

Toxicity Evaluation of Surface Waters from Artificial Lakes (Shihwa, Asan, Busa) and Mankyung River using Sea Urchin (*Strongylocentrotus nudus*) Sperm and Embryo

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등근성게 (*Strongylocentrotus nudus*) 정자와 수정란을 이용한 인공 호수(시화, 아산, 부사) 및 만경강 표층수의 독성 평가

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요 약

연안의 방조제 건설은 하천수와 바다물의 교환을 인위적으로 차단하고, 방조제 내부에는 호수를 형성하게 된다. 이렇게 형성된 인공호수의 수질은 호수로 유입되는 하천의 수질에 크게 영향을 받을 수 있으며, 인공호수의 수질이 악화되면 인근 해양생태계에 영향을 미칠 수 있다. 본 연구는 인공호수의 물이 바다로 방류될 때 해양생태계에 어떠한 영향을 미칠 것인지를 예측하고자 수행되었다. 방조제 건설로 이미 형성되어 있는 시화호, 아산호, 부사호와, 현재 건설중인 새만금 방조제로 인하여 형성될 호수의 유입 하천인 만경강에서 표층수를 채수하여 등근성게 (*Strongylocentrotus nudus*)의 정자와 수정란을 이용한 생물검정을 실시하였다. 담수호의 수질을 해양생물을 이용하여 평가할 수 있도록 하기 위하여 시료의 염분을 조절하면서 실험을 실시하였다. 정자를 이용한 수정 실험 결과, 시화호에서는 수정률에 영향을 미치지 않았고, 아산호와 부사호에서는 약간의 영향이 있었으나 그 정도는 미약하였다. 반면, 만경강의 경우 수정률의 저해가 상당히 크게 나타났다. 수정란을 이용한 발생 실험 결과, 시화호, 아산호, 부사호에서는 약간의 영향이 나타난 반면, 만경강의 경우는 매우 큰 영향이 나타났다. 이들 실험 결과를 종합하면, 시화호, 아산호, 부사호의 수질은 대체로 양호하나, 만경강의 수질은 크게 우려되는 수준이었다. 따라서, 앞으로 건설될 새만금 호수와 인근 해양생태계를 보전하기 위해서는 만경강의 수질 개선을 위한 대책이 마련되어야 할 것이다.

Key words : *Strongylocentrotus nudus*, toxicity, artificial lake, Mankyung river, salinity

INTRODUCTION

The demand for lands in Korea caused active recla-

mation of shallow coastal areas including tidal flats (Koh, 1997). Many of reclamations have been done by dyke constructions. As a consequence of dyke constructions, artificial lakes were formed between the mouth of river and sea, which prevents the exchange and mixing of water mass and its dissolved

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and particulate components between river water and seawater. In this case, river water does not flow directly to the sea but stay for some time in artificial lake. The only chance for this lake water to flow out and to contact with seawater is the sluice operation of water gate. Therefore, the water quality of lake water tends to be influenced by the water quality of inflowing river water. If the lake water is contaminated by the river water, it subsequently affect the sea water and marine ecosystem. In other words, the water quality of lake water before being discharged to the sea is crucial for the environmental safety of nearby coastal ecosystem. Here, we set the purpose of this study to know whether there are adverse effects in lake water to marine organism.

We evaluated surface waters from 3 artificial lakes (Shihwa, Asan, and Busa) those were already formed by dykes, plus a river (Mankyung) that flows out to Saemankeum area where a new dyke is being constructed. Especially, lake Shihwa has a notorious pollution history. Just after the completion of dyke construction in 1994, the heavy metal concentration of lake Shihwa increased by effluents from adjacent industrial complexes (Kim *et al.*, 2002), and anoxic layer was formed in the bottom of the lake (Han and Park, 1999). To enable the evaluation of freshwater with marine organism, we adopted salinity adjustment technique, which is commonly used in the toxicity tests of effluents with marine organisms (USEPA, 1996; 2002).

