Bioassay Using Daphnia Daphnia Trajectories

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journal or publication title	Proceedings, International Symposium of the Kanazawa University 22st-Century COE Program
volume	1
page range	369-370
year	2003-03-16
URL	http://hdl.handle.net/2297/6431

Proceedings: International Symposium of the Kanazawa University 21st-Century COE Program Vol. 1 (2003)
Edited by Naoto KAMATA

Bioassay Using *Daphnia* Trajectories

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Abstract - The effect of cupper salt concentration on the trajectories of *Daphnia magna* was investigated, and the significant difference was observed between 0.02 mg l^{-1} cupper solution and the control.

I. Introduction

Daphnia species has been employed extensively in the environmental monitoring of toxic chemicals [1, 2]. Shimizu et al. have shown the possibility of the application of *Daphnia* trajectories to bioassay [3]. In this paper, we investigated the applicability of *Daphnia* trajectory analysis to the detection of toxicity of cupper ions.

II. Materials and Methods.

A. *Culture Methods*

A single clone of *Daphnia magna* was used in this study. Culture conditions were adapted from protocols for ASTM standards (American Society of Testing and Materials, 1986). The culture medium was synthetic freshwater (48 mg of $NaHCO_3$, 30mg of $CaSO_4 \cdot 2H_2O$, 30 mg of $MgSO_4$, and 2 mg of KCl per liter of deionized water adjusted to a pH of 7.4) with a hardness of 45 mg of $CaCO_3$ per litter. Culture vessels were 3-L clear glass jars that were filled with approximately 2 L of culture medium. The density of *Daphnia* in the vessel was kept below 30 animals per litter. *Daphnia* were fed daily with a mixture of commercial fish food and dry yeast, prepared according to an ASTM protocol. *Daphnia* were kept under a light:dark period of 16 h:8 h at 20±1 °C. All salt reagents were of analytical grade.

B. *Analysis of Swimming Trajector*

Experimental setup is shown in Fig. 1. *Daphnia magna* less than 24 h old was chosen from the stock culture and transferred to a temperature controlled (20±1 °C, with EYELA: CTP101) observation chamber (30 mm in diameter), filled with 4 ml of the ASTM solution containing various amount of cupper salt ($CuSO_4 \cdot H_2O$; Wako Pure Chemical Industries, Osaka, Japan). *Daphnia* swimming behavior was analyzed with a digital motion analysis system, consisting of digital microscope (KEYENCE : VH-6300), a video capturing board, and a personal computer with motion analysis software.

At one consecutive measurement, the position of *Daphnia magna* (*x*, *y* coordinate) was recorded for 60 seconds at the interval of 0.1 seconds (600 frames). This procedure was repeated at the interval of 5 to 15 minutes.

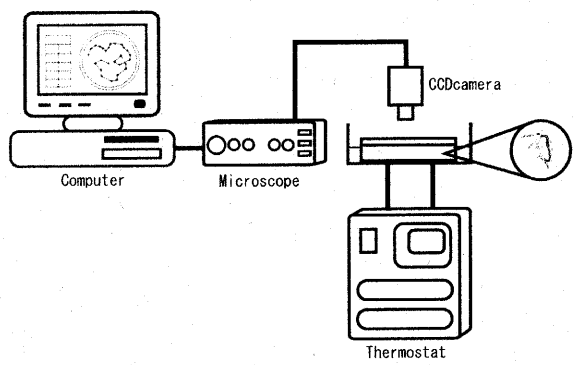

Fig. 1 Experimental apparatus.

C. *Calculation of Fractal Based Parameters*

We used a modified dividers method for the calculation of the fractal dimension of the Daphnia trajectories.

In the original dividers method, the length of the system *L* was evaluated by the number of the steps n_ε required to cover the system by the divider of length ε, then the length *L* as a function of ε is expressed as follows.

$$L(\varepsilon) = n_\varepsilon \varepsilon \tag{1}$$

If the system is a fractal, there is a relationship between $L(\varepsilon)$ and the ε as follows.

$$L(\varepsilon) = A\varepsilon^{1-D} \tag{2}$$

where *D* is the fractal dimension and *A* is a constant.

