Computational analysis of movement behaviors of medaka (*Oryzias latipes*) after the treatments of copper by using fractal dimension and artificial neural networks

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Abstract

Response behaviors of medaka were computationally analyzed before and after the treatments of copper at low concentration (1.0 mg/L). Parameters (e.g., speed, stop time, turning rate, etc) of the movement patterns were used as input for training the Multi-Layer Perceptron. Detection rates of the movement patterns such as ‘Slow movement’ and ‘No movement’ increased after the treatments. However, a higher degree of variation was observed in detection rates. Fractal dimension calculated from the movement data of individual specimens decreased consistently after the treatments. Higher consistency in fractal dimension was further achieved by using the data for collective rearing. Feasibility of behavioral monitoring was discussed in assessing toxic chemicals in environment.
Keywords: response behavior, medaka, copper, fractal dimension, Artificial Neural Network, behavioral monitoring.

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1 Introduction

Recently automatic detection of response behaviors of animals has been considered as an efficient tool for bio-monitoring of aquatic ecosystems [1]. Dutta *et al* [2] suggested that a behavioral bioassay would be more sensitive than other types of testing methods. A numerous accounts of behavioral research on effects of toxic chemicals at low concentrations have been reported in various taxa, including crustaceans [3, 4], snails [5], fish [6] and insects [7, 8]. Recently Oshima *et al* [9] observed suppression of sexual behavior in male medaka exposed to estradiol. However, these studies are mostly based on observation of single or combinations of single behaviors mainly with qualitative descriptions. Not much computational research has been carried out for automatically detecting behavioral changes from continuous recording.

Behaviors, however, have been regarded as difficult for analysis due to complexity residing in the data. Theoretical studies have been carried out on analyzing movement data regarding correlation function [10], random walk [11], etc. Recently fractal dimension has been considered as an efficient parameter to quantitatively express behavioral states. Fractal dimension has been widely used for analyzing non-linear phenomena in biological and ecological sciences such as geographical features, morphology, etc, [see 12]. Johnson *et al* [13] and Weins *et al* [14, 15] used fractal dimension for analysis of insect movement to quantitatively characterize behavioral states that might not be available through absolute measures of pathway configurations. Alados *et al* [16] used fractal dimension for detecting response behaviors of parasitic infection in Spanish ibex. In this study we used fractal dimension to reveal behavioral states of indicator specimens in response to toxic substances.

Along with fractal dimension, we also implemented Artificial Neural Networks (ANNs) to address pattern changes in response behaviors. While fractal dimension quantitatively compresses behavioral changes as one parameter, ANNs are useful for dealing with local information and for revealing specific behavioral patterns explicitly. ANNs have been widely used for analyzing complex data in computer and electronics engineering [see 17, 18] and have been recently implemented to ecological sciences in various aspects such as forecasting, input-output relationships, data organization, classification, etc [see 19, 20]. Recently ANNs have been applied to behavioral monitoring. Self-Organizing Map was applied to classification of response behaviors of indicator organisms treated with diazinon [21, 22]. Multi-Layer Perceptron (MLP) was used to automatically detect behavioral changes in organisms such as medakas and chionomids in response to toxic chemicals [23, 24].

In this study we intend to extract local and global information residing in behavioral data and to propose a system to quantitatively characterize response behaviors in both explicit (i.e., MLP) and compressed (i.e., fractal dimension) forms. Initially MLP was applied to detection of changes in specific movement patterns after the treatments of toxic substances. Subsequently we elucidated fractal dimension as a means of minimizing the variability of behavioral data to be a reliable parameter to detect changes in behavioral states.

2 Materials and methods

2.1 Test specimens and observation system

Medakas (*Oryzias latipes*), the "*or*" strain originally developed by Bioscience Center, Nagoya University, Japan, were obtained from Toxicology Research Center, Korea Research Institute of Chemical Technology (KRICT; Taejeon, Korea) for testing. The stock populations were maintained in a glass tank, and were reared with artificial dry diet (Tetramin®) under the light regime of L10:D14 in temperature ranging 25±1°C. In photo-phase, a fluorescent lamp (20 W) was used as the light source and was located above the observation aquarium with 30 cm apart. In scoto-phase, a red light (20 W) was provided at the same position.

