Modern Physics Letters B, Vol. 25, Nos. 12 & 13 (2011) 1143–1149
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DOI: 10.1142/S0217984911026851

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UNRAVELING MARKOV PROCESSES IN MOVEMENT PATTERNS OF INDICATOR SPECIES IN RESPONSE TO CHEMICAL STRESSORS

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Revealing biological responses of organisms in responding to environmental stressors is the critical issue in contemporary ecological sciences. Markov processes in behavioral data were unraveled by utilizing the hidden Markov model (HMM). Individual organisms of daphnia (*Daphnia magna*) and zebrafish (*Danio rerio*) were exposed to diazinon at low concentrations. The transition probability matrix (TPM) and the emission probability matrix (EPM) were accordingly estimated by training with the HMM and were verified before and after the treatments with 10^{-6} tolerance in 10^3 iterations. Structured property in behavioral changes was accordingly revealed to characterize dynamic processes in movement patterns. Parameters and sequences produced through the HMM training could be a suitable means of monitoring toxic chemicals in environment.

Keywords: Hidden Markov model; movement behaviors; zebrafish, daphnia.

1. Introduction

Revealing biological responses of organisms in responding to environmental stressors (e.g. pollution, climate change) garners special attention in ecological sciences recently. Due to complexity and the large amount of data, however, continuous behavioral recordings are difficult to analyze. Parametric and statistical methods have been conventionally applied to analysis of response behaviors, covering auto-correlation coefficients,[1] fractal dimensions,[2] and statistical discrimination of motion.[3] Recently, heuristic methods were also implemented to information extraction with multi-layer perception,[4] self-organizing map (SOM),[5] and wavelet.[6]

In order to elucidate dynamic processes in behavioral changes, the hidden Markov model (HMM) has been applied to behavioral changes of indicator species.[7,8] The advantage of HMMs is to predict the optimal state sequence (i.e., behavioral changes) from the observed data[9] (i.e. measurable values). HMMs have been reported in various fields including speech recognition,[9] and bioinformatics.[10] Since Chatfield and Lemon[11] applied HMMs to behavioral sequences, HMMs have been applied to ecology, ranging from communities,[12] populations,[13] to individuals (behavior).[7,8,14]

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Recently, Liu *et al.*[8] applied the HMM to analysis of response behaviors of daphnia after exposure to an insecticide. A heuristic method (i.e. SOM) was used for determining behavioral states, and linear speed was considered as events in Liu *et al.*[8] In this study, we focused on defining behavioral states with more basic components in movement, linear and angular speed, and movement direction as behavioral events. Based on the basic components of movement, structured properties in dynamic processes of behavioral changes were disclosed before and after the treatments of the chemicals.

2. Material and Method

2.1. *Test species and observation*

Zebrafish (*Danio rerio*) and daphnia (*Daphnia magna*) were used as test species in this study. Both species are sensitive to chemicals and have been widely used for monitoring in risk assessment.[15] Diazinon (O, O-diethyl-O-(2-isopropyl-6-methyl-pyrimidine-4-yl) phosphorothioate) was selected as the test chemical because it has been suspected of being one of the major sources of insecticides that contaminate natural waters.[16] Rearing and observation conditions of zebrafish and daphnia followed Refs. 8 and 17.

The trajectories of zebrafish and daphnia in two dimensions were continuously recorded in observation aquariums ($40\times20\times10$ cm^3 for zebrafish, and $6\times5\times1$ cm^3 for daphnia) for 24 hours before and 24 hours after the treatment of diazinon at concentration of 1.0 ppb. The positions of test organisms were recorded at 0.25 s time intervals according to Refs. 2, 8 and 17. In total, 50 individuals were observed for zebrafish and daphnia separately. We randomly chose 10,000 continuous movement segments (0.25 s) from 50 individuals separately before and after the chemical treatments for both species.

2.2. *HMMs*

HMMs are used to model a pair of complementary stochastic processes.[18,19] The first process is represented by a set of unobserved states (S_i), also called hidden or internal states. The probability distribution that models states transition is represented via a transition probability matrix (TPM) $A=\{a_{ij}\}$ with $a_{ij}=P(q_{t+1}\mid q_t=S_i), 1\le i,j\le N$ in which N is the number of states, q_t is the current state of the system, and is the set of hidden states. The second stochastic process presents the probability of observing or measuring some predetermined values given that the system is in a specific hidden state. A sequence of T observations is represented by a set $O=O_1,O_2,O_3,\ldots,O_t,\ldots,O_T$ where each element O_t is a member of the symbols set $V = v_1,v_2,\ldots,v_M$. The Emission probability matrix (EPM) of any symbol given an internal state j is defined by a matrix $B=\{b_i(k)\}$ with $b_j(k)=P(O_t=v_k\mid q_t=S_i), 1\le j\le N, 1\le k\le M$.

