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Change in Spatial Dispersion of *Daphnia magna* (Cladocera: Daphniidae) Populations Exposed to Organophosphorus Insecticide, Diazinon

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유기인계 살충제(다이아지논)에 대한 물벼룩, *Daphnia magna* (Cladocera: Daphniidae) 개체군의 공간분산 변이

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ABSTRACT

We explored collective behaviors of indicator species to elucidate the effect of the chemical stress. After the treatments of an insecticide, diazinon, at low concentrations (1.0 and 10.0 μg/L), spatial dispersion patterns of *Daphnia magna* were checked in a test chamber. The *I*-index was used to characterize the movement data before (0～1 h) and after (1～2 h) the treatments in laboratory conditions. The slopes of the frequency distribution of *I*-index in semi-log scale decreased significantly, and the test populations appeared to be more dispersed with a lower degree of aggregation after the treatments. The index was feasible in indicating decrease in the ability of the specimens to keep desirable distances with neighbor individuals under chemical stress and showed a possibility of monitoring presence of toxic chemicals in environment through group behavior measurement.

Key words : *Daphnia magna*, spatial dispersion, *I*-index, biomonitoring, response behavior, multi-individual distance

INTRODUCTION

The response behaviors of indicator specimens have been reported to be an efficient bio-monitoring tool for detecting the presence of toxic agents in aquatic ecosystems (Lemly and Smith, 1986; Dutta *et al.*, 1992; Kwak *et al.*, 2002; Chon *et al.*, 2005; Park *et al.*, 2005, Ji *et al.*, 2007). Behavioral research on the effects of toxic chemicals at low concentrations has been conducted with various taxa such as crustaceans

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(Abgrall *et al.*, 2000; Roast *et al.*, 2000), aquatic insects (Gerhardt *et al.*, 2005), snails (Ibrahim *et al.*, 1992), and fish (Lorenz *et al.*, 1996; Staaks *et al.*, 1999; Oshima *et al.*, 2003).

In particular, *Daphnia* spp. have been considered to be useful indicators for ecotoxicological monitoring in aquatic ecosystems since they are sensitive to the presence of a wide variety of chemicals in environment and readily respond to the presence of pollutants in multiple ways (Dodson *et al.*, 1995). The specimens of *Daphnia* have been used for checking chronic response at the population level (e.g., checking survival rate, reproduction) (see Baillieul *et al.*, 1993, Muyseen and Janssen, 2007) and behavioral response at the individual level (e.g., abnormal behaviors) (see Dodson *et al.*, 1995; Lechelt *et al.*, 2000).

Regarding behavioral tests with *Daphnia*, parameter estimation has been mainly conducted by analyzing movement data. Wolf *et al.* (1998) and Untersteiner *et al.* (2003) measured average swimming velocity to detect changes in the locomotory behavior of *Daphnia magna* after exposure to heavy metals. Dodson *et al.* (1995) reported that measures such as velocity, turning angle, and upward and downward angles during hops could be successfully used to characterize response behaviors of *Daphnia pulxe* aferr testing with carbaryl. Shimizu *et al.* (2002) observed increases in fractal dimension in *Daphnia* motion under acute toxicity of various toxic chemicals including an organophosphorus insecticide, dichlorvos. By checking various parameters concurrently, Watson *et al.* (2007) successfully detected volatile organic compounds in freshwater by synthetically analyzing swimming behavior of *Daphnia magna* recently.

The previous studies were conducive to understanding the behavioral responses of test specimens exposed to chemical stress. However, the methods were mainly based on the parameters (e.g., velocity) regarding movement of single organisms. Not much research has been conducted regarding information on multiple organism behaviors. Lechelt *et al.* (2000) reported that properties of group behavior such as speed distribution and fractal dimension are stable across time for monitoring behavioral changes caused by the chemical treatments. This type of multi-individual monitoring has been delayed until recently due to technical difficulty in measuring and quantifying the relational information between individuals (Michels *et al.*, 1999).

