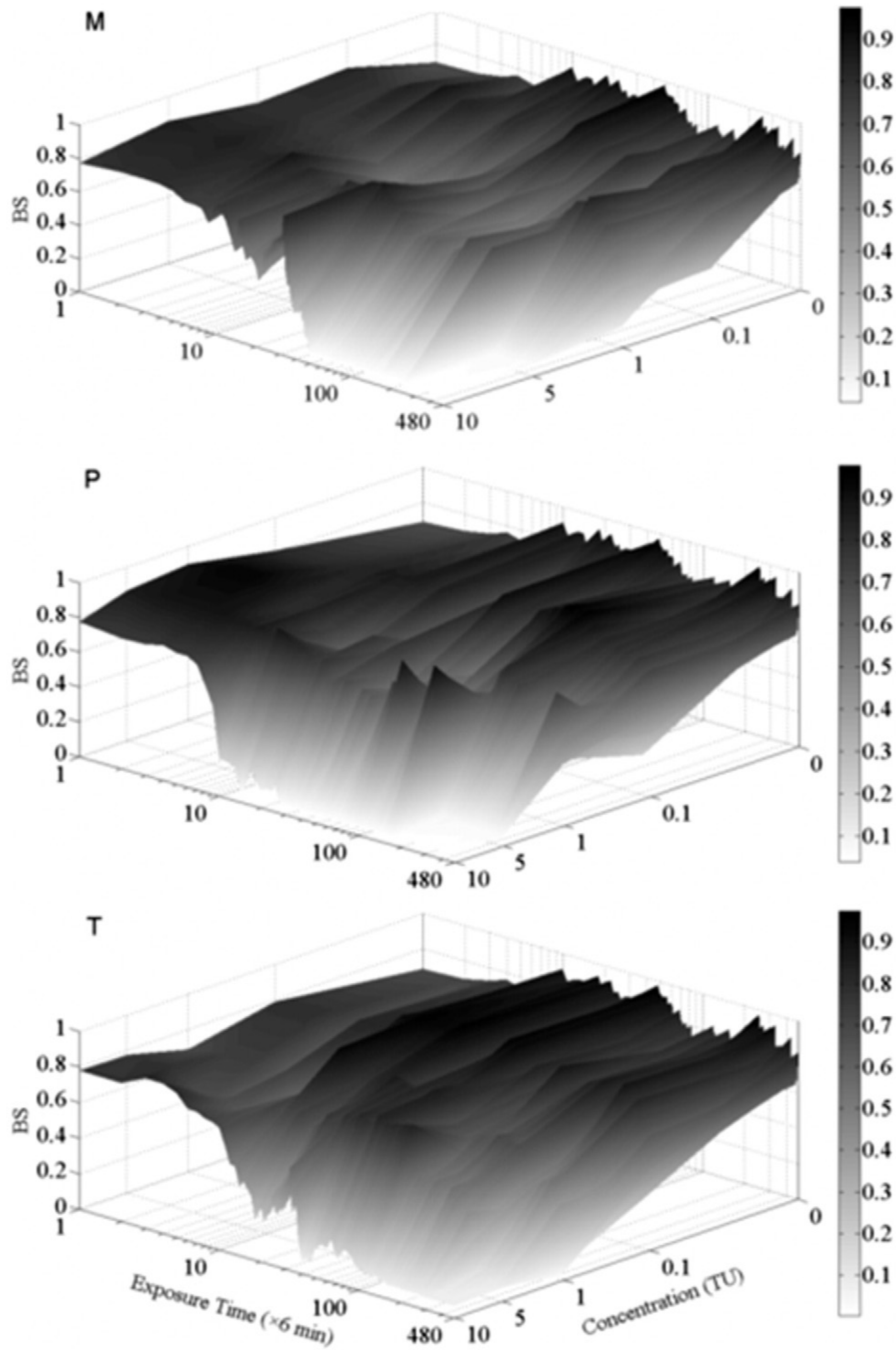

Fig. 1. Response patterns of Behavior Strength (BS) in 3 dimensions across different concentrations as the time progresses in different chemicals. Exposure time was shown in log scale. M, P, and T standing for malathion, parathion, and trichlorfon, respectively.

The measured BS values from OMS showed high levels of noise in raw data. In preliminary tests various data treatments including Fourier transformation (high frequency cut-off) and Kalman filtering were applied to the time-series data to minimize noise. However, most filtering methods were not successful in obtaining useful information out of complex BS data. Among various methods, integrating time-series BS values was found most efficient in filtering the data to address biologically meaningful message while minimizing noise in the data concurrently. After integration, the integrated data were statistically fitted to linear model regression. The linear properties (e.g., slope) presented

