

OPTIMUM CULTURING AND TESTING CONDITIONS FOR ENVIRONMENTAL TOXICITY TEST WITH *SIMOCEPHALUS MIXTUS*

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Abstract : A water flea, *Simocephalus mixtus*, was collected from a wetland and evaluated the effects of laboratory culturing method and toxicity test procedure on the survival and reproduction as an environmental toxicity test organism. Temperature, feed concentration, test chamber size, water hardness, toxicity sensitivity and other factors were evaluated to determine their effects on individual culturing success and toxicity test result. Neonate reproduction and sensitivity of toxicity test were influenced by temperature during four-weeks culturing, and at 24 and 48 hrs toxicity test. Reproduction rate and toxicity sensitivity of *Simocephalus mixtus* at 25°C were higher than at 20°C. A wide range of media hardness (reconstituted hard water as 80 - 180 mg CaCO₃/L) was acceptable for culturing *Simocephalus mixtus* for use in toxicity tests. This is one of great advantages as a test organism, because daphnids are generally very sensitive to media hardness. Two unicellular green algae (*Chlorella* and *Scenedesmus*) were tested for an adequate culture as food with different size of beakers. There was no significant difference between two foods. However, the optimum condition of food concentration was found to be 2.5 mg/L/day as TOC in a beaker of 50-mL/individual. Other test parameters like lighting, air supply and culture maintenance followed Standard methods for *Daphnia*. We found that *Simocephalus mixtus* could be easily cultured and maintained using the facilities found in the majority of test laboratories.

Key Words : *Simocephalus mixtus*, toxicity test, culturing, survival, reproduction

INTRODUCTION

The increase use of whole effluent toxicity test and monitoring surface waters with crustaceans are expected. Crustaceans are herbivores that have a very important role in aquatic ecosystems. The freshwater cladoceran, *Daphnia*, commonly known as the water flea, is used as a freshwater toxicity test organism. The cladocerans, *Daphnia magna*, *Ceriodaphnia dubia* or *Daphnia pulex* are the test species approved for

use in acute toxicity test. *Simocephalus mixtus* which also belongs to the class Crustacea was collected from Woopo wetlands in Korea and examined as a test organism of acute toxicity test. This species are ubiquitous in nature and can be readily obtainable from freshwater in Korea. *Simocephalus mixtus* attains a length of approximately 0.5 - 2.0 mm (Fig. 1).

There is a need to refine and standardize the culturing method and the testing approach. This study evaluated the effects of laboratory culturing and toxicity test procedures on the survival and reproduction of *Simocephalus mixtus*.

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Figure 1. *Simocephalus mixtus* ($\times 40$).

Phylum : Arthropoda
 Class : Crustacea
 Order : Cladocera
 Family : Daphnidae
 Genus : *Simocephalus mixtus*

MATERIALS AND METHODS

This research was conducted for a new species, *Simocephalus mixtus*, to optimize culturing condition and toxicity test using the simplest and most widely used methods that are found in the majority of testing laboratories. *S. mixtus* used in tests were originally collected from Woopo wetland in Korea as a test organism of toxicity test. *S. mixtus* are ubiquitous in nature and can be readily obtainable from freshwater in Korea. The distribution and identification of this species was investigated and confirmed by Yoon and Kim¹⁾. *S. mixtus* was cultured in an environmental chamber at $25 \pm 2^\circ\text{C}$ with a photoperiod of 16:8 hr light:dark. Hard Reconstituted Water Medium²⁾ was used for general laboratory culturing because it is already a widely used procedure. The cultures of *S. mixtus* maintained in 1L glass beakers with a hardness of 120 ~ 140 mg/L as CaCO_3 corresponding to the natural condition of Woopo wetland. The diet of *Scenedesmus* plus YTC was feed for culturing *S. mixtus* adjusting 0.5 ~0.7 mg/L(day of TOC by renewal of culture media three times a week.

RESULTS AND DISCUSSIONS

S. mixtus attains a maximum length of approximately 2.0 mm, which is smaller than

Daphnia magna (5.0 ~ 6.0 mm)but larger than *Ceriodaphnia dubia* (1 ~ 2 mm). The average life span of *S. mixtus* is about 50 days at 25°C and 60 days at 20°C . Four distinct life-cycle periods are recognized: egg, juvenile, and adolescent, and adult, as well as the life span of *Daphnia*. The time required to grow adult varies from 6 to 8 days under the culturing condition of 120 mg/L of hardness as CaCO_3 and 25°C of temperature. Under laboratory conditions, a clutch of 6 to 8 eggs is released into the brood chamber. Each neonate of *S. mixtus* (<24 h old) was maintained at 20°C and 25°C in 100 ml solutions. Other test conditions described in general laboratory culture methods applied to the temperature effect test. The effect of culture temperature on pregnancy rate and reproduction (neonate/female) was shown in Fig. 2. Culturing at 25°C appeared to have a growth of bigger size, higher pregnancy rate, and more reproduction.

