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The Effects of Nonylphenol on Freshwater Phytoplankton and Zooplankton Communities

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Recent studies reveal that the endocrine disrupter nonylphenol can also influence the growth of planktonic organisms. To clarify the effect of nonylphenol on the whole planktonic community, we monitored planktonic abundances after addition of nonylphenol using small-scale microcosms in a laboratory. Nonylphenol was added at final concentrations of 1.25 and 2.5 μg L^{-1}, close to the EC50 for the growth of the rotifer, *Brachionus calyciflorus*. Chlorophyll *a* concentration increased significantly between 2 to 5 days after nonylphenol treatment compared to the control. The abundance of the predominant phytoplankton, *Stephanodiscus hantzschii*, followed the same pattern as chlorophyll *a* concentration. While there was no negative effect on the abundance of ciliates and rotifers, crustacean zooplankton abundance was higher in nonylphenol treatments. Although the relationship did not reach significance, the growth rate of rotifers tended to decline with increasing nonylphenol dosing. It is likely that the decreased rotifer grazing on *S. hantzschii* caused significant increase in their abundance. This study emphasizes the importance of considering indirect effects of environmental pollutants when predicting the response of biological community to toxicant exposure.

Key words : nonylphenol, phytoplankton, bloom, planktonic community

INTRODUCTION

Lakes and rivers receive many anthropogenic chemicals that can impact natural communities. Nonylphenol, one such chemical, is an endocrine disrupter (Lech *et al.*, 1996; Keith *et al.*, 2001; Jobling *et al.*, 2004), and the degradation product of nonylphenol polyethoxylates widely used as surfactants in domestic and industrial detergents. This chemical enters natural freshwater environments through urban and industrial discharges, where it persists for a long time due to low decomposition rates compared to its parent compounds. This environmental pollutant can also impact planktonic community.

The effect of nonylphenol on the population growth of planktonic organisms has been studied, especially for zooplankton (Preston *et al.*, 2000; Marcial *et al.*, 2003; Lee *et al.*, 2007). For example, EC50 for *Daphnia magna*, the concentrations of nonylphenol that reduces the intrinsic growth rate by 50% were 7 μg L^{-1} by Lee *et al.* (2007). Rotifers seems to be more sensitive than crustacean zooplankton (Severin *et al.*, 2003; Lee *et al.*, 2007). The growth of *Brachionus calyciflorus* was inhibited at 5 μg L^{-1} nonylephenol, and the EC50 was 2.49 μg L^{-1} (Lee *et al.*, 2007). Moreover, heterotrophic flagellates are also sensitive to nonylphenol; the EC50 for the heterotrophic nanoflagellate *Diphylleia rotans* is 3.49 μg L^{-1}. Nonylphenol also affects asexual reproduction in

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phytoplanktonic species, although EC50s of phytoplankton are higher than those of zooplankton. For noxious phytoplankton of the genus *Microcystis*, nonylphenol influences growth between 1 and 2 mg L^{-1}. The response of each planktonic functional group against nonylphenol loading is very different.

Preston (2002) and Fleeger *et al.* (2003) both point out the significance of the indirect effects of toxicity on aquatic ecosystems. An indirect effect may be a significant factor influencing the manner in which ecosystem structure and function respond to anthropogenic stressors (Preston, 2000). The differences in sensitivity to nonylphenol suggest that its discharge into natural environments has the potential to disturb the planktonic food web structure. Indeed, Wang *et al.* (2007) and Lee *et al.* (2007) predicted that nonylphenol loading into freshwater environments stimulates *Microcystis* bloom. Hense (2003) examined the effects of nonylphenol on the whole planktonic community using microcosm and mesocosm studies and demonstrated a significant increase in phytoplankton assemblages in nonylphenol treatments. However, information on the effects of nonylphenol on natural phytoplankton communities remains limited.