In this study changes in behaviors caused by toxicological disturbance were hypothesized to affect individual-individual distances. Distribution of distances between individuals would be consequently changed at the population level after the treatments (Vicsek *et al.*, 1995; Tu, 2000; Lee *et al.*, 2006). Distance indices, based on individual-to-individual or point-to-individual positions, has a long tradition in ecology, being initialized with phytosociological sampling (Cottam and Curtis, 1956; Greig-Smith, 1964). Many different distance indices have been proposed in the literature to detect non-randomness in animal and plant populations in spatial dispersion (Pielou, 1977, Southwood, 1978). Degrees of dispersion could be measured in two aspects: (1) counting the number of individuals in the sampled quadrats, and (2) recording distances between individuals or between points and individuals. The first method counting densities in the quadrats provided various parameters including departure from Poisson distribution (Davies, 1971), "*k*" in the negative binomial (Hairston, 1959), Taylor's power law (Taylor *et al.*, 1978), Morisita index (1971), Mean crowding (Lloyd, 1967), and Iwao's index (1972). Detailed discussion on these parameters is out of scope in this study and could be referred to Pielou (1977) and Southwood (1978). The parameters used in this method are mainly useful for measuring dispersion in stationary species and could present degree of aggregation in different species and in different habitats. However, the indices obtained in this method may be prone to errors caused by variation in quadrate size and would require appropriate ranges of densities for more efficient estimation.

The second methods measuring distances between individuals (or between points and individuals) could be feasible in checking mobile species, although the method could be still applicable to stationary species.

The method has been recently more promising along with the recent development of computer interfacing techniques for automatic recognition of individuals. The method could be roughly divided into 3 types regarding measurements: (1) the distance between the nearest neighbors (Greig-Smith, 1964), (2) the distance between points to the closest individuals (Keuls *et al.*, 1963), and (3) addition of the distances up to the *n*th nearest individuals for (1) and (2) (Morisita 1954, Thompson 1956, Keuls *et al.*, 1963). The first measurement checking the nearest neighbors (Greig-Smith, 1964), which was originally used for estimating mean densites (Clark and Evans, 1954), is simple to measure, but randomness may not be guaranteed (Pielou, 1977; Southwood, 1978). However, considering the practical difficulty of numbering all the individuals in advance for obtaining the truly randomness, this process is still preferred for practical purpose (Seber, 1973; Pielou, 1977; Southwood, 1978). The second method taking the distance between the point and the nearest individual may be more efficient in obtainment of randomness, but the method would have a problem when the points are closer to the boundary in measuring the distances to individuals (Southwood, 1978). The third method for obtaining the additional distances up to the *n*th order neighbors would be more accurate, and dispersion patterns could be detected over a large area. But some ecological effects (e.g., competition) may be not clearly presented due to spatial heterogeneity in large area. In addition automatic recognition may not be simple to recognize all the additional neighbors in continuous recording.

Considering various conditions, including simplicity of measurement and practical efficiency in expressing degree of dispersion, we chose the methods for measuring the distance between the nearest neighbors. The *I*-index proposed by Johnson and Zimmer (1985) appears to be a powerful test for spatial patterning (Gonzalez-Andujar and Saavedra, 2003). Given a sample of random individuals the distance to the nearest individual could be collectively used to define spatial patterns covering, uniform, random and aggregated dispersion (Johnson and Zimmer, 1985). We checked degree of dispersion of indicator species with the *I*-index under the stressful conditions of the chemical treatments.

MATERIALS AND METHODS

1. Daphnia culture

Specimens of *Daphnia magna* were obtained from the Toxicology Research Center, at the Korea Research Institute of Chemical Technology, in Daejeon, Republic of Korea. Initial population density of the specimens was one *Daphnia* per 100 mL and was cultured in M4 medium (Elendt and Bias, 1990) in 2-L containers under a 14 hours light, 10 hours dark cycle at 24°C (±1)°C. Green algae, *Chlorella vulgaris*, were used as their food source. Twenty mL of the algal culture with a concentration of approximately 10^7 cells/mL was added to each *Daphnia* culture three times a week. The neonates were separated daily from the adults using sieves. Twenty-four hour old *Daphnia* were used for observation in a test chamber (9.0 cm wide × 9.0 cm deep × 1.0 cm height (water level)).

