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Effect of Temporary Loading of Nonylphenol on a Summer Planktonic Community in a Eutrophic Pond

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Recent studies reveal one of the representative endocrine disrupters of nonylphenol affects on the composition of a planktonic community. Since nonylphenol is sometimes discharged into eutrophic waters, we monitored planktonic community composition of a eutrophic pond after receiving nonylphenol when cyanobacterium *Microcystis aeruginosa* mainly dominated. The experiment was carried out two times using small-scale microcosms in a laboratory. In both two experiments, ciliate abundances significantly decreased when nonylphenol was added. On the seventh day, the ciliate abundances in 10 μg L^{-1} added treatments decreased by 36.9% in the first experiment and 33.6% in the second, when compared to the control. The response of other planktonic groups was less obvious to nonylphenol addition. In particular, in the first experiment, Chl. *b*/Chl. *a* and Chl. *c*/Chl. *a* significantly increased with the addition of nonylphenol, while total Chl. *a* concentration did not change. Indeed, bacillariophyceae and chlorophyceae abundances tended to increase with nonylphenol dosing. From these results, we tentatively hypothesized that nonylphenol loading positively affects on abundances of edible phytoplankton such as *Scenedesmus* spp. and diatoms by releasing from grazing pressure due to decrease in ciliate abundances. The present study emphasizes that the indirect effect of endocrine disrupters should be paid more attention when freshwater resources are polluted by them.

Key words : endocrine disrupting chemicals, nonylphenol, phyto-zooplankton community, *Microcystis aeruginosa* bloom

INTRODUCTION

Nonylphenol is an endocrine disrupter (Lech *et al.*, 1996; Keith *et al.*, 2001; Jobling *et al.*, 2004), and a degradation product in alkyphenol and nonylphenol polyethoxylates that are widely used as surfactants in domestic and industrial detergents throughout the world (Giger *et al.*, 1984). The source of pollutants in urban and industrial discharges has remained in the water column for a long time due to the low decomposition. The pollutants do also impact not only the reproductive behavior of fish (Gray *et al.*, 1997; Yadetie *et al.*, 2002), but also the growth of the planktonic community (Radix *et al.*, 2002; Lee *et al.*, 2007). A variety of ecotoxicological tests have been designed to assess several effects, since the toxicity impacts at multiple levels including molecules, tissues, organs, individuals, populations and communities (Hanazato, 1998a, b, 2001; Lee *et al.*, 2007). However, accurate prediction of the response of the planktonic community to nonylphenol pollution is difficult because natural ecosystems are diverse and the effects are also complicated.

In the Han River water in Korea, the nonylphenol concentration has been measured to be

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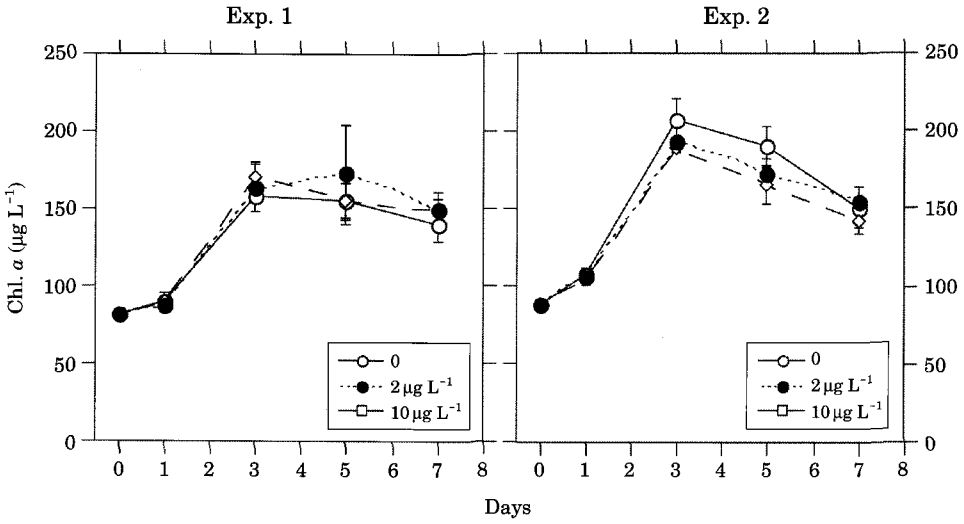

Fig. 1. Changes in chlorophyll *a* concentration in microcosm experiments. Values are the mean of triplicates, and error bars represent the standard deviation.