In this study, different levels of linear and angular speed were considered as criteria for determining behavioral states of test organisms, while the movement directions (left, right, and straight) were considered as events.[7,20] Four states were classified according to the level of linear and angular speed for two species (Table 1). The initial probability in TPM assumed equal for each state (0.25). Initial EPM based on the experimental observation was obtained from 10,000 segments separately before and after the treatments (Table 2). The left, right, and straight movements were defined by the positive, negative, and 0 angular speeds. The parameters (TPM and EPM) were resolved by the Forward-Backward algorithms.[19] The HMM toolbox in Matlab (MATLAB 7.8, The MathWorks, R2009) was used to calculate the estimated TPMs and EPMs. The estimated TPMs and EPMs were evaluated with the tolerance level equal to 10^{-6} in 10^{3} iterations according to Ref. 10.

Table 1. The definition of behavioral states depending on linear and angular speed in movement segment in 0.25 s.

Species	State Component	1	2	3	4
Daphnia	Linear (mm/s)	≥ 7.5	≥ 7.5	< 7.5	< 7.5
	Absolute angular (rad/s)	≥ 5	< 5	≥ 5	< 5
Zebrafish	Linear (mm/s)	≥ 100	≥ 100	< 100	< 100
	Absolute angular (rad/s)	≥ 5	< 5	≥ 5	< 5

Table. 2. Experimental EPM (*L* – left, *R* – right, *S* – straight) when the movement data were combined before and after the treatments: (a) Daphnia, (b) Zebrafish.

(a)

Event State	*L*	*R*	*S*	Total
1	0.38	0.62	0.00	1.00
2	0.11	0.89	0.00	1.00
3	0.45	0.39	0.16	1.00
4	0.24	0.34	0.42	1.00

(b)

Event State	*L*	*R*	*S*	Total
1	0.47	0.53	0.00	1.00
2	0.14	0.86	0.00	1.00
3	0.43	0.37	0.20	1.00
4	0.25	0.33	0.42	1.00

3. Results

The histograms of turning angles of zebrafish and daphnia accordingly presented the effect of chemical treatments (Fig. 1). The shape of histogram followed the Gaussian distribution before the treatments for both species (Goodness of fit: *R-square* 0.9939 for daphnia, 0.9934 for zebrafish). After the treatments, however, the Gaussian shape disappeared (Fig. 1). The relationships between linear and angular speed were presented in Fig. 2. The movement segments with linear speed in high range (> 7.5 mm/s for daphnia and > 100 mm/s for zebrafish) were observed before treatment, but disappeared in a substantial number after the treatments. In addition, the number of movement segments with positive angular speed higher than 5 rad/s markedly increased after the treatments (Fig. 2). The scatter plots between linear and angular speed were similar for both species.

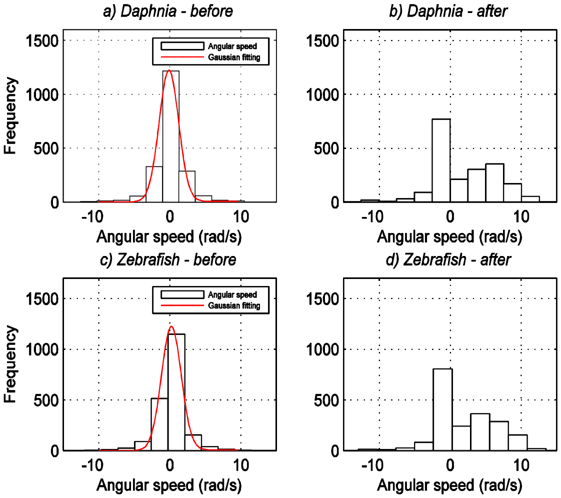

Fig. 1: Histograms of angular speed before and after the treatments.

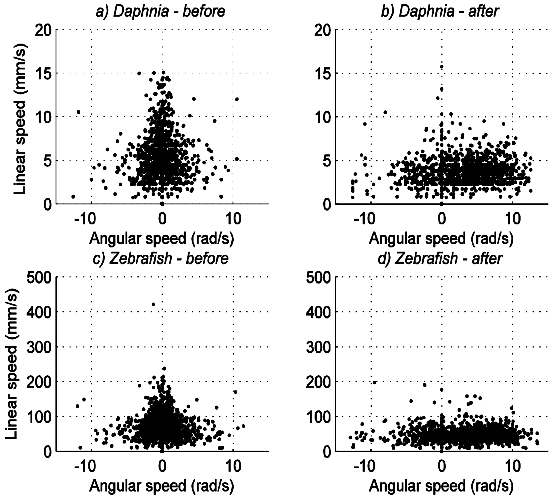

Fig. 2: Scatter plots of linear and angular speed before and after the treatments.

After training with the HMM, the estimated TPMs and EPMs were accordingly obtained (Table 3). The estimated TPMs for two species were similar before the treatments. It was notable that all the transition probabilities were high on the diagonal line after the treatment for both species (Table 3). This indicated that the organisms tended to keep the current states after the treatments rather than changing to other behavioral states. Estimated EPMs were also similar for both species (Table 3).

Table 3. Estimated TPMs and EPMs for daphnia and zebrafish before and after the treatments.