The position of the test specimens of medaka (age: 6–12 months) was recorded by using an observation system consisting of an observation aquarium, a camera and software for image recognition. Individuals or groups of medakas were placed in a glass aquarium (volume of water: 40 cm ×20 cm ×10 cm), and their position was scanned from the side view at 0.25 s intervals using a CCTV camera (Kukjae Electronics Co. Ltd.; IVC-841®) for four days (two days before the treatments and two days after the treatments). The analog data captured by the camera were digitized by using a video overlay board (Sigmacom Co., LTD.; Sigma TV II®), and were sent to the image processing system to locate the target organisms in two dimension. The software for recognition of the movement tracks and other supporting mathematical programs were provided according to [23].

During the period of observation, disturbances in experimental conditions were minimized: oxygen, fresh water and food were not supplied to test specimens to simplify observation conditions [23]. Before monitoring, the specimens were acclimated to the observation aquarium for 1–2 days. Environmental factors such as light and temperature were maintained to the same condition for rearing stock populations.

2.2 Experimental procedure

Copper was treated to medaka fishes in this study. The level of LC_{50} for copper to medaka population was reported as 5 mg/L [25]. After two days of observation without treatment, reaction behaviors were also recorded for two days after the treatments of copper (1 mg/L).

Initially, we observed 10 medaka fishes individually before and after the treatments. The parameters were extracted from the segmented data in every 30 s. Based on previous research on the movement tracks [23], the following parameters were selected to characterize the movement patterns and were subsequently used as input data for training MLP (see section 2.3):

1) Speed (mm/s): average in movement distance of the fish during the observation time.

2) *Y*-position (mm/sec^2): the average distance in *Y*-axis measured from the surface during the observation time; as the specimens was located close to the surface, the *Y*-position was decreased
3) Stop number: the total frequency in which specimen did not move.
4) Stop duration (*total time of stops:* s): the total duration in which the specimen did not move.
5) Turning rate (rad/s): the sum of angle changes in radian in absolute values divided by the cumulated time duration of movement.
6) Meander (rad/mm): the sum of angle changes in radian in absolute values divided by the path length.

For calculating statistics of the parameters of the movement tracks, we selected 10 sample segments (30 s) by visual observation for each movement pattern. This process was repeated for 10 specimens. In total 100 segments were obtained for each pattern. Among the selected samples 30 segments were randomly chosen for statistical analysis. Subsequently 10 samples were independently selected for the MLP training by random sampling. For testing the trained MLP, the whole sequence of the movement data for four days (2 days separately for each 'before' and 'after' the treatments) were provided to the trained MLP. The data segments in every 30 s interval were continuously provided to be recognized by the trained network.

Medaka fishes were also reared in groups with four specimens in the same conditions applied to individual rearing in 10 replications. Fractal dimension (see section 2.4) was calculated for the movement data for specimens in individual and group rearing.

2.3 Multi-Layer Perceptron (MLP)

The MLP [26] was trained with the data for the movement tracks. Training proceeds to minimize the mean square error between the actual input and desired output (or target value) according to the back-propagation algorithm (Fig. 1) [18, 26]. In this study the parameters characterizing the movement tracks were used as input data (6 nodes), while the decision of the movement patterns were given in the binary form as matching output (6 nodes).

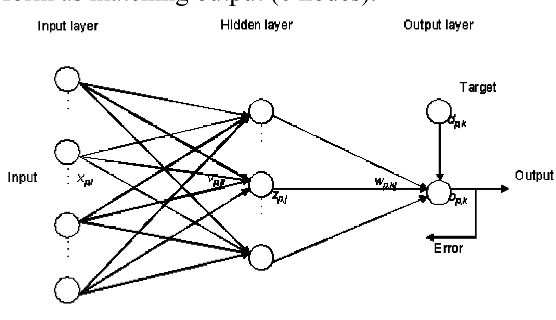

Figure 1: The schematic diagram of MLP.