As a tool for evaluating toxicity of lakes- and river waters, bioassays using the sperm and embryo of Korean purple sea urchin (*Strongylocentrotus nudus*) were applied. The standard bioassay protocols for *S. nudus* were prepared by Lee (2000), which was based on those proposed by USEPA (1995). Since the sperm and embryo bioassay using sea urchin has relatively high sensitivity (Nacci *et al.*, 1996), simple test procedure (Dinnel *et al.*, 1987), short exposure duration (USEPA, 1995), and needs less volume of sample (USEPA, 1995) compared with other marine bioassays, these are widely applied to evaluate the toxicity of chemicals (Pagano *et al.*, 1982), industrial

effluents (Cherr *et al.*, 1987), and the toxicity identification evaluation (TIE) of effluents (Bailey *et al.*, 1995). This study is the first trial to evaluate the toxicity of lake water with a marine organism, and can provide useful information about the change in toxicity during the discharge of lake water to the sea.

MATERIALS AND METHODS

Sample preparation

Surface water samples were collected at 3 artificial lakes and a river (Fig. 1), i.e. lake Shihwa (37° 19'N, 126° 40'E), lake Asan (36° 54'N, 126° 54'E), lake Busa (36° 10'N, 126° 32'E), and Mankyung river (35° 55'N, 126° 56'E) in May 1999. At each site, 1 L of surface water was collected, filtered in situ with GF/F filter (Whatman), frozen immediately by dry ice, transferred to the laboratory, and then kept at -20°C. Two days before the experiment, samples were

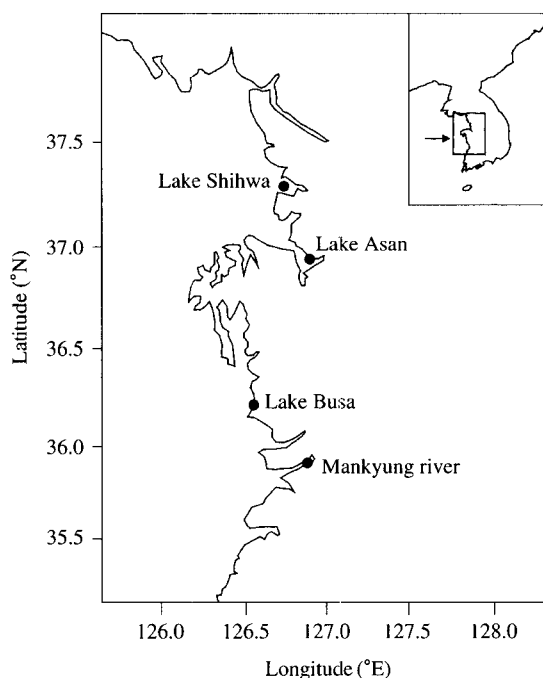


Fig. 1. Locations of sampling sites for surface water toxicity assessment in 1999.

thawed at room temperature for 24 hr and test solutions were prepared by salinity adjustment. Artificial seawater salt (Instant Ocean[®]) was added to samples to make test solutions with target salinities. Target salinities for fertilization test were 20, 22, 24, 26, 28, 30, and 32 psu, and those for development test were 26, 27, 28, 29, 30, 31, and 32 psu. Controls for each treatment was prepared by the same procedure using de-ionized water. After preparation, test solutions were capped, allowed at 5°C for 24 hr, then the final salinity was measured by a salinometer (YSI).

Test organism

Adults of *Strongylocentrotus nudus* were collected at a subtidal area (ca. 10 m in depth) off Pohang by SCUBA diving during natural spawning period (July–August) and reared in the laboratory. They were maintained with continuous aeration and fed dried kelp (*Laminaria* sp.). Seawater for rearing sea urchins was pre-filtered through 1 µm mesh. Temperature and salinity was controlled within 18~22°C and 30~31 psu, respectively.

Preparation of gametes

Spawning was induced by injecting 1 mL of 0.5 N KCl into coelomic cavity (Strathmann, 1987). Ten individuals were used in the spawning induction. Mature sea urchins shed their gametes within 5 min after the injection. Males released white- or cream-colored sperms and females released yellow- or orange-colored eggs. Sperms released from the gonopores were transferred directly into a 1.5-mL microcentrifuge tube using a Pasteur pipette, then the tube was kept in a refrigerator (5°C) before use. Eggs were collected by placing each female with oral side up on a 100-mL beaker filled with GF/F filtered seawater (FSW, salinity: 31 psu) for 30 min. Egg suspension was passed through a 100 µm mesh screen to remove fecal materials and larger particles. Eggs were rinsed with FSW 3 times, then kept at the experimental temperature (20°C) before use. Experiments began within 30 min after the collection of both

gametes.