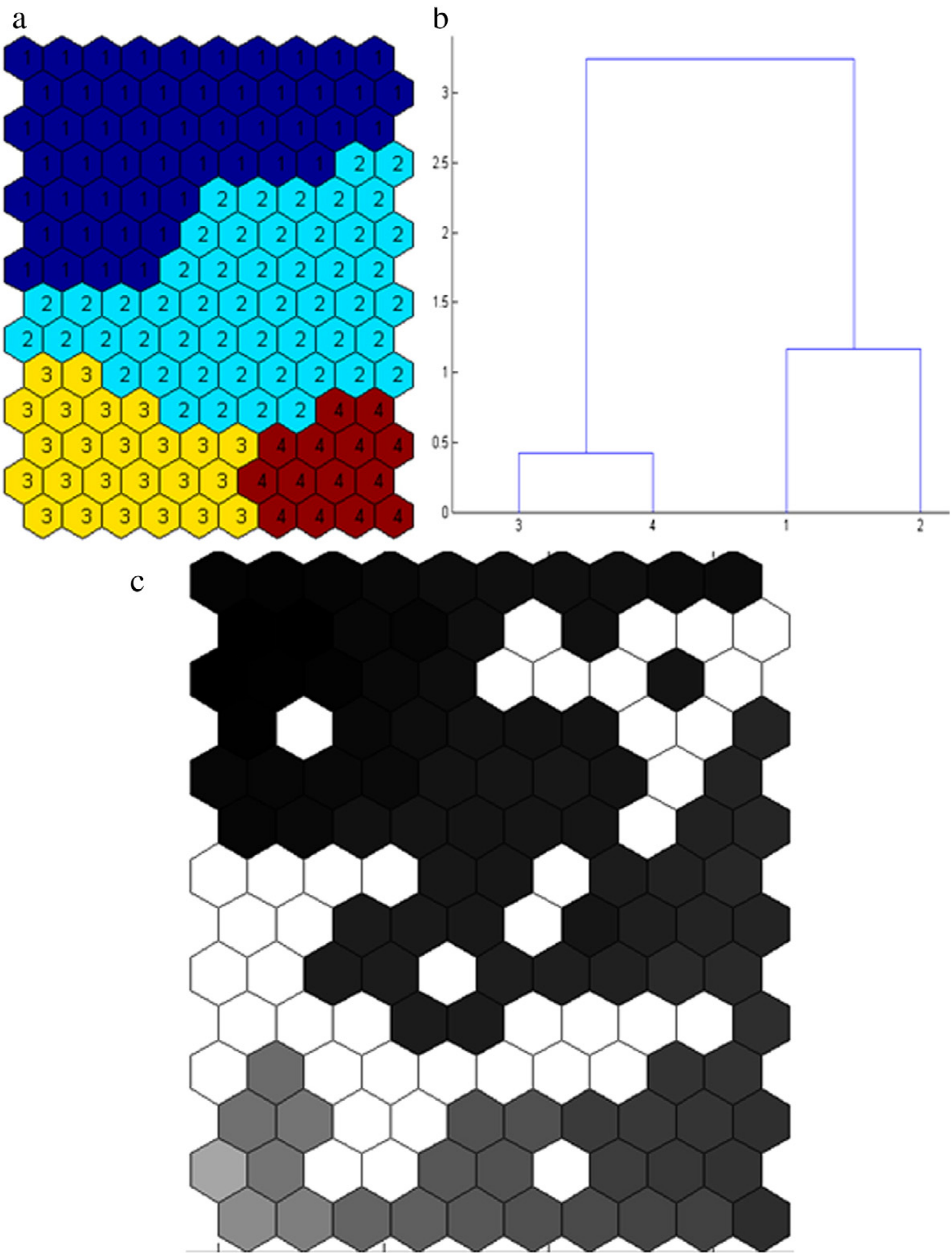

Fig. 2. Clustering of BS values in association with different chemicals and concentrations by the SOM. (a) Four clusters classified by the SOM; (b) cluster distances according to dendrogram by the Ward's linkage method; (c) profile of average observation times superimposed on the SOM (Fig. 2-a). The gray levels indicate the exposure time (the maximum of 48 h (the darker level indicating the later time)), and (d) profiles of BS values visualized on the SOM according to different chemicals and concentrations. (The blank nodes (white) in Fig. 2-c indicate no sample units clustered within these nodes. The alphabet and number used for the title of subfigures in Fig. 2-d stand for chemicals and toxic units, respectively. For example, "T0.1" means treatment of trichlorfon at 0.1 TU, and "C" stands for the control. Values in the vertical bar in Fig. 2-d present the range of mean BS values.).

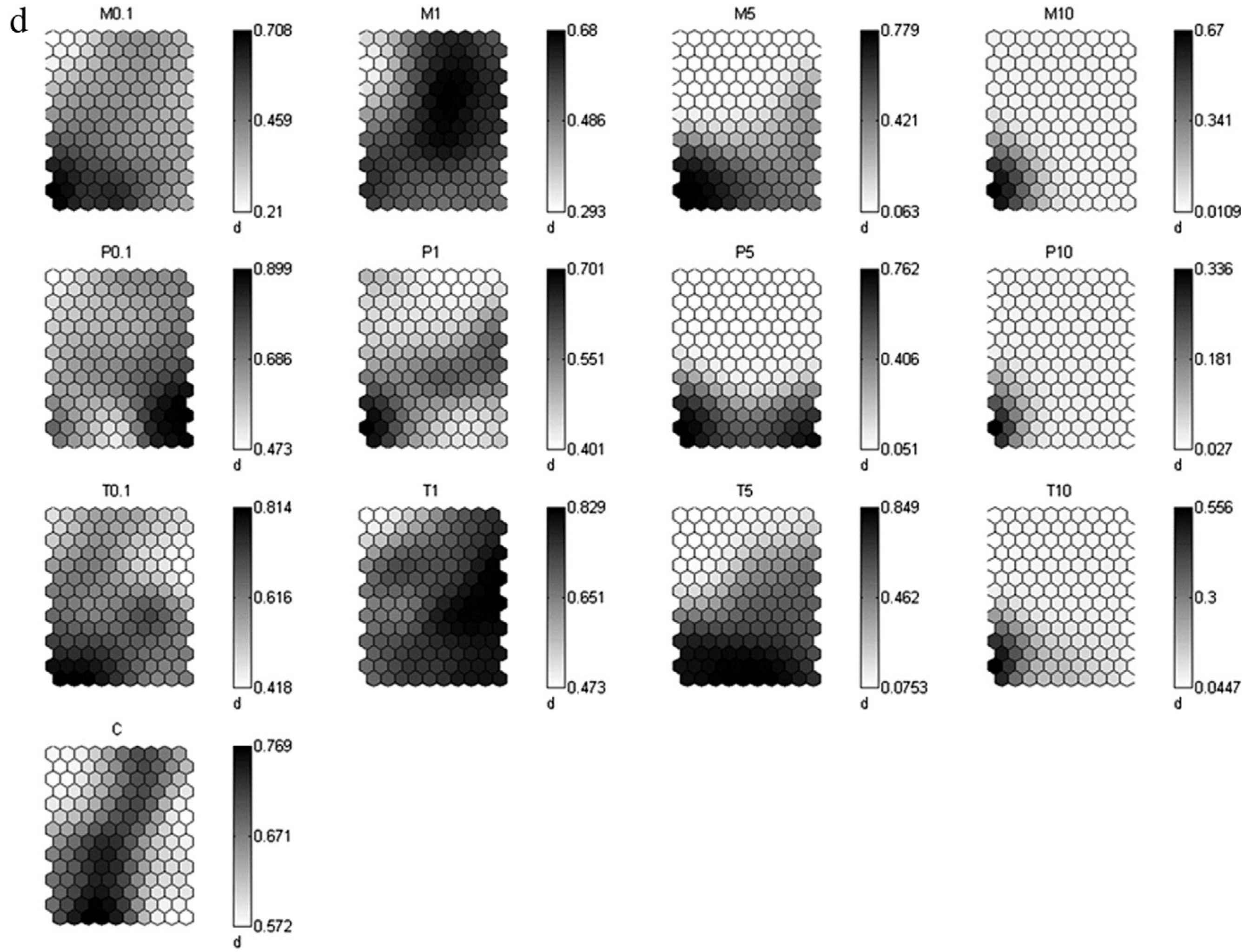

Fig. 2 (*continued*).

overall impact of chemical concentrations (i.e., different levels of TU). Subsequently residual curves of integral BS values from the linear fitting were produced to define the stepwise phases (i.e., acclimation, adjustment) of test fish during the course of behavioral responses to chemicals.

3. Results

3.1. BS patterns on 3-D surface plot

The changes in BS analyzed by 3-D surface plot are shown in Fig. 1 in different concentrations as the time progresses after exposure to different chemicals, malathion, parathion, and trichlorfon. Overall response patterns of test organisms were reflected from surface shapes of BS values in 3-D. Parathion appeared to be most variable in presenting response behaviors: early decrease in exposure to maximum dose (10 TU) and numerous short phases of recoveries with variable degrees in BS at lower concentrations during the course of response to chemicals after exposure (Fig. 1). Malathion was weak in expressing stress responses compared to parathion and showed a slow decrease in BS at the maximum concentration. Variability in recovery, however, was also similarly found at lower concentrations. Trichlorfon presented simple responses compared to other chemicals by showing a smoother 3-D response surface (Fig. 1). The degree of decrease in BS at the maximum concentration of trichlorfon was in between parathion and malathion.

The BS values in the control were invariably observed as about 0.8 in average. This high value of 0.8 was mostly observed at the initial period (approximately first 6 min) of every exposure for all chemicals except for 10 TU (Fig. 1). At 10 TU some lower values around 0.6 were observed in the similar initial period. Stress response was differentiated according to concentrations. With a minimal concentration of 0.1 TU, overall response was not severe and somewhat similar among different chemicals, ending up with 0.3–0.6 at the last observation phase (48 h after exposure) (Fig. 1; see also Fig. 4). However, small scale recovery and intoxication periods were variably observed according to chemicals as the time progressed as stated above.

With higher concentrations, the BS values decreased earlier compared to lower concentrations, although there were variations (i.e., temporary recovery) during the course of stepwise response according to chemicals, confirming the postulation that an organism displayed a time-dependent sequence of different regulatory or compensatory behavioral stress responses above their respective thresholds of resistance (Selye, 1973). At 1 TU, the BS values decreased gradually as the time progressed. Partial increase and decrease in BS values were observed during the course of toxic response, including 4 phases in BR: stimulation, acclimation, adjustment (readjustment), and toxic effect (Zhang et al., 2012). The values of BS greater than 0.0 were observed variably according to different chemicals at the end of observation period at 1 TU: around 0.2, 0.4 and 0.1 for malathion, parathion and trichlorfon, respectively (Fig. 1; see also Fig. 4). At 5 TU the values mostly decreased to 0.0 finally at the end of exposure time. At 1 day after exposure, however, BS values still showed values greater than 0.0 (around 0.3–0.5). At 5 TU, stress response showed maximum variation with BS values broadly ranging between 0.1 and 0.8 during the course of toxic response. All different phases in BR were also observed at 5 TU. With maximal 10 TU, BS decreased rapidly around 6 min after exposure, and decreased to 0.0 (death) within 10 h for all chemicals (Fig. 1). Overall, the results demonstrated that BS values accordingly presented both common (i.e., overall decrease along with increase in TU values) and variable responses in BR according to different chemicals and time after exposure (i.e., difference in recovery patterns) (Fig. 1; see also Fig. 4).