The net input ($NET_{p,j}$) to neuron j of the hidden layer for pattern p is calculated as the summation of each input layer output ($X_{p\ i}$; input value of parameter) multiplied by weight (v_{ji}). The similar calculation is provided for the neuron k of the output layer being linked by summation of each hidden layer output ($Z_{p,j}$). An activation function (logistic function in this case) is applied to calculate the output of neuron j of the hidden layer ($Z_{p,j}$) and the output of neuron k of the output layer ($O_{p,k}$), according to the following eqn. (1):

$$f(NET) = \frac{1}{1+\exp(-\lambda NET)} \tag{1}$$

where λ is the activation function coefficient. NET is expressed either in $Z_{p,j}$ or $O_{p,k}$ as follows, eqn. (2), (3):

$$z_{p,j} = f\left(\sum_i x_{p,i} v_{ji}\right) \tag{2}$$

$$o_{p,k} = f\left(\sum_j z_{p,j} w_{kj}\right) \tag{3}$$

where v_{ji} and $w_{k,j}$ are the connection weight between neuron i of the input layer and neuron j of the hidden layer, and the connection weight between neuron j of the hidden layer and neuron k of the output layer, respectively.

The back-propagation algorithm adjusts the connection intensities (weights (v_{ji}) and ($w_{k,j}$)) of the network in a way that minimizes error. The sum of the errors in each neuron for pattern p, Err_p, is calculated as follows, eqn. (4):

$$Err_p = \frac{1}{2}\sum_k (d_{p,k} - o_{p,k})^2 \tag{4}$$

where $d_{p,k}$ is the target value corresponding to pattern p at neuron k. The value of the activation function coefficients, λ, used in this study was 1.0, and the learning coefficient, which updates the weights in iterative calculation, was set at 0.01. The level of error tolerance was 1.0, and the threshold for determining the binary level for the activation function was 0.5. Network pruning was not required during the training process in this study. Details of using MLP can be found in the related bibliographies [17, 18, 26, 27].

2.4 Fractal dimension

Fractal dimension, D, was measured on location of specimens in individual rearing. The points recorded in every 0.25 *sec* in 1-hour segment of the movement tracks were used for calculation based on the Box-Counting method (MATLAB® 5.3.), eqn. (5):

$$N(r) = (1/r)^D, \qquad D = \frac{\log N}{\log(1/r)} \tag{5}$$

where $N(r)$ is the number of points observed within the box sized as r^2. The two values, $N(r)$ and r are presented as a linear form by the double logarithmic graph.

In each 1-hour segment of the movement tracks, overlapping was allowed for 30 min.

Fractal dimension was also measured for the data from group rearing. The data points (0.25 s interval) of four specimens in the 15-minute segments were used for calculating fractal dimension. In each segment of the movement tracks overlapping was allowed for 30 min and 7.5 min for individual and group rearing respectively.

3 Results

3.1 Characterization of behavioral patterns

Behaviors of fish have been reported to show typical patterns, including stationary movement, up-down swimming with circular motion, eating, agonistic behavior, hiding, etc [28]. In this research we also observed some clear movement patterns of medakas under the experimental conditions. Figure 2 shows the typical movement patterns of the tested specimens.

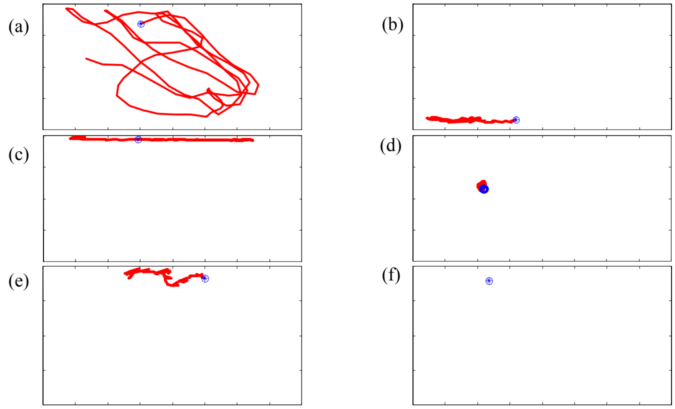

Figure 2: The movement tracks (side view) showing the behavioral patterns of medaka specimens in 30 s segments (a); Swimming, (b); Feeding, (c); Surface movement, (d); Slow movement, (e); Frequent stop, (f); No movement).

