

## ***Interactive comment on “Development and evaluation of new behavioral indexes for a biological early warning system using *Daphnia magna*” by T. Y. Jeong et al.***

**T. Y. Jeong et al.**

sdkim@gist.ac.kr

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<General comment>

C1. To improve the balance in the discussion, disadvantages and weaknesses of the system and new indexes should be also included in the manuscript.

A: Because we conducted only exposure tests for evaluating performance of indexes without any tests or analysis for comparing this system and other, we could not discuss about the disadvantages and weakness much. We tried to explain weakness and reason of apparent problems in exposure tests and to suggest further studies from line

C55

290 to 301 with several references.

C2. The conclusion regarding applicability of the system and indexes in the field should be further clarified.

A: The sort of studies regarding applicability will be clarified more in revised version. The kinds of study will be calibration method of signal against to regional water characteristics, optimization of the system in terms of stability of test organism's behavior, and data analysis of fluctuating index data.

<Specific comment>

C1. Please comment how the reduction of the number of chambers from six to two affected the value of the TI index? Furthermore, this research used 10 daphnia per test chamber whereas in the paper by Jeon et al. (2008) each test chamber contained one *Daphnia magna*, and in the preliminary test 30 daphnia were followed.

A: We could not directly compare TI indexes derived from previous and recent system because the comparison test was not conducted. It can be assumed there are no differences of TI value except increasing statistical power by increasing total number of *Daphnia* used for system, because number of *Daphnia* in a chamber was increased from 1 to 10 instead of reducing number of chamber from 6 to 2 without differences in calculation and recording method. In Jeon's study, they collected control data for student's t-test by detecting behavior of 30 *Daphnia* in preliminary exposure period. In our study, we expressed same period as the 'unexposed period' and 20 *Daphnia* were used.

C2. Please specify for the “effluents” whether the municipal waste water is treated, and if so, what type of treatment is applied. It would be helpful providing information about general water quality parameters from the effluents.

A: The Yesan effluent was treated with extended aeration process and the Iksan and Yeosu effluents were treated with conventional activated sludge process. The parame-

C56

ters estimated at the point of exposure study were described in table 1 of supplement.

C3. Please describe the preliminary 24-h whole effluent toxicity test, or at least provide the reference.

A: This study followed US EPA's method. The title of reference is "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms"

C4. Yeosu is salt water. Please provide the value of saline concentration. How was the effect of saline concentration included in the values of various indexes?

A: We did not estimate ion concentration and effect of saline concentration on indexes. However, Kim et al. (2009) reported a result of Toxicity Identification Evaluation of the effluent from Yeosu. The ion concentration estimated by Kim et al. (2009) is described in table 2 of supplement.

C5. Please discuss the results of the individual indexes for the effluents, and if not relevant, please explain why they are not discussed.

A: Higher power of combined index compared to individual indexes was already confirmed at the copper exposure test stage. As mentioned at line 171, the effluent test was conducted to estimate applicability of combined index to field, so the analysis focused on performance of combined index. Performance analysis for individual indexes was not conducted in effluent exposure test.

C6. The correlation test is actually prepared only for the Yesan effluent. How many replicas were carried out for each dilution?

A: There was no replicate. We tried to test applicability of one system at a time. From the results of this study, employment of more systems or more chambers seems needed for making reliable alarm in field using present system.

C7. If the number of replications is the only limiting factor of the system to detect

C57

precise toxicity level, please provide information what is the minimal number of replica that will provide precise toxicity level. If number of replicas is not the only limiting factor, please address other limiting factors of the system to detect precise toxicity level.

A: Limiting factor causing low power of this system is not only replication. If we develop more powerful behavioral parameters, recording methods and calculation algorithm those are other major limiting factors, performance of the system will be improved. We wanted to imply replication is only limiting factor without modifying our system or algorithm. Additionally, we did not conduct tests for optimizing replication number, so we can not define proper replication number in this study.

C8. The first conclusion is that DI shows the best performance among three indexes is demonstrated only for the copper solution, but not for the effluents. Please consider reformulating the conclusion.

A: We will reformulate it for only copper solution.

C9. In the third conclusion you suggest that further research is needed for the application in the field. Does it imply that the system is not applicable for the field study at this point? It would improve the quality of the paper if you can include what exactly can be further optimized for enhancement of accuracy and applicability of BEWS in the field. Consider reformulating the third conclusion.

A: It implies this system is not quite concise for field application yet. We will reformulate the sentence and include what kinds of studies related to applicability improvement are necessary in revised version as mentioned in answer for second general comment.

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Interactive comment on Drink. Water Eng. Sci. Discuss., 6, 39, 2013.

C58

<Table>

Sampling site	Dissolved oxygen(mg/L)	pH	Conductivity(mS/cm)
Bisan	4.67	7.24	5.99
Yeosu	3.70	8	15.77
Yesan	4.32	6.81	0.399

Table 1. Parameters of effluents used in this study

Ion	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Na <sup>+</sup>	F <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Br <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>
Concentration (mg/L)	501.5	52.61	137.3	3724.1	29.9	6284.2	2270.3	60.5	124

(Kim et al., 2009)

Table 2. Ion concentration of Yeosu effluent

<References>

Kim, S. D., Ra, J. S., Kim, K. T., Kim, J. Y., Park, J. E., Kim, H. D., and Kim, E. Y. Identification of ecotoxicity from wastewater treatment facilities and investigation of sources of toxicity. Ministry Of Environment, Republic of Korea, 2009.

Fig. 1.