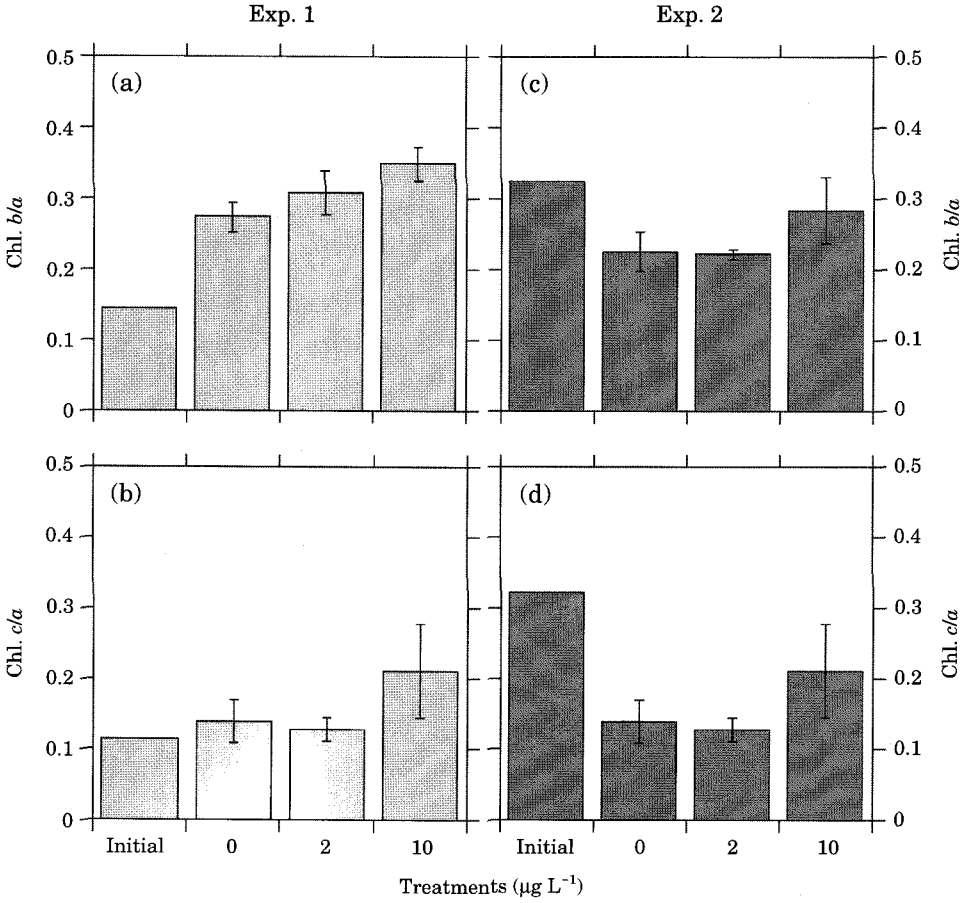

Fig. 2. Changes in chlorophyll composition of Exp. 1 (left) and Exp. 2 (right) in microcosm experiments. Chl. *b*/Chl. *a* and Chl. *c*/Chl. *a* ratio in the initial day compared to the in the final day. Values are the mean of triplicates, and error bars represent the standard deviation.

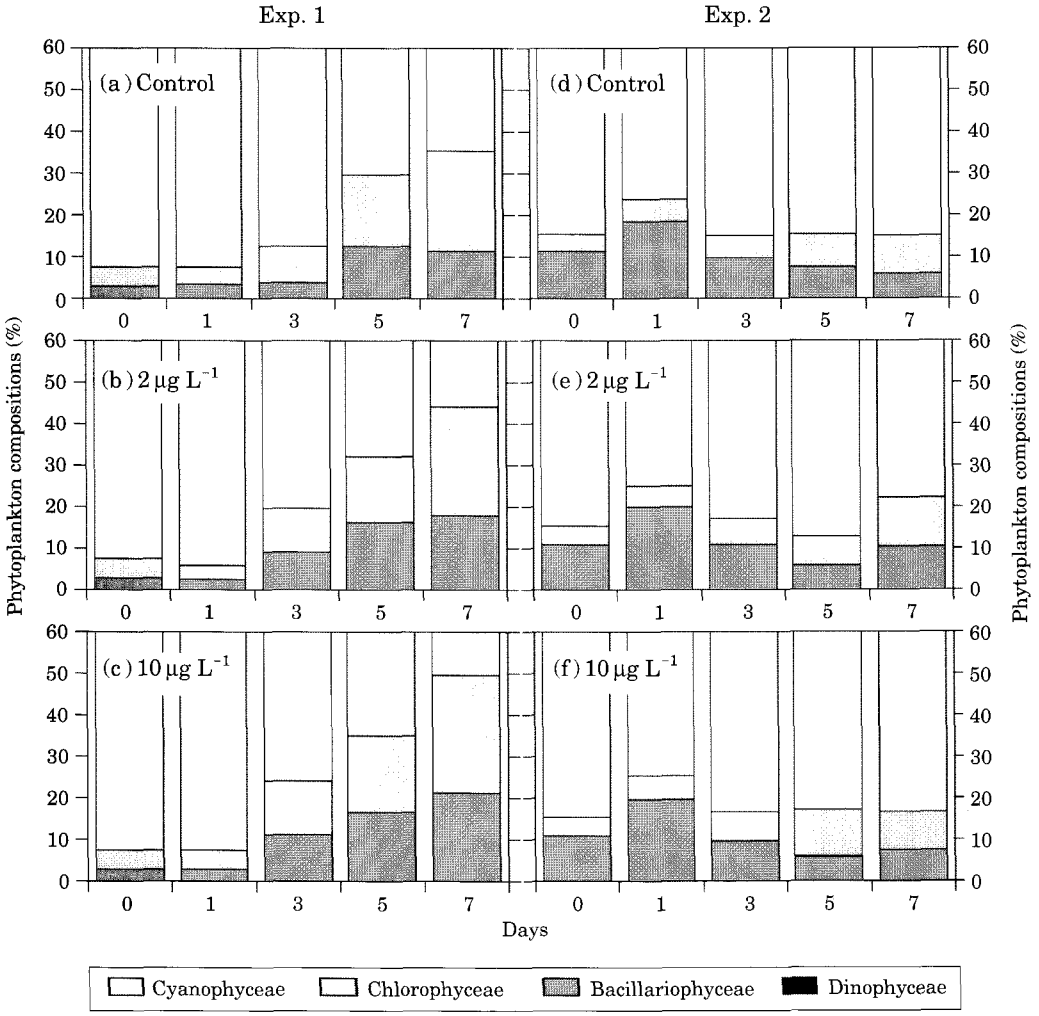

Fig. 3. Changes in dominant phytoplankton composition of Exp. 1 (left) and Exp. 2 (right) in microcosm experiments.

from 23.2 to 187.6 ng L^{-1}, which is a negligible level (Li *et al.*, 2004a). In particular, the total concentration of nonylphenolic compounds ranged from 0.015 to 36. 4 μg L^{-1} (average: 1.0 μg L^{-1}) in Masan Bay (Li *et al.*, 2008). However, a much higher concentration of nonylphenol, 41.3 μg L^{-1}, has been measured in Lake Shihwa, which is one of the most polluted in Korea. The high pollution is largely a result of the industrial environments surrounding the lake, although the nonylphenol concentration in the lake typically ranges between 33 and 275 ng L^{-1} (Li *et al.*, 2004b, c). These reports imply that plankton communities in such environments are intermittently exposed to large nonylphenol discharges, making it important to understand their plankton response to nonylphenol.

In the present study, we aimed to understand the impact of nonylphenol in freshwater, especially in the water's phyto-zooplankton community. In the summer, a freshwater phytoplankton community is mainly dominated by *Microcystis aeruginosa* (Kim *et al.*, 2003, 2006). The simplicity of the summer phytoplankton community made it easier to study the response of the planktonic community to nonylphenol. Here, we examined using small microcosms to indicate the possible direct and indirect effects of nonylphenol on a freshwater phytoplankton community, even at relatively low concentrations.

MATERIALS AND METHODS

One water sample was collected at two dif-

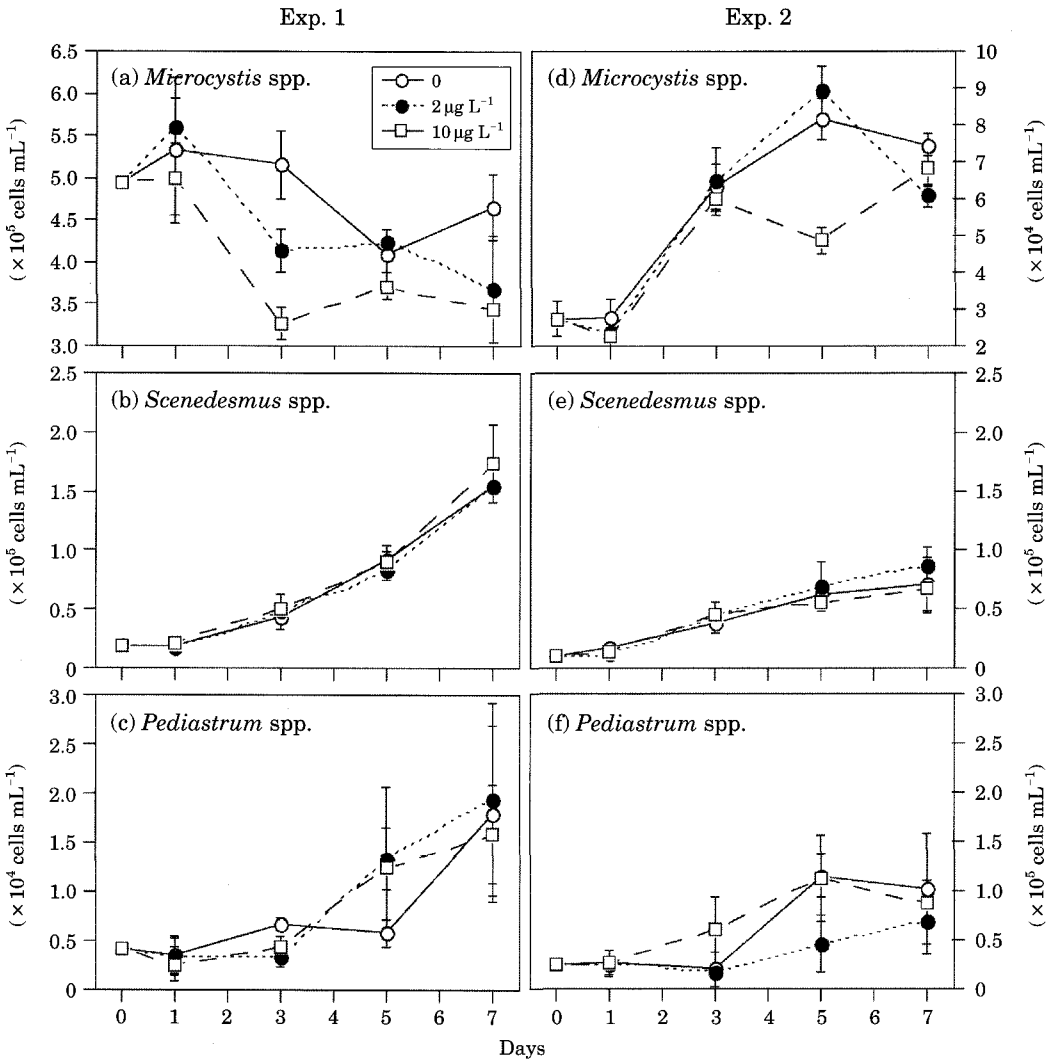

Fig. 4. Changes in *Microcystis* spp., *Scenedesmus* spp., and *Pediastrum* spp., abundances in microcosm experiments. Values are the mean of triplicates, and error bars represent the standard deviation.

ferent times from Lake Ilgam: one was taken on 5 September 2007 for Exp. 1, and the second was taken on 14 September 2007 for Exp. 2. Lake Ilgam is a eutrophic lake located on the campus of Konkuk University, Korea (37° 32′ 22″N, 127° 04′36″E). It has a surface area of 66,116 m^2 and a maximum depth of c.a. 2 m. It is densely populated by cyanobacteria, such as *Microcystis aeruginosa*, from early summer to fall each year (Kim *et al.*, 2003).

The water temperature, DO, and the pH of each water sample were around 25°C, 9.0 mg L^{-1} and 8.0, respectively. Although the nutrient levels of the water samples did not determine, we adjusted the C medium based on the N 100 μM (final concentrations) in both experiments (Lee *et al.*, 2005, 2007). Also, the pH in the Exp. 2 was only adjusted by 9.5, which was the optimum condition for culturing *M. aeruginosa* (Kim *et al.*, 2006; Lee *et al.*, 2007). A total of nine 6 L plastic containers for each experiment were prepared. Five liters of the lake water were poured into each container and incubated at 25°C under 70 μmol photons m^{-2} sec^{-1} with a 12 L : 12 D cycle. Nonylphenol in each time was adjusted with concentrations of control (0), 2, and 10 μg L^{-1} according to the method of Lee *et al.* (2007) in which the nonylphenol stock solutions were made by dissolving acetone in distilled water. The stock solutions directly added to the test water of 6 L plastic containers.

Table 1. Result of ANOVA test in Exp. 1. Values are means at final day of the incubation ± standard deviation.

Item	Control (a)	2 μg L^{-1} (b)	10 μg L^{-1} (c)	*F*	*P*
Chl. *a* (μg L^{-1})	140.0±11.4	149.5±7.0	150.0±11.2	0.93	0.44
Chl. *b* (μg L^{-1})	38.4±5.5	46.0±3.8	52.5±6.9	4.88	0.05
Chl. *c* (μg L^{-1})	21.9±4.0	28.0±1.7	33.0±6.4	4.70	0.05
Chl. *b*/Chl. *a*	0.27±0.02	0.31±0.03	0.35±0.02	6.45	0.03 (a vs c)*
Chl. *c*/Chl. *a*	0.16±0.02	0.19±0.01	0.22±0.03	8.22	0.02 (a vs c)*
Phytoplankton (cells mL^{-1})					
Total phytoplankton	893,000±76,000	833,000±59,300	869,000±74,400	0.56	0.60
M. aeruginosa	466,000±96,300	368,000±62,300	344,000±80,400	6.78	0.03 (a vs c)*
Pediastrum spp.	18,000±8,970	19,300±9,820	15,800±5,000	0.14	0.87
Nitzschia spp.	1,560±1,020	667±667	0	3.7	0.09
Scenedesumus spp.	155,000±14,600	154,000±2,780	174,000±32,800	0.86	0.47
Total bacillariophyceae	80,700±56,600	117,000±15,900	145,000±13,100	2.59	0.15
Total dinophyceae	26.3±3.4	30.2±5.2	28.5±0.64	0.88	0.46
Total chlorophyceae	173,000±13,700	174,000±7,670	190,000±33.800	0.57	0.58
M. aeruginosa/total phyto-	0.52	0.44±0.05	0.40±0.04	9.39	0.01 (a vs c)*
Diatom/total phyto-	0.78±0.04	0.69±0.04	0.76±0.05	3.67	0.09
Chlorophyceae/total phyto-	0.2±0.03	0.21±0.02	0.22±0.02	0.614	0.57
Ciliate (cells mL^{-1})					
Small ciliate (<40 μm)	322±11.7	220±20.4	201±4.9	63.2	0.001 (a vs b, c)**
Large ciliate (>40 μm)	0	2.0±0.8	1.5±0.6	8.82	0.01 (a vs b)*
Rotifer (ind. L^{-1})					
Brachionus spp.	130±37	115±77	190±136	0.54	0.60
Kellicottia spp.	218±88	35±48	75±54	1.30	0.34
Keratella spp.	120±40	95±9	130±31	1.11	0.38
Polyarthra spp.	45 ±30	35±23	5±8	2.6	0.15
Crustacean (ind. L^{-1})					
Bosmina spp.	710±112	625±31	660±52	1.00	0.42
Nauplius	120±30	40±45	30 ±15	6.79	0.02
Copepod	595±70	810±79	550±15	14.6	0.004 (b vs a, c)**

*$P<0.05$, **$P<0.001$; the difference between control and treatments.

Since acetone was highly diluted (78.8 μg L^{-1} in nonyphenol 10 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 acetone added treatment as control. All experiments were carried out in triplicate. Water samples were collected 0, 1, 3, 5 and 7 days after the addition of nonylphenol. A portion of water sample was fixed with Lugol solution at a final concentration of 1% to measure phytoplankton and ciliates population densities. Another portion of sample was fixed with buffered formalin for zooplankton counting. These fixed samples were stored at 4°C in dark condition. Then a 50 mL portion of each water sample was filtered through a GF/F filter to estimate the chlorophyll concentration of the water. The filter was soaked with 90% acetone overnight under dark conditions at 4°C. The extracted chlorophyll (Chl) *a*, *b* and *c*, were measured with a spectrophotometer and quantified according to the equation of Jeffrey and Humphery (1975).

The cell density of cyanophyceae *M. aeruginosa* was enumerated using a haemocytometer under a light microscope. Bacillariophyceae and chlorophyceae were directly counted under a light microscope at magnifications of ×200 and ×400, respectively Ciliates and dinophyceae were enumerated using a light microscope with magnification ×200 after the samples were densely collected by 4 times. Using a light microscope with ×200 magnification, zooplanktons, including rotifer, were counted after the 500 mL sub-samples were densely collected by 10 times after gentle reverse filtration through a 20 μm Nitex mesh.

A one-way analysis of variance (ANOVA) was carried out to test for differences among the treatments. Multiple comparisons were subsequently carried out using Tukey's test with a discrimina-

Table 2. Result of ANOVA test in Exp. 2. Values are means at final day of the incubation ± standard deviation.

Item	Control (a)	2 μg L^{-1} (b)	10 μg L^{-1} (c)	*F*	*P*
Chl. *a* (μg L^{-1})	151±13.1	142±8.7	154±3.0	1.49	0.30
Chl. *b* (μg L^{-1})	34±6.6	31±1.0	40±1.0	4.25	0.07
Chl. *c* (μg L^{-1})	21.1±5.6	18.4±3.5	32.6±9.7	3.72	0.09
Chl. *b*/Chl. *a*	0.23±0.03	0.22±0.01	0.26±0.01	4.76	0.06
Chl. *c*/Chl. *a*	0.14±0.03	0.13±0.02	0.21±0.07	3.24	0.11
Phytoplankton (cells mL^{-1})					
Total phytoplankton	960,000±87,900	878,000±25,500	896,000±28,800	1.83	0.24
M. aeruginosa	746,000±30,900	609,000±29,200	681,000±44,900	11.0	0.009 (a vs b, c)*
Pediastrum spp.	10,200±5,670	68,900±3,420	86,700±2,400	0.50	0.63
Nitzschia spp	889±1,020	667±667	222±385	0.63	0.56
Scenedesumus spp.	71,300± 23,400	87,600±14,400	68,400±22,200	0.77	0.51
Total bacillariophyceae	51,300±7,420	80,200±22,900	60,400±17,700	2.20	0.19
Total dinophyceae	41.8±3.6	60.4±20.2	29.5±9.7	4.22	0.07
Total chlorophyceae	81,600±26,000	94,400±1,710	77,100±21,600	0.51	0.62
M. aeruginosa/total phyto-	0.78±0.04	0.69±0.04	0.76±0.05	3.67	0.09
Diatom/total phyto-	0.05±0.01	0.09±0.03	0.07±0.02	3.09	0.12
Chlorophyceae/total phyto-	0.08±0.02	0.11±0.02	0.09±0.02	1.18	0.37
Ciliate (cells mL^{-1})					
Small ciliate (< 40 μm)	131±8.0	104±6.1	88±4.8	41.1	0.001 (a vs b, c)**
Large ciliate (> 40 μm)	3.3±1.9	1.1±1.1	0.4±0.6	4.00	0.07
Rotifer (ind. L^{-1})					
Brachionus spp.	135±15	220±85	190±92	1.05	0.41
Kellicottia spp.	15±15	30±26	10±8.6	1.00	0.42
Keratella spp.	455±105	455±95	400±113	0.27	0.77
Polyarthra spp.	85±34	75±45	110±60	0.42	0.67
Crustacean (ind. L^{-1})					
Bosmina spp.	325±17	330±75	430±151	1.09	0.39
Nauplius	0	15±15	15±15	1.50	0.29
Copepod	360±119	420±143	220±73	2.36	0.18

*$P < 0.05$, **$P < 0.001$; the difference between control and treatments.

tion level of $p=0.05$.

RESULTS

Nonylphenol dosing experiments were carried out twice (Exp. 1 and Exp. 2). The concentration of chlorophyll *a* (Chl. *a*) in Exp. 1 increased from one day to three days, and then reached plateaus until the end of the experiment. In the Exp. 2, after Chl. *a* concentrations also increased dramatically until the third day, and then the concentrations gradually decreased in all treatments. Although the Chl. *a* concentration in nonylphenol treatments in Exp. 1 was relatively higher than that in controls, the response of Chl. *a* to the nonylphenol addition in Exp. 2 was opposite to that observed in Exp. 1 (Fig. 1). The compositions of chlorophyll in the initial and final day are shown in Fig. 2. In Exp. 1, Chl. *b*/Chl. *a* and Chl. *c*/Chl. *a* ratios in the final day compared to the initial day, showed a gradual increase with increasing nonylphenol dosing. As reflected in the total chlorophyll compositions, phytoplankton composition was also changed by nonylphenol dosing (Fig. 3). Cyanophyceae on the initial day of Exp. 1 and Exp. 2 occurred over 92% and 85% of total phytoplankton abundances, respectively. In Exp. 1, cyanophyceae in nonylphenol treatment decreased from 3 to 7 days than those of the control, whereas those of chlorophyceae and bacillariophyceae gradually increased with increasing of nonylphenol dosing. However, such trend was not observed in Exp. 2, as above described.

Microcystis aeruginosa dominated the water sample of both experiments. Among the phytoplankton communities, population densities of *Synedra* spp., *Scenedesumus* spp., *Nitzschia* spp., *Cryptomonas ovata, Chlamydomonas* spp., *Pediastrum* spp., and *Peridinium* spp. were relatively

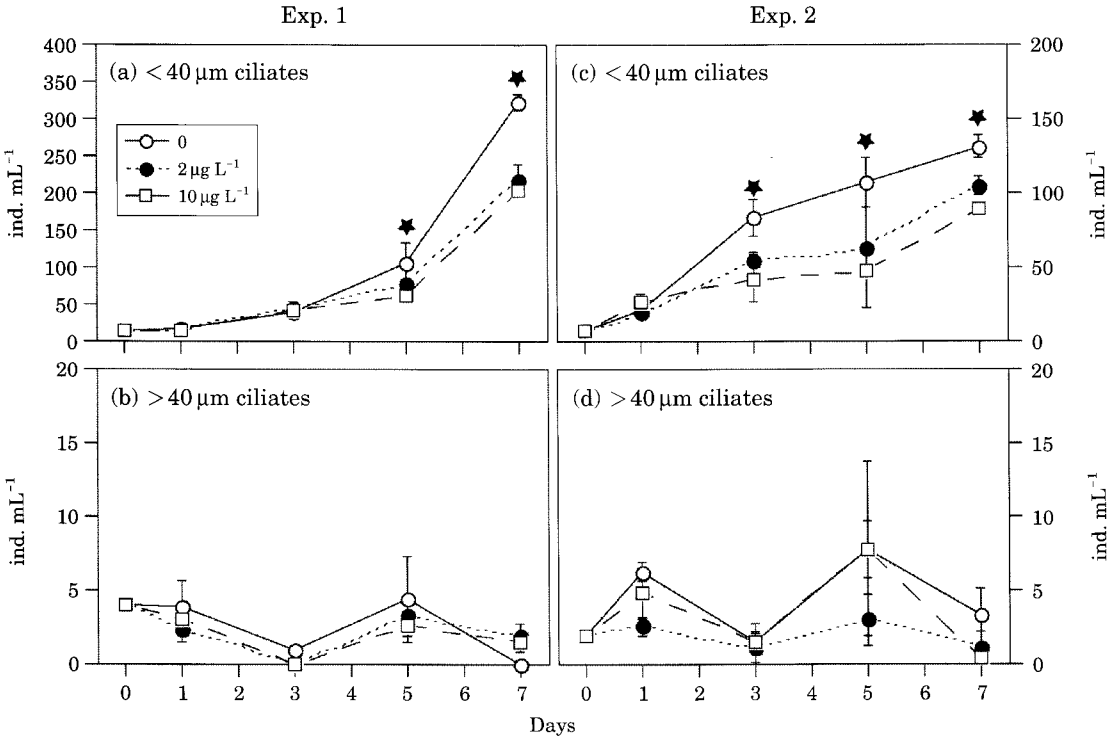

Fig. 5. Changes in small ciliate (< 40 μm) and large ciliate (> 40 μm) abundances in microcosm experiments. Values are the mean of triplicates, and error bars represent the standard deviation. Star marks indicate significant difference (ANOVA) from treatments ($p < 0.01$).

higher. The response of *M. aeruginosa* was quite similar to changes in the Chl. *a* concentration (Figs. 1 and 4). Phytoplankton communities including *M. aeruginos* were not directly affected, even at 10 μg L^{-1}, which was the maximum dosing concentration used in this study (Figs. 4 and 5).

Small ciliates (< 40 μm) grew better in the control than did those in 2 and 10 μg L^{-1} treatments in both experiments. In contrast, the abundance of large ciliates (> 40 μm) gradually declined in all treatments, suggesting no effects of nonylphenol on large ciliates (Fig. 5).

Among the rotifers, *Brachionus* spp., *Kellicottia* spp. *Keratella* spp., *Polyarthra* spp., and *Trichocerca* spp. were dominant in both experiments (Fig. 6). The genus *Brachionus* grew better in all treatments during incubation. In contrast, the abundance of rotifers other than *Brachionus* gradually decreased in the later incubation period. Most rotifers and crustacean-zooplankton did not respond to the nonylphenol dosing. Abundances of small crustacean-zooplankton, like *Bosmina* and copepod, gradually increased, whereas abundances of nauplius declined to the lowest level after 7 days. This implied that the nauplius may have become to the adult.

The ANOVA of these results are shown in Tables 1 and 2. A high abundance of *M. aeruginosa* was detected in the control of Exp. 1, although a difference of the abundance of *M. aeruginosa* was not observed between control and treatments in Exp. 2. Although large ciliates responded in nonylphenol dosing the difference was not significant, effects of nonylphenol dosing in small ciliates communities were significantly observed. Also, significant differences of the abundance of most rotifer species and crustacean-zooplankton were not observed between control and nonylphenol dosing treatments.

DISCUSSION

The effects of toxicants for nonylphenol are typically evaluated intensively in individual species. Such evaluations provide important information in determining the risk of chemical exposure. In particular, the primary producer, including cyano-

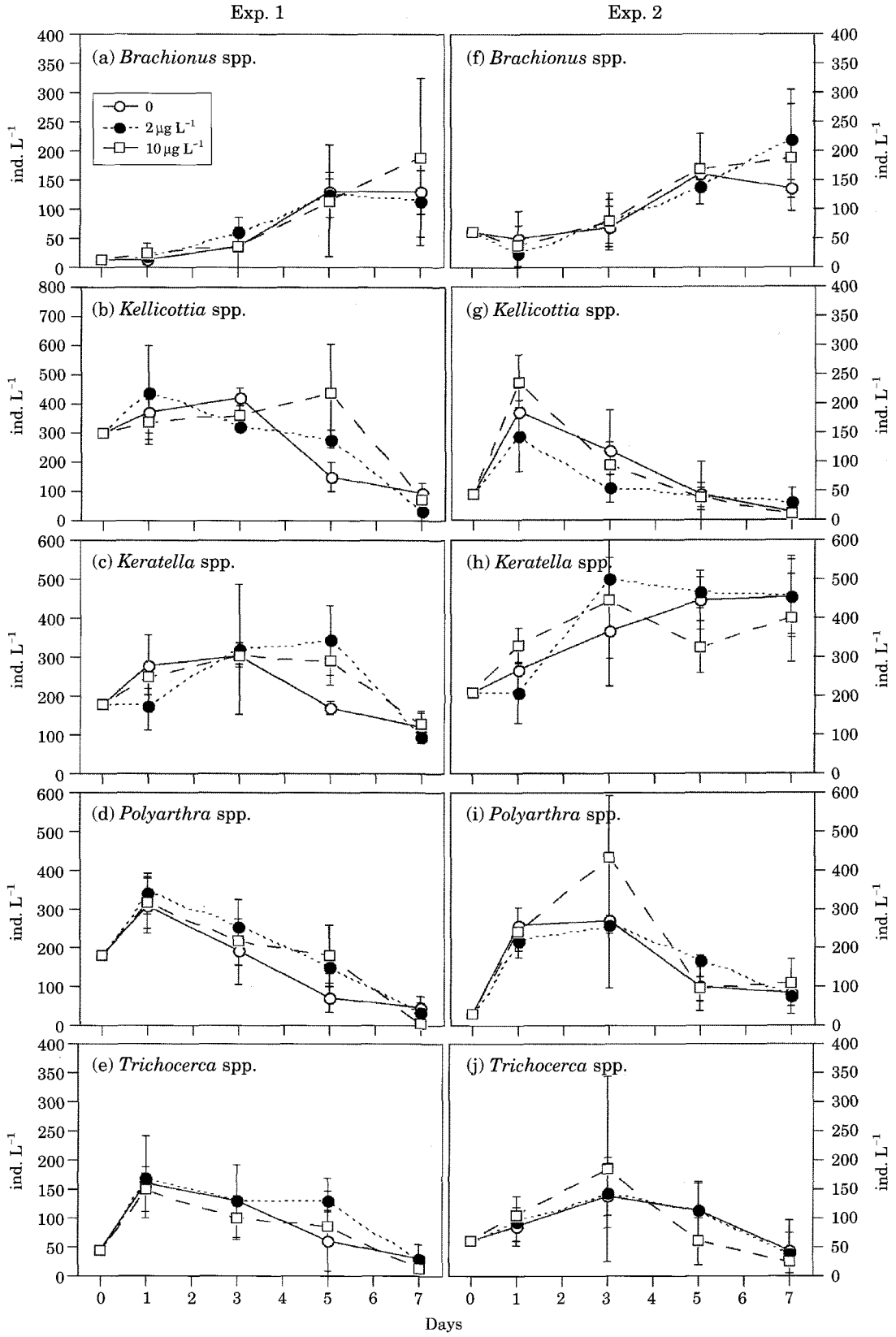

Fig. 6. Changes in rotifer abundances in microcosm experiments. Values are the mean of triplicates, and error bars represent the standard deviation.

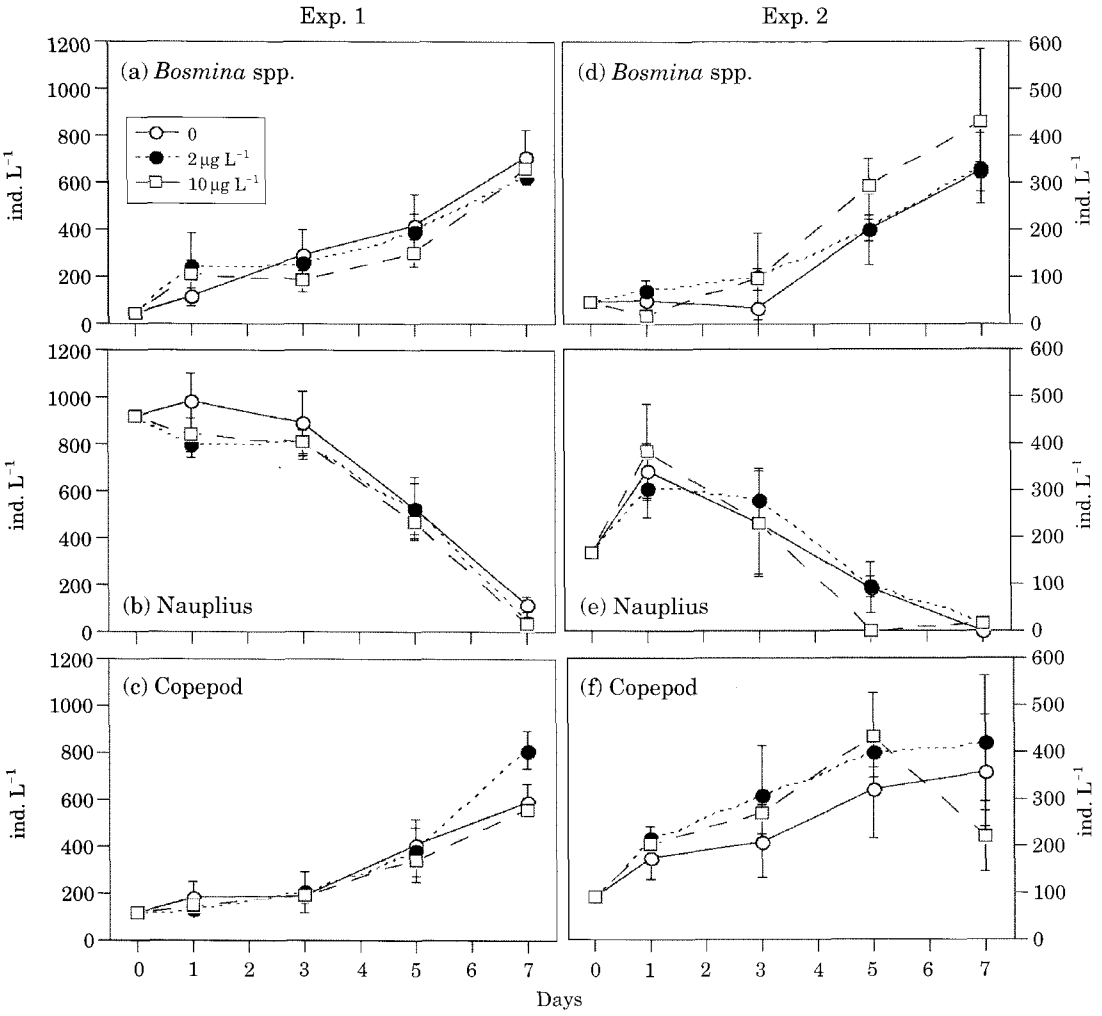

Fig. 7. Changes in crustacean zooplankton abundances in microcosm experiments. Values are the mean of triplicates, and error bars represent the standard deviation.

phyceae, tends to tolerate nonylphenol concentrations better than high tropic level organisms such protists or zooplankton (Hanazato, 2001; Macial *et al.*, 2003; Lee *et al.*, 2007; Wang *et al.*, 2007), suggesting that nonylphenol does cause a shift in a planktonic community structure. The present study leads to such a hypothesis.

Among the planktonic groups evaluated in the present study, small ciliates seemed to be sensitive to nonylphenol loading. Small ciliates (<40 μm) in the nonylphenol dosing treatment did not grow well compared to the control (Fig. 5). Since ciliates play a significant role in the planktonic food web (Pierce and Turner, 1992), the sensitivity of small ciliates possibly causes the shift in composition of planktonic community.

In the present study, inedible phytoplankter of *Microcystis* dominated the tested pond water. Wang *et al.* (2007) proposed that growth of *Microcystis* cells was quite suitable enough for creating a low-nonylphenol concentration. Lee *et al.* (2007) also reported that *M. aeruginosa* grew well, even at 50 μg L^{-1}, at relatively higher nonylphenol levels. They also observed an EC50 for *M. aeruginosa* that was at least seven-folds higher than other planktonic organisms. Thus, it is likely that the growth of *Microcystis* was not affected by the nonylphenol dosing. Indeed, the abundance of *Microcystis* and Chl. *a* concentration in both experiments were not affected by the addition of nonylphenol.

However, we found significant shifts in the phytoplankton community composition. Chl. *b*/Chl. *a* and Chl. *c*/Chl. *a* increased significantly com-

pared to the control. Moreover, bacillariophyceae and chlorophyceae, abundances slightly increased in nonylphenol-added treatments although the difference was insignificant. The observed shift in the composition of phytoplankton in both experiments (Figs. 2 and 3) may be caused indirectly, since *Microcystis* spp. growth was not affected by nonylphenol, as mentioned above.

The genus of *Scenedesumus* is a representative phytoplankton species in eutrophic waters and it is a suitable food source for ciliates and zooplankton. Unidentified diatoms found in the present study were mostly small and edible. Thus, we hypothesized that 1) nonylphenol negatively affects ciliate abundance, 2) decreased ciliate abundance caused lowering of grazing pressures on edible phytoplankters, such as bacillariophyceae and chlorophyceae including *Scenedesmus* spp., and 3) the composition of phytoplankton shifted by the increase in abundance of fast growing but edible species. Although experimental analysis is required to prove this hypothesis, we emphasize that biological interactions such as competition and predation play significant roles in indirect impacts induced by nonylphenol in the planktonic community. Consequently, such indirect toxicity effects are important in predicting the response of an ecosystem structure and function to anthropogenic contamination.

The significance of the indirect effects of toxicity in aquatic ecosystems was reviewed by Preston (2002) and Fleeger *et al.* (2003). The differences in their organism sensitivity to nonylphenol might be triggered to the change of the potential community succession through the planktonic food web structure in the aquatic ecosystem. According to Wang *et al.* (2007) and Lee *et al.* (2007), cyanophyceae *Microcystis* bloom was stimulated with nonylphenol loading into freshwater environments. However, heterotrophic flagellates are also sensitive to nonylphenol, especially for *Diphylleia rotans* (EC50 for growth rate; 3.49 $\mu g\ L^{-1}$, as negligible level) (Lee *et al.*, 2007). Accordingly, the effects of nonylphenol in natural planktonic communities, as complex assemblages, are not well understood because most study was remained single species tests.

In conclusion, the present study suggests that the biological interaction sometimes amplifies the impact of environmental pollutants on a certain functional group of a plankton community. In determining a phytoplankton species composition, biological interactions, such as competition and predation, sometimes play significant roles compared to direct effect. Therefore, more attention should be paid to indirect effect to predict the response to anthropogenic inputs.

ACKNOWLEDGEMENTS

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