독성물질 아치사량 처리에 대한 생물개체의 행동 모니터링 및 이동궤적에 대한 수리적 분석

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Behavioral Monitoring and Computational Analysis of the Locomotive Tracks of Organisms in Response to Sub-Lethal Treatments of Toxic Chemicals

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SUMMARY

Recently behavioral responses to sub-lethal doses of toxic chemicals have drawn attentions regarding *in situ* biomonitoring of toxic chemicals. The response behaviors were characteristically variable, depending upon the different chemicals and test animals. When medaka was treated with diazinon (0.1 mg/L), the fish showed some typical patterns such as irregular up-and-down movement and repetitive back-and-forth movements with irregular turns. Characteristic patterns also appeared after treated with carbofuran (0.1 mg/L) such as the patterns of saw teeth, burst swimming with nipping, etc. The larvae of an aquatic midge, *Chironomus flaviplumus*, showed more frequent movement patterns of ventilation after treated with carbofuran (0.1 mg/L). The complex data for the locomotive tracks were further analyzed through the Two-Dimensional Fast Fourier Transform and the different characteristics of the movement observed with the different insecticides were effectively represented through the transform. Behavioral characteristics recognized by the track recognition system could be an alternative tool to detect chemicals in environment.

INTRODUCTION

Recently the researches on the sub-lethal effects of toxic substances have been accumulated rapidly, and have been reported on various taxa, crustaceans (St-Amand, 1999; Roast *et al.*, 2000), snails (Ibrahim *et al.*, 1992) and fish (Gray *et al.*, 1999). Behavioral responses have been reported to be sensitive to sub-lethal exposures to various chemical pollutants (Dutta *et al.*, 1992; Lemly and Smith, 1986).

In parallel with behavioral study, techniques of quantitative measurements also have been rapidly developed recently. The movement tracks of fish have been continuously observed for the signs of toxic substances through video taping based on characters such as rheotaxis disorientation. Lorenz *et al.* (1995) recorded movement to characterize locomotive patterns to detect the effect of atrazine on fish. Lee and Lee (1996) developed a system to detect irregular movement of carp to an acute level of diazinon. However, these studies were mainly based on simple activity and positional data such as the average movement velocity, occupation time at certain positions, etc.

In actual field or semi-natural conditions, however, there is a high degree of variability and complexity in the total shape observed in locomotive tracks. In this case the information based on simple velocity and position measurement are not sufficient to characterize the overall shape of the observed locomotive tracks. They do not carry further information on expressing attributes on the locomotive tracks produced by responding organisms such as how the locomotive tracks would look like, circular, linear, curved, etc. In this study we tried to emphasize to extract information out of overall shape of movement tracks and how it could be verified in detecting the impact of external stimuli to organisms by implementing a computational method.

MATERIALS AND METHODS

Medaka stock populations were fed flake diet (Tetramin) at the water temperature 23±1℃ in a glass tank. Test organisms (age: 6-12month) were randomly chosen and placed individually in the 5 liter glass aquarium (25×10×25 cm3), and were continuously observed in vertical position through the observation system for 26 hours, for 2 hours without treatment and subsequently for 24 hours after the treatment of the insecticides.

Diazinon (O,O-diethyl O-2-isopropyl-4-methyl-6-pyrimidyl thiophosphate) and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzo-furanol methylcarbamate) were applied in sublethal doses to medaka in semi-natural conditions. With preliminary tests we selected lowest level of observing response behavior against the treatments of diazinon and carbofuran. Diazinon (LC50 5 mg/L; Kim *et al* 1999), dissolved in DMSO (0.025 mg/l), was applied directly into an aquarium accommodating an individual of medaka adult at the concentration of 0.1 mg/L. In the similar manner, carbofuran, dissolved in water, was applied at the concentration of 0.1 mg/l. Thirteen individuals were observed at the given concentrations of the insecticides respectively.