'Swimming' presented the active state of specimens (Fig. 2(a)), being characterized by the highest speed (65.82 mm/s) and wide circling in the observation aquarium (Table 1). 'Feeding' showed the horizontal movement along the bottom of the observation aquarium in the limited range (Fig. 2(b)). The *Y*-position (180.02 mm) of 'Feeding' is higher than any other patterns (Table 1). In the observation system, the position on *Y*-coordinate movement is higher as it is closer to the bottom of the aquarium. 'Surface movement' showed horizontal activity near the top area of the aquarium (Fig. 2(c)). In contrast to the

'Feeding', *Y*-position (15.56 mm) of 'Surface movement' was lower than any other patterns (Table 1). 'Slow movement' showed the lower phase of activity: the stop time (20.92 s) was longer than any other patterns (Table 1). 'Stop' is defined as the specimens maintaining the same position for the duration of 0.25 s in this study. Stop time is calculated as summation of the time duration for each stop. 'Frequent stop' is another pattern showing slow phase of activity. The specimens repeated the 'stop' and 'short advancement'. Stop number in 'Frequent stop' was observed as frequently as in 'Slow movement', however stop time was distinctively shorter in 'Frequent stop' (6.2 s) than in 'Slow movement' (20.92 s) (Table 1). Overall, 'Frequent stop' presented somewhat more active states compared with 'Slow movement'.

Table 1: Parameters characterizing the different movement patterns of medaka specimens before and after the treatments of copper (*n*=30 for each parameter for each pattern, (a); Swimming, (b); Feeding, (c); Surface movement, (d); Slow movement, (e); Frequent stop).

Parameters	Speed (*mm/s*)		Y-position (*mm*)		Stop time (*sec*)		Stop number		Turning rate (*rad/s*)		Meander (*rad/mm*)	
Patterns	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
a	65.8	12.4	90.7	16.4	0.3	0.5	0.7	1.2	1.7	0.3	0.0	0.0
b	11.2	2.4	180.2	3.4	7.7	4.0	11.7	2.7	4.4	0.8	0.3	0.1
c	17.5	9.8	15.6	7.2	7.2	5.0	11.2	6.0	2.9	1.2	0.2	0.1
d	3.6	1.1	97.9	82.1	20.9	3.3	13.1	4.8	5.9	1.0	0.5	0.1
e	10.0	2.0	107.6	39.3	6.2	3.5	14.2	4.8	5.0	1.4	0.4	0.1

Table 2: Analysis of variance (ANOVA) and Tukey test for multiple comparisons of parameters characterizing the different movement patterns of medaka specimens before and after the treatments (*n*=30 for each parameter for each pattern, (a); Swimming, (b); Feeding, (c); Surface movement, (d); Slow movement, (e); Frequent stop).

Parameters	F[1]	P	Comparison of parameters[2] (Tukey test, α=0.05)
Speed (*mm/s*)	368.01	<0.001	$a \neq c \neq b = e \neq d$
Y-position (*mm*)	59.38	<0.001	$b \neq e = d = a \neq c$
Stop time (*sec*)	133.75	<0.001	$d \neq b = c = e \neq a$
Stop number	48.99	<0.001	$e = d = b = c \neq a$
Turning rate (*rad/s*)	80.11	<0.001	$d \neq e = b \neq a = c$
Meander (*rad/mm*)	112.23	<0.001	$d \neq e = b \neq c = a$

[1] $F_{0.05(2), 5, 150} = 2.66$

[2] Patterns were listed in the increasing order from left to right.