An insecticide, diazinon, was diluted with dimethyl sulfoxide to the level of 1.0 μg/L and 10.0 μg/L and was applied directly to the test chamber. According to Burkepile *et al.* (2000), the 48 h LC50 to *Daphnia magna* was 2.39 μg/L. In this study, observations were carried out within one hour after the treatments, and no specimens were observed to die during the observation periods. Observation before and after the chemical treatments was replicated fifteen times.

2. Data acquisition and analysis

After transferring 20 specimens from a culture container to the test chamber, we allowed the specimens to adapt to new environment for 30 min. After this stabilization period, their movements were continuously recorded from above through a CCD camera (256 gray levels, resolution 512 × 512) connected to a PC via a frame grabber (Fig. 1) for one hour before and one hour after the treatments. Snapshots were taken at 0.25 second intervals. The height of water level for observation was set to a low level (10 cm), and observation was conducted with 2 dimensions for simplicity of recording. These snapshots included information of test specimens' coordinates on screen. To characterize the spatial distribution of the individuals, we used *I*-index proposed by Johnson and Zimmer (1985). Given a sample of *N* individuals with random distribution (with *x* and *y* co-ordinates), then *I*-index of dispersion is defined as:

$$I=(N+1)\frac{\sum_i (x_i^2)^2}{\left[\sum_i x_i^2\right]^2},$$

where x_i is the distance to the nearest neighbor for individual *i*. The index *I* has an expected value of approximately 2 for random distributions, less than 2 toward uniform distributions (i.e., uniform distribution if the value is close to 1), and greater than 2 toward clumped distributions; the more clumped the distribu-

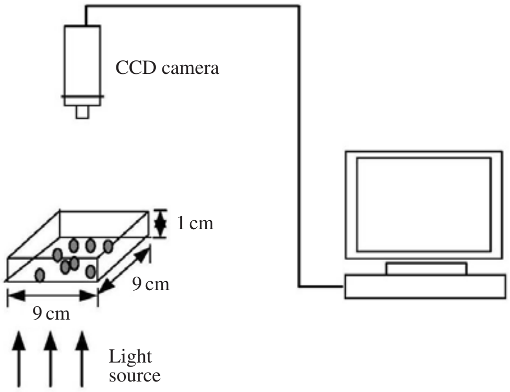

Fig. 1. The schematic diagram of observation system.

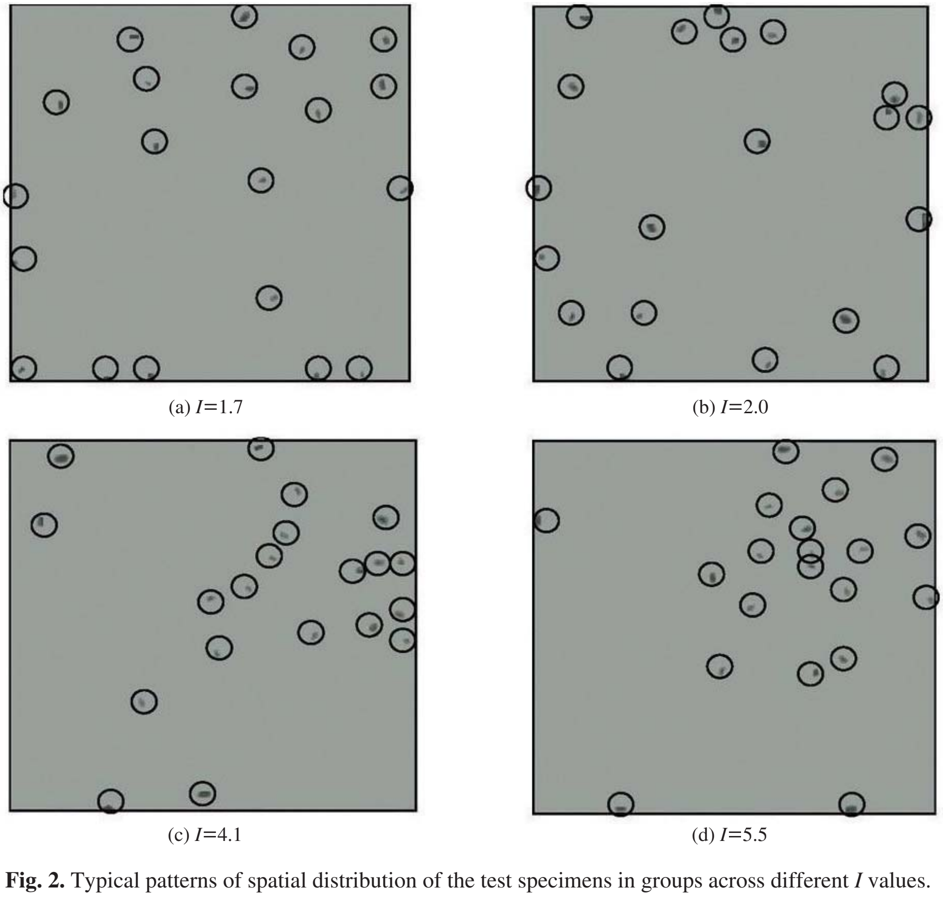
(a) *I*=1.7
(b) *I*=2.0
(c) *I*=4.1
(d) *I*=5.5