Activities of a midge, *Chironomus flaviplumus* (Chironomidae), were also traced after the treatment of carbofuran (0.1 mg/L) for four days (1 day: before treatment, 3 days: after treatment). The fourth instar larvae were placed in an observation cage (6×7×2.5cm3) at temperature of 18℃ and at the light condition of 10LL:14DD. The tracking system was devised to detect the instant, partial movement of the insect body in the case of chironomid.

The observation system consists of test aquarium, camera, time generator, A/D interface, and a series of software for image recognition system (Fig. 1). The test organisms observed with CCTV camera were scanned continuously every 0.25 second during the observation time. Analog data captured by the camera were digitized by using a video overlay board, and were passed to the image recognition system to locate the target organism in spatial and time domain.

The Locomotive tracks of the tested individuals were recorded at 0.25 sec intervals and analyzed for every continuous segments for 2 and 10 minutes to medaka and chironomid respectively. Two-Dimensional Fast Fourier Transform (2D-FFT) was further implemented to quantitatively represent response behavior of locomotion. 2D-FFT transformed the data recorded in time domain to frequency domain to bring out and emphasize the specific characters of the locomotive traces in two-dimensional images. The intensity of image expressed in gray level *X(k1, k2)* at the location of *k*1 and *k*2 on the *x* and *y* coordinate is transformed as follows:

$$X(k_1,k_2)=\sum_{n_1=0}^{N_1-1}\sum_{n_2=0}^{N_2-1}x(n_1,n_2)e^{-j(2\pi/N_1)k_1n_1}e^{-j(2\pi/N_2)k_2n_2}$$

$$0\le k_1\le N_1-1,\quad 0\le k_2\le N_2-1$$

where *N1* and *N2* represent size of image in locomotive tracks in two-dimension in frequency domain, *n1* and *n2* are coordinates in Frourier transform. Details of Fourier Transform could be referred to Oppenheim and Schafer (1975).

RESULTS AND DISCUSSION

Although the responses were variable, it was possible to observe some characteristic patterns of the treated specimens. When the test individuals of medaka were not treated with insecticides, the movement patterns (in vertical view) appeared to be generally smoother and linear. When the fish moves actively, it usually spanned a larger area in the observation aquarium without a large number of intermittent stops (Fig. 2a). The untreated individuals also showed more active phase, frequently crossing the aquarium in a diagonal shape and following similar routes.

Fig. 2c shows an example pattern when the test fish was treated with diazinon. The locomotive tracks were contrasted with the irregular down-and-up movements and small-scale irregular turns at the corners of the tracks. In some case, the locomotive tracks resembled "entanglements of thread" with repeated irregular turns. The treated individuals also characteristically stayed for a longer time at the upper layer of the test aquarium, and sometimes the range of movement was shortened.

The treatment of carbofuran also showed distinctive patterns of locomotion. The treated individuals swam frequently in a small area of the test aquarium. One example of typical response pattern was the motions of stop-and-nipping, and this consequently produced rougher traces, a shape like the saw teeth (Fig. 2e). Another characteristic pattern appeared with the carbofuran treatment was the rapid up-and-down movements. At down position, the fish concentrated on one corner of the observation aquarium.

In case of midge, activities were in general high and spanned a large area of the observation cage (Fig. 3a). After the treatment these typical patterns generally disappeared while the ventilation activity, appearing as the shape of compressed zig-zag, was frequently observed in photo- and scoto-phases. One typical example was the directional movement continuously shaken by the small back-and-forth movement (Fig. 3c). The activity of the test individuals was generally depressed after the chemical treatment.