The parameters mostly appeared to be statistically different in different movement patterns according to the Tukey test [29] (Table 2). Regarding speed, ‘Swimming’, ‘Surface movement’ and ‘Slow movement’ were different among the patterns, but ‘Frequent stop’ and ‘Feeding’ were in the same range. ‘Feeding’ and ‘Frequent stop’ were also similar in the other parameters including stop time, stop number, turning rate and meander. *Y*-position, however, was different between the two patterns. The other movement patterns were uniquely distinguished each other and were statistically different (Table 2).

3.2 MLP applied to individual data

The parameters characterizing the movement patterns were effectively learned by MLP with the training rates mostly over 92%. As stated before the whole data set was used for testing. The pattern of input segment (30 s) was recognized by the trained MLP. Detection rates were calculated as the number of correct recognition divided the number of the total recognition for each individual. The detection rate for each specimen was subsequently averaged with 10 specimens. Table 3 shows changes in detection rates (%) for each pattern in averages for 10 specimens before and after the treatments.

Table 3: Detection rate (%) of different movement patterns of medaka specimens before and after the treatment of copper (a; Swimming, b; Feeding, c; Surface movement, d; Slow movement, e; Frequent stop, f; No movement). (n=10).

Treatments	Patterns	Day		Night		All	
		Mean	SD	Mean	SD	Mean	SD
Before Treatment	a	31.48%	30.21	10.79%	17.93	21.13%	26.41
	b	13.10%	11.24	7.91%	6.84	10.50%	9.44
	c	6.56%	10.21	7.74%	5.07	7.34%	7.73
	d	5.80%	5.25	10.70%	5.68	8.25%	5.89
	e	4.24%	3.75	9.12%	9.12	6.68%	7.23
	f	6.19%	7.89	20.26%	19.55	13.22%	16.21
After Treatment	a	12.51%	13.25	3.51%	3.49	8.01%	10.5
	b	8.21%	5.93	3.23%	2.72	5.72%	5.17
	c	3.56%	3.94	2.45%	1.72	3.01%	3.01
	d	14.91%	7.33	19.45%	5.18	17.18%	6.6
	d	9.21%	5.87	8.77%	5.81	8.99%	5.69
	f	21.88%	11.37	37.93%	9.41	29.21%	13.07

Before the treatments, detection rate of ‘Swimming’ pattern was high with 21.13%, but the rate decreased to 8.01% after the treatments (Table 3). The ‘Feeding’ (from 10. 50% to 5.72%) and ‘Surface movement’ (from 7.34% to

3.01%) patterns also accordingly decreased. In contrast, detection rates for 'Slow movement' (from 8.25% to 17.18%), 'Frequent stop' (form 6.68% to 8.99%) and 'No movement' (from 13.22% to 29.21%) increased after the treatments of copper (Table 3). In general, detection rates for the patterns representing high activity (e.g., 'Swimming', 'Feeding' etc) were decreased after the treatments.

Higher variation was observed in the detection rates obtained by the trained network. Standard deviations in detection rates were in the higher range, and all the parameters were not distinguished with statistical tests. Table 4 shows the comparison of detection rates (%) before and after the treatments based on the *t*-test (n=10 for each pattern) in different light phases. The patterns of 'Slow movement' and 'No movement' were statistically significant accordingly in photo- and scoto-phase, and the total periods (Table 4). Although the average values showed differences, 'Swimming', 'Feeding' and 'Frequent stop' were not statistically different before and after the treatments. The trends of behavioral changes were similar in scoto- and photo-phases. 'Surface movement' was only different at night before and after the treatments (Table 4).

Table 4: Comparison of detection rates (%) in different movement patters before and after the treatments based on the t-test (n= 10 for each pattern, a; Swimming, b; Feeding, c; Surface movement, d; Slow movement, e; Frequent stop, f; No movement).