Fig. 2. Typical patterns of spatial distribution of the test specimens in groups across different *I* values.

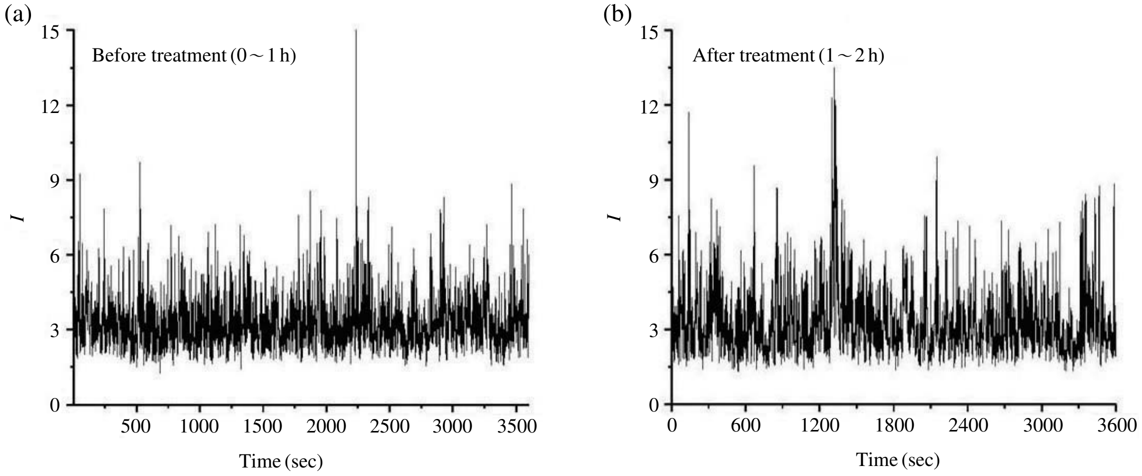

Fig. 3. Time series of I values before (‘0 ~ 1 h’) and after (‘1 ~ 2 h’) the treatments of diazinon (1.0 μg/L) in 0.25 sec interval.

tion, the higher I value. Time series of I values obtained from the consecutive snapshots were transformed to frequency distributions, and plotted on semi-log coordinates. The slopes of the frequency distributions were determined using linear regression to facilitate comparison between observations taken before and after the treatments. The paired t-test (Zar, 1999) was conducted to compare the slopes of regression analysis before and after the treatments (or between the treatments) for the fifteen replications.