3.2. Patterning of BR

Overall patterns of BR in different chemical treatments were shown on the SOM based on training with mean values of BS in association with different experimental conditions (chemicals and concentrations) as the time progressed (Fig. 2). According to Ward's clustering method, four clusters were identified (Fig. 2-a, b) along the vertical gradient. The vertical gradient was mainly in accordance with the time: the late phase at the top area (clusters 1 and 2) (16 h later) and the early phase at the bottom area (clusters 3 and 4) (Fig. 2-c). In the early phase, time difference was further observed: the left cluster 3 (before 4 h) was earlier than the right cluster 4 (between 4 h and 16 h).

Visualization of the BS profile on the SOM effectively presented the time and chemical effects (Fig. 2-d). The values in the vertical bar in Fig. 2-d indicate the range of mean BS values after visualization during the observation period. In the control, BS values were in the range of 0.57–0.77 (see subfigure titled as "C" at bottom left corner of Fig. 2-d). It was noteworthy that the BS profile was in the maximal range along the diagonal line from the bottom-left to upper-right corner on the SOM in the control. By matching with Fig. 2-c, BS changes showed an evident rhythm in the control: higher BS values observed in the photophase (0–4 h, 14–30 h, and 38–48 h) along with diagonal line while the lower values were found in the scotophase (4–14 h and 30–38 h) away from the diagonal line in the SOM (subfigure "C" in Fig. 2-d). Meanwhile, circadian rhythm diminished as toxic effects increased along with increase in concentration. In minimum concentration the periodicity was partially traceable; BS profiles in the control matched those with 0.1 TU in the early period, especially in malathion and trichlorfon (i.e., higher BS levels at the bottom left corner of the SOM in subfigures "M0.1" and "T0.1" in Fig. 2-d). It was noteworthy that variability among different chemicals was also observed in BS values. Percents of lower BS values comparing with the control at 0.1 TU were maximum with parathion (47.9%), followed by trichlorfon (41.8%) and malathion (21.1%). Periodicity decreased as the degree of concentration increased. With high concentrations, 5 TU and 10 TU, periodicity was not observed and BS profiles were similar among different chemicals (Fig. 2-d). With the maximum concentration at 10 TU the highest BS values were observed narrowly in the limited area in the SOM regardless of chemicals (bottom right corner in subfigures of M10, P10 and T10, Fig. 2-d). This was in accordance with high BS values during the brief early period after exposure before severe intoxication process started.

3.3. Filtering by integration

According to integration of time series BS values, we were able to obtain corresponding Integral BS (Int. BS) for the control (Fig. 3-b black curve) and the treatments (Fig. 5) as the time progressed. Remarkably the highly fluctuating nature of original BS values (Figs. 3-a and 4) was filtered efficiently in the integrated time series of BS values to show linear development of toxic behavior responses. The linear gradients of Int. BS were observed markedly according to toxic concentrations regardless of different chemicals (Fig. 5). The highest slope (i.e., least toxic effect) was observed for the control. Subsequently Int. BS values decreased as concentration increased (Fig. 5). The curves for 5 TU and 10 TU were distinguished with the minimal ranges of slope by showing 10 TU for the strongest toxic effect. Since death mode appeared at the final phase of the observation period at these concentrations, the curves appeared to be flat lines. However the lines increased minutely during the course of observation in death mode due to low saturation line observed in OMS. At higher concentrations of 5 TU and 10 TU chemicals were consistent in showing different levels of toxicity. Parathion presented the strongest toxic effect (i.e., lowest slopes) whereas the trichlorfon had the weakest toxic effect (i.e., steepest slopes) as the time progressed at higher concentrations. For 0.1 TU and 1 TU, however, the curves were somewhat mixed, especially in the early period after exposure. From the mid phase on, toxic response to 0.1 TU became weaker comparing with 1 TU (Fig. 5). It was also noteworthy that parathion showed the least toxic effect (i.e., higher slope) at the minimum toxic level of 0.1 TU after the initial exposure period.

Linear fitting was further conducted to the time series of Int. BS values. The red lines in Figs. 3-b and 6 were the linear fitted lines for Int. BS for the control and the treatments, respectively. Since death mode was observed at 5 TU and 10 TU (see Section 2.3 "Experimental setup"), the fitting was conducted after excluding the period of death mode. Slopes and elevations in the fitted linear regression models are presented in Fig. 7 (see also Appendix 1 for parameters). Due to decrease in BS values (y-axis), slopes of lines decreased as the concentration increased at the final stage of observation similarly across different chemicals (Figs. 6 and 7). Although the slopes were in a similar range except for variability shown at the minimum level of 0.1 TU, statistical significance ($p < 0.05$) according to multiple comparison of slopes (Zar, 1999) was mostly observed among different chemicals across different levels of toxic concentrations due to small ranges in standard deviations (Fig. 7). Only slopes of parathion and trichlorfon at 10 TU were not significant. For the case of elevations, the gradient along the increase in concentrations was not clearly observed. Elevations were in the maximum range at 5 TU regardless of chemicals although the difference was not substantial, whereas the minimum range was observed with 0.1 TU. Variation in elevations was higher at 1 TU and 5 TU among chemicals. Similar to the case of slopes, a significant difference ($p < 0.05$) was observed among different chemicals in different concentrations except elevations between parathion and trichlorfon at 10 TU (Fig. 7).

Degree of linear fitting was differentiated according to toxic concentrations. Linear fitting was remarkably close to Int. BS curves for low concentrations of 0.1 TU and 1 TU all through the observation period

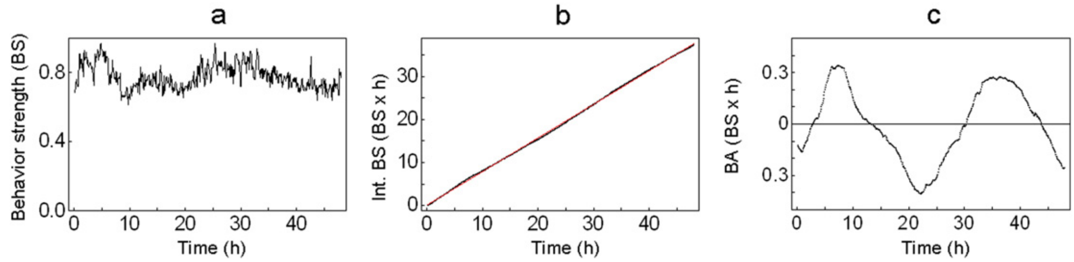

Fig. 3. Filtering by integration conducted on time series of BS values for the control. (a) BS values as the time progressed, (b) integral of BS values (black) and linear fitting (red), and (c) residual curve based on the fitted line.