	Photophase		Scotophase		Total period	
	t	*P*	t	*P*	T	*P*
a	1.818	n.s.	1.259	n.s.	1.666	n.s.
b	1.215	n.s.	2.012	n.s.	1.646	n.s.
c	0.992	n.s.	3.123	$0.01<P<0.02$	1.815	n.s.
d	3.193	$0.01<P<0.02$	3.599	$0.005<P<0.01$	3.836	$0.002<P<0.005$
e	2.255	n.s.	0.100	n.s.	0.912	n.s.
f	3.587	$0.005<P<0.01$	2.576	$0.05<P<0.02$	3.293	$0.005<P<0.01$

$t_{0.05(2),9} = 2.262$

3.3 Fractal dimension applied to individual rearing

In contrast to the results from MLP, fractal dimension showed more consistency in revealing changes in behavioral states of medaka specimens after the treatments of copper (Fig. 3). Although there were individual variations, decrease in fractal dimension appeared consistently for all the tested specimens. The average in fractal dimension was 1.62 ± 0.10 before the treatments, but decreased to 1.42 ± 0.16 after the treatments. The Nested ANOVA indicated that the values of fractal dimension were statistically different between 'before' and 'after' the treatments (df = (1, 18), F = 6.2, $0.02<P<0.05$). The sub-group of individual specimens, however, was different (df = (18, 600), F = 3.07, $P<0.001$). This indicated that individual variation existed in the values of fractal dimension.

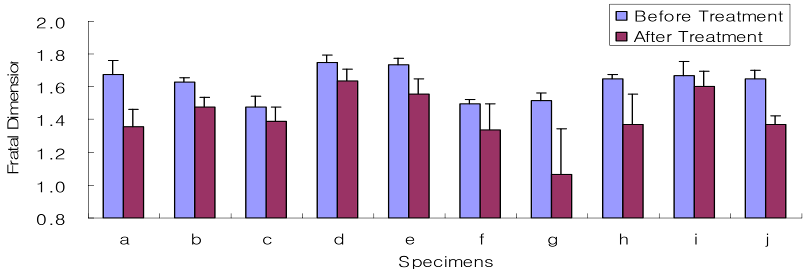

Figure 3: Fractal dimension of the movement points in different specimens of medaka obtained from individual rearing before and after the treatments of copper, 1 mg/L.

3.4 Fractal dimension applied to group rearing

We further analyzed fractal dimension of the movement points when the specimens were reared in groups of 4 specimens (Fig. 4). Fractal dimension consistently decreased after the treatments of copper (1 mg/L) in different groups, being similar to the case of individual rearing. The average of fractal dimension was 1.63 ± 0.02 before the treatments and 1.46 ± 0.07 after the treatments. The values of fractal dimension from group rearing were more consistent compared with individual rearing. The Nested ANOVA showed that the values of fractal dimension were significantly different between the treatments (df = (1, 18), F = 23.35, $P<0.001$). In contrast to the case of individual rearing, however, the values of fractal dimension were also in the similar range between the tested groups: the sub-group difference was not significant (df = (18, 820), F = 0.05, $P>0.5$). This indicated that individual variation in fractal dimension could be minimized through group rearing of fishes.

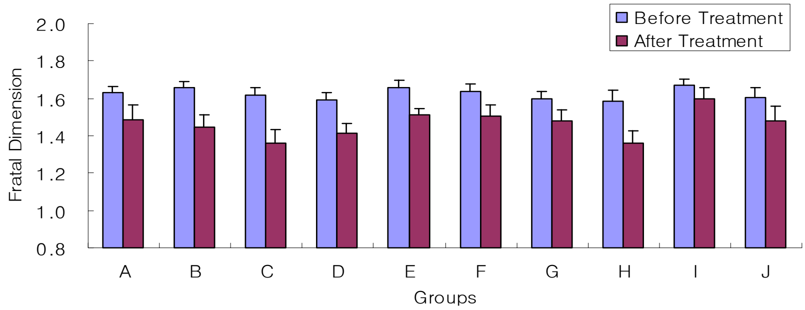

Figure 4: Fractal dimension of the movement points in different groups of the 4 medaka specimens before and after the treatments of copper, 1.0 mg/L.