In our experiments, however, we were not able to use dividers of constant lengths. Instead we used the intervals of segment number, *n*, for the measure of the length. The length of the *Daphnia* trajectories are evaluated via

$$L = \sum_{j=1}^{N/n} \left\{ (x_{nj} - x_{n(j-1)})^2 + (y_{nj} - y_{n(j-1)})^2 \right\}^{0.5} \qquad (3)$$

where N is the total number of the segments, and was 600 in this research, x_i and y_i are the coordinate of the *Daphnia* at *i*th segment.

Then, there is a relationship between n and L such that

$$L = Bn^{1-D'} \qquad (4)$$

where B is a constant and D' is a parameter which can be related to D as follows.

$$D = \frac{1}{2 - D'} \qquad (5)$$

III. Results and Discussion

Fig. 1 shows the relationship between the intervals of segment number n and the length of the system evaluated by Eq. (3). For n smaller than 10, the relationship in double-log plot showed linearity, and we were able to estimate the parameter D' from the slopes in double-log plots.

Figs. 2 shows the change in parameter D' at various concentration of cupper salt. It is clearly seen that the parameter D' began to increase tens of minutes after *Daphnia magna* was put into the solution containing the cupper salt. Also, it is seen that it took more time before the parameter D' showed significant difference from the control for solutions having less amount of cupper salt.

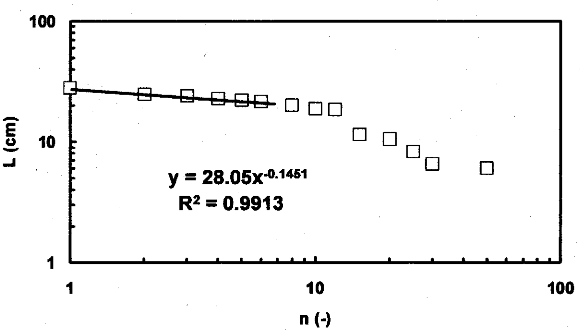

Fig. 1. Determination of parameter D'.

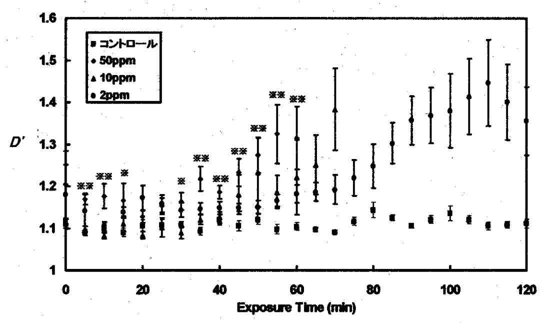

Fig. 2. Change in D' at various cupper concentration.

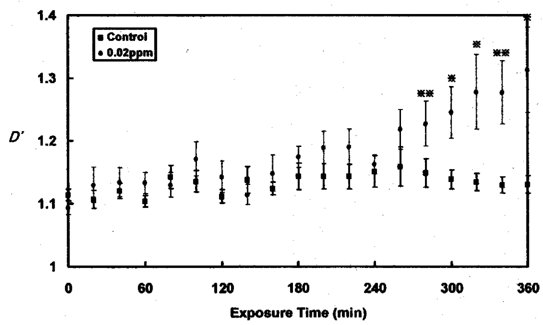

Fig. 3. Change in D' at 0.02 ppm of cupper.

Reported EC_{50} concentrations of cupper detected by *Daphnia magna* were at around 0.02 ppm. Fig. 3 shows that the difference of D' between the cupper solution of this concentration and control became significant about 5 hours after *Daphnia magna* was put into the toxic solution. (Note that the traditional EC_{50} test will take 48 hours before getting results.) This suggests that by using *Daphnia* trajectories, we may be able to establish a more efficient bioassay than EC_{50} analysis.

References

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[3] N. Shimizu, C. Ogino, T. Kawanishi, Y. Hayashi, "Fractal analysis of *Daphnia* motion for acute toxicity bioassay," *Environmental Toxicology* Vol. 17, pp. 441-448, 2002.