(Fig. 6). Linear fitting was further observable at higher toxic concentrations, 5 TU and 10 TU, in the early period before the death mode (gray shade in Fig. 6). At 0.1 TU the Int. BS values were almost similar to linear lines, although minute variation existed according to different chemicals. In order to describe the behavioral state, we defined the behavior activity (BA) as Int. BS subtracted by its linear fitted value (Fig. 8). If BA is in the positive range, the test organism would be in "active" state. The BA values were mostly negative at initial stage regardless of chemicals. Only at 1 TU treated with malathion, the BA values were positive in the initial period (Fig. 8). Mostly in the middle phase during the observation period, BA values were positive. This indicated that activity as expressed by BS was higher in the middle phase although some fluctuations were observed according to chemicals and concentrations. It was also noteworthy that the Int. BS curves were broken during the course of stress response, more strongly with trichlorfon treatment (see arrows in Fig. 6). The time for break points generally matched step-like decrease in time series BS values (Fig. 4). At the maximum concentration of 10 TU the observed Int. BS values rapidly increased and were quickly close to the fitted lines before death mode (gray shade in Fig. 6) regardless of chemicals, most early with parathion around 2 h and lately around 10 h with trichlorfon.

Responses of test organisms were more closely examined with BA curves (Fig. 3-c for the control and Fig. 6 for the treated). The BA curve in the control showed two peaks in positive values, showing the period of 4–14 h and the period of 30–38 h after exposure (Fig. 3c). These periods reflected active phase of test fishes in accordance with photophase in an experiment during the observation period. The positive BA values were still observed in the similar period at low values (around 0.5) at 0.1 TU regardless of chemicals, clearly with malathion and weakly with parathion (Fig. 8). In parathion treatment, positive BA value was observed with only one phase. It was also noteworthy that the period of negative values in between two positive phases were minimized in malathion and trichlorfon (Fig. 8). The results indicated that the toxic effects and activity were mixed at 0.1 TU as expressed by BA values. It was remarkable that toxic responses were variable according to chemicals. Toxic effect appeared to be strongest in parathion treatments according to BA values at 0.1 TU since activity in association with circadian rhythm was not observed with the parathion treatment when compared with other chemicals; BA values tended to be high even though in scotophase in the parathion treatment (left panel, Fig. 8).

At higher values of 1 TU variability in toxic response was also observed. Parathion showed a higher variability in responses at 1 TU compared to 0.1 TU (Fig. 8). With the trichlorfon treatment at l TU the response was simple with a strong break point, although the response was complex at a lower level of 0.1 TU. At higher concentrations, 5 TU and 10 TU, responses (excluding death mode) became simple in most cases (Fig. 8). With parathion at 5 TU, however, the response was still variable although death mode appeared earlier; positive and negative phases were repeatedly found, even more strongly when compared with the case of 1 TU. This indicated that parathion caused complex responses in the intermediate range of toxic concentrations. At the maximum concentration of 10 TU, however, the responses became simple before death mode. It was noteworthy that death mode appeared most lately in the trichlorfon treatment (Fig. 8).

3.4. Behavior modes

The crossing times of BA between positive and negative values were commonly observed (Fig. 8). Peak (negative and positive) and crossing times in BA values during the course of behavior response are listed in Appendix 2. The crossing time was more variable at low concentrations than at high concentrations. The crossing times from negative to

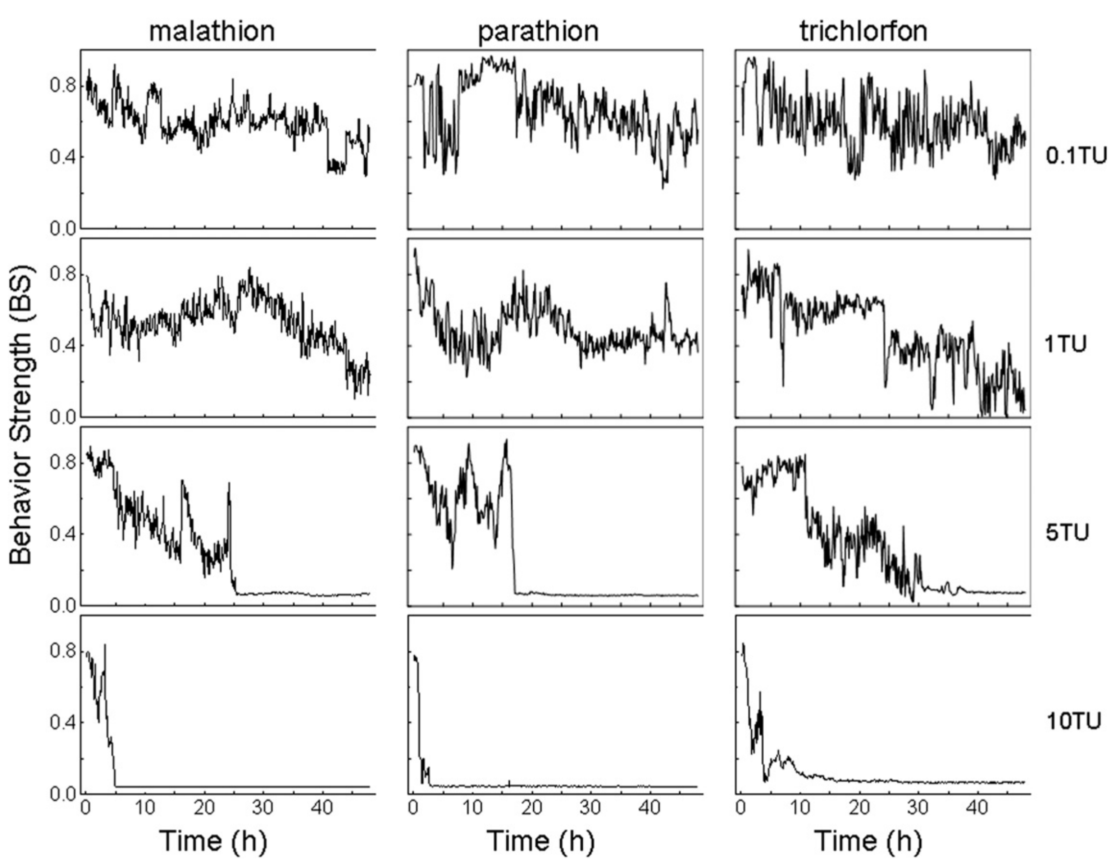

Fig. 4. Time series of BS values in different treatments for malathion (left panel), parathion (center panel), and tricholorfon (right panel) across different concentrations. Concentrations are 0.1 TU, 1 TU, 5 TU and 10 TU listed from top to bottom.

positive (or vice versa) values in Fig. 8 were indicative of the response state of test organisms to chemicals. If the BA value is positive, the organisms are in the active state, indicating that they adjust themselves to gain activity (as expressed by BS values) against intoxication effects. If the BA value is negative the reverse situation would occur, indicating lower activity.

At the minimum concentration of 0.1 TU, crossing time patterns were variable, being highly diverse with trichlorfon and malathion at 0.1 TU (Appendix 2). The treatment showed three acclimation–adjustment modes additionally including early acclimation–adjustment and re-acclimation–adjustment modes, starting at 6.6, 21.3, and 37.6 h, respectively for the case of trichlorfon treatment (Appendix 2). Although the periods of early adjustment and acclimation were short, a similar pattern was observed with the malathion treatment at 0.1 TU, starting and ending at 10.3 and 22.4 h, respectively (Fig. 8). In contrast, parathion only showed single acclimation–adjustment modes, starting at 13.8 h. At the higher value of 1 TU, crossing times were also observed diversely. Especially with parathion the crossing time was markedly variable compared to the low level, 0.1 TU (Fig. 8). The first crossing time was observed as early as 3.1 h, being followed by additional positive phases (Fig. 8, Appendix 2). Trichlorfon in contrast showed simple pattern showing one positive phase between 11.9 and 38.2 h at 1 TU, although the chemical showed the most diverse pattern at the low level of 0.1 TU as stated above (Appendix 2).

At higher toxic concentrations, 5 TU and 10 TU, death mode appeared as stated above and the crossing time was generally simple (Fig. 8, Appendix 2). With parathion at 5 TU, however, complex responses were still observed as stated above. Five crossing times were found whereas four crossing times were shown at 1 TU. It was noteworthy that the last phase immediately before death mode in fact did not cross the zero value, being nearly close to zero before death mode. The organism faced its death without acclimation mode with the parathion treatment at 5 TU. Overall the results indicated that concentration–response relationships are differently expressed depending upon chemicals, concentrations, and exposure time.