The observed locomotive traces were further transformed in 2-D FFT mapping in frequency domain. Generally when the untreated medaka was in active phase the transformed pattern appeared as a radial shape (Fig. 2b). When the treated fish showed down-and-up movements with the diazinon treatment (Figs. 2c), the transformed pattern appeared in an unbalanced radial shapes, which indicated movements in partial direction (Fig. 2d). The treatment of carbofuran (Fig. 2e) also showed distinctive patterns of the transformed map. The radiation unevenly covered the map area, and the radiation lines consisted of collection of small spots (Fig. 2f). The transformed map before (Fig. 3a) and after (Fig. 3b) treatment of carbofuran to midge also characteristically different. While the map for the untreatment showed more spots (Fig. 3b), the map for the treatment was compacted with linear components.

The tested insecticides are anticholinesterase and affects nervous system of tested organisms. The results clearly showed that locomotive behavior consequently reflected disturbed status of nervous system of the test organisms after the treatment of insecticides. The response patterns in the locomotive tracks appeared differently according to different insecticides, although their toxic mechanisms are similar as anti-choline esterase as shown in Figs. 2 and 3. This study also demonstrated that behavioral characteristics detected through computational method could effectively characterize the response patterns.

REFERENCES

Dutta, H., J. Marcelino, and Ch. Richmonds. 1992. *Arch Intern Physiol Biochem Biophys.* 100(5):331-334.

Gray, M.A., K.L. Teather, and C.D. Metcalfe. 1999. *Environ Toxicol Chem.* 18(11):2587-2594.

Ibrahim, W.L.F., P. Furu, A.M. Ibrahim, and N.Ø. Christensen. 1992. *J Helminthol.* 66:79-88.

Kim, J.S., S.C. Koh, S.K. Lee, and T.S. Chon. 1999. *Kor J Environ Toxicol.* 14(3):81-85.

Lee, S.E., and S.K. Lee. 1996. *J KSWO* 12(2):119-126.

Lemly, A.D., and R.J. Smith. 1986. *Ecotoxicol Environ Saf.* 11(2):210-218.

Lorenz, R., O.H. Spieser, and C. Steinberg. 1995. *Acta Hydrochim Hydrobiol.* 23:197-201.

Oppenheim, A.V., and R.W. Schafer. 1975. *Digital Signal Processing* (Prentice Hall Inc.) pp. 87-135.

Roast, S.D., J. Widdows, and M.B. Jones. 2000. *Aquat Toxicol.* 47:227-241.

St-Amand, L., R. Gagnon, T.T. Packard, and C. Savenkoff. 1999. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol.* 112:33-43.

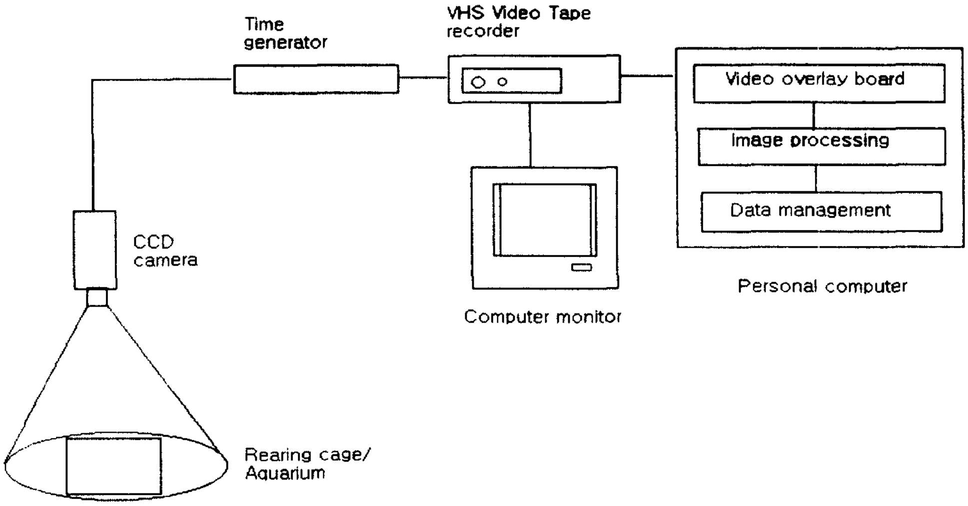

Fig. 1. An observation system designed for automatic detection of the locomotive tracks of organisms in response to sub-lethal toxic substances.