Fertilization test

Sea urchin fertilization test was performed based on the standard protocol of USEPA (1995). Pretests were performed prior to the main test to determine the optimal dilution ratio of sperm. First, 20 µL of sperm was suspended in 5 mL of FSW (1/250 suspension). From this suspension, 8 different dilution ratios ranging from 1/1000 to 1/16,000 were prepared. Fifty µL of each sperm suspension was added to triplicate scintillation vials (Wheaton) filled with 5 mL of artificial seawater (31 psu), allowed for 20 min, and then 1,000 eggs in 100 µL of FSW were added to each vial. After another 20 min, 50 µL of formaldehyde was injected to each vial. Fertilization rate was measured by examining 100 eggs under a compound microscope (×40, Olympus). Fertilized eggs were easily distinguished from unfertilized ones by the presence of fertilization membrane around the egg mass. The optimal dilution ratio was determined as the lowest ratio at which the fertilization rate was more than 80%.

In the experiment with surface water samples, 5 mL of each test solution was transferred to triplicate vials. Fifty µL of sperm suspension with the optimum dilution ratio determined from the pretest was added to each vial. The rest of test procedure and microscopic observation was the same as above.

Development test

Sea urchin development test was performed based on the standard protocol of USEPA (1995). Eggs and sperms were prepared with densities of ca. 5×10^3 eggs/mL and 1.5×10^7 sperms/mL, respectively. Fertilization was achieved by mixing two suspensions. After 10 min, the fertilized eggs were collected on a 40 µm mesh screen allowing excessive sperm to pass through. Fertilized eggs were rinsed with FSW 5 times. Approximately 500 fertilized eggs were transferred to triplicate vials filled with 5 mL of test solutions to which Penicillin G (10 units/mL, Sigma)

were added. Vials were capped and incubated at 20°C. After 48 hr, test was terminated by injecting 50 µL of formaldehyde. A hundred embryos from each vial were examined under a compound microscope (× 100, Olympus) to determine the proportion of normal larvae. Determination of normality of larval shape of *S. nudus* was based on Lee (2000). Dead embryos, embryos with suspended development, or deformed larvae were regarded as abnormal.

Data analyses

Fertilization and development rate data for different salinity treatments in each sample were compared with those at control (ASW) by the one-tailed *t*-test (Zar, 1984) using the SPSS program. For all analyses, a significance level of $\alpha = 0.05$ was used.

Fertilization and development rate data for each treatment were fitted to a logistic function with 3 parameters as:

$$F \text{ (or } D) = a / (1 + e^{-b(S-c)}) \quad (\text{Eq. 1})$$

where, *F* is the proportion of fertilized eggs in fertilization test, *D* is the proportion of normally developed larvae in development test, *S* is salinity, and *a*, *b*, *c* are parameters estimated by the curve fitting. Parameter *a* indicates the theoretical maximum of *F* or *D*, *b* indicates the slope of curve, and *c* indicates the salinity at which the *F* or *D* becomes 50% of the theoretical maximum.

RESULTS

Fertilization and development in control

The fertilization rate of *Strongylocentrotus nudus* in control (artificial seawater: ASW) increased as salinity increased (Fig. 2A). It was lower than 80% when salinity was 20~24 psu, but was higher than 80% when salinity was 26~32 psu. Therefore, the threshold salinity for successful fertilization of *S. nudus* under uncontaminated condition is 26 psu. The development rate also increased as salinity increased