If the BA value is negative the activity is diminishing. Consequently intoxicating effects would be acclimated in test organisms. If the BA value is positive, however, test organisms would regain activity. These two phases could be considered as acclimation and adjustment, respectively, as defined for BS modes in Zhang et al. (2012) (Fig. 9). Also the final phase with negative values before death mode could be further considered as toxic modes. The matching modes are shown on the BA curves according to different chemicals and concentrations (different shades in Fig. 8). Through filtering by integration the state of test organisms was more precisely addressed in response to chemicals. For parathion, for instance, response was complex at 1 TU and 5 TU compared to 0.1 TU; early acclimation and adjustment were found additionally before the acclimation and adjustment periods (Fig. 8). Similarly, a complex response pattern was observed with trichlorfon treatment at 0.1 TU: both early and late phases of acclimation–adjustment were found as stated above.

It was also noteworthy that the BA values were positive at the initial phase for the case of malathion at 1 TU, showing stimulation before occurrence of early acclimation (see arrow at 1 TU, malathion treatment in Fig. 8). This indicated that test organisms somewhat maintained high levels of activity in the initial phase. Negative values were observed for other chemicals, parathion and trichlorfon, in the initial period (Fig. 9). In summary, different modes in responses including stimulation, acclimation, adjustment, and toxic modes were accordingly found in association with negative and positive phases in the BA curves.

In the BA curves, peaking time was found in between crossing times with both positive (local maximum) and negative (local minimum) values (Fig. 8 and Appendix 2). Consequently inclining and declining phases were clearly observed between peaks regardless of chemicals and concentrations. Dotted arrows (inclining and declining) with the parathion treatment in Fig. 8 show an example of intoxicating and recovering tendencies. During the period of declining phase, test organisms would have a tendency in loosing BA values continuously until the values reach the minimum peak, and this declination period would represent intoxication due to a continuous decrease in BA values in test organisms. Similarly the BA values would continuously increase to reach the maximum in the period of inclination (Fig. 8). A continuous increase in the BA values would indicate recovery from intoxication. Both intoxicating and recovering tendencies passed through all acclimation and adjustment modes (i.e., crossing time) during the course of response and were consistently observed across different concentrations regardless of chemicals (Fig. 8). Consequently, new states of test organisms were defined as intoxicating and recovering tendencies corresponding to the inclining and declining phases of BA values, respectively, based on the experimental conditions in this study.

Fig. 10 shows frequency profiles for presenting the period of different behavior modes according to crossing times (Fig. 8, Appendix 2). Overall response landscapes were illustrated according to chemicals and concentrations. Each chemical showed a different landscape, presenting unique characteristics depending upon concentrations. It was noteworthy that parathion showed diverse repertoire at 5 TU whereas malathion and trichlorfon presented highly diverse modes at 0.1 TU. In the treatment of trichlorfon the behavior modes rapidly decreased when the concentration was higher than 0.1 TU, monotonously showing single acclimation–adjustment modes before toxic mode. In contrast, the response was rather simple at 0.1 TU but became diverse at higher level of 1 TU and 5 TU in the case of parathion as stated above. The death mode appeared strongly at higher concentrations, 5 TU and 10 TU (Fig. 10). It was noteworthy that frequency of death mode was minimized with the trichlorfon treatment at these concentrations. Overall, toxic response landscapes were accordingly differentiated in relation with concentrations and exposure time in different chemicals.

4. Discussion

By 3-D plots followed by the SOM and filtering by integration, complexity of response behaviors was accordingly elucidated to confirm different BR modes (Figs. 8 and 9). We were further able to define states of intoxicating and recovering tendencies in test organisms in this study (Fig. 8). By utilizing 3-D surface plots initially, overall profiles were efficiently illustrated (e.g., complex in parathion, simple in trichlorfon; Fig. 1) to provide a comprehensive impression on toxic effects of different chemicals. Subsequently by the SOM the degree of time and concentration effects were identifiable; circadian rhythm started to diminish from low concentration (0.1 TU) and toxic effects were strongly detected at higher concentrations of 5 TU and 10 TU (Fig. 2).

Finally by filtering based on integration, different behavior states could be effectively addressed during the course of responses after exposure (Figs. 3–10). Filtering by integration provided a basis for computationally defining BR modes (e.g., acclimation, adjustment)

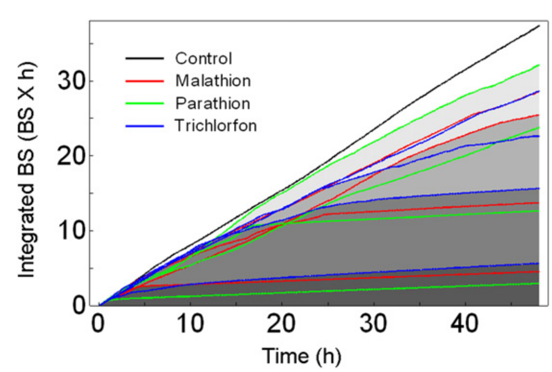

Fig. 5. Integral BS values as the time progressed according to malathion (red), parathion (green), and trichlorfon (blue) in different concentrations.

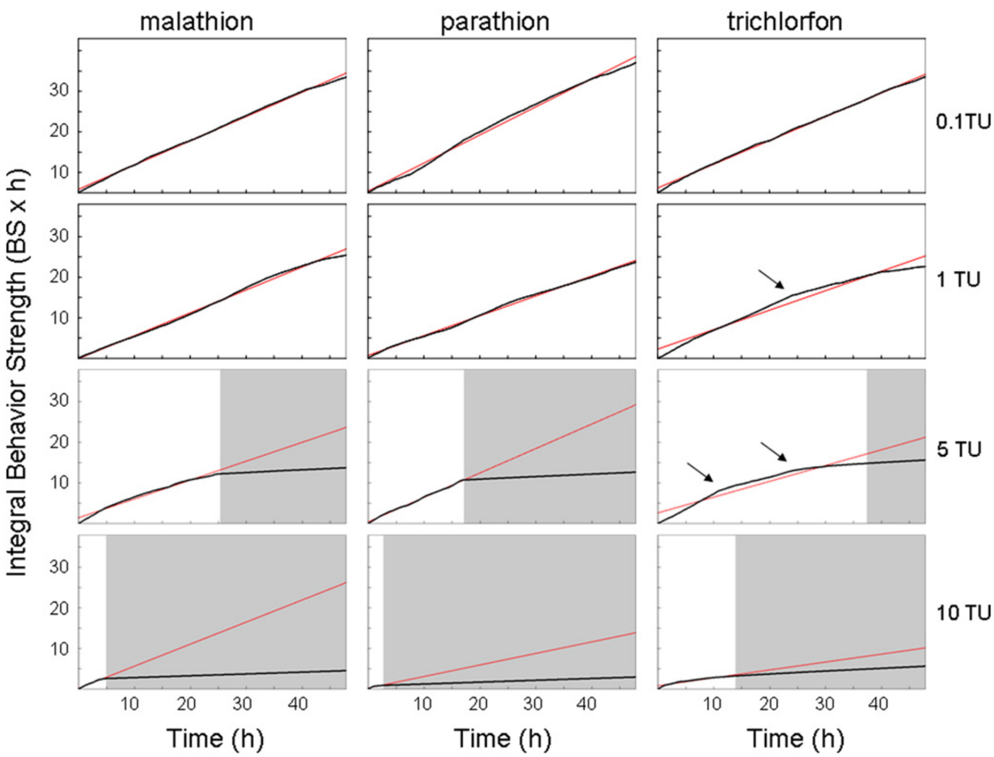

Fig. 6. Integral BS values (black) with linear fitting (red) as the time progressed for malathion (left panel), parathion (center panel), and tricholorfon (right panel) across different concentrations. Concentrations are 0.1 TU, 1 TU, 5 TU and 10 TU listed from top to bottom and the gray shaded regions indicate death mode. Arrows stand for break points.

proposed by empirical data (Zhang et al., 2012). The empirically defined modes were more precisely illustrated according to chemicals and concentrations through BA values in this study (Figs. 8 and 9). For instance, highly variable BR modes were found at 0.1 TU with the trichlorfon treatment including three phases of acclimation–adjustment periods, whereas response modes were highly simplified at higher concentrations with this chemical (Fig. 8). It was also noteworthy that parathion showed diverse modes at the intermediate toxic levels of 1 TU and 5 TU comparing with 0.1 TU.