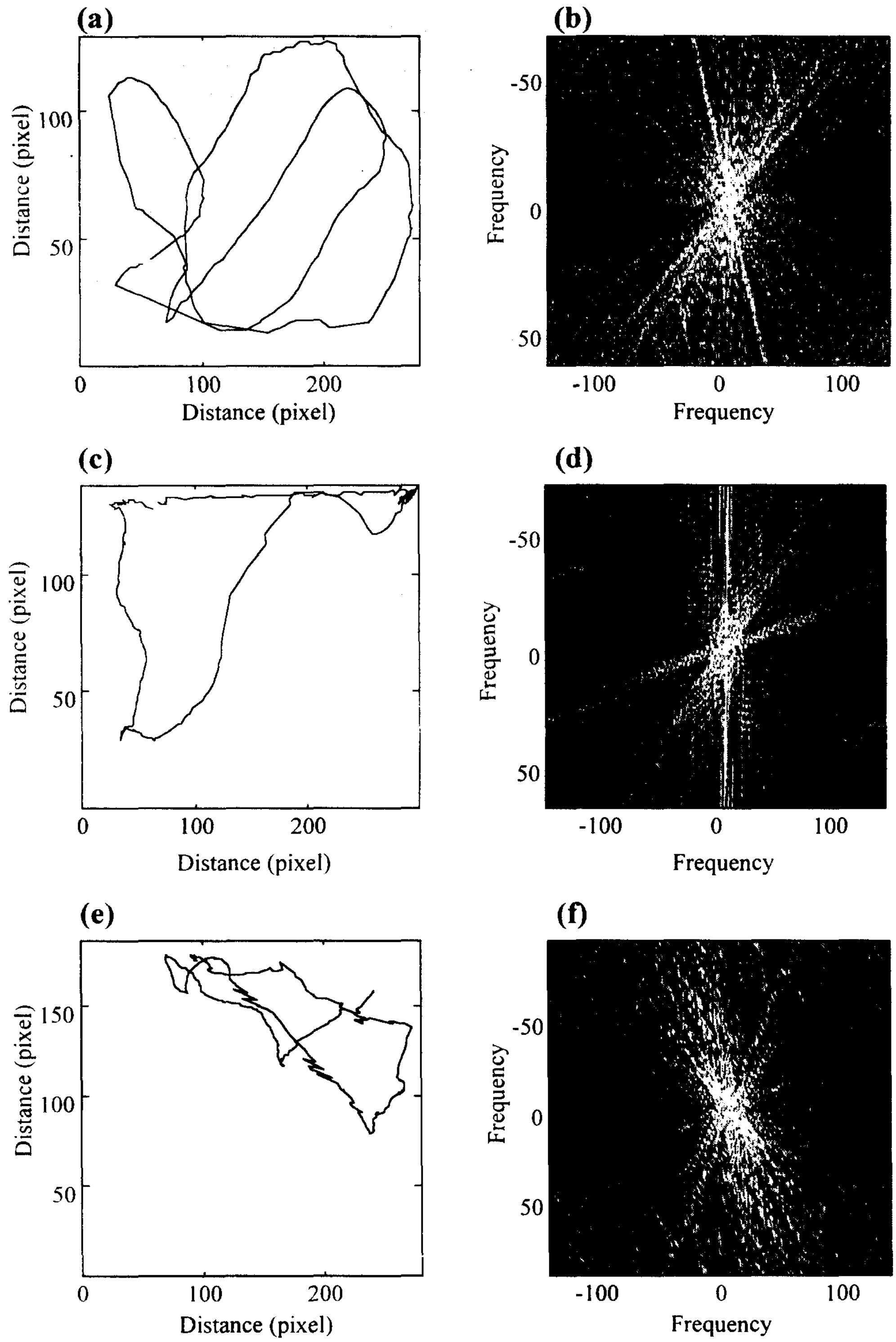

Fig. 2. The 2-Dimension image of locomotive tracks (a, c, and e) of medaka (*Oryzias latipes*) and their corresponding 2D-FFT mapping (b, d, and f). (a)-(b) when not treated with the insecticides, (c)-(d) when treated with 0.1 mg/L diazinon, (e)-(f) when treated with 0.1 mg/L carbofuran.