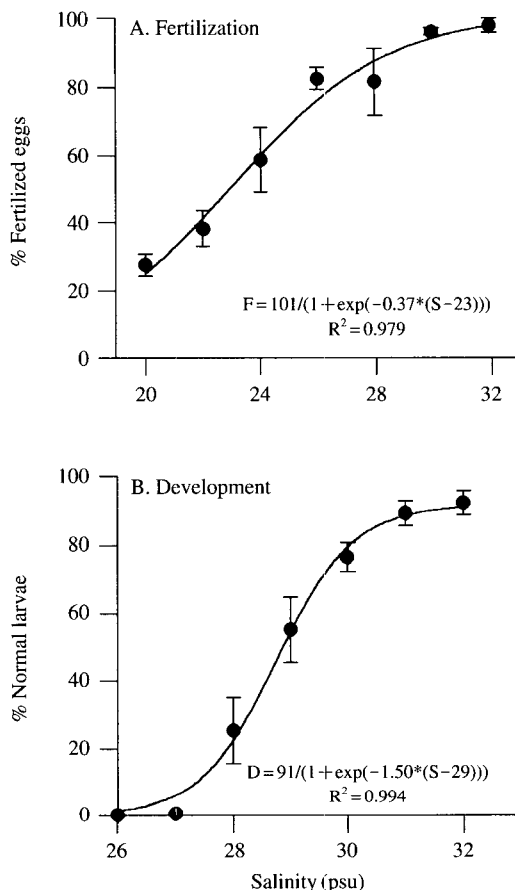


Fig. 2. Changes in the proportion of fertilized eggs (A) and normally developed larvae (B) of *Strongylocentrotus nudus* with increasing salinity of control (artificial seawater). Symbol represents mean \pm SD. Fertilization and development data were fitted to a logistic function of salinity.

(Fig. 2B). There were no normal larvae when salinity was 26~27 psu. The proportion of normal larvae increased rapidly as salinity increased from 28 to 30 psu. It was higher than 80% when salinity was 31~32 psu. Therefore, the threshold salinity for normal development of *S. nudus* is 30 psu, which is higher value than that of fertilization.

Fertilization in lakes and river water samples

The fertilization rates in salinity adjusted surface water samples showed similar trends to that of con-

Table 1. Changes in the proportion of fertilized eggs (% , mean ± SD, n = 3) of *Strongylocentrotus nudus* with increasing salinity of artificial lakes and river water. Values with asterisk in each row are significantly lower (p < 0.05) than that of control.

Salinity (psu)	Control	L. Shihwa	L. Asan	L. Busa	Mankyung R.
20	27.3 ± 3.2	40.3 ± 4.2	22.7 ± 3.5	19.0 ± 9.5	22.0 ± 9.5
22	38.0 ± 5.2	53.0 ± 5.6	23.7 ± 4.7*	32.7 ± 8.3	24.0 ± 2.6*
24	58.3 ± 9.6	77.0 ± 4.6	28.3 ± 5.9*	39.0 ± 9.5	40.7 ± 9.0
26	82.3 ± 3.2	77.0 ± 7.0	48.3 ± 7.2*	50.3 ± 7.5*	64.3 ± 2.5*
28	81.3 ± 9.8	93.0 ± 2.6	69.0 ± 9.2	73.7 ± 9.0	64.3 ± 3.2*
30	96.0 ± 1.0	93.7 ± 2.5	83.7 ± 4.2*	85.3 ± 5.9*	69.0 ± 3.6*
32	97.7 ± 2.1	91.3 ± 5.5	83.7 ± 5.9*	86.3 ± 3.1*	67.0 ± 9.5*