Moreover, the stimulation mode was additionally found at 0.1 TU with the malathion treatment (Fig. 8). Considering BA values was positive in this case, we could conjecture that test organisms were more active and this over-activity in the early period after exposure would be due to stimulation in behaviors; test organisms would respond sensitively to the chemical at specific concentration. These examples demonstrated that the filtering by integrated BS was feasible in finding the behavior states precisely.

By taking account of continuously inclining and declining directions in the BA values, the intoxicating and recovering tendencies were further defined in this study. It is noteworthy that test organisms repeat the intoxicating and recovering tendencies during the course of response to chemicals before death regardless of chemicals (Fig. 8). Along with BR modes, intoxicating and recovering tendencies could be a general basis for profiling overall toxic behavior responses in animals including humans after exposure to different chemicals in different concentrations. The behavior modes and tendencies could be useful for establishing a reference system for diagnosing response behavior states of test organisms along the course of response to stress. To the best of the authors' knowledge, filtering by integration was first applied to toxic response behavior of animals. More in depth studies are warranted in revealing toxic mechanisms in relation with internal (e.g., physiological network) and external (e.g., environmental condition) factors.

The landscapes according to frequencies of different BR modes (Fig. 10) demonstrated how each chemical could be differently outlined in characterizing toxic responses in the same OP across different toxic concentrations, although the overall toxic response was similar from low to high TU values. For instance, diverse response was observed with trichlorforn only at the minimum concentration, 0.1 TU, although overall toxic response landscape was highly simple at higher concentrations with this chemical. Parathion was unique in showing a diverse response at the

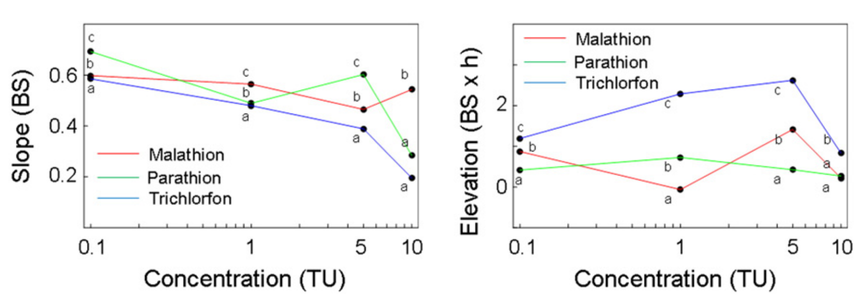

Fig. 7. Slopes and elevations for linear regression fitting to integral BS values in relation with different concentrations for malathion (red), parathion (green) and trichlorfon (blue). Different alphabets represent statistical significance ($p < 0.05$) of slopes and elevations among different chemicals according to multiple comparison test (Zar, 1999).

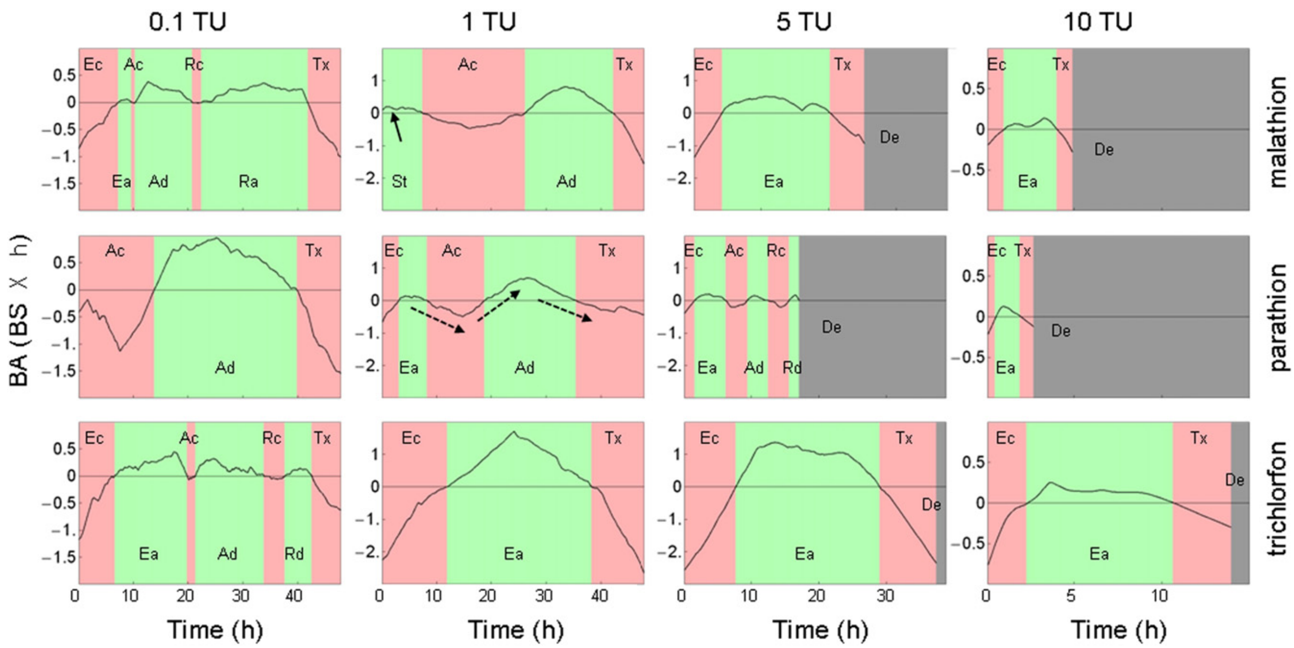

Fig. 8. BA values and different BR modes for malathion (top), parathion (middle) and trichlorfon (bottom) across different concentrations, 0.1 TU, 1 TU, 5 TU, and 10 TU. Abbreviations, St, Ec, Ea, Ac, Ad, Rc, Ra, Tx and De, represent stimulation, early acclimation, early adjustment, acclimation, adjustment, reacclimation, readjustment, toxic effect and death, respectively. Dotted arrows shown at parathion, 1 TU, mean intoxication (declining) and recovering (inclining) tendencies, respectively. The solid arrow shown at malathion, 1 TU, indicates stimulation mode initially, i.e., the positivity of BA value.

intermediate concentrations, 1 TU and 5 TU (Fig. 10). This type of response landscape would be also useful for establishing an overall toxic referencing system for animals responding to different chemicals.

A possibility for future study on behavior mechanism may be of concern if there is any threshold for causing this type of behavior change. A "threshold" was proposed to cause the effects of stress to decide the tendency of BR in test organisms (Selye, 1973). The threshold may be a special type of behavior. As reported in Zhang et al. (2012), the "avoidance behavior" was observed after "no effect", followed by a continuous weakening of BS (acclimation, toxic effects) or adjustment. After "avoidance behavior", a trend for behavior modulation to maintain a stable internal environment and to diminish dependence on an external environment (Putman and Wratten, 1984) was observed in associating with the phases "acclimation" and "adjustment (readjustment)". This type of behavioral mechanism should be explored in detail for checking different BR modes in relation with intoxicating and recovering tendencies in the future (Figs. 8 and 10).