Paltang Reservoir, constructed in the Han River in the eastern part of Seoul in Korea, is a quite important freshwater resource. This reservoir provides drinking water and industrial water for more than 12 million. The nonylphenol concentration in the Han River water has been measured at 23.2~187.6 ng L^{-1} (Li *et al.*, 2004c), a negligible level. However, much higher concentration of nonylphenol (1,533 ng L^{-1}) has been reported for Lake Shihwa, which is one of the most polluted limnological resources in Korea, although the nonylphenol concentration typically ranges between 33~275 ng L^{-1} (Li *et al.*, 2004a, b). These results suggest that plankton communities can be exposed intermittently to high concentrations of nonylphenol in effluents, making it important to understand their response to nonylphenol.

The present study aimed to clarify the impact of nonylphenol, especially on the phytoplankton community in Paltang Reservoir. In the winter season, the phytoplankton community is dominated by the diatom *Stephanodiscus hantzschii* (Hong *et al.*, 2002). The simplicity of the winter phytoplankton community made it easier to study the response of the planktonic community to nonylphenol. Accordingly, we carried out incubation experiments in the laboratory. We monitored the abundance of the planktonic community after the addition of nonylphenol to the water. We observed that temporal input of nonylphenol caused significant increases in phytoplankton abundance. The possibility of reducing feeding activity in response to nonylphenol is discussed.

MATERIALS AND METHODS

Water for the incubation experiment was collected at the surface layer of Paltang reservoir on 30 January 2007. The water temperature was 3.0°C. The sample was treated within 2 hours after the collection. Five liters of the water were poured into 6 L cages made of polypropylene and incubated at 4°C under 70 μmol photons m^{-2} sec^{-1} with a 12 : 12=L : D cycle. Totally, nine cages were prepared. After one day of stabilization, nonylphenol was added to three cages at final concentrations of 1.25, and to the other three at 2.5 μg L^{-1}. Remained three cages were prepared as the control without nonlyphenol. Nonylphenol dissolved to the acetone were diluted according to Lee *et al.* (2007). Since aceton was highly diluted (19.7 μg L^{-1} in nonyphenol 2.5 μg L^{-1} treatment) and the previous results (Lee *et al.*, 2005) revealed that 3 mg L^{-1} of nonylphenol did not effect on the growth of planktonic community, we did not prepare aceton added treatment as control.

Water samples were collected on 0, 2, 5, and 7 days after the addition of nonylphenol. A portion of the samples was fixed with Lugol solution at a final concentration of 1% and stored at 4°C under dark conditions until cell counting. To estimate chlorophyll *a* concentration, the 100 mL portion of the water samples was filtered through a GF/F filter. The filter was soaked with 90% acetone overnight under dark conditions at 4°C. The extracted chlorophyll *a* was measured with a spectrophotometer and quantified according to the equation of Jeffrey and Humphery (1975).

The cell number of *S. hantzschii* in the 400 μL portion of each sample was directly counted under ×400 magnification of a light microscope. *Cryptomonas ovata, Asterionella formosa, Synedra* sp., and ciliates were enumerated in the 400 μL portion of samples which was concentrated by 5~6 times using natural sedimentation for one day under ×200 magnification of a light microscope.

Rotifers and crustacean zooplankton were also enumerated in the 4 mL of the sample, which was concentrated by 5~6 times under ×40 magnification of a light microscope. The growth rate of the zooplankton were calculated under the assumption of exponential growth with the following equation; $\mu=\ln(N_f/N_0)/t$, where N_0 and N_f are cell densities at 0 and 7 days, respectively, and t is the incubation period of 7 days.

A one-way analysis of variance (ANOVA) was used to test for differences among the treatments. Multiple comparisons were subsequently carried out using Tukey's test with a discrimination level of $p=0.05$.