Figure 2. The effect of culturing temperature on neonate reproduction.

Acute toxicity tests were conducted at 20°C and 25°C using neonate (<24 h old) of *S. mixtus* with 500 $\mu\text{g/L}$ of Cu, Cd and Zn. Ten neonates of *S. mixtus* were exposed to each heavy metal in 100 mL test solution. At 25°C , survivals were less than 50% with 1 hr-Cd exposure, 6 hr-Cu exposure, and 24 hr-Zn exposure, whereas survivals at 20°C were more than 75% under the same condition(Fig. 3). This high mortality indicates that toxicity test at 25°C with *S. mixtus* is more sensitive to heavy metals.

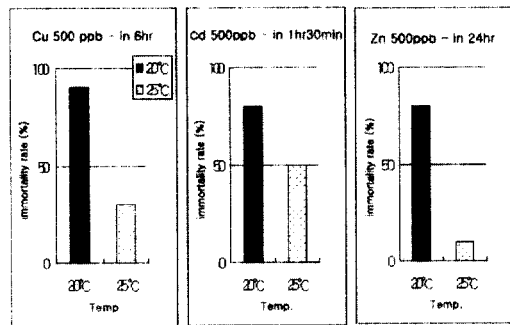


Figure 3. Temperature effect on the sensitivity of toxicity test.

Water type is an important factor that influence on the survival and reproduction of water flea. In this experiment, five water types were prepared according to standard formulations². Intermediate hard water (120 ~ 140 mg/L as CaCO₃) was added to this as culture water. Reproduction and survival was observed during 21 days at 25°C with renewal of culture media every two days. The highest pregnancy rate was observed with intermediate water type (120 ~ 140 mg/L as CaCO₃). Survivals with very soft water (10 ~ 13 mg/L as CaCO₃) and very hard water (280 ~ 320 mg/L as CaCO₃) were 40% and 60%, respectively. These two types of water are not adequate for *S. mixtus* culturing because the survival criterion is generally >80% survival of test animals.³⁾

However, the range of 40 ~ 180 mg/L of hardness did not have any effect on the survival of *S. mixtus* at all as shown in Fig. 4. Water hardness influenced on the reproduction of *S. mixtus* as appeared in Fig. 5. Reproduction with the culture media of MHW, IHW and HW was higher than that with the others. *S. mixtus*

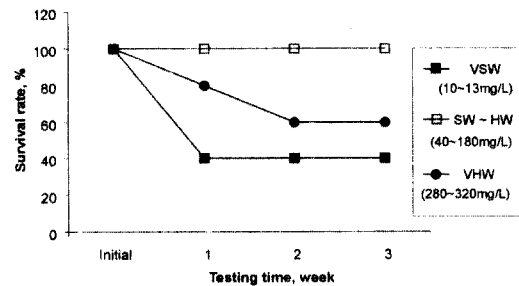


Figure 4. The effect of water hardness on survival rate.

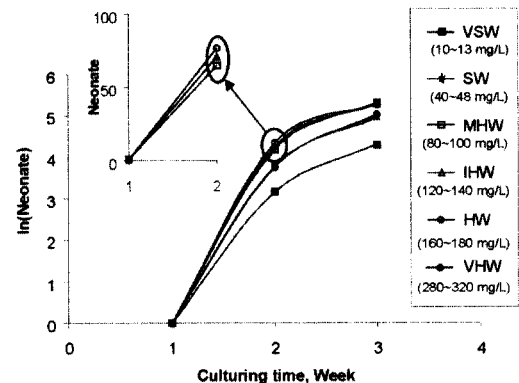


Figure 5. Reproduction at different hardness during culture.

reproduction in MHW, IHW and HW was not much different, but HW was more favorable water type to this species.

The effects of food type, food concentration, and cultural density on reproduction of *S. mixtus* were evaluated. Experimental variables and conditions are summarized in Table 1.