RESULTS

Degree of aggregation was accordingly reflected by I-index. Fig. 2 shows the typical patterns of spatial distribution of *Daphnia* individuals in the test chambers across different I values. As the organisms were located in more dispersed patterns, I values tended to decrease. Fig. 3 shows an example of the time-varying I values measured with the sequential capture interval before (Fig. 3(a)) and after (Fig. 3(b)) the treatments. Most I values were lager than 2, indicating that *Daphnia* tended to be more clustered than random distribution. Peaking in I-values was intermittently observed during the observation periods, indicating that the organisms strongly aggregated for the short periods of time. However, the values were variable in a great degree as the time progressed. Due to fluctuations observed in I values, however, average values of the index did not appear to be different ‘before’ and ‘after’ the treatments (Fig. 3).

In order to differentiate the data structure before and after the treatments, intermittency of I values was investigated during the observation periods. We transformed the data of I values into frequency distributions and analyzed slopes on semi-log coordinates through regression analysis of frequency distribution in relation with I values. We checked initially whether variation existed in the slopes for the specimens without treatment during the whole observation periods (‘0 ~ 1 h’ and ‘1 ~ 2 h’) (Fig. 4). The slopes (-0.39 in averages with $\mathrm{SD}=0.23$) of the regression line in the first hour (‘0 ~ 1 h’) were in a similar range to the slopes (-0.39 in averages with $\mathrm{SD}=0.05$) in the second hour (‘1 ~ 2 h’). The overall difference before and after the treatments was not significant (t-test, $t=0.61$, $p=0.55$) when all the slopes for the 15 cases for the first hour were compared with those for the second hour using the t-test (Fig. 4 (b)).

After the treatments, however, the slopes decreased. A higher frequency was observed at the lower values of I after the treatments (i.e., lower slopes) than before the treatments. Fig. 5 shows an example of regression

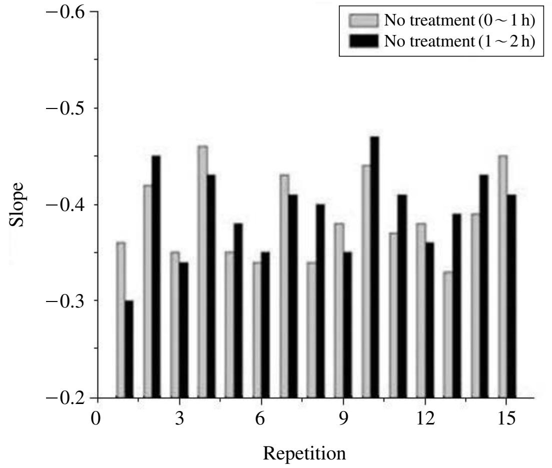

Fig. 4. Slopes of the frequency distribution in fifteen replications based on regression analysis of *I* values for '0 ~ 1 h' and '1 ~ 2 h' before the treatments.

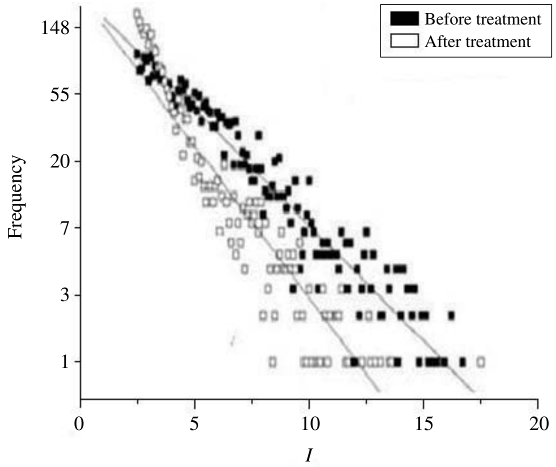

Fig. 5. Frequency distribution of *I* values based on regression analysis before and after the treatments of diazinon at 1.0 μg/L (slopes in this example: −0.33 for 'before treatment' and −0.45 for 'after treatment').