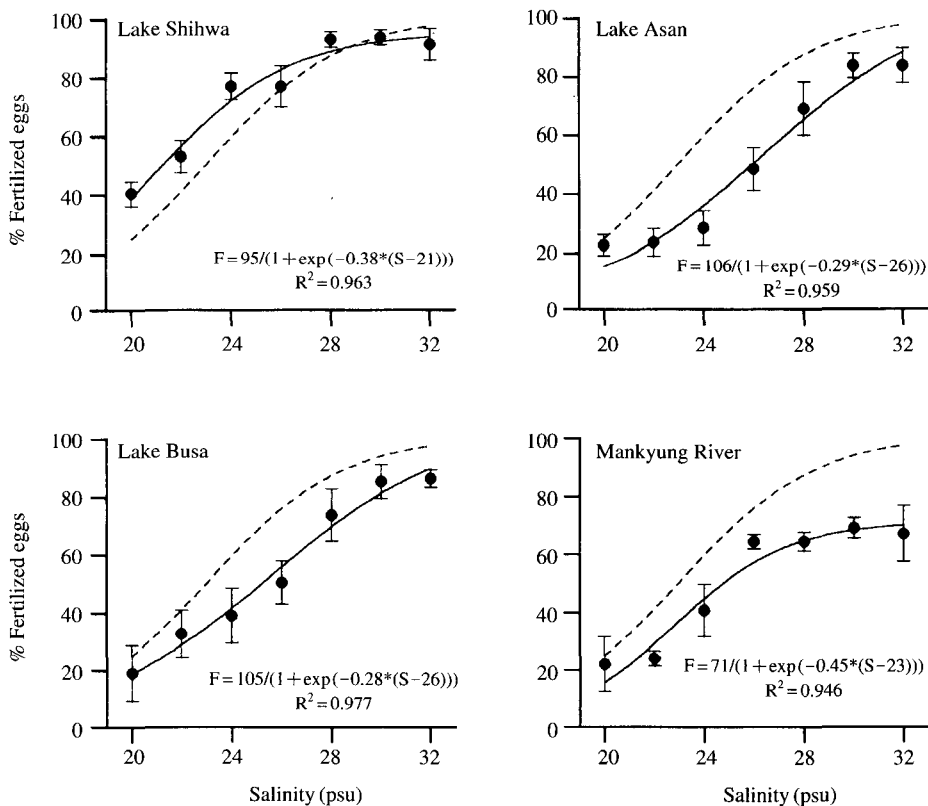


Fig. 3. Changes in the proportion of fertilized eggs of *Strongylocentrotus nudus* with increasing salinity of artificial lakes and river water. Symbol represents mean ± SD. Dotted line: fitted curve for control from Fig. 2A.

control (Fig. 3, Table 1), but the fertilization rate at each salinity treatment were different among samples. In lake Shihwa water, fertilization rate was higher than that of control when salinity was 20~26 psu. There were no significant differences in fertilization rate

with that of control at all salinity treatments. The fitted curve for lake Shihwa water located at higher position than that of control (Fig. 3). In contrast, decreases in fertilization rate relative to control were found in water samples from lake Asan, lake Busa,

Table 2. Changes in the proportion of normally developed larvae (% , mean \pm SD, n = 3) of *Strongylocentrotus nudus* with increasing salinity of artificial lakes and river water. Values with asterisk in each row are significantly lower ($p < 0.05$) than that of control.

Salinity (psu)	Control	L. Shihwa	L. Asan	L. Busa	Mankyung R
26	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
27	0.3 \pm 0.6	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
28	25.3 \pm 9.7	4.3 \pm 5.1*	0.0 \pm 0.0*	0.0 \pm 0.0*	0.0 \pm 0.0*
29	55.0 \pm 9.6	49.3 \pm 4.5	31.7 \pm 9.9*	42.7 \pm 7.5	0.0 \pm 0.0*
30	76.3 \pm 4.2	86.7 \pm 4.7	85.0 \pm 7.0	85.0 \pm 3.0	0.0 \pm 0.0*
31	89.0 \pm 3.6	87.7 \pm 2.5	88.0 \pm 2.6	88.7 \pm 1.5	4.0 \pm 5.3*
32	92.0 \pm 3.5	88.7 \pm 2.5	90.3 \pm 1.5	87.7 \pm 2.5	10.7 \pm 6.0*