RESULTS

Chlorophyll *a* concentration in nonylphenol treatments was significantly higher than in controls (Fig. 1). Two days after nonylphenol addition, chlo-

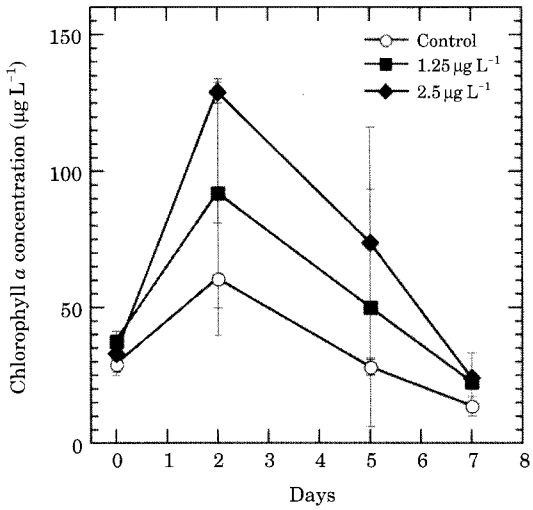

Fig. 1. Changes in chlorophyll *a* concentration in the microcosm experiment. Values are the mean of triplicates, and error bars represent the standard deviation. Open circles (0 μg L^{-1}), closed squares (1.25 μg L^{-1}), and closed diamonds (2.5 μg L^{-1}).

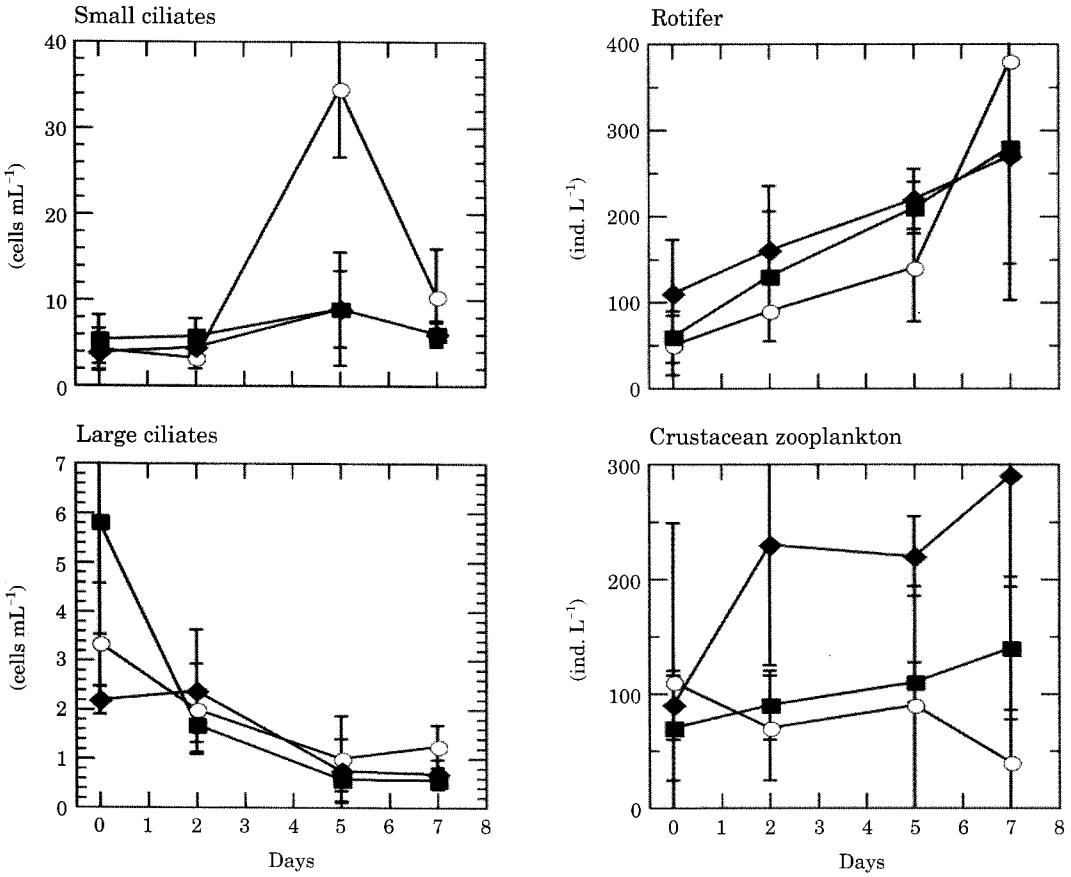

Fig. 2. Changes in dominant phytoplankton abundance in the microcosm experiment. Values are the mean of triplicates, and error bars represent the standard deviation. Open circles (0 μg L^{-1}), closed squares (1.25 μg L^{-1}), and closed diamonds (2.5 μg L^{-1}).