In this study, we explored the filtering method with only one species. Future study may be required to test with other indicator species in exposure to diverse chemicals to confirm BR modes. Also we used three replications in this study according to a previous report (Zhang et al., 2012). Since the modes were more precisely found in this study (Figs. 8 and 10), additional tests with more replications would be required to confirm precision in determining modes (e.g., stimulation, acclimation, adjustment) and tendencies (i.e., intoxicating, recovering) in the future. Regarding field applications for practical purpose, however, not many replications could be allowed for monitoring, considering the limit in observation and measurement resources. Consequently a sampling study regarding how many replications should be minimally required to guarantee a certain level of precision should be additionally investigated for reliable assessment in the future.

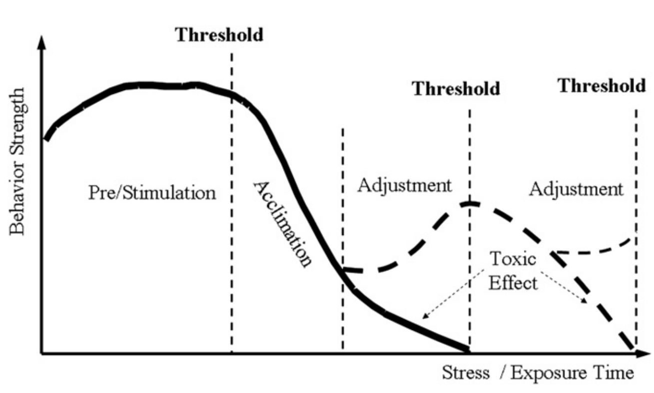

Fig. 9. Different BR modes including stimulation, acclimation, adjustment and toxic effect according to empirical observation (Zhang et al., 2012).

5. Conclusions

The different BR modes of medaka in response to chemical stress, including acclimation, adjustment (readjustment) and toxic modes, were elucidated with computational approaches by 3-D plots followed by the SOM and filtering by integration. By utilizing 3-D surface plots overall profiles were efficiently illustrated (e.g., complex in parathion, simple in trichlorfon). By the SOM the degree of time and concentration effects were identifiable; circadian rhythm started to diminish from low concentration (0.1 TU) and toxic effects were strongly detected at higher concentrations of 5 TU and 10 TU. The highly fluctuating nature of original BS values was filtered efficiently based on integration. BA curves were suitable in determining different BR modes proposed by empirical observations: the positive and negative phases were in accordance with acclimation and adjustment, respectively. Stimulation and re-acclimation–adjustment were also found based on BA curves. New states of test organisms were further defined to present intoxicating and recovering tendencies according to inclination and declination trends observed in BA curves. The landscapes according to frequencies of different BR modes demonstrated how chemicals had different characteristics in showing response behaviors (e.g., complexity at 0.1 TU with trichlorfon and malathion treatments, and 1 TU and 5 TU with parathion treatment) in the same OP, although the overall toxic response showed a gradient from low to high toxic concentrations. The response landscapes would be useful for establishing an overall toxic referencing system for animals responding to different test chemicals. This type of behavior modes and response landscapes would be useful

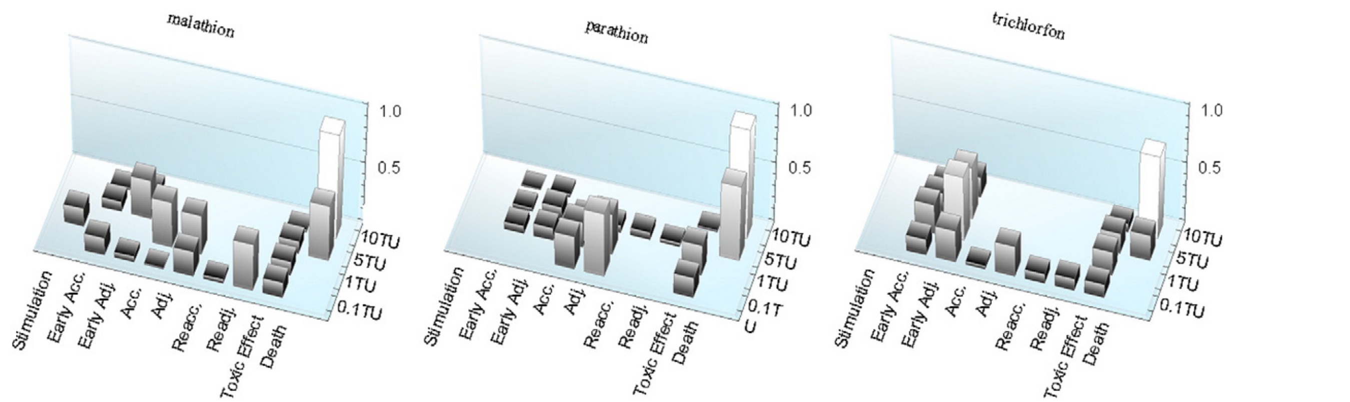

Fig. 10. Frequencies (rate) profiles of different BR modes for test organisms based on BA curves in response to malathion, parathion, and trichlorfon (the higher in frequency, the lighter in gray level.).

for establishing overall toxic referencing system for animals responding to different chemicals. More tests with diverse species and chemicals are warranted in field conditions to confirm precision and feasibility in diagnosing behavior modes and tendencies of test organisms for monitoring.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ecoinf.2014.11.008.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (21107135). The research was also supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0012960). We appreciate greatly Dr. Tuyen Van Nguyen for his assistance with statistical analysis.

References

Azizullah, A., Richter, P., Häder, D.P., 2011. Comparative toxicity of the pesticides carbofuran and malathion to the freshwater flagellate *Euglena gracilis*. Ecotoxicology 20 (6), 1442–1454.

Beitinger, T., 1990. Behavioral reactions for the assessment of stress in fishes. J. Great Lakes Res. 16, 495–528.

Billoir, E., Alexandre, R.R.P., Charles, S., 2007. Integrating the lethal and sublethal effects of toxic compounds into the population dynamics of Daphnia magna: a combination of the DEBtox and matrix population models. Ecol. Model. 203, 204–214.

Breiman, L., Friedman, J.H., Olshen, R.A., Stone, C.G., 1984. Classification and Regression Trees. Wadsworth International Group, Belmont, California, USA.

Cho, E., Chon, T., 2004/2006. Application of wavelet analysis to ecological data. Ecol. Inform. 1 (3), 229–233.

Chon, T., 2011. Self-organizing maps applied to ecological sciences. Ecol. Inform. 6, 50–61.

Chon, T., Park, Y.S., Ross, M.H., 1998. Activity of German cockroach, Blattella germanica (L.) (Orthoptera: Blattellidae), at different microhabitats in semi-natural conditions when treated with sublethal doses of pesticides. J. Asia Pac. Entomol. 1, 77–83.

Coffin, M.R.S., Drolet, D., Hamilton, D.J., Barbeau, M.A., 2008. Effect of immersion at low tide on distribution and movement of the mud snail, *Ilyanassa obsoleta* (Say), in the upper Bay of Fundy, eastern Canada. J. Exp. Mar. Biol. Ecol. 364, 110–115.

Dutta, H., Marcelino, J., Richmonds, C., 1993. Brain acetylcholinesterase activity and optomotor behavior in bluefills, *Lepomis macrochirus* exposed to different concentrations of diazinon. Arch. Int. Physiol. Biochim. Biophys. 100 (5), 331–334.

Eto, M., 1974. Organophosphorous pesticides: Organic and biological chemistry. CRC, Ohio.

Farr, A.J., Chabot, C.C.T., Douglas, H., 1995. Behavioral avoidance of fluoranthene by fathead minnows (*Pimephales promelas*). Neurotoxicol. Teratol. 17, 265–271.

Fulton, M.H., Key, P.B., 2001. Acetylcholinesterase inhibition in esturaine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environ. Toxicol. Chem. 20, 37–45.