4 Discussion and conclusions

A computational system was developed for automatically detecting the movement states of medaka specimens in this study. Although individual variation occurred, MLP was useful for detecting movement patterns explicitly. The specific patterns such as 'Slow movement' and 'No movement' were statistically different before and after the treatments (Table 3). These patterns could be used as indicator patterns of medakas for detecting presence of toxic substance in environment.

We further showed that the higher variation in individuals could be decreased by using fractal dimension. The values of fractal dimension appeared to consistently decrease for all the tested specimens after the treatments (Fig. 3). The group testing, consisting of 4 medaka fishes, further minimized the variation of fractal dimension by showing no statistical difference among different groups (Fig. 4). Consistency in the measurement of fractal dimension was revealed in comparing Coefficient of Variation (CV: standard deviation divided by mean) (Fig. 5). CVs for group rearing were lower for both 'before' and 'after' the treatments. The difference between individual and group rearing was more clearly observed after the treatments with the statistical significance (df= (1, 18), $F = 6.01$, $0.02<P<0.05$). The statistical difference was not observed for CVs between individual and group rearing before the treatments (df= (1, 18), $F = 0.66$, $P>0.5$), however the average value was lower for group rearing (Fig. 5).

This study indicated that fractal dimension based on group rearing could be used as a reliable parameter to indicate behavioral changes of medakas after the treatments of copper. Another advantage of fractal dimension is the flexibility in recording data points in group rearing. In the image processing system, it is in general difficult to trace the movement tracks for each specimen in group rearing especially if the specimens are small in size. Fractal dimension, however, was measured from the positions of the specimens collectively, and tracing each individual movement was not necessary in this case.

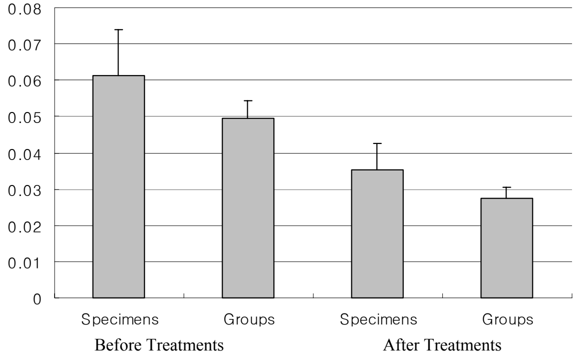

Figure 5: Comparison of CVs of fractal dimension in individual and group rearing before and after the treatments of copper, 1.0 mg/L.

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In real situation, MLP and fractal dimension could be used in combination for providing more practical information for warning system in risk assessment. While fractal dimension would provide information on the global change in behavioral states more consistently in a compressed form, MLP would reveal differences in the specific patterns, thus providing more detailed information on explicit response behaviors. The two methods could be combined to produce an efficient monitoring system for *in-situ* risk assessment in aquatic systems in the future.

In this study we used copper as toxic substances. Copper plays an essential role in mitochondrial function, detoxification of free radicals, neurotransmitter synthesis, cross-linking of connective tissue, and cellular iron metabolism. Copper causes mutations in genes encoding “P-type” transport ATPase and induces neurotic disease such as Lou Gehrig’s and Wilson’s disease [30]. The toxicological impact would consequently produce stressful responding behaviors of the organisms. Toxic responses to copper have been reported on some indicator species. Activity accordingly decreased in *Daphnia magna* and *Gammarus* [31, 32]. Not much quantitative research, however, has been conducted on behavioral changes especially on vertebrates such as fish. In this study, we demonstrated that computational methods such as MLP and fractal dimension could be efficiently used for monitoring contamination of copper by using fish as indicator specimens.

In conclusion, MLP could accommodate local information on response behaviors and would be useful for detecting changes in specific patterns. The consistency in behavioral detection was achieved by fractal dimension especially through group rearing, and the parameter could be useful source of a reliable indicator in determining behavioral states of specimens exposed to toxic chemicals. MLP and fractal dimension could be used in combination as an efficient means of *in-situ* monitoring by providing both ‘local and more specific’ (i.e., MLP), and ‘global and more consistent’ (i.e., fractal dimension) information concurrently.

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