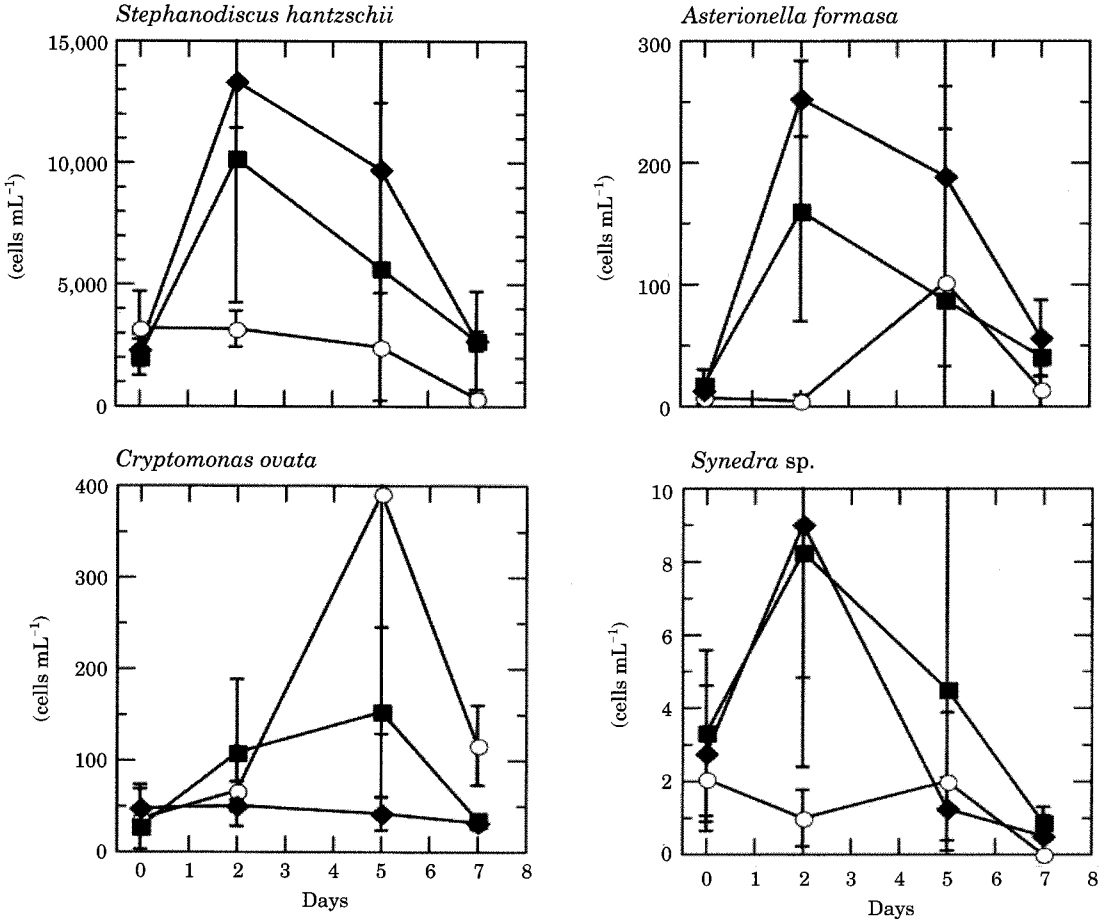

Fig. 3. Changes in ciliate, rotifer, and crustacean zooplankton abundances in the microcosm experiment. Values are the mean of triplicates, and error bars represent the standard deviation. Open circles (0 μg L^{-1}), closed squares (1.25 μg L^{-1}), and closed diamonds (2.5 μg L^{-1}).

rophyll *a* concentrations increased to 92.1 μg L^{-1} (1.25 μg L^{-1} nonylphenol), and 129.1 μg L^{-1} (2.5 μg L^{-1}). After five days, chlorophyll *a* concentrations began to gradually decrease to 22.6 (1.25 μg L^{-1} nonylphenol) and 24.2 μg L^{-1} (2.5 μg L^{-1}) at 7 days after treatment.