analysis before and after the treatments when diazion was treated with 1.0 μg/L. A substantial decrease in slopes was observed at both concentrations of 1.0 μg/L and 10.0 μg/L. When diazinon was treated with 10.0 μg/L, the slopes decreased to −0.42 in averages (SD =0.08) from −0.31 (SD=0.07) before the treatments in averages with fifteen replications (Fig. 6(a)). At the lower concentration of 1.0 μg/L, the slopes also decreased in a substantial amount (Fig. 6(b)). Decrease in the slopes was consistently observed with 15 replications with −0.44 (SD=0.05) in averages compared with −0.36 (SD=0.06) before the treatments. Statistical significance was observed for both concentrations before and after the treatments (*t*-test, $t=7.22$, $p=0.0001$ for 1.0 μg/L treatment, and $t=3.68$, $p=0.0009$ for 10.0 μg/L). This indicated that the treated groups showed somewhat a higher tendency of dispersion after exposure to diazinon. When the slopes between two concentrations were compared by using the t-test, however, there was no statistical difference between 1.0 μg/L and 10.0 μg/L (*t*-test, $t=1.35$, $p=0.1888$).

DISCUSSION AND CONCLUSIONS

In the present study, we hypothesized that a desired level of distance will be maintained between different individuals in the test populations and that the stability of keeping the distance between individuals would be disturbed in some degree after exposure to toxic chemicals. We demonstrated that a parameter indicating the totality of distance between test individuals is stable in presenting toxic effects (Figs. 5, 6). Although the time series data for the index, *I*, were not conspicuous in differentiating effects of the treatments in averages (Fig. 3), the intermittency analysis was feasible in illustrating changes in spatial distribution patterns of the test populations in a stable manner. The change in spatial distribution of *Daphnia* was reported by Kleiven *et al.* (1996). The authors found that *Daphnia* were able to adjust their spatial distribution in response to other kinds of cues; these included light chemical substances, food levels, etc. The individuals affected by chemical stress could not continuously maintain the distance after the treatments. Maintenance of distance could be disturbed by various causes such as escaping, adaptation or protection reactions (Wolf *et al.*, 1998). The reactions could be more directly related with behaviors such as increased swimming activity, which can cause more dispersed pattern in spatial distribution

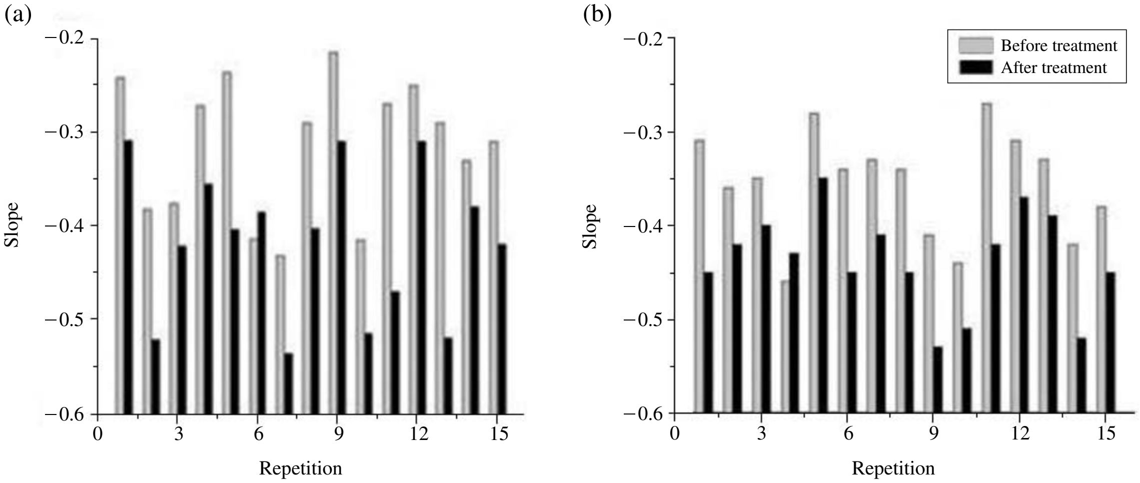

Fig. 6. Slopes of the frequency distribution in fifteen replications based on regression analysis of *I* values before and after the treatments of diazinon: (a) 10.0 μg/L, and (b) 1.0 μg/L.

of the organisms (Ferrando and Andreu, 1993; Wolf *et al.*, 1998). In this study, the effect of disturbance on maintenance of spatial distribution among individuals was confirmed under chemical stress. The detailed relationships between distance change and swimming behaviors under stress could be further checked in the future.