Gerhardt, A., ClostermannM, M., FridlundM, B., Svensson, E., 1994. Monitoring of behavioral patterns of aquatic organisms with an impedance conversion technique. Environ. Int. 20 (2), 209–219.

Gray, M.A., Teather, K.L., Metcalfe, C.D., 1999. Reproductive success and behavior of Japanese medaka (*Oryzias latipes*) exposed to 4-tera-octylphenol. Environ. Toxicol. Chem. 18, 2587–2594.

Guilhermino, L., Diamantino, T., Silva, M.C., Soares, A.M.V.M., 2000. Acute toxicity test with *Daphnia magna*: an alternative to mammals in the prescreening of chemical toxicity. Ecotoxicol. Environ. Saf. 46, 357–362.

Ibrahim, W.L.F., Furu, P., Ibrahim, A.M., Christensen, N.O., 1992. Effect of the orgaophosphorous insecticide, chlorpyrifos (Dursban), on growth, fecundity and mortality of *Biomphalaria aexandrina* and on the production of *Schistosoma mansoni* cercariae in the snail. J. Helminthol. 66, 79–88.

Inan, O.T., Marcu, O., Sanchez, M.E., Bhattacharya, S., Kovacs, G.T.A., 2011. A portable system for monitoring the behavioral activity of *Drosophila*. J. Neurosci. Methods 202, 45–52.

Ji, C., Lee, S., Choi, K., Kwak, I., Lee, S., Cha, E., Lee, S., Chon, T., 2007. Monitoring of movement behaviors of chironomid larvae after exposure to diazinon using fractal dimension and self-organizing map. Int. J. Ecodyn. 2 (1), 27–38.

Kavathekar, D., Mueller, T., Fagan, W.F., 2013. Introducing animal movement visualizer, a visualization tool for animal movement data from satellite collars and radiotelemetry. Ecol. Inform. http://dx.doi.org/10.1016/j.ecoinf.2012.12.005.

Kim, C., Kwak, I., Cha, E., Chon, T., 2006. Implementation of wavelets and artificial neural networks to detection of toxic response behavior of chironomids (Chironomidae: Diptera) for water quality monitoring. Ecol. Model. 195, 61–71.

Kong, F., Yin, D., Yan, G., 2000. Environmental Biology. Higher Education Press, Beijing (In Chinese).

Kwak, I., Chon, T., Kang, H., Chung, N., Kim, J., Koh, S., 2002. Pattern recognition of the movement tracks of medaka (*Oryzias latipes*) in response to sub-lethal treatments of an insecticide by using artificial neural networks. Environ. Pollut. 120, 671–681.

Lemly, A.D., Smith, R.J., 1986. A behavioral assay for assessing effects of pollutants of fish chemoreception. Ecotoxicol. Environ. Saf. 11 (2), 210–218.

Liu, Y., Lee, S., Chon, T., 2011. Analysis of behavioral changes of zebrafish (*Danio rerio*) in response to formaldehyde using self-organizing map and a hidden Markov model. Ecol. Model. 222 (14), 2191–2201.

Moore, A., Waring, C.P., 1996. Sublethal effects of the pesticide diazinon on olfactory function in mature male Atlantic salmon parr. J. Fish Biol. 48, 758–775.

Nimkerdphol, K., Nakagawa, M., 2008. Effect of sodium hypochlorite on zebrafish swimming behavior estimated by fractal dimension analysis. J. Biosci. Bioeng. 105 (5), 486–492.

Nishiuchi, Y., Hashimoto, Y., 1967. Toxicity of pesticide ingredients to some fresh water organisms. Sci. Pest. Control. 32, 5–11.

Park, Y., Chung, N., Choi, K., Cha, E., Lee, S., Chon, T., 2005. Computational characterization of behavioral response of medaka (*Oryzias latipes*) treated with diazinon. Aquat. Toxicol. 71, 215–228.

Polansky, L., Wittemyer, G., Cross, P.C., Tambling, C.J., Getz, W.M., 2010. From moonlight to movement and synchronized randomness: fourier and wavelet analyses of animal location time series data. Ecology 91 (5), 1506–1518.

Putman, R.J., Wratten, S.D., 1984. Principles of Ecology. Groom Helm, London and Canberra.

Ren, Z., Wang, Z., 2010. The differences in the behavior characteristics between *Daphnia magna* and Japanese madaka in an on-line biomonitoring system. J. Environ. Sci. 22 (5), 703–708.

Ren, Z., Li, Z., Zha, J., Ma, M., Wang, Z., Gerhardt, A., 2007. The early warning of aquatic organophosphorus pesticide contamination by on-line monitoring behavioral changes of *Daphnia magna*. Environ. Monit. Assess. 134, 373–383.

Ren, Z., Zhang, X., Wang, X., Qi, P., Zhang, B., Zeng, Y., Fu, R., Miao, M., 2015. AChE inhibition: one dominant factor for swimming behavior changes of *Daphnia magna* under DDVP exposure. Chemosphere 120, 252–257.

Richard, O.D., Peter, E.H., David, G.S., 2001. Pattern Classification. 2nd edn. Wiley Interscience, USA.

Ripley, B.D., 1996. Pattern Recognition and Neural Networks. Cambridge University Press, Cambridge, UK.

Roast, S.D., Widdows, J., Jones, M.B., 2000. Disruption of swimming in the hyperbenthic mysid *Neomysis integer* (Peracarida: Mysidaces) by the organophosphate pesticide chlorpyrifos. Aquat. Toxicol. 47, 227–241.

Rodriguez Jr., M., Sanders, C.A., Greenbaum, E., 2002. Biosensors for rapid monitoring of primary-source drinking water using naturally occurring photosynthesis. Biosens. Bioelectron. 17, 843–849.

Sandbacka, M., Christianson, I., Isomaa, B., 2000. The acute toxicity of surfactants on fish cells, *Daphnia magna* and fish — a comparative study. Toxicol. in Vitro 14, 61–68.
Schulz, R., Liess, M., 1999. A field study of the effects of agriculturally derived input on stream macroinvertebrate dynamics. Aquat. Toxicol. 46, 155–176.
Selye, H., 1973. The evolution of the stress concept. Am. Sci. 61, 692–699.
Smith, G.J., 1987. Pesticide Use and Toxicity in Relation to Wildlife: Organophosphorus and Carbamate Compounds. United States Department of the Interior, Fish and Wildlife Service, Resource Publication, Washington, DC.
Sun, R.Y., Li, B., Zhuge, Y., Shang, Y.C., 1993. General Ecology. Higher Education Press, Beijing.
Tahedl, H., Häder, D.-P., 2001. Automated biomonitoring using real time movement analysis of *Euglena gracilis*. Ecotoxicol. Environ. Saf. 48, 161–169.
Tsuda, T., Kojima, M., Harada, H., Nakajima, A., Aoki, S., 1997. Acute toxicity, accumulation and excretion of organophosphorous insecticides and their oxidation products in killifish. Chemosphere 35 (5), 939–949.
Vesanto, J., Himberg, J., Alhoniemi, E., Parhankangas, J., Team, S., Oy, L., 2000. Som toolbox for matlab. Techn. Ber. Helsinki University of Technology.
Wang, Y., Liu, X., Hu, J., Li, L., Li, Y., 2007. The simulation and experimental study of conductance sensor's electric field distribution. Petrol. Instrum. 1 (21), 16–18 (in Chinese).
Zar, J.H., 1999. Biostatistical Analysis. Prentice Hall, Upper Saddle River, USA.
Zhang, G., Chen, L., Chen, J., Ren, Z., Wang, Z., Chon, T.-S., 2012. Evidence for the stepwise behavioral response model (SBRM): the effects of carbamate pesticides on medaka (*Oryzias latipes*) in an online monitoring system. Chemosphere 87, 734–741.