S. hantzschii predominated in the water sample. *Cryptomonas ovata, Asterionella formosa, Synedra* sp., *Scenedesumus* spp. *Chlamydomonas* sp., *Fragillaria* sp., and *Peridinium* spp. were also observed. The response of *S. hantzschii* on nonylphenol was quite similar to changes in chlorophyll *a* concentration (Figs. 1 and 2). Higher cell densities of *S. hantzschii* were detected two days after treatment, gradually declining to the lowest level after 7 days. The highest chlorophyll *a* concentration and *S. hantzschii* cell densities were detected in the 2.5 μg L^{-1} treatment, followed by 1.25 μg L^{-1} treatments. *A. formosa* and *Synedra* sp. responded similarly to *S. hantzschii* (Fig. 2). In contrast, *C. ovata* was presented at greater abundance in the control treatment after five days (Fig. 2). The abundance of *C. ovata* did not increase after 2.5 μg L^{-1} nonylphenol treatment.

Protists and zooplankton abundances were different from each other (Fig. 3). Large ciliates decreased in abundance in all treatments, including the control, suggesting no effects of nonylphenol on large ciliates. Small ciliates grew only in the control treatment. In this experiment, most rotifers belonged to genus *Polyarthra* and grew well in all treatments during the incubation. Crustacean zooplankton also increased in abundance in nonylphenol treatments, while abundance dropped slightly in control conditions. Inter-

Table 1. Result of ANOVA test. Values were means from 2 to 7 days of the incubation±standard deviation. Letters in parentheses denote significant differences at 5% among treatments as determined by Tukey's test.

	Control (0 μg L^{-1})	1.25 μg L^{-1}	2.5 μg L^{-1}	F	p
Chlorophyll *a* (μg L^{-1})	34.14±23.31 (a)	54.90±42.89 (a)	75.73±50.38 (a)	2.37	0.11
Phytoplankton (cells mL^{-1})					
S. hantzschii	1,972±1,724 (a)	6,162±5,573 (ab)	8,585±6,075 (b)	4.26	0.03
C. ovata	191.6±201.7 (a)	98.7±80.0 (a)	41.5±13.0 (a)	3.28	0.06
A. formosa	39.9±93.5 (a)	96.0±98.8 (ab)	165.8±118.7 (b)	3.29	0.05
Synedra sp.	1.00±1.34 (a)	4.55±5.84 (a)	3.59±4.61 (a)	1.59	0.22
Zooplankton (ind. L^{-1}) & ciliates (cells L^{-1})					
Crustacean zooplankton	66.7±65.1 (a)	113.4±41.9 (ab)	246.9±80.5 (b)	18.92	<0.01
Rotifer	203.5±147.7 (a)	206.9±102.3 (a)	216.7±104.9 (a)	0.03	0.97
Large ciliates (>30 μm)	1,418±808 (a)	943±607 (a)	1,272±1,098 (a)	0.84	0.44
Small ciliates (<30 μm)	16,060±15,010 (a)	6,930±3,830 (a)	6,490±3,100 (a)	3.15	0.06

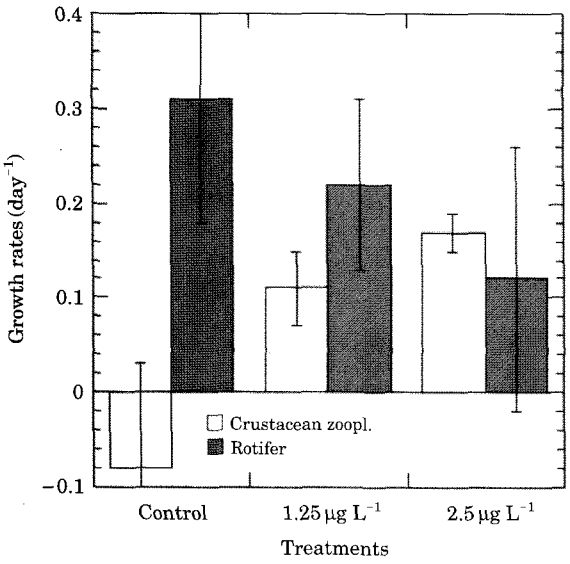

Fig. 4. Growth rates for rotifer (gray bars) and crustacean zooplankton (white bars). Values are the mean of triplicates, and error bars represent the standard deviation.