The toxic mechanism of diazinon is based on cholinesterase inhibition and affects organisms' neural systems (Kozolvskaya and Mayer, 1984; Ferslew *et al.*, 1992; Burkepile *et al.*, 2000). These neurotoxic effects of diazinon were presented by response behaviors in indicator species (Chon *et al.*, 2005; Park *et al.*, 2005). Toxic effects of diazinon on groups of *Daphnia* were further demonstrated through dispersion patterns in this study.

We intended to show that values of the index would correspond to the gradients of the concentrations. However, the parameters between two concentrations (1.0 μg/L and 10.0 μg/L) (i.e., slopes of the frequency transformation) were not significant (Fig. 6). This may be partly due to variation of behaviors of the test population and partly due to the fact that the size of test chamber may dilute the degree of dispersal in the treated concentrations. In fact we also tested with 100.0 μg/L, but most of the testing individuals were dead in a short time period after the treatments. In the future, additional experimental tests would be required to check toxic effects at detailed levels of concentrations. Although the gradients in indices were not observable as stated above, the difference of the slopes between before and after treatment was consistently observed (Fig. 6). The results were congruent in two different concentrations (1.0 μg/L and 10.0 μg/L). Since behavioral data are complex and are difficult to characterize the differences in concentrations in effective low ranges; the toxic effects are mostly reported with one level of concentration in continuous behavioral monitoring (Kwak *et al.*, 2002; Park *et al.*, 2005; Ji *et al.*, 2007).

Regarding efficiency in *in-situ* monitoring compared with other measurements of physico-chemical factors, our findings can be still useful for in-situ monitoring as a means of early warning. The samples early detected by behavioral monitoring could be further checked with more detailed physico-chemical or biochemical tests afterwards. Using *I*-index in group observation presents another practical advantage for monitoring. For checking group activity, for instance, movement data for each individual in test populations would be required continuously for the observation period. Tracking of individual data, however, would require an extremely large amount of computation resources and would be additionally prone to noise in various types during data processing for dealing with individual data. This would be impractical if monitoring is carried out continuously in real-time *in situ*. On the other hand, measuring *I*-index does not require considerable computation time and memory because only position data for the individuals would be necessary for calculating *I* values. This type of practicality in measurement and the straightforward calculation of *I* index would be feasible in its implementation to field situations.

During the experiment, boundary effects on individual's behavior were also observed. When some individuals reached the boundary of the arena, they stayed in this area for a certain time. This type of behavior, however, did not affect significantly the results presented in this study. Boundary locations of the test populations usually contributed to changes in frequency of *I* values in either minimal or maximal range. Changes in the frequency in low or high range were not critical in determining the slopes of the frequency table in this study, since frequency in the medium range of *I* values were substantially high and the slopes were mainly determined by the points observed in this range. For this reason, we did not make such boundary effects a focus of our investigation in this study. Behaviors in the boundary area, however, would be different on the individual basis under the spatially constrained conditions, although boundary behaviors would not be differently illustrated on monitoring distances between individuals at population level. A close look at boundary behaviors would be needed to fully characterize response behaviors of individuals to the treatments of toxic chemicals in the future.

Besides boundary behaviors other related behaviors (e.g., contact behaviors between individuals) in the arena could also provide additional information in describing inter-individual response behaviors. The impact of other biological (e.g., food) and abiological variables (e.g., water flow, temperature) on organisms' response behaviors could be further checked in revealing the factor-response causality relationships in behavioral monitoring.

In conclusion, intermittent analysis of inter-individual distance was feasible in extracting information from complex data of collective behavior of *Daphnia* under chemical stress. The index presenting the degree of dispersion could be utilized as an alternative tool for monitoring presence of chemicals in environment.

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