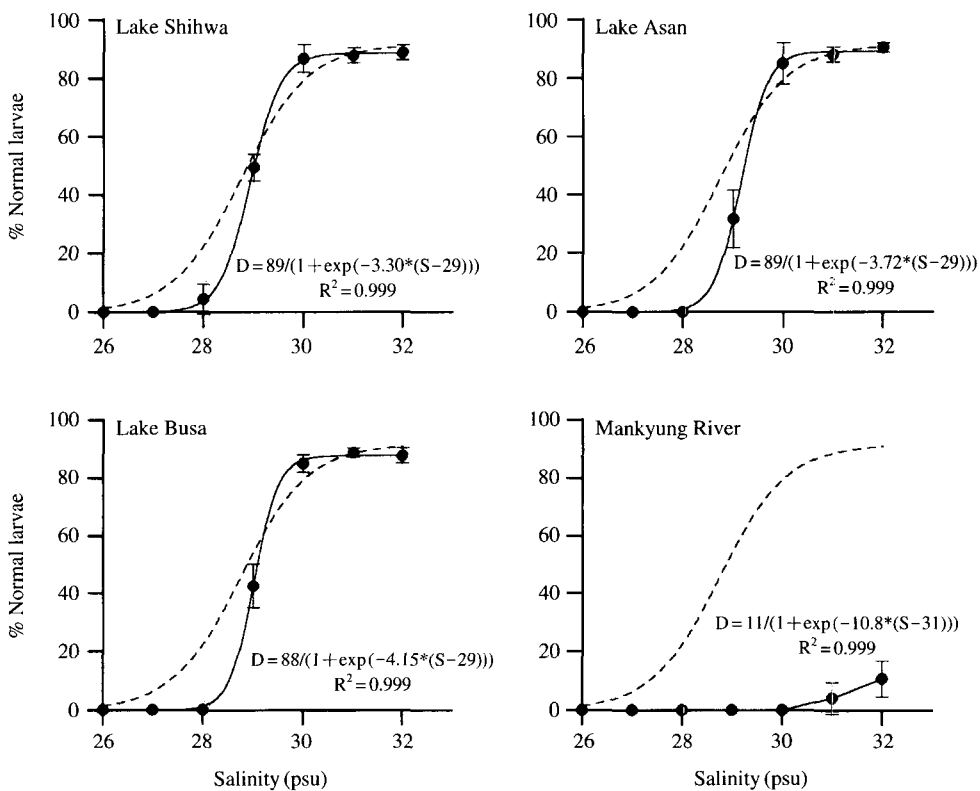


Fig. 4. Changes in the proportion of normally developed larvae of *Strongylocentrotus nudus* with increasing salinity of artificial lakes and river water. Symbol represents mean \pm SD. Dotted line: fitted curve for control from Fig. 2B.

and Mankyung river. Fertilization rates were significantly lower than those in control at 5, 3, and 5 salinity treatments in lake Asan, lake Busa, and Mankyung river, respectively (Table 1). Therefore, the fitted curves for these water samples located at lower position than that of control (Fig. 3). The maximum

fertilization rate was 93.7% for lake Shihwa, 83.7% for lake Asan, 86.3% for lake Busa, and 69.0% for Mankyung river. These results indicate that the adverse effects of surface waters on the fertilization of *S. nudus* was nil for lake Shihwa, slight for lakes Asan and Busa, and large for Mankyung river.

Development in lakes and river water samples

The proportion of normally developed larvae in salinity adjusted surface water samples from lakes Shihwa, Asan, and Busa showed somewhat similar trends (Fig. 4). In these 3 lake waters, the proportion of normal larvae was less than 20% when salinity was 26~28 psu. But it increased rapidly as salinity increased further, and reached over 80% when salinity was 30~32 psu. Development rates were significantly lower than those in control at only 1, 2, and 1 salinity treatments (28~29 psu) in lake Shihwa, lake Asan, and lake Busa, respectively (Table 2). The fitted curves for these lake waters located at lower position than that of control when salinity was 26~29 psu, but the curves overlapped with the control when salinity was 30~32 psu (Fig. 4). However, the development rates in Mankyung river water appeared differently. The proportion of normal larvae was 0% until salinity reached 30 psu. It increased only to 10.7% as salinity increased to 32 psu (Fig. 4). Development rates were significantly lower than those in control at 5 treatments (Table 2). The maximum development rate was 88.7% for lake Shihwa, 90.3% for lake Asan, 87.7% for lake Busa, and 10.7% for Mankyung river. These results indicate that the adverse effects of surface waters on the development of *S. nudus* was slight for lakes Shihwa, Asan, and Busa, while it was great for Mankyung river.

DISCUSSION

The threshold salinity for fertilization and development in negative control (artificial seawater) was 26 and 30 psu, respectively. Lee (2000) reported that the acceptable range of salinity for fertilization and development of *Strongylocentrotus nudus* was 26~38 and 28~34 psu, respectively. The threshold salinity for fertilization in this study is in good accordance with that of Lee (2000), but that for development is slightly higher. This difference seems due to the different incubation medium. Lee (2000) used natural seawater as control but we used artificial seawater. Since sur-

face water sample for toxicity tests in this study was freshwater (with zero salinity), artificial seawater salt should be added to the samples. The negative control for these samples should also contain the same amount of artificial seawater salt. Therefore, we should use inevitably artificial seawater as a control. According to USEPA (1991), the sensitivity of the larvae of sheepshead minnow (*Cyprinodon variegatus*) in artificial seawater could increase over 3 times than that in natural seawater. Therefore, slightly increased salinity threshold (decreased tolerance limit) in artificial seawater can be regarded as a general phenomenon.

In this study, we applied the salinity adjustment technique to assess freshwater with marine organism. Salinity adjustment was done to the samples in many studies on the bioassays with industrial effluents and toxicity identification evaluation (USEPA, 1996; 2002). To assess accurately the potential toxicity of a sample to marine organisms, it is highly important to know the effect of salinity adjustment itself on the toxicity of sample. Ho *et al.* (1995) reported that toxicity of salinity adjusted effluents to *Mysidopsis bahia* and *Menidia beryllina* did not change significantly over 40 days. They also emphasized that effluent stored with brine had fewer significant changes in toxicity than did effluent stored without brine. Krassoi (1995) also reported that the addition of artificial seawater salt to effluents did not significantly affect the results of toxicity test with 2 bivalve larvae (*Chlamys asperimus* and *Saccostrea commercialis*). Therefore, salinity adjustment is a quite useful technique when the salinity of sample is out of the acceptable range for conducting the bioassay with marine organisms.