estingly, the increase in abundance was more apparent in the 2.5 μg L^{-1} treatment.

Results of one-way ANOVA are shown in Table 1. Chlorophyll *a* concentrations gradually increased along with nonylphenol dose, although differences were insignificant. Abundances of *S. hantzschii*, *A. formosa*, and *Synedra* sp. changed in a pattern similar to that of chlorophyll *a* concentration. Among these phytoplanktonic species, significant differences were detected for *S. hantzschii* and *A. formosa*. As mentioned above, the response of *C. ovata* differed from that of diatoms. Although a higher abundance was detected in the control treatment, the difference was not significant. Abundances of crustacean zooplankton were significantly higher in nonylphenol treatments. On the other hand, there was no difference in the abundances of rotifers and large ciliates. Small ciliates responded in the opposite manner to crustacean zooplankton with higher abundances in the control treatment.

Because rotifer and crustacean zooplankton grew positively in most treatments, growth rates were compared among the treatments (Fig. 4). The growth rate of crustacean zooplankton tended to increase along with nonylphenol concentration. On the other hand, rotifers showed an opposite pattern of higher growth was found in control treatment in the absence of nonylphenol. Indeed, rotifer abundances were higher in nonylphenol treatments after seven days (380±105 ind. L^{-1}, control; 280±135 ind. L^{-1}, 1.25 μg L^{-1}; 270±167 ind. L^{-1}, 2.5 μg L^{-1}).

DISCUSSION

Pollution of limnetic environments with anthropogenic chemicals is a prominent issue in the conservation of freshwater resources. High levels of nonylphenol have been intermittently recorded in lakes or rivers where industrial wastewaters directly deposit (Li *et al.*, 2004a, b). Several studies have demonstrated that nonylphenol is an endocrine disrupter that also affects the growth of asexually reproducing planktonic organisms (Li *et al.*, 2007; Wang *et al.*, 2007). Sensitivity to nonylphenol varies among planktonic species; phytoplankton tend to tolerate nonylphenol better than protists or zooplankton. As a result, nonyl-

phenol can cause a shift in planktonic community structure. We observed that a sudden increase in nonylphenol concentration could cause algal blooms, implying that temporal nonylphenol discharge into lakes or rivers, even at nonlethal concentrations, can cause significant changes in planktonic community structure.

The effects of toxicants are typically evaluated intensively in individual species. Such studies provide important information in determining the risk of chemical exposure. At the same time, toxicants also affect on biological interactions such as competition and predation, causing indirect impacts on the planktonic community. Consequently, indirect toxicity effects are also important in predicting the response of ecosystem structure and function to anthropogenic contamination. Lee *et al.* (2005) demonstrated that *S. hantzschii* growth was not affected by nonylphenol concentrations below 1 mg L^{-1} (Lee *et al.*, 2005). However, this phytoplankter quickly responded to the inoculation of nonylphenol at low levels, suggesting that nonylphenol indirectly affects phytoplankton by releasing grazing pressure.

The growth rate of rotifers declined with increasing nonylphenol concentration, although significant differences were not detected between treatments. The growth rate in the 2.5 μg L^{-1} treatment was almost half that of the control, which is consistent with the results of previous work (Lee *et al.*, 2007). The EC50 of nonylphenol for the rotifer *Brachionus* was 2.5 μg L^{-1}, indicating the potential sensitivity of that rotifer to nonylphenol. If true, such low feeding activity by the rotifer could possibly explain why *S. hantzschii* abundance increased in nonylphenol treatments.