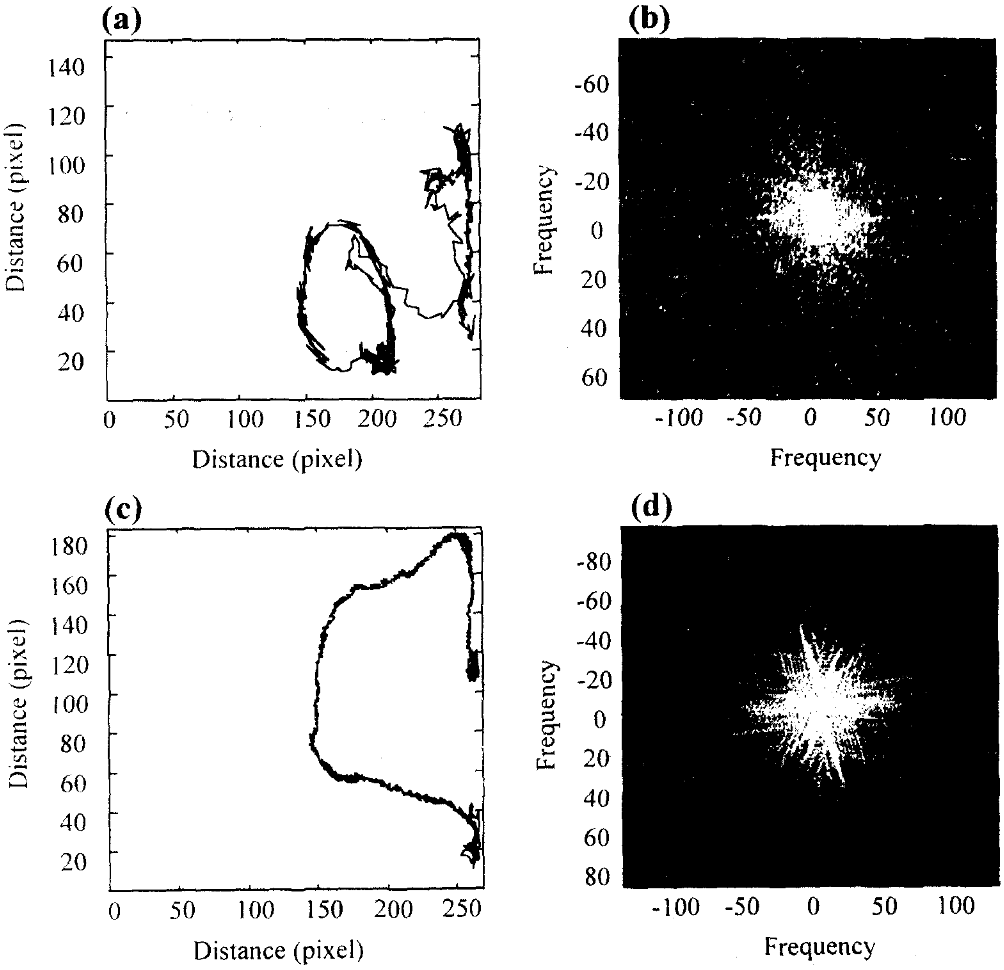

Fig. 3. The 2-Dimension image of locomotive tracks (a and c) of midge (*Chironomus flaviplumus*) and their corresponding 2D-FFT mapping (b and d). (a)-(b) when not treated with the insecticide, (c)-(d) when treated with 0.1 mg/L carbofuran.