We used a 3-parameter logistic function to express the salinity-response relationships. This function fitted well to the rates of fertilization and development of *S. nudus* against the salinity. On the whole, the coefficients of determination (R^2) were higher than 0.9. Therefore, it is highly recommended to apply this logistic function in calculating the EC_{xx} values from sea urchin fertilization and development data.

Table 3. Comparison of parameters from curve-fitting (logistic function) of data from sea urchin (*Strongylocentrotus nudus*) fertilization and development in control (artificial seawater: ASW), and surface waters from 3 artificial lakes and a river. The fitting equation is F (or D) = $a/(1 + e^{(-b(S-c)})}$.

Sample	Fertilization test			Development test		
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>
Control (ASW)	101	0.37	23	91	1.50	29
Lake Shihwa	95	0.38	21	89	3.30	29
Lake Asan	106	0.29	26	89	3.72	29
Lake Busa	105	0.28	26	88	4.15	29
Mankyung River	71	0.45	23	11	10.8	31

Additional advantage of this function is that the parameters estimated by curve fitting explain well the characteristics of toxic responses. We can compare the toxicity of samples by comparing the estimated parameters. Parameter *a* in Eq. 1 indicates the theoretical maximum of the biological response. In case of sea urchin fertilization (or development), this value should be 100%, if there is more than 1 treatment with no adverse effect. This values in our samples clearly showed that the surface water from Mankyung river was more toxic than those from 3 lakes (Table 3). The parameters *b* indicates the slope of curve, which is less indicative of toxic strength but may have information about the property of toxicant. Parameter *c* indicates the salinity at which the response becomes 50% of *a*, which *per se* can be regarded as the same concept of EC₅₀, if *a* value approaches 100%. But this parameter is strongly dependent on *a*. Therefore, the comparison of *c* is reasonable among samples with similar *a* values.

From the results of this study, we can conclude that the effect of surface water samples on the fertilization and development of *S. nudus* was strong in the following order: Mankyung river > (lake Asan ≈ lake Busa) > lake Shihwa. On the whole, the effects of surface water samples from 3 lakes were somewhat similar and far less than that from Mankyung river. Therefore, we can expect that ecological impacts of these lake waters will be small if they are

discharged to the sea. It is noticeable that surface water from lake Shihwa—which receives effluents from adjacent industrial complexes—has little adverse effects of sea urchin fertilization and development. This may be related to the improvement of water quality owing to gate operation beginning at 1997 (Kim *et al.*, 2002).

However, the Mankyung river has great potentials to adversely affect the receiving waters. At the mouth of Mankyung river, there are huge tidal flats (Saemankeum area) which may play a key role to reduce toxicity and to disperse pollutants contained in overlying water. Therefore, the reclamation of Saemankeum area will ultimately raise the probability of environmental risks to the coastal ecosystems, if the water quality of Mankyung river remains at the present level. It is necessary to improve the water quality of Mankyung river. But, if we consider the nature of spatial and temporal variability of river water, the result from this study only may not be sufficient; it has no information about the toxicants in the surface water. Therefore, it is strongly needed to investigate and to monitor the chemical properties of Mankyung river water and its impacts on adjacent coastal ecosystems.

CONCLUSION

We evaluated the toxicity of surface water from lakes Shihwa, Asan, Busa, and Mankyung river using sperm and embryo of sea urchin, *Strongylocentrotus nudus*. To enable the toxicity test of freshwater with marine organism, salinity adjustment technique was applied. In general, the proportion of fertilized eggs and normally developed larvae increased as the salinity of sample increased. In fertilization test, the fitting curve of lake Shihwa sample located higher than that of control, but those from other samples lower. Surface water from Mankyung river showed the highest toxicity among samples tested in this study. In development test, samples from lakes Shihwa, Asan and Busa showed similar trends and the adverse effects

were slight. But, in Mankyung river water, the highest rate of development was only 10.7%, indicating great effects on the development. Consequently, it is necessary to improve the water quality of Mankyung river to maintain the ecosystem health of lake Saemankeum and adjacent coastal area.

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