Triplicate result in Table 2 shows that there is no significant difference between two food types ($P < 0.05$). *S. mixtus* was fed once a day with algae of *Scenedesmus sp.* during next

Table 1. Experimental conditions for optimization of culture

Factors	Test variables							
	<i>Chlorella sp.</i>				<i>Scenedesmus sp.</i>			
Food type								
Feed conc. (mg/L as TOC)	0.2	0.5	0.8	1.0	1.5	2.5	3.0	4.0
Test chamber size (mL)	50				100			
Cultural density*	1/50			1/100		2/100		

*Cultural density: number of daphnids per chamber size(mL)

Temperature: 25°C, Hardness: 120 - 140 mg CaCO₃/L, Solution- renewal frequency: daily

Feeding frequency: daily, Neonate age: < 24 hour, Light - Dark: 16hr - 8hr, Light level: < 800 Lux

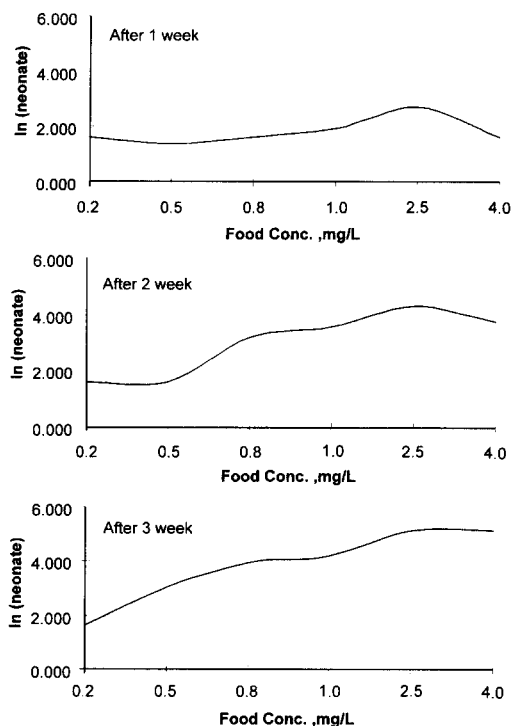


Figure 6. The effect of food concentration on neonate reproduction after one to three weeks culturing.

experiments. Food concentration should be sufficient to support normal growth and reproduction. A reproduction criterion for Daphnids provided by the American Society for Testing and Materials^{3,4} is 60 neonates per a female in a 21-day chronic test as the minimum number. The time for *S. mixtus* to reach sexual maturity appeared to be 6 days. The usual reproduction of *S. mixtus* averaged around 5 young per female per reproduction day. Six food concentrations of *Scenedesmus* sp. were tested for its optimum feeding condition. *S. mixtus* fed with 2.5 mg C/L of food concentration had the highest accumulative reproduction in 21 days.

Table 2. The effect of testing density on reproduction rate

Food Conc. (mg/L)	2.5			3.0		
	1/50	1/100	2/100	1/50	1/100	2/100
Testing density (mL ⁻¹)	1/50	1/100	2/100	1/50	1/100	2/100
First brood (No)	6	5	4	6	5	5
Juvenile time (day)	4	4	4	4	4	4
Neonate/female/21 day	60	86	61	70	78	65

Results obtained and shown in Table 2 indicate that the effect of culture density (number of daphnids per chamber) on reproduction. Neonates produced under all the conditions exceeded 60 young/ female in 21-day culture. It appeared that no clear relation between beaker size and reproduction was found. *S. mixtus* fed daily in the larger beaker (100 mL) had slightly higher reproduction than that fed in 50 mL beaker. 50 mL of beaker size per water flea required to produce acceptable number of neonate is sufficient for culturing this species.

A comparison of *Daphnia magna*, *Ceriodaphnia dubia* and *Simocephalus mixtus* toxicity test was performed to study the relative sensitivity and discrimination abilities. The results as shown in Table 3 indicated that *Simocephalus mixtus* was considerably sensitive in heavy metals toxicity test.

Table 3. A comparison of *D. magna* and *S. mixtus* in LC₅₀(48hr)

(unit : µg/L)

	Cd	Zn	Cu
<i>D. magna</i> ⁵⁾	97(118)	59(68)	47(54)
<i>S. mixtus</i>	44	56	35
<i>C. dubia</i> ⁶⁾	(110)	(76)	(27)

(5) D. I. Mount and T. J. Norberg, (1984)

(6) Margaret W. Toussaint, Tommy R shedd, willian, H. Van der schalie, (1995)

CONCLUSIONS

Water fleas are herbivores occupying a key position in ecosystem. One of water fleas, *Simocephalus mixtus*, which is ubiquitous in fresh water, was collected from Woopo wetlands in Korea and examined as a toxicity test organism. Reproduction rate and toxicity sensitivity of *Simocephalus mixtus* at 25°C were higher

than at 20°C. A wide range of media hardness (reconstituted hard water as 80 - 180 mg CaCO₃/L) was acceptable for culturing *Simocephalus mixtus* for use in toxicity test. This is one of great advantages as a test organism, because daphnids are generally very sensitive to media hardness. The optimum food concentration was found to be 2.5 mg/L/day as TOC in a beaker of 50mL/individual with green algae of either *Chlorella* or *Scenedesmus*.

ACKNOWLEDGMENT

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