The response of crustacean zooplankton abundance was positive to nonylphenol loading. As Lee *et al.* (2007) demonstrated, the EC50 for *Daphnia magna* was higher than the highest nonylphenol concentration in our experiment, suggesting that nonylphenol does not affect the feeding activity and growth of that species. In such a case, abundance may depend on algal prey density, as shown in the present study. Thus, these zooplankton probably experience an indirect effect when nonylphenol loading is below a lethal level.

As demonstrated by the present and previous studies, phytoplanktonic organisms are less sensitive to nonylphenol than zooplankton. Wang *et al.* (2007) proposed that a low concentration of nonylphenol may favor survival of *Microcystis* cells in their environment. Lee *et al.* (2007) investigated the EC50 for heterotrophic nanoflagellates, rotifers, and crustacean zooplankton. They observed an EC50 for *Microcystis aeruginosa* that was at least seven times higher than other planktonic organisms, leading to the prediction that nonylphenol can cause *Microcystis* blooms via release from grazing pressure. Our results support this hypothesis. In addition, release from grazing pressure alters the fitness of phytoplankton, promoting the dominance of fast-growing phytoplankton under nonylphenol stress.

Preston (2002) described one of the common indirect effects of toxicants as the release of tolerant/resistant species from competition and/or predation, resulting in a shift in ecosystem structure. Such indirect effects of toxicants are well known for zooplankton communities (Hanazato, 2001). Large zooplankton such as *Daphnia* are sensitive to pesticides, but are superior in competition between other zooplankton. In contrast, small zooplankton such as *Bosmina* and rotifers are tolerant to chemicals, but still inferior to *Daphnia*. When pesticides contaminate lakes in relatively low concentrations, small zooplankton species became dominant, while large *Daphnia* were damaged (Hanazato, 1998). The dominance of rotifers after the destruction of *Daphnia* by pesticides has been observed (Papst and Boyer, 1980; Kaushik, 1985; Yasuno *et al.*, 1988). Boyle *et al.* (1996) further described an example of the impact of environmental pollutants on interactions between zoo- and phytoplankton. They found that the pesticide diflubenzuron directly destroyed zooplankton, indirectly causing an increase in algal abundance due to reduced zooplankton grazing (Boyle *et al.*, 1996). Logically, such an indirect effect on phytoplankton communities by toxicants should also exist.

In the present study, we did not measure nonylphenol concentration in the water. However, the nonylphenol concentration in the Han River has been reported as 23.2~187.6 ng L^{-1} (Li *et al.*, 2004c). They found that nonylphenol concentration in the river water tended to lower in colder season than those in warmer season, probably due to lower microbial activity under low temperature. Moreover, the concentration increased along down to the river. Sampling station of the present study was located upstream from stations investigated in Li *et al.* (2004c). Accordingly, we

believe that the nonylphenol concentration in the river water tested was negligible compared to the added nonylphenol.

As in the Han River, the nonylphenol concentration in Lake Shihwa appears to be generally negligible. However, Li *et al.* (2004b) reported a high concentration of nonylphenol in the lake once during a year of monitoring. This observation shows that planktonic communities that include sensitive species can experience a sudden increase in nonylphenol. The present study emphasizes that short exposure to environmental pollutants, even in low concentrations, can cause shifts in the planktonic community structure.

The response of *C. ovata* to nonylphenol was very different from that of *S. hantzchii*. *Cryptomonas* is known to be mixotrophic (Jones, 2000). Autotrophic organisms appear to be less sensitive than heterotrophic ones. It is possible that consumption of prey containing nonylphenol is lethal. Differences in sensitivity in relation to trophic mode should be clarified in the future.

In general, biological interactions such as competition and predation can play a significant role in determining phytoplankton community structure. Differences in sensitivity to toxicants influence these interactions and cause indirect effects on community structure and function. The result of the present study provides an example to explain how a toxicant affects on phytoplankton community composition and size. Further study is needed to generalize the indirect effect and to predict the effect of toxicants on biological community.

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