TPM

Daphnia	State	1	2	3	4	Total
Before	1	0.54	0.35	0.11	0.00	1.00
	2	0.66	0.00	0.22	0.12	1.00
	3	0.33	0.16	0.00	0.51	1.00
	4	0.14	0.06	0.11	0.69	1.00
After	1	0.80	0.06	0.00	0.14	1.00
	2	0.04	0.93	0.00	0.03	1.00
	3	0.00	0.00	0.72	0.28	1.00
	4	0.05	0.05	0.14	0.76	1.00

EPM

Event State	L	R	S	Total
1	0.65	0.35	0.00	1.00
2	0.00	1.00	0.00	1.00
3	0.51	0.00	0.49	1.00
4	0.00	0.01	0.99	1.00
1	0.48	0.52	0.00	1.00
2	0.05	0.95	0.00	1.00
3	0.24	0.76	0.00	1.00
4	0.01	0.01	0.98	1.00

TPM

Zebrafish	State	1	2	3	4	Total
Before	1	0.54	0.35	0.11	0.00	1.00
	2	0.66	0.00	0.21	0.13	1.00
	3	0.33	0.16	0.00	0.51	1.00
	4	0.14	0.06	0.11	0.69	1.00
After	1	0.80	0.06	0.00	0.14	1.00
	2	0.04	0.92	0.00	0.04	1.00
	3	0.00	0.00	0.72	0.28	1.00
	4	0.06	0.05	0.14	0.76	1.00

EPM

Event State	L	R	S	Total
1	0.65	0.35	0.00	1.00
2	0.00	1.00	0.00	1.00
3	0.52	0.00	0.48	1.00
4	0.00	0.01	0.99	1.00
1	0.48	0.52	0.00	1.00
2	0.05	0.95	0.00	1.00
3	0.25	0.75	0.00	1.00
4	0.01	0.00	0.99	1.00

Table 4. TPMs in different values of criteria for linear (v_0) and angular (φ_0) speed for determining states: (a) $v_0 = 100$ mm/s, $\varphi_0 = 2.5$ rad/s; (b) $v_0 = 50$ mm/s, $\varphi_0 = 5$ rad/s for zebrafish after the treatments.

(a)

State	1	2	3	4	Total
1	0.92	0.04	0.00	0.04	1.00
2	0.06	0.81	0.00	0.13	1.00
3	0.00	0.00	0.72	0.28	1.00
4	0.05	0.05	0.14	0.76	1.00

(b)

State	1	2	3	4	Total
1	0.44	0.46	0.04	0.06	1.00
2	0.46	0.47	0.04	0.03	1.00
3	0.02	0.01	0.77	0.20	1.00
4	0.02	0.03	0.17	0.78	1.00

Considering the TPMs were remarkably similar for both species, we checked if the consistency in the TPMs would disappear by adjusting the criteria for determining states of zebrafish. With changes in angular speed (from 5 rad/s to 2.5 rad/s), the similarity remained with the distinctive diagonal line as long as the symmetry in angular speed was chosen (Table 4(a)). When criteria for linear speed was changed (from 100 mm/s to 50 mm/s), however, high probabilities on the diagonal line disappeared (Table 4(b)). When we changed the criteria for determining behavioral states around initial values of v_0 and φ_0 for two species, the TPMs were still similar between two species, although the diagonal line with high probabilities disappeared.

4. Discussion and Conclusion

Markov processes in movement behaviors of different species, zebrafish and daphnia, were demonstrated under normal and stressful conditions in this study. In Liu *et al.*,[8] the states of HMM were heuristically determined by the SOM and the events were defined as different levels of the linear speed. This study used more basic components in movement (i.e. linear and angular speed) for determining behavioral states and movement direction as events. The estimated TPMs and EPMs were remarkably similar between two species (Table 3), presenting more structured property in complex dynamic processes of behavioral changes. This consistency in TPMs and EPMs may be partly due to basic properties of linear and angular speed in movement commonly shown in both species (Fig. 2). The linear and angular speed was clearly divided according to distinctive values ($v_0 = 7.5$ mm/s for daphnia, and $v_0 = 100$ mm/s for zebrafish) and ($\varphi_0 = 5$ rad/s for both species). Considering substantial differences in behaviors were observed according to these values (Figs. 1 and 2) and consistent TPMs and EPMs were obtained correspondingly for both species (Table 3), the values in linear and angular speed carry structured property in movement behaviors and were meaningful in determining states and events of indicator species (Table 1).

The EPMs of daphnia and zebrafish illustrated that the animals turns right more than left after the treatments (Table 3). Currently, no clear mechanisms on why right turns were more produced after intoxication. Further study may be needed in conjunction with physiological processes.

In conclusion, the HMM was feasible in revealing Markov processes in behavioral changes when behavioral states were defined by the linear and angular speed and were matched to movement direction as events. Structured property in transition probability such as similarity of TPMs in different species and consistence in diagonal line in TPMs were observed. Parameters and events produced by the HMMs could be an alternative monitoring tool to detect the effect of chemicals in aquatic ecosystems.

Acknowledgments

This work was supported by the Bio-Scientific Research Grant funded by the Pusan National University (PNU, Bio-Scientific Research Grant) (PNU-